A. A. Mohamed Hatha
P. Lakshmanaperumalsamy
Editors

A Closer Look at Actinomycetes

BACTERIOLOGY RESEARCH DEVELOPMENTS

NOVA

BACTERIOLOGY RESEARCH DEVELOPMENTS

A CLOSER LOOK AT ACTINOMYCETES

BACTERIOLOGY RESEARCH DEVELOPMENTS

Additional books and e-books in this series can be found on Nova's website under the Series tab.

BACTERIOLOGY RESEARCH DEVELOPMENTS

A CLOSER LOOK AT ACTINOMYCETES

A. A. MOHAMED HATHA
AND
P. LAKSHMANAPERUMALSAMY
EDITORS

nova
science publishers
New York

Library of Congress Cataloging-in-Publication Data

Names: Hatha, A. A. Mohamed, editor. | Lakshmanaperumalsamy, P., editor.
Title: A closer look at actinomycetes / [edited by] A.A. Mohamed Hatha, P. Lakshmanaperumalsamy.
Description: New York : Nova Science Publishers, 2020. | Series: Bacteriology research developments | Includes bibliographical references and index. |
Identifiers: LCCN 2019058239 (print) | LCCN 2019058240 (ebook) | ISBN 9781536170467 (hardcover) | ISBN 9781536170474 (adobe pdf)
Subjects: LCSH: Actinobacteria.
Classification: LCC QR82.A35 C57 2020 (print) | LCC QR82.A35 (ebook) | DDC 579.3/21--dc23
LC record available at https://lccn.loc.gov/2019058239
LC ebook record available at https://lccn.loc.gov/2019058240

Published by Nova Science Publishers, Inc. † New York

The actinomycetes play a key role in different environmental processes which are mediated by their ability to produce a wide range of metabolites. While recognized as a source of antibacterial agents, the potential applicability of actinomycetal metabolites is immense, varying from agricultural use as plant growth promoter to possible applications in cancer therapy. "A Closer Look at Actinomycetes" explores this fascinating and amazing bacterial genus providing a comprehensive approach to these microorganisms and their metabolites.

Joaquim Ruiz
Researcher
Universidad Científica del Sur
Lima, Peru

CONTENTS

PREFACE

Actinomycetes are a versatile group of Gram positive bacteria widely distributed in the terrestrial and aquatic environments. The specialty of the actinomycetes is that they have a mycelial appearance unlike most bacteria. This group of bacteria is well known for their ability to produce a range of bioactive molecules, including antibiotics and various kinds of enzymes. As they are known for their ability to produce various antibiotics, the actinomycetes are widely explored by various research groups in search of novel drug molecules. Since the cultivation and maintenance of actinobacteria are not that easy as in the case of other bacteria, they are rather underexplored.

With the frequent emergence of multidrug resistant bacteria, which are outpacing the discovery of new antibiotics, there is a renewed interest in actinomycetes from special habitats such as extreme habitats in the marine environment, salt pans, geothermal springs, permanently frozen polar environments etc. Endophytic actinomycetes are also attracting the attention of current researchers in this field. This book titled "A Closer Look at Actinomycetes" is a compilation of articles which deals with interesting topics such as "actinomycetes as microbial drug factories", endophytic fungi from special habitats of Pakistan as well as strategies for exploration of actinomycetes diversity and the taxonomy of actinomycetes should be of

great interest to those who are interested in Actinomycetes research. People with interest in general microbiology will also find it an interesting read.

Chapter 1 - Natural products possess the novel sources of diverse microorganisms that have potential medicinal values. Among them, actinomycetes demonstrate a promising group. Actinomycetes are prokaryotes and ubiquitous, producing aerobic spore, which belong to the order Actinomycetales characterized with high G+C DNA content in their genome. They unveil an array of morphological differentiation because of their mycelial lifestyle. Owing to their large genome with numerous transcription factors controlling various gene expression, they are stable and possess persistent metabolic diversity. Actinomycete is a good model for the production of important metabolites and enzymes which are achieved as a result of their unique metabolic and physiological capabilities. About 45% of the total biologically active compounds are produced by actinomycetes. They are also the most significant synthesizers of economically and biotechnologically important metabolites. In the past decades, a large number of actinomycetes have been isolated and screened as potential source, accounting for 70%-80% of the relevant bioactive compounds that are commercially available from various geographic regions around the world. The bioactive metabolites of actinomycetes have important applications in human medicine because of their therapeutic superiority against diverse disorders. Members of this group can be considered the source of a large pool of antibacterial (streptomycin, tetracycline, chloramphenicol), antifungal (nystatin), immunosuppressive (rapamycin, cyclosporine), antiviral (tunicamycin), antitumor (actinomycin, mitomycin C, anthracyclines), antidiabetogenic (bafilomycin, streptozotocin), antiparasitic (avermectin), hypercholesterolemia (statins such as lovastatin and mevastatin) and enzyme inhibitory (clavulanic acid) compounds. Additionally, actinomycetes have tremendous potential for producing clinically proven antitumor drugs such as anthracyclines (aclarubicin, daunomycin, and doxorubicin), aureolic acids (mithramycin), enediynes (neocarzinostatin), peptides (bleomycin and actinomycin D), carzinophilin, antimetabolites (pentostatin), and mitomycins. This indicates that the products of actinomycetes possess pharmacokinetic properties, thus

occupying a prominent position in clinical research and development. Their proven ability to produce complicated compounds exhibiting excellent bioactive potency has attracted the scientific community to further explore and understand actinomycetes species, which are dealt with in detail in the chapter.

Chapter 2 - The Indo-Pak region of the subcontinent that includes Pakistan and India has a long history of traditional medicines. In Pakistan, Unani-Tibb (Graeco-Arabic) medicine system has been in practice for centuries with the local population still relying on the Unani practitioners. In India, the Ayurveda system of medicine has deep historical roots. Both the systems rely on ethnobotany i.e., traditional knowledge of local plants.

With the authors' recent understanding of endophytes that are the commensal symbionts residing within the plants, the medicinal use of such plants is connected as much as to these migrant organisms as to their biochemistry. One of such symbionts are the actinomycetes, the well-known producers of antibiotics that have been extensively isolated from soil; the researchers have now turned their attention towards those that have moved inside the plants. If the source plant has been used in traditional medicines for healing purposes then the actinomycetes residing within them are more likely candidates for novel drug sources. The most notable example of this hypothesis is the potent peptide antibiotic munumbicins produced by the *Streptomyces* NRRL-30562 resident of the endosphere of the Snake Vine plant, *Kennedia nigricans*. In this chapter, the authors will focus on the important medicinal plants in the Indo-Pak region and the recent studies on the diversity of their endophytic actinomycetes. Besides, the authors will emphasize the diverse metabolites produced by them and their bioactivities as well.

Chapter 3 - *Amycolatopsis* strains are aerobic, Gram-positive, non-acid-fast, filamentous, squarish and rod-shaped fragments on the substrate and aerial mycelia. *Amycolatopsis* species are alkaliphilic, mesophilic, thermophilic and pathogenic bacteria. They were distributed in soils, plants, freshwater, salt lakes, rock, ocean sediments, clinical samples from humans and horses, sugar cane bagasse, natural caves, a mine and a catacomb, equine placenta, volcanic soil, rhizospheric soil, polluted sediment, and arid soil.

Amycolatopsis strains are generally isolated by the serial dilutions of soil sample on actinomycetes isolation agar, humic acid-vitamin (HV) agar, starch-casein agar, casein mineral agar, oatmeal agar, asparagine agar, ISP2 medium, brain heart infusion agar, GTY agar, medium MY10S, NY medium, SM1, SM2 and SM3 agar. They can be distinguished from other genera by using morphological and chemotaxonomic characteristics and by using genus-specific oligonucleotide primers based on 16S rRNA gene sequences. *Amycolatopsis* strains produced various bioactive compounds such as antibacterial, antifungal, antiviral, immunosuppressant and antitumor compounds, especially *A. mediterranei* and *A. rifamycinica* (ansamycin-type antibiotic rifamycin, tolypomcins), *A. orientalis* (glycopeptide antibiotic vancomycin, muraceins, orienticin, quartromicin, balhimycins, etc.), *A. azurea* (azureomycins and octacosamicins) and *A. lurida* (benzathrins, ristocetin). The ansamycin and glycopeptide classes have efficient to medicine. In addition, they have ability to degrade biopolymers such as poly (L-lactic acid) (PLA), poly(ε-caprolactone) and poly(β-hydroxybutyrate). *A. orientalis* subsp. *orientalis*, *A. thailandensis* and *A. samaneae* produced three novel PLA-degrading enzymes named PLAase I, II and III. The copper-resistant *Amycolatopsis* strain was applied in bioremediation biotechnologies. *Amycolatopsis* strains are known to have chitinase for antifungal, cellulase, and xylanase for biofuel production, in addition, has reported producing lipase and keratinase. This chapter describes the recent status of *Amycolatopsis* species on taxonomy, secondary metabolites and their other applications

Chapter 4 - Terrestrial actinomycetes were extensively studied and screened since the 1950s to study its effect on human health and diseases. And several studies reported terrestrial actinomycetes with anti-cancer and anti-infective properties. Initially, actinomycetes in the marine ecosystems were largely neglected assuming little incentives in isolating marine strains in discovering new drugs. However, in the last two decades, the focus was shifted towards marine actinomycetes owing to their structural diversity as well as high medicinal value. Studies have paid high dividends to researchers exploring marine ecosystems for the presence of novel strains of actinomycetes. These marine actinomycetes harbored many active

principles that are effective against various diseases. Marine actinomycetes are often referred to as goldmine due to the presence of diverse secondary metabolites. Marine actinomycetes research can lead to the discovery of many novel drug candidates that are effective against various deadly diseases like cancer, malaria, and several drug-resistant infections.

Chapter 5 - *Actinomycetes* are known for their ability to produce several antibiotics. They are widely distributed in soils, compost, freshwater, marine and plants, especially *Streptomyces* strains are abundant in soils. In recent years, *Streptomyces* strains have attracted the interest of researchers as a source of biocontrol agents and use in agriculture. Several new species have been proposed, and their abilities to control plant diseases and promoted plant growth have been uncovered. In agriculture, numerous *Streptomyces* strains have demonstrated the abilities to control the growth of bacterial and fungal phytopathogens, such as *Fusarium* wilt by *Fusarium oxysporum*, anthracnose by *Colletotrichum gloeosporioides*, leaf spot by *Alternaria brassicicola*. Moreover, their ability in term of plant growth promoting based on indole acetic acid (IAA) production of *Streptomyces rochei* and *Streptomyces sundarbansensis* strains have been proved in various economic crops such as in wheat. The active compounds, natamycin produced from *Streptomyces lydicus*, actinomycin D from *Streptomyces mutabilis*, chrestoxanthone A from *Streptomyces chrestomyceticus* strains have been reported. In particular, some pesticides developed for agricultural management belonged to *Streptomyces*, such as actinovate® from *S. lydicus* WYEC108 and mycostop® from *S. griseoviridis* K61 were registered as commercial fungicides in Europe, Canada and USA. This chapter describes the potential of *Streptomyces* strains against plant pathogens, their isolation and cultivation methods, taxonomic information, bioactive compounds and their applications in agriculture.

Chapter 6 - Actinomycetes are aerobic, Gram-positive, filamentous bacteria presented true branching mycelia and high mol% guanine and cytosine (G+C) content in the genome. Over the last decade, actinomycetes which lived in unexplored habitats have obtained significant attention because of their large biodiversity and proper metabolites with pharmaceutical, agricultural and industrial values. Plant endosphere is an

enormous micro-ecosystem where different niches can be resided by numerous different microorganisms, and it contributed a valuable source of actinomycetes. Endophytic actinomycetes that inhabit living root tissues of plants are a relatively untapped source of novel species and potential bioactive metabolites. Based on the 16S rRNA gene sequences, the endophytic actinomycetes are belonged to members of *Streptomycetaceae*, *Streptosporangiaceae*, *Micromonosporaceae*, *Thermomonosporaceae*, *Pseudonocardiaceae* and *Actinosynnemataceae*. Presently, the strains associated with plant roots were *Streptomyces*, *Micromonospora*, *Microbispora*, *Nocardia*, *Nocardioides*, *Pseudonocardia* and *Streptosporangium*; and the new genera were *Plantactinospora*, *Phytohabitans*, *Actinophytocola* and *Allostreptomyces*. Furthermore, many groups of secondary metabolites were produced by plant-derived actinomycetes such as peptides, flavonoids, polyketides, macrolides, terpenes and alkaloids. These metabolites exhibited various biological activities: antibacterial, antifungal, anti-phytopathogens, immunosuppressant, anti-tumour and anti-cancer. This chapter highlights the achievement of isolation, identification, diversity and bioactive metabolites from endophytic actinomycetes associated with plant roots.

Chapter 7 - Actinomycetes are Gram- positive bacteria having high GC content in their genome. They are crucial from industrial perspective as they have great ability for production of bioactive secondary metabolites. Compounds produced by them possess diverse biological activities such as anticancer, antifungal, antibacterial, antiviral and belong to distinct chemical classes. Members of genus *Streptomyces* are well known producers of bioactive compounds. Due to emergence of drug resistant pathogens, there is a dire need for the discovery of new compounds having unique modes of action. During isolation and screening programme of actinomycetes carried out in the authors' laboratory, a biologically active strain was isolated from agricultural soil, Dhanaura, Uttar Pradesh, India and designated as Strain 51. Morphological and biochemical studies revealed that Strain 51 belongs to genus *Streptomyces* and it showed 100% 16S rRNA gene sequence homology with *Streptomyces griseochromogenes*. Present investigation was undertaken as an effort to extract and characterize potent compounds from

Strain 51 which are responsible for higher bioactivity. Extraction of bioactive metabolites was performed using cold extraction method taking ethyl acetate as solvent. Minimum inhibitory concentration (MIC) of compounds against *Bacillus cereus* was determined by microdilution method taking industrial antibiotic- chloramphenicol as positive control. Crude extract of Strain 51 showed inhibition of *Bacillus cereus* growth at 0.0050 mg/ml while chloramphenicol suppressed growth at 0.0075 mg/ml. Metabolomic studies were carried out for identification and structural elucidation of bioactive molecules using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS/MS) techniques. GC-MS analysis of strain 51 extract showed the presence of thirty three volatile organic constituents, out of which some are reported in literature to have diverse biological activities. Extract of Strain 51 was also subjected to LC-MS analysis which gave several sharp peaks in the spectrum. Metabolites identified in LC-MS data showed molecular ion peaks at m/z 228, 758, 548, 784 and 803. The structure was elucidated and confirmed for each peak through their mass fragmentation patterns; as a result structure of compounds were confirmed as thiolutin, streptothricin D, antimycin A, rifaximin and fujimycin. PCR was performed for detection of biosynthetic gene clusters responsible for production of bioactive compounds in Strain 51. Amplification of polyketide synthase-I (PKS-I) and non- ribosomal peptide synthetases (NRPS) was observed, the amplicons were purified and sequenced. The gene sequences were submitted in NCBI-GenBank database under accession numbers MK355718 and MK355717. In future studies, the authors' aim is to produce a novel compound by introducing genetic manipulations in these genes.

Chapter 8 - As emergence of multi drug resistant bacteria has become more frequent, the search is on for novel antibiotics. Under explored and unique habitats are being prospected for novel strains of Actinomycetes which have the potential to produce new antibiotics. Accordingly, the present study has been taken up with an objective to explore the diversity of actinomycetes from Vembandu estuary, the biggest and one of the 3 Ramsar sites in Kerala. Sediment samples were collected seasonally, using van veen grab from selected stations for a period of one year. Actinomycetes were

isolated using Kusters agar and characterized by polyphasic taxonomy. Physiological capabilities of isolates were evaluated and the antibacterial activities against a range of pathogens were studied using standard methods. Various genera of Actinomycetes such as *Actinobispora*, *Actinokineospora*, *Actinosynnema*, *Catellospora*, *Kibdelosporangium*, *Micromonospora*, *Nocardiopsis*, *Rhodococcus*, *Saccharopolyspora*, *Streptoalloteichus*, *Streptomyces*, *Streptosporangium*, *Thermoactinomyces* and atypical *Nocardia asteroids* were encountered in the lake sediments. More than 90% of the actinomycete isolates revealed good antibacterial activity against pathogenic bacteria. While 81% of them were able to produce protease, 56% of them decomposed hypoxanthine and tyrosine. Ability to decompose xanthine was relatively low (11%). Molecular identification of potential Actinomycete strains were carried out based on 16s rRNA gene, which revealed their identity as *Streptomyces olivaceus*, *Streptomyces costaricanus, Nocardiopsis flavescens* and *Nocardiopsis alkaliphila*. This study revealed that lake sediments could be good source of potential actinomycetes and reconfirms the need for exploring under explored and unexplored habitats for diverse actinomycetes which could probably yield novel antibiotics to fight the emerging threat of multidrug resistant pathogens.

Chapter 9 - Actinomycetes are Gram-positive, filamentous bacteria that formed true branching mycelia and high mol% guanine and cytosine (G+C) content in chromosomal DNA. They are widely distributed in terrestrial, aquatic and marine ecosystems and played important role in recycling of complex organic matters resulting in humus formation. Actinomycetes are normally isolated by the standard dilution plate technique using different media, for instance, humic acid-vitamin (HV) agar, starch casein nitrate (SCN) agar, glycerol arginine agar, glucose asparagine agar, Gauze mineral medium no. 1, arginine-vitamin (AV) agar, soil extract agar, water agar (WA), Küster's agar and glycerol yeast extract agar. The identification of actinomycetes are based on phenotypic and chemotaxonomic characteristics: isomers of diaminopimelic acid, whole-cell sugars, fatty acid profiles, polar lipid patterns and isoprenoid quinones, involving 16SrRNA gene sequences analysis and DNA-DNA hybridization. Presently, next-

generation sequencing (NGS) has given a rapid and cost-effective approach to obtain whole-genome sequences (WGS) of actinomycete strains. Whole genome sequence analyses of the actinomycetes were based on average nucleotide identity (ANI), and *in silico* genomic similarity with the optimum threshold ranges appropriate for species delineation. The genomic analysis not only provides reliable taxonomic position, but also gives invaluable insights into the biology of actinomycetes.

In: A Closer Look at Actinomycetes ISBN: 978-1-53617-046-7
Editors: A. A. Mohamed Hatha et al.

Chapter 1

ACTINOMYCETES: MICROBIAL DRUG FACTORY

S. Aruna Sharmili[1,*], Jayashree Shanmugam[1] and Mayakkannan Gopal[2]

[1]Department of Biotechnology, Stella Maris College (Autonomous), Chennai, Tamilnadu, India

[2]NOCH International Sdn Bhd. Kuala Lumpur, Malaysia

ABSTRACT

Natural products possess the novel sources of diverse microorganisms that have potential medicinal values. Among them, actinomycetes demonstrate a promising group. Actinomycetes are prokaryotes and ubiquitous, producing aerobic spore, which belong to the order Actinomycetales characterized with high G+C DNA content in their genome. They unveil an array of morphological differentiation because of their mycelial lifestyle. Owing to their large genome with numerous transcription factors controlling various gene expression, they are stable and possess persistent metabolic diversity. Actinomycete is a good model for the production of important metabolites and enzymes which are

* Corresponding Author's Email: arsharmilis@gmail.com.

achieved as a result of their unique metabolic and physiological capabilities. About 45% of the total biologically active compounds are produced by actinomycetes. They are also the most significant synthesizers of economically and biotechnologically important metabolites. In the past decades, a large number of actinomycetes have been isolated and screened as potential source, accounting for 70%-80% of the relevant bioactive compounds that are commercially available from various geographic regions around the world. The bioactive metabolites of actinomycetes have important applications in human medicine because of their therapeutic superiority against diverse disorders. Members of this group can be considered the source of a large pool of antibacterial (streptomycin, tetracycline, chloramphenicol), antifungal (nystatin), immunosuppressive (rapamycin, cyclosporine), antiviral (tunicamycin), antitumor (actinomycin, mitomycin C, anthracyclines), antidiabetogenic (bafilomycin, streptozotocin), antiparasitic (avermectin), hypercholesterolemia (statins such as lovastatin and mevastatin) and enzyme inhibitory (clavulanic acid) compounds. Additionally, actinomycetes have tremendous potential for producing clinically proven antitumor drugs such as anthracyclines (aclarubicin, daunomycin, and doxorubicin), aureolic acids (mithramycin), enediynes (neocarzinostatin), peptides (bleomycin and actinomycin D), carzinophilin, antimetabolites (pentostatin), and mitomycins. This indicates that the products of actinomycetes possess pharmacokinetic properties, thus occupying a prominent position in clinical research and development. Their proven ability to produce complicated compounds exhibiting excellent bioactive potency has attracted the scientific community to further explore and understand actinomycetes species, which are dealt with in detail in the chapter.

Keywords: Actinomycetes, Bioactive metabolites, Antibiotics, Drugs

1. INTRODUCTION

Novel products from nature contribute in a big way as important sources for drug discovery. For decades, they form the landscape of new molecular entities, new drug leads and analogs, representing successfully a substantial share in pharmaceutical market and still used widely as frontline therapies in clinical practice (Newman and Cragg, 2016). The average life expectancy of humans was significantly increased by these therapeutic drugs. Natural resources *viz.*, microbes, plants, and animals contribute about 60% of the approved therapeutic drugs (Tan et al., 2018). Among these, microbial-

derived natural products particularly members of phylum Actinomycetes stand out as a significant source of approved drugs (Bérdy, 2012). So far, about 70% of more than 22,000 known biologically active secondary metabolites were obtained from actinomycetes (Berdy 2005). Approximately 10-20 secondary metabolites produced by each strain of actinomycetes has innate capability (Bentley et al., 2002). Actinomycetes are heterogeneous extremely diverse group of aerobic prokaryotic, nonmotile, filamentous, Gram-positive saprophytic bacteria with high guanine (G) and cytosine (C) contents in their DNA (70–80%) (Goodfellow and Williams, 1983). This group represents one of the largest taxonomic units recognized within the bacterial domain including Rubrobacteria, Thermoleophilia, Coriobacteriia, Acidimicrobiia, Nitriliruptoria, and Actinobacteria, 5 subclasses, 6 orders, and 14 suborders (Ludwig et al., 2012). They constitute one of the largest ubiquitous bacterial phyla, most are free-living and widely distributed in terrestrial and aquatic (including marine) ecosystems (Macagnan et al., 2006). Approximately 70% of the pharmaceutically active natural secondary metabolites that are in clinical use are contributed by actinomycetes (Ibrahim et al., 2018). They are the notable and profitable producers of great number of unique and chemically diverse bioactive compounds acting mainly as antiviral, immunosuppressant, antifungal, anti-trypanosomal, antileishmanial, antimalarial, antibacterial, cytotoxic, antioxidant, cardio-vascular, enzyme inhibitors and anti-inflammatory drugs (Passari et al., 2017) (Figure 1). Among the actinomycetes, the most widely studied genus *Streptomyces*, with 843 species and 38 subspecies (LPSN, 2018), have been considered as an economically important extraordinary producers of antibiotics. They are exceptionally rich source contributing approximately 50–55% of known antibiotics (Subramani and Williams, 2013). The genus of Streptomyces covers array of chemical structures such as peptides, macrolides, lactones, indoles, terpenes, and quinines. They occupy the center stage of various drug discovery program and determine the fate of pharmaceutically active drugs (Molinski et al., 2009). In the past few decades, actinomyces have contributed significantly as cancer therapeutics (Ravikumar et al., 2012; Subramani and Williams, 2013). The unusual structures and properties of

the metabolites are due to their extremely large genome with hundreds of transcription factors that control gene expression responsible for metabolic diversity, allowing response to specific needs (Shantikumar et al., 2006; Sharma et al., 2014). These metabolites are produced from fermented broth under controlled conditions (Sivalingam et al., 2019). The need for new drugs to circumvent the problem of resistant pathogens and combat life-threatening disease leads to the hunt for new species and compounds (Ekwenye et al., 2007). However, the re-isolation of known compounds have subsequently increased hence the decrease in the discovery rate of new compounds from actinomycetes due to the exhaustion of the usual terrestrial sources (Watve et al., 2001). Various unique environmental niches are explored for actinobacteria, such as marine sources, hydrothermal vent, deserts, tropical rain forest, deep sea, cryoenvironment, volcanic environment, endophytes from plants and microbial symbionts from animal hosts (Ezra et al., 2004; Clardy et al., 2006), so that rare species with therapeutic excellence are discovered. It is therefore evident that the underexplored habitats are to be searched continuously for novel natural microbial products with greater therapeutic efficiency.

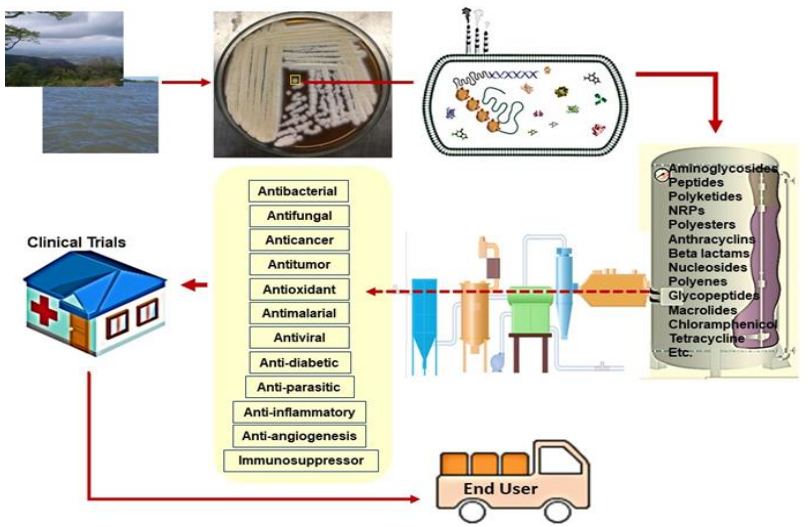


Figure 1. An illustration representing the Actinomycete Factory Producing Potential Biological Active Metabolites for Pharma Industry.

2. ACTINOMYCETES AS A SOURCE OF ANTIBIOTICS

Antibiotics are agents that kill or inhibit the growth of microorganisms (Dorlands, 2010) and are produced by bacteria, fungi, actinobacteria, algae, lichens and plants. They target bacterial structure or function such as cell wall synthesis, transcription, translation, DNA replication and synthesis. The major classes of antibiotics produced by actinobacteria are as follows: Aminoglycosides (streptomycin, gentamycin, kanamycin, neomycin,), Angucyclines (auricin, ladomycin, moromycin), Ansamycins (gldanamycin, rifamycin), Anthracyclins (daunorubicin-antitumor agent), Beta lactams (cephamycins), Beta lactamase inhibitors clavulanic acid, Chloramphenicol, Glutarimides (cyclohexamide), Glycopeptides (teichoplanin, vancomycin), Lipopeptides (daptomycin), Lantibiotics (mersacidin, actagardine), Macrolides (erythromycin, clarithromycin, tylosin), Oxazolidinones (cycloserine), Streptogramins (Streptogramin), tetracyclines (Barka et al., 2016). Of the total 30 antibiotics launched worldwide since 2000, 12 were reported from the members of actinobacteria. They were either natural products or derivatives of natural products (Subramani and Detmer, 2019). Aminoglycosides inhibit protein synthesis and alter the bacterial cell wall integrity (Vakulenko and Mobashery, 2003). Streptomycin is an aminoglycoside isolated from *Streptomyces griseus* and was effective against *Mycobacterium tuberculosis* as a monotherapy until the development of resistant strains. It is now used as a combination drug for treating tuberculosis. Streptomycin is water soluble and combines irreversibly with the 30S subunit of the 70S ribosome found in bacteria. It also binds to the S12 protein involved in the initiation of protein synthesis, thus blocking N-formylmethionine of the tRNA to the ribosome (Mahajan and Lakshmi, 2012). It is also known to disrupt the cell membrane of bacteria (Das et al., 2009). Commercial brands of streptomycin include ABBISTRYN-S® vial, CIPSTRYN-S ® vial, ISOS® vial, Strepto-Fatol®, and Estreptomycina®. Beta lactam binds to penicillin-binding proteins and interferes with the structural cross-linking of peptidoglycans, thereby preventing terminal transpeptidation in the cell wall of bacteria and leading to cytolysis due to increased osmotic pressure (Mandell, 2005).

Chloramphenicol was derived from *Streptomycesvenezuelae* and was the first antibiotic to be entirely manufactured synthetically. It has affinity for the peptidyl transferase of the 50S ribosomal subunit of the 70S ribosome, thereby the peptide chain elongation is prevented which leads to the inhibition of protein synthesis (Schwarz et al., 2004). It displays both bacteriostatic and bactericidal activities against a variety of microbes. It is commercially marketed as Chloromycetin®, paraxin®, Titomycine® and chemicetina®. Tetracyclins are broad-spectrum antibiotics belonging to a subclass of polyketides. Chlortetracycline was the first group of antibiotics secreted by *Streptomyces aureofaciens*. Oxytetracyclins were from *Streptomyces rimosus.* Tertacyclins exhibit time-dependent concentration-enhanced killing. They reversibly bind to the 16S part of the 30S ribosomal subunit inhibiting aminoacyl t-RNA binding, thereby blocking protein synthesis (Speer et al., 1992). They do not directly kill bacterial cells hence are classified as bacteriostatic agents. Glycopeptides bind to dipeptide D-alanly-D alanine within the cell wall of Gram-positive bacteria, thereby preventing the addition of new units to peptidoglycan and inhibiting the synthesis of the peptidoglycan (Klare et al., 2003). Vancomycin is a glycopeptide obtained as a metabolite from *Amycolatopsis orientalis*, which is adopted as the last line of treatment against Gram-positive infections when penicillin and cephalosporin fail. Telavancin (VIBATITVTM) is a synthetic derivative of vancomycin that is used to treat complicated scaled skin and soft tissue infections. Macrolides bind to the 50S ribosomal subunit and inhibit protein synthesis (Roberts, 2002). Erythromycin was the macrolide antibiotic reported first from *Streptomyces erythreus*. It is a group of compounds that exhibit time-dependent killing with a post-antibiotic effect (PAE). Clarithromycin, azithromycin, dirithromycin, roxithromycin, flurothromycin and telithromycin are the novel erythromycin-based antibiotic drugs (Ying et al., 2010). Ery-C®, Ery-Tab®, E-mycin®, dispertab®, PCE® are some of the commercial brands of erythromycin. Quinolone targets the action of topoisomerase IV and DNA gyrase, thereby inhibiting DNA replication (Chaudhary et al., 2013). Sulphonamides target the p-aminobenzoic acid and interfere with the metabolic process (Fukuda et al., 2009). Table 1 illustrates the various antibiotics produced by

actinobacteria between 2011 and 2018. Platensimycin from *Streptomyces platensis* inhibits the key enzyme beta ketoacyl synthases I/II (Fab F/B) involved in the production of fatty acids required for the synthesis of bacterial cell membrane (Palanichamy and Krishna, 2010).

Table 1. Antibiotics from actinomycetes between 2011 and 2018

Source	**Compounds (Antibiotics)**	**Chemical class**	**Biological activity**	**References**
Streptomyces asenjonii strainKNN 42.f	Asenjonamide A-C	Di-ketone polyketide	Gram positive/Negative bacteria	Abdelkader et al., 2018
Micromonospora sp	Phocoenamicins B and C	Spirotetronates	Gram positive	Perez et al., 2018
Allostreptomyces sp. K12-0794	Hamuramicin A & B	Macrolide	Antibacterial activity	Suga et al., 2018
Amycolatopsis sp. K16-0194	Dipyrimicin A & B		Antibacterial activity	Izuta et al., 2018
Mumia sp. YSP-2-79	Mumiamicin	Furan fatty acid	Antibacterial activity	Kimura et al., 2018
*Streptomyces*sp. DA 3-7	Pyridine-2,5-diacetamide	Pyridine alkaloid	Antibacterial activity	Nithya et al., 2018
Streptomyces cyaneofuscatus M-169	Anthracimycin B	Macrolide	Antibacterial activity	Rodriguez et al., 2018
Pseudonocardia carboxydivorans M-227	Branimycins B and C	Macrolide	Antibacterial activity	Brana et al., 2017
Streptomyces subflavus subsp. irumaensis	Bisoxazolomycin AM-3603		Antibacterial activity	Koomsiri et al., 2017
Streptomyces ghanaensis TXC6-16	Ghanamycin A and B	Gamma-butyrolactones	Antibacterial activity	Xu et al., 2017
Micromonospora harpali	Tetrocarcin P, Microsporanates A–F	Spirotetronate glycosides	Antibacterial activity	Gui et al., 2017
Streptomyces fradiae MM456MmF7	Fradiamine A	Siderophore	Antibacterial activity	Takehana et al., 2017

Table 1. (Continued)

Source	Compounds (Antibiotics)	Chemical class	Biological activity	References
Streptomyces griseus OS-3601	Iminimycin A & B	Indolizidine alkaloid	Antibacterial activity	Nakashima et al., 2016a Nakashima et al., 2016b
Actinomadura sp KC 191	Actinomadurol	Norditerpenoids	Antibacterial activity	Shin et al., 2016
Micromonospora sp	Micromonohalimanes A and B	Diterpenoids	Gram positive	Zhang et al., 2016a
Micromonospora sp	Quinolone Alkaloid	Alkaloid	Antibacterial activity	Thi et al., 2016
Micromonospora sp	1,4-dioxane derivative	Dioxane	Antibacterial activity	Thi et al., 2016
Pseudonocardia sp	Pseudonocardides A-G	Gamma - butyrolactones	Antibacterial activity	Zhang et al., 2016b
Pseudonocardia sp	Curvularin macrolides1-5	Macrolides	Antibacterial activity	Ye et al., 2016
Verrucosispora sp	Glycerol 1-hydroxy-2,5-dimethyl benzoate	Salicyclic derivative	Anti-MRSA activity	Huang et al., 2016
Streptomycesspin overrucosus	Spithioneines A and B		Antibacterial activity	Fu et al., 2015
Streptomyces sp	Salinamide F	Depsipeptide	Gram positive / Negative bacteria	Hassan et al., 2015
Streptomyces monomycini	Argolaphos A/B	Phosphonopeptide	Gram positive / Negative bacteria	Ju et al., 2015
Salinospora afghaniensis	Thiolactomycin	Thiotertonic acids	Gram positive bacteria	Tang et al., 2015
Streptomyces WAB9	2-Amino-N-(2-amino-3-phenylpropanoyl)-N hydroxyl 3-phenylpropanamide	Hydroxamic acid	Gram positive bacteria	Yekkour et al., 2015
Micromonospora rosaria	Difluoststin A	Angucycline	Gram positive bacteria	Yamanaka et al., 2014
Lentzea kentukyensis	Lassomycin	Cyclic peptide	*Mycobacterium tuberclosis*	Gavrish et al., 2014
Saccharomonas pora sp	Taromycin A	Lipopeptide	Gram positive bacteria	Yamanaka et al., 2014
Solwaraspora sp	Solwaric acids A and B	Trialkyl-substituted aromatic acids	Antibacterial activity against MDR pathogens	Ellis et al., 2014
Salinospora pacifica	Enterocin	Polyketide	Gram positive bacteria	Bonet et al., 2014

Source	Compounds (Antibiotics)	Chemical class	Biological activity	References
Micrococcus sp	Microluside A	Ortho-glycosylated xanthone	Antibacterial activity	Eltamany et al., 2014
Streptomyces scopuliridis SCSIO ZJ46	Desotamides B-D	Peptides	Antibacterial activity	Song et al., 2014
Streptomyces sp	Anthracimycin	Tricarboxilic	Gram positive bacteria	Jang et al., 2013
Streptomyces sp	Ohmyungsamycin A and B	Cyclic peptides	Antibacterial activity	Um et al., 2013a
Streptomyces sp 12A35	Lobophorins H and I	Spirotetronate	Antibacterial activity	Pan et al., 2013
Streptomyces sp SCSIO 01127	Lobophorins E and F	Spirotetronate	Antibacterial activity	Niu et al., 2011
Streptomyces sp	Chaxamycins	Ansamycins	Antibacterial activity	Rateb et al., 2011

3. Mechanism of Antimicrobial Compound Production

The life cycle of *Streptomyces* starts with the germination of the spore that grows from the vegetative hyphae. The hypha grows and branches out resulting in a branched mycelium. Under nutrient depletion/stress, accumulation of ppGpp (guanosine pentaphosphate) occurs which leads to growth cessation and the vegetative mycelium differentiates into aerial hypha (Van et al., 2011). It is at this moment in the life cycle of *Streptomyces* that the transition occurs from vegetative to aerial growth, resulting in the production of most antibiotics. *Streptomyces* is sessile and when nutrient depletion occurs, the vegetative or substrate mycelium is autolytically degraded by a programmed cell death (PCD)-like mechanism (Miguelez et al., 1999). Repression of the nutrient sensory DasR protein is achieved by the cell wall-derived metabolites. PCD results in the accumulation of amino acids, sugars, lipids, nucleotides around the lysing substrate mycelium (Rigali et al., 2006) (Figure 2). This attracts motile competing microbes in the habitat. Antibiotics are produced at this moment to protect the nutrients. The evidence of link between PCD and antibiotic production was based on

the observation that cell wall-derived N-acetylglucoseamine (GlcNAc) acts as a signal for the onset of development of the molecule for antibiotic production. Supplementing with GlcNAc when the nutrient depletion occurs, accelerates both the onset of development and antibiotic production. On the contrary, in nutrient-rich media, high concentration of GlcNAc blocks the development and antibiotic production and there is only vegetative growth (Rigali et al., 2008). GlcNAc that acts as an important signaling molecule is derived from chitin in nutrient-rich soil or from *Streptomyces* cell wall during PCD. The key behind this dual signaling resides on the nature of the sugar transporters. Monomeric GlcNAc enters the cell via the NagE2 permease which is a part of the PEP-dependent phosphate system (PTS). Chitobiose (dimeric GlcNAc), a subunit of chitin enters via ABC transporter DAS ABC of Hgc EFC. The internalized GlcNAc is converted into glucose amine 6 phosphate (GlcN-6-P) by the coenzymes Nag A and Nag B. This GlcN-6-P acts as an allosteric effector of the GntR family regulator DasR which controls the production of antibiotics besides siderophores. GlcNAc-dependent nutrient signaling is most likely to be mediated through changes to the level of GlcN-6-P. GlcN-6-Phosphate binds to DasR leading to depression of Das R-mediated control of antibiotic production. Antibiotic biosynthesis and secretion can be induced by adding GlcNAc to minimal media with a poor carbon source.

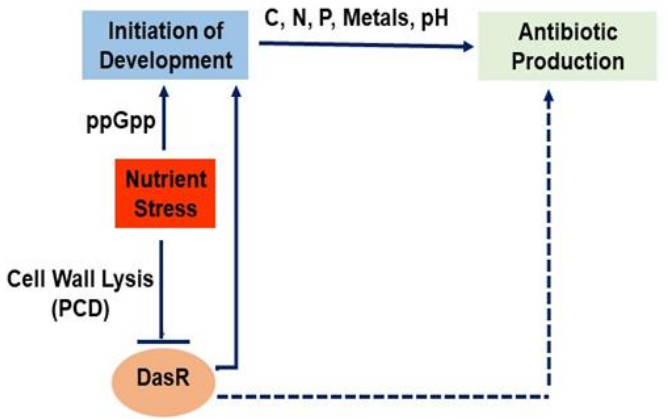


Figure 2. Antibiotic Production in Actinomycetes.

4. ANTICANCER/ANTITUMOR COMPOUNDS

Cancer remains a great challenge despite the progress made in its diagnosis and treatment. Although many scientists and oncologists have succeeded in cancer treatment for the last decades, tumour diseases constitute one of the main reasons of deaths worldwide. Globally, there was an estimated 9.6 million cancer-related deaths in 2018 and 1 in 6 deaths occur due to cancer (www.who.int/news-room/fact-sheets/detail/cancer). By 2030, the mortality in the modern world due to cancer is expected to rise to 17 million out of approximately 26 million new cancer cases (Thun et al., 2010). Across the globe, the rapid development of resistance to multiple chemotherapeutic drugs and the associated high toxicity with their undesirable side effects is always a concern leading to the search of novel natural drugs and their derivatives that are more effective and less toxic with greater therapeutic efficiency (Demain and Sanchez, 2009). Among microorganisms, members of actinobacteria are extraordinary source of novel anticancer and antitumor metabolites and gained a significant place in pharma industry. With the encouraging expansion in the fields of high-throughput screening and fermentation, mining of genomes for cryptic pathways, and combinatorial biosynthesis to generate new secondary metabolites related to existing pharmacophores progress has been made recently in the research field on drug discovery from actinomycetes (Baltz, 2008). The emerging evidence suggests marine actinomycetes are more likely a great source of anticancer and antitumor drugs than their terrestrial counterparts (Busi and Pattnaik, 2018). Major secondary metabolites producing actinobacteria belongs to the genus *Streptomyces*, *Actinomyces*, *Arthrobacter*, *Corynebacterium*, *Frankia*, *Micrococcus*, *Micromonospora* and several others (Passari et al., 2015). The mentioned members secrete clinically useful antitumor and anticancer compounds with diverse chemical structures including peptides, macrolides, lactones, indoles, terpenes and quinones (Han et al., 2012). The secondary metabolites produced by actinomycetes used in chemotherapy includes anthracyclines (aclarubicin, daunomycin, and doxorubicin), peptides (bleomycin and actinomycin D), aureolic acids (mithramycin), enediynes (neocarzinostatin), antimetabolites

(pentostatin), carzinophilin, mitomycins, pentostatin and resistomycin (Schwartsmann et al., 2001). Table 2 shows some of the secondary metabolites isolated from actinomycetes from last decade that have potential anticancer and antitumor activities.

Table 2. Secondary metabolites isolated from actinomycetes from 2010-2019 that have potential anticancer and antitumor activities

Source	Compound	Chemical Structure	Biological Activity	References
Dermacoccus	Dermacozines A-G	Phenazine derivative	Antitumor	AbdelMohsen et al., 2010
Salinispora arenicola	Arenimycin	Quinones	Anticancer	Asolkar et al., 2010
Actinoalloteichus cyanogriseus WH1-2216-6	Caerulomycin (F,G,H,I,J,K)	Polyketides	Anticancer	Fu et al., 2011a
Actinoalloteichus cyanogriseus WH1-2216-6	Cyanogrisides A,B,C,D	Polyketides	Anticancer	Fu et al., 2011b
Streptomyces sp.	Padanamide A, B	NRPS	Anticancer	Williams et al., 2011
Streptomyces sp. NPS853	Usabamycin A-C	Indolocarbazole	Anticancer	Sato et al., 2011
Micromonospora sp.	Levantilides A and B	Macrolides	Anticancer	Gartner et al., 2011
Pseudonocardia sp.	Pseudonocardians A–C	Quinones	Anticancer	Li et al., 2011
Amycolatopsis alba (DVR D4)	1(10-aminodecyl) Pyridinium	Indolocarbazole	Anticancer	Dasari et al., 2012
Micromonospora haikouensi	Menaquinones	Quinones	Antitumor	Xie et al. 2012
Nocardia dassonvillei.	N-(2-hydroxyphenyl)-2-phenazinamine (NHP)	Phenazine derivative	Anticancer	Gao et al., 2012
Streptomyces sp. strain FMA	Stretocarbazoles A,B	Indolocarbazole	Anticancer	Fu et al., 2012
Streptomyces sp. WBF16	Aureolic acid (Chromomycin)	Indolocarbazoles	Antitumor	Lu et al., 2012
Streptomyces lusitanus	Grincamycins B–F	Glycoside	Anticancer	Huang et al., 2012
Streptomyces sp.	Spiroindimicins A–D	Alkaloids	Anticancer	Zhang et al., 2012

Source	**Compound**	**Chemical Structure**	**Biological Activity**	**References**
Actinomadura sp. BCC 24717	1-ethyl-β-carboline-3-carboxylic acid, 1-methyl indole-3-carboxamide, 1-vinyl-β-carboline-3-carboxylic acid	Indolocarbazole	Anticancer	Kornsakulkarn et al., 2013
Micromonospora sp.	Levantilide C	Macrolides	Antitumor	Fei et al., 2013
Streptomyces sp.	Ohmyungsamycins A and B	NRPS	Anticancer	Um et al., 2013a
Streptomyces sp	Sungsanpin	Lasso peptide	Antitumor	Um et al., 2013b
Streptomyces sp. CHQ-64	Drimentine G; Indotertine B	Isoprenoids	Anticancer	Che et al., 2013
Nocardiopsis alba	Methoxyneihumicin	Diketopiperazine	Anticancer	Zhang et al., 2013
Serinicoccus sp.	Seriniquinone	Quinones	Anticancer	Trzoss et al., 2014
Streptomyces sp. SCSIO 03032	Spiroindimicins A-D, Indimicin B	Indolocarbazole	Anticancer	Zhang et al., 2014
Actinomycetospora chlora	Thiasporine A	Thiazole	Anticancer	Fu and MacMillan, 2015
Streptomyces strain, MUM256	Pyrrolopyrazine	Alkaloid	Anticancer	Tan et al., 2015
Microbacterium sediminis	Microbacterins A and B	Peptides	Antitumor	Liu et al., 2015
Streptomyces sp. SCSIO 11594	Marangucycline B	Glycoside	Anticancer	Song et al., 2015
Microbacterium sp.	Microindolinone A	Novel indole	Antitumor	Niu et al., 2017
Nocardiopsis sp.	Nocazines F and G	Diketopiperazine	Antitumor	Sun et al., 2017
Streptomyces sp. AGM12-1	Cyclo (S-Pro-S-Val)	Diketopiperazine	Antitumor	Ahmad et al., 2017
Streptomyces sp. KCB13F003	Ulleungdin	Lasso peptide	Anticancer	Son et al., 2018
Streptomyces bingchenggensis ULS14	Staurosporine	Indolocarbazole	Anticancer	Davies-Bolorunduro et al., 2019

The mechanism of action to control different cancer or tumor cells by actinomycetes metabolites is diverse including apoptosis, mitochondrial permeabilization, blockage of signal transduction pathways, disturbance in

cellular differentiations, tumor-induced angiogenesis or by intercalating with duplex DNA and inhibiting the DNA-dependent RNA polymerase activities (Wadkinset al., 1998; Olano et al., 2009a). These secondary metabolites are synthesized by different biosynthesis pathway: (1) the peptide pathway, (2) the polyketide synthase (PKS) pathway, (3) the nonribosomal polypeptide synthase (NRPS) pathway, (4) the hybrid (nonribosmial polyketide synthetic) pathway and (5) the Shikimate pathway and mainly organized by gene clusters encoding polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPS). Polyketides are natural metabolites assembled by sequential set of reactions catalyzed by multifunctional polyketides synthases (PKS) enzymes. The PKS is categorized into three different groups such as types I, II, and III facilitating the condensation reactions of acyl-CoA precursors to produce wide range of pharmacologically active polyketides with multiple hydroxyketone or hydroxyaldehyde functional groups. This class is often highly oxygenated (Rocha-Santos and Duarte, 2014) and comprises a highly diverse class of chemical structures viz., Anthracyclines, Aureolic acids, Tetracenomycins, Angucyclines, Pentangular polyphenols, Aryl-C-glycosides, Spiro-tetronates, Macrolactams, Enediynes, Phoslactomycins, and Polyethers (Olano et al., 2009b). Non-ribosomal peptides comprise small molecules synthesized by non-ribosomal peptide synthetases. They are not involved at the level of ribosomes (Harir et al., 2018). The enzyme condenses aminoacyl-AMP to produce different peptides and then modified by epimerization, oxidase, reductase or methyltransferase activities (Gomathi and Gothandam, 2016). The NRPS uses proteinogenic and non-proteinogenic amino acids as monomeric building blocks including D-amino acids and other carboxylic acids. They also have peptide with unique structural heterocyclic elements and deoxy sugars (Fischbach and Walsh, 2006). The products of these multifunctional enzymes such as Heterocyclic quinones, Depsipeptides, Actinomycins, Myxochelins and Benzodiazepines have a broad spectrum of biological activities. Hybrid/Mixed peptides are multifunctional proteins organized into modules. The combined action of type I PKSs and NRPSs assembles amino acids and short-chain carboxylic acids peptide compounds. Peptides, namely, Bleomycins, Macrolactams,

Salinosporamide and Azinomycins belong to this class and are currently used in several clinical trials as well as in chemotherapy for the treatment of several malignancies (Olano et al., 2009a). 2-C-methyl-Derythritol 4-phosphate (MEP) pathway modifies five-carbon precursor molecule isopentenyl diphosphates (IPP) into isoprenoids, namely, Altemicidin, Furaquinocin A, Furanonaphthoquinone, Sesquiterpenoid pentalenolactone, Brasilicardin A, Neomarinones, Glaciapyrroles, Drimentine G and Indotertine B possess antitumor and anticancer activities (Gomathi and Gothandam, 2016). They are similar to terpenes and based on the number of C5 units classified as monoterpenes (C10), sesquiterpenes (C15) and diterpenes (C20) (Dairi, 2005). Indolocarbazoles constitute a new class of secondary metabolites with antitumor activity. These distinct family has a characteristic indolo[2,3-a] pyrrolo[3,4-c]carbazole core attached with sugars derived from two units of tryptophan, glucose and methionine. This unique class of compounds causes DNA-damage targeting on topoisomerases I and II, and inhibition of protein kinases, including serine/threonine and tyrosine kinases (Sanchez et al., 2006). Apart from the mentioned types, actinomycetes also secrete important compounds with other chemical structure that has biological activities against many carcinomas (Gao et al., 2012; Karpińskiand Adamczak, 2018).

5. Other Compounds from Actinobacteria

Trioxacarin A, B and C obtained from *Streptomyces ochraceus* and *Streptomyces bottropensis* showed potant antiplasmodial activity (Maskey et al., 2004). Salinos poramide A isolated from *Actinomycetes tropica* was a potent inhibitor of human malarial parasite *in vivo* and *in vitro* (Alharbi et al., 2016). Antiviral compound pimprinethine isolated from Streptomyces sp exhibited antiviral activity against EV71 and ADV-7. The compound showed slight activity against CVB3, HSV-1 and H1N1, though there were exceptions *in vivo*. The activity of these compounds may be at early stages of EV71 replication period (Wei et al., 2014). Forazoline A, a polyketide compound isolated from Actinomadura sp exhibited anti-fungal activity

against *Candida albicans* through disruption of membrane integrity (Wyche et al., 2014). Nesterenloniane, a novel cyclic ether isolated from deep sea derived *Nesterenkonia flava* showed anti-allergic activity (Xie et al., 2017). Halomadurones derived from Actinomadura sp exhibited potent nuclear factor E2-related factor antioxidant response element (Nrf2-ARE) activation which is used as therapeutic for neurodegenerative diseases (Wyche et al., 2013). Voglibose, acarbose, valienamine, adiposin-1 and trestatin-B isolated from *Streptomyces hygroscopicus-limoneus*, *Streptomyces calvus* and *Streptomyces dimorphogenes* showed alpha-glucosidase inhibitor activity (Alharbi et al., 2016). Pyrizinostin, an inhibitor of pyroglutamyl peptidase was isolated from *Streptomyces sp* SA-2289 (Aoyagi et al., 1995). Pyrostatin A and B produced by *Streptomyces sp* SA- 350 exhibited inhibition of the enzyme N-acetyl – beta – glucosaminidase (Imada, 2005).

6. Drugs Clinically Approved and in Trials

Actinomycetes, one of the most accepted and proved prolific producers of high-impact secondary metabolites, are intensively exploited by pharma industries. The drugs and analogs of these secondary metabolites are tremendously introduced in the market and currently used in clinical practice. Ertapenem isolated from *Streptomyces cattleya* has higher stability for renal dehydropeptidase enzymes hydrolysis located in the brush border of the kidneys with broad spectrum of antibacterial activity against *Escherichia coli*, *Morganella morganii*, *Klebsiella* sp., *Proteus* sp., *Citrobacter* sp., *Enterobacter* sp., and *Serratia marcescens* (Sader and Gales, 2001). Clinically approved drug, Telithromycin, a semi-synthetic derivative erythromycin A from *Saccharopolyspora erythraea* exhibits antibacterial effect on respiratory tract pathogens resistant to other macrolides (Zhanel et al., 2002). Biapenem, a carbapenem analog isolated from *Streptomyces cattleya*, acts as an antibacterial agent against both Gram-negative and Gram-positive bacteria including species producing β-lactamases (Perry and Ibbotson, 2002). *Streptomyces*-derived daptomycin was discovered in 1980s and approved by the US Food and Drug Regulatory

Administration (US FDA) for clinical use in 2003. It still represents the first-line drug against MRSA bacteremia (Choo and Chambers, 2016). Tigecycline derivative of minocycline exhibited potent activity against tetracycline-resistant organisms and clinically utilized in an injection formulation (Zhanel et al., 2004). Plazomicin (ACHN-490) a semi-syntheic derivative of sisomicin active against Gram-positive and -negative bacteria is in the pipeline (Aggen et al., 2010). The FDA-approved drug Ceftaroline has a high affinity toward the PBP2a of MRSA and belongs to the cephalosporin antibiotics (Saravolatz et al., 2011). Fixdaomicin, approved for the treatment of *Candida difficile*-mediated colitis in 2011 is derived from tiacumicin (Genilloud, 2017). Antibiotic Surotomycin (MK-4261, CB-183,315) a semi-synthetic daptomycin derivative was active against daptomycin-resistant *S.aureus, E.faecalis, E. faecium* and *C. difficile* (Snydman et al., 2012). Some of the newly approved drugs are synthetic analogs of antibiotics produced by *Streptomyces* (De Lima Procópio et al., 2012; Kumar and Chopra, 2013). Semi-synthetic lipoglycopeptides derived from vancomycin, telavancin and oritavancin were approved in 2009 and 2011 (Bouza and Burillo, 2010). Dalvabacin an analog of teicoplanin like glycoprotein A40926 produced by Nonomuraea sp was approved in 2014 for the treatment of Gram-positive acute bacterial skin and skin structure infection (ABSSSI) with *S. aureus, Enterococus faecalis* and different species of Streptococci (Genilloud, 2017). Omadacycline an aminomethylcycline with broad spectrum of antibacterial activity in the later stage of clinical development (Honeyman et al., 2015). Sarecycline (PTK-AR01) is being evaluated in phase III trials for the treatment of acne vulgaris (Knight et al., 2016). Antibiotic Surotomycin (MK-4261, CB-183,315), a semi-synthetic daptomycin derivative is active against daptomycin-resistant *S.aureus.* Ceftazidime-avibactam and ceftolozane-tazobactam, the new cephalosporin siderophore S649266, omadacycline (phase 2 trial) and eravacycline are in clinical trials (Fernandes and Martens, 2017). Previously isolated Fosfomycin from *S. fradiae* is currently undergoing comparative phase 3 clinical trials in the treatment of chronic urinary tract infection and acute pyelonephritis in hospitalized adults (Fernandes and Martens, 2017). Everolimus produced from *Streptomyces hygroscopicus* an orally active

derivative of rapamycin exhibits immunosuppressive effect by blocking growth factor and non-hematopoietic cells to arrest the cell cycle at the G_1/S phase (Chapman and Perry, 2004). Miglustat, an analog of nojirimycin isolated from the broth filtrate of *Streptomyces lavendulae*, has been approved for patients unable to receive enzyme replacement therapy as a therapeutic drug for type I lysosomal storage disorder associated with pathological accumulation of glucosylceramide in cells of the monocyte/macrophage lineage (Pastores et al., 2005). Gemtuzumab ozogamicin was discovered from fermentation products produced by *Micromonospora echinospora* sp. *calichensi*. This prodrug of calicheamicin has been found effective in the treatment of patients with acute myeloid lymphoma (Lee et al., 1987). Amrubicin hydrochloride is a completely synthetic derivative of doxorubicin isolated from *Streptomyces peucetius* var *caesius*, which demonstrated potent antitumor activity against human tumor xenografts of breast, lung and gastric cancer (Sugiura et al., 2005). Pimecrolimus is a novel analog of ascomycin from *Streptomyces hygroscopicus* var *ascomyceticus*, which was approved for the treatment of inflammatory skin diseases such as allergic contact dermatitis and atopic dermatitis (Gupta and Chow, 2003). Dactinomycin the most common anticancer drug isolated from *Streptomyces parvulus* was used in the treatment of a variety of cancer including Wilms tumor, rhabdomyosarcoma, Ewing's sarcoma, trophoblastic neoplasm, testicular cancer, and certain types of ovarian cancer. Another anticancer drug produced by *Streptomyces plicatus* is plicamycin, which was also in clinical usage but the production was discontinued in 2000 (Li, 2017). Actinomycin D, Anthracyclines (Daunorubicin, Doxorubicin, Epirubicin, Pirirubicin and Valrubicin), Bleomycin, Mitosanes (Mitomycin C), Anthracenones (Mithramycin, Streptozotocin and Pentostatin) and Enediynes (Calcheamycin) are the other approved drug candidates for cancer chemotherapy (Demain and Vaishnav, 2011). Sungsanpin, a new lasso peptide obtained from Streptomyces sp. SNJ013, has inhibitory activity against lung cancer and currently in preclinical trials for cancer treatment (Russo et al., 2015). Chartreusin, an aromatic glycosylated polyketide from *S. chartreusis* currently in phase II clinical trials possesses significant chemotherapeutic activity against various

tumor cell lines (Butler, 2008). Salinosporamide A produced by *S. tropica* strain CNB-392 is currently in phase I clinical trials for the treatment of human colon carcinoma (Olano et al., 2009a). Apart from this, actinomycete compounds such as Elsamitrucin (elsamicin A), Brostallicin (an α-bromoacryloyl derivative of distamycin A), Geldanamycin (a polyketide natural), Sirolimus, Staurosporine, Doxorubicin and Pladienolide D have oncological effect and are currently under various phases of clinical evaluation (Portugal, 2003; Bisht et al.,2003; Broggini et al., 2004; Butler, 2008). Intensive research, systematic new platforms, multidisciplinary approach to drug discovery and discrete pharmaceutical applications will continue to pave way for sustained production of promising novel human drug templates from actinomycetes.

CONCLUSION

The growing major challenges of emerging diseases, established diseases and resistance to drugs have led to the quest for potent novel drugs from microbial resources. Undoubtedly, actinomycetes are interesting sources of biologically active metabolites with promising chemical characteristics and medical significance. This group has been the frontier for the discovery of vast collection of pharmacologically important natural products such as antibiotics, enzyme inhibitors, immunomodifiers, anticancer, antitumor and many other compounds valuable to mankind (Fiedler et al., 2004). Although complex, combinatorial chemistry offered analogs of these natural products with minor modifications and new scaffolds to work on (Baltz, 2007; Kekuda et al., 2010). However, the discovery of new compounds was declined for last decades and there was a need to expand the search more. Recently, research has been directed towards the investigation of biosynthesis-like gene clusters which remains silent under standard laboratory culture conditions and to get activated. This activation could pave way to harness the biological capabilities of actinomycetes for sustained discovery of bioactive compounds for therapeutic use (Xu et al., 2018). Fast genome sequencing, genome mining,

combinatorial biosynthesis and whole-genome sequence (WGS) analysis of the potent strains could provide knowledge on gene clusters, bioactive compound-associated regulatory pathways and insights on the modification of the already existing metabolites (Greule et al., 2017). Adaptation of advanced cultivation techniques would create positive impact in obtaining detailed information on the taxonomy, ecology and chemical characteristics of uncultivable or rare actinomycetes from unexploited microbial niches in the hope of acquiring novel metabolites (Rath et al., 2011). Consequently, extensive, integrated, multifaceted research collaborations across the borders are needed to unravel the new platforms for the discovery of natural product from actinomycetes with improved therapeutic properties for the development of new medications.

REFERENCES

[1] Newman, DJ., Cragg, GM. Natural products as sources of new drugs from 1981 to 2014. *Journal of Natural Products* 79, no.3 (2016): 629-661.

[2] Tan, LT., Chan, KG., Chan, CK., Khan, TM., Lee, LH., Goh, BH. "Antioxidative Potential of a Streptomyces sp. MUM292 Isolated from Mangrove Soil." *Biomedical Research International* 1; (2018): 4823126.

[3] Bérdy, J. "Thoughts and facts about antibiotics: where we are now and where we are heading." *The Journal of Antibiotics* (Tokyo) 65, no. 8 (2012): 385-395.

[4] Bérdy, J. "Bioactive microbial metabolites." *The Journal of Antibiotics* (Tokyo) 58, no. 1 (2005): 1-26.

[5] Bentley, SD., Chater, KF., Cerdeño-Tárraga, AM., Challis, GL., Thomson, NR., James, KD et al. "Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2)." *Nature* 417, no. 6885 (2002):141-147.

[6] Goodfellow, M., Williams, ST. Ecology of actinomycetes.*Annual Reviw Microbiology* 37, (1983): 189-216.

[7] Ludwig, W., Euzéby, J., Schumann, P., Buss, HJ., Trujillo, ME., Kämpfer, P., Whiteman, WB. “Road map of the phylum Actinobacteria. In: Goodfellow, M., Kämpfer, P., Busse, HJ., Trujillo, ME., Suzuki, KI., Ludwig, W., Whitman, WB. (eds) Bergey’s manual of systematic bacteriology (2012). Springer, New York, pp 1–28.

[8] Macagnan, D., Romeiro, RDS., de Souza, JT., Pomella, AWV. “Isolation of actinomycetes and endospore-forming bacteria from the cacao pod surface and their antagonistic activity against the witches’ broom and black pod pathogens.” *Phytoparasitica* 34, no. 2 (2006):122–132.

[9] Ibrahim, AH., Desoukey, SY., Fouad, MA., Kamel, MS., Gulder, TAM., Abdelmohsen, UR. “Natural Product Potential of the Genus Nocardiopsis.” *Marine Drugs* 16, no. 5 (2018): 147.

[10] Passari, AK., Mishra, VK., Singh, G., Singh, P., Kumar, B., Gupta, VK., Sarma, R K., Saikia, R., Donovan, AO., Singh, BP. “Insights into the functionality of endophytic actinobacteria with a focus on their biosynthetic potential and secondary metabolites production.” *Scientific Reports* 7, no. 1 (2017):11809.

[11] LPSN. “Genus Streptomyces.” (2018). http://www.bacterio.net/streptomyces.html.

[12] Subramani, R., William, A. "Culturable rare Actinomycetes: diversity, isolation and marine natural product discovery." *Applied microbiology and biotechnology* 97, no. 21 (2013): 9291-9321.

[13] Molinski, TF., Dalisay, DS., Lievens, SL., Saludes, JP. “Drug development from marine natural products.” *Nature Reviews. Drug Discovery* 8, no. 1 (2009): 69-85.

[14] Ravikumar, S., Fredimoses, M., Gnanadesigan, M. "Anticancer property of sediment actinomycetes against MCF–7 and MDA–MB–231 cell lines." *Asian Pacific journal of tropical biomedicine* 2, no. 2 (2012): 92-96.

[15] Shantikumar, L., Baruah, I., Bora, TC. “Actinomycetes of loktak habitat: isolation and screening for antimicrobial activities. *Biotechnology* 5, no. 2 (2006): 217-221.

[16] Sharma, M., Pink, D., Meenakshi, C. "Actinomycetes: source, identification, and their applications." *Int J Curr Microbiol App Sci* 3, no. 2 (2014): 801-832.

[17] Sivalingam, P., Kui, H., John, Pote., Kandasamy, P. "Extreme Environment Streptomyces: Potential Sources for New Antibacterial and Anticancer Drug Leads?" *International Journal of Microbiology* (2019).

[18] Ekwenye., Uchechi N., Erinma, K. "Investigation of plasmid DNA and antibiotic resistance in some pathogenic organisms." *African Journal of Biotechnology* 6, no. 7 (2007): 877-880.

[19] Watve, MG., Rashmi, T., Maithili, MJ., Bhalachandra, DB. "How many antibiotics are produced by the genus Streptomyces?" *Archives of microbiology* 176, no. 5 (2001): 386-390.

[20] Ezra, D., Uvidelio, FC., Gary, AS., Wilford, MH., Heidi, P., James B J., Margaret, AM., Condron., David, BT., Joseph, S., Michelle, M., Michelle, H., Barbara, W., Debbie, Y. "Coronamycins, peptide antibiotics produced by a verticillate Streptomyces sp.(MSU-2110) endophytic on Monstera sp." *Microbiology* 150, no. 4 (2004): 785-793.

[21] Clardy, Jon, Michael A. Fischbach, and Christopher T. Walsh. "New antibiotics from bacterial natural products." *Nature biotechnology* 24, no. 12 (2006): 1541.

[22] Dorland, NW. "Dorlands medical dictionary: antibacterial." *Archived from the original on (2010 Nov 17), Retrieved (2010 Oct 29)* (2010).

[23] Barka, EA., Parul, V., Lisa, S., Nathalie, GV., Cedric J., Hans, PK., Christophe, C., Yder, O., Gilles, P W. "Taxonomy, physiology, and natural products of Actinobacteria." *Microbiol. Mol. Biol. Rev.* 80, no. 1 (2016): 1-43.

[24] Subramani, R., Detmer, S. "Marine rare actinomycetes: A promising source of structurally diverse and unique novel natural products." *Marine drugs* 17, no. 5 (2019): 249.

[25] Vakulenko, SB., Shahriar, M. "Versatility of aminoglycosides and prospects for their future." *Clinical microbiology reviews* 16, no. 3 (2003): 430-450.

[26] Mahajan, GB, Lakshmi, B. "Antibacterial agents from actinomycetes-a review." *Front Biosci (Elite Ed)* 4, no. 4 (2012): 240-53.

[27] Das, R., Pushpa, G., Pushpendra, S., Chauhan, DS., Kiran, K., Katoch, VM. "Association of mutations in rpsL gene with high degree of streptomycin resistance in clinical isolates of *Mycobacterium tuberculosis* in India." *Indian Journal of Medical Research* 129, no. 1 (2009): 108-111.

[28] Mandell, D. "Bennett's Principles and Practice of Infectious Diseases." (2005).

[29] Schwarz, S., Corinna, K., Benoit, D., Axel, C. "Molecular basis of bacterial resistance to chloramphenicol and florfenicol." *FEMS microbiology reviews* 28, no. 5 (2004): 519-542.

[30] Speer, BS., Nadja, BS., Abigail, AS. "Bacterial resistance to tetracycline: mechanisms, transfer, and clinical significance." *Clinical microbiology reviews* 5, no. 4 (1992): 387-399.

[31] Klare, I., Carola, K., Dietlinde, B., Guido, W., Wolfgang, W. "Occurrence and spread of antibiotic resistances in *Enterococcus faecium*." *International journal of food microbiology* 88, no. 2-3 (2003): 269-290.

[32] Roberts, MC. "Resistance to tetracycline, macrolide-lincosamide-streptogramin, trimethoprim, and sulfonamide drug classes." *Molecular biotechnology* 20, no. 3 (2002): 261-283.

[33] Ying, L., Datong, T. "Recent advances in the medicinal chemistry of novel erythromycin-derivatized antibiotics." *Current topics in medicinal chemistry* 10, no. 14 (2010): 1441-1469.

[34] Chaudhary, HS., Bhavana, S., Anju, RS., Saurabh, S. "Diversity and versatility of actinomycetes and its role in antibiotic production." *Journal of Applied Pharmaceutical Science* 3, no. 8 (2013): S83-S94.

[35] Fukuda, K., Takashi, T., Yuko, S., Yuta, M., Kenji, I. "Enhanced production of the fluorinated nucleoside antibiotic nucleocidin by a rifR-resistant mutant of *Streptomyces calvus* IFO13200." *Actinomycetologica* 23, no. 2 (2009): 51-55.

[36] Palanichamy, K., Krishna, PK. "Discovery and syntheses of “superbug challengers”—platensimycin and platencin." *Chemistry–An Asian Journal* 5, no. 4 (2010): 668-703.

[37] Abdelkader, MSA., Thomas, P., Juan, A.A., Alan, TB., Michael, G., Rainer, E., Marcel, J., Mostafa, ER. "Asenjonamides A–C, antibacterial metabolites isolated from *Streptomyces asenjonii* strain KNN 42. f from an extreme-hyper arid Atacama Desert soil." *The Journal of antibiotics* 71, no. 4 (2018): 425.

[38] Pérez, BM., Daniel, OC., Mercedes, C., Maria, K., Jesús, M., Francisca, V., Olga, G., Fernando, R. "Phocoenamicins B and C, New Antibacterial Spirotetronates Isolated from a Marine Micromonospora sp." *Marine drugs* 16, no. 3 (2018): 95.

[39] Suga, T., Tōru, K., Yuki, I., Masato, I., Kenichi, N., Akira, T., Atsuko, M., Yōko, T, Satoshi, O., Takuji, N. "Hamuramicins A and B, 22-membered macrolides, produced by an endophytic actinomycete Allostreptomyces sp. K12-0794." *The Journal of antibiotics* 71, no. 7 (2018): 619.

[40] Izuta, S., Shohei, K., Makoto, K., Rei, M., Hirotaka, M., Atsuko Matsumoto, K N., Yōko, T., Satoshi, O., Takuji, N. "Dipyrimicin A and B, microbial compounds isolated from Amycolatopsis sp. K16-0194." *The Journal of antibiotics* 71, no. 5 (2018): 535.

[41] Kimura T., Tajima, A., Inahashi, Y., Iwatsuki, M., Kasai, H., Mokudai, T., Niwano, Y., Shiomi, K., Takahashi, Y., Ōmura, S., Nakashima, T. "Mumiamicin: Structure and bioactivity of a new furan fatty acid from Mumia sp. YSP-2-79." *The Journal of general and applied microbiology* 64, no. 2 (2018): 62-67.

[42] Nithya, K., Chinnasamy, M., Bhaskar, B., Naiyf, SA, Shine, K., Jamal, MK., Dharumadurai, D. "Desert actinobacteria as a source of bioactive compounds production with a special emphasis on Pyridine-2, 5-diacetamide a new pyridine alkaloid produced by Streptomyces sp. DA3-7." *Microbiological research* 207 (2018): 116-133.

[43] Rodríguez, V., Jesús, M., Aida, S., Mercedes, C., Luis, G., Gloria, B., Fernando, R. "Anthracimycin B, a potent antibiotic against gram-positive bacteria isolated from cultures of the deep-sea actinomycete

Streptomyces cyaneofuscatus M-169." *Marine drugs* 16, no. 11 (2018): 406.

[44] Braña, AF., Aida, S., Ignacio, P., Luis, O., Jonathan, F., Juan, JP., Jesús Martín., de la Cruz, M., Díaz, C., Vicente, F., Reyes, F., García, L A., Blanco, G."Branimycins B and C, antibiotics produced by the abyssal actinobacterium *Pseudonocardia carboxydivorans* M-227." *Journal of natural products* 80, no. 2 (2017): 569-573.

[45] Koomsiri, W., Yuki, I., Tōru, K., Kazuro, S., Yōko, T., Satoshi O., Arinthip, T., Takuji, N. "Bisoxazolomycin A: A new natural product from '*Streptomyces* subflavus subsp. *Irumaensis*' AM-3603." *The Journal of antibiotics* 70, no. 12 (2017): 1142.

[46] Xu, J., Kang, G., Dao, JZ., Yuan, GL., Li, T. "Ghanamycins A and B, two novel γ-butyrolactones from marine-derived *Streptomyces ghanaensis* TXC6-16." *The Journal of antibiotics* 70, no. 6 (2017): 733.

[47] Gui, C., Shanwen, Z., Xiangcheng, Z., Wenjuan, D., Hongbo, H., Yu, G., Yanwen, D., Jianhua, J. "Antimicrobial spirotetronate metabolites from marine-derived *Micromonospora harpali* SCSIO GJ089." *Journal of natural products* 80, no. 5 (2017): 1594-1603.

[48] Takehana, Y., Maya, U., Masaki, H., Chiaki, K., Ryuichi, S., Masayuki, I. "Fradiamine A, a new siderophore from the deep-sea actinomycete *Streptomyces fradiae* MM456M-mF7." *The Journal of antibiotics* 70, no. 5 (2017): 611.

[49] Nakashima, T., Rei, M., Hirotaka, M., Masato, I., Tatsuya S., Yoshinori, K., Kazuro, S., George, A P., Yōko, T., Satoshi, O. "Absolute configuration of iminimycin B, a new indolizidine alkaloid, from *Streptomyces griseus* OS-3601." *Tetrahedron Letters* 57, no. 30 (2016a): 3284-3286.

[50] Nakashima, T., Rei, M., Masato, I., Tatsuya, S., Toru, K., Yukihiro, A., Yoshinori, K., Kazuro, S., George, AP., Yōko, T., Satoshi, Ō."Iminimycin A, the new iminium metabolite produced by *Streptomyces griseus* OS-3601." *The Journal of antibiotics*69, no. 8 (2016b): 611.

[51] Shin, B., Byung, YK., Eunji, C., Ki-Bong, O., Jongheon, S., Michael, G., Dong-Chan, O. "Actinomadurol, an Antibacterial Norditerpenoid from a Rare Actinomycete, Actinomadura sp. KC 191." *Journal of natural products* 79, no. 7 (2016): 1886-1890.

[52] Zhang, Y., Navid, A., Doug, RB., Gregory, AE., Kenneth, JB., Shirley, PN., Ilia, AG., Tim, SB. "Micromonohalimanes A and B: Antibacterial halimane-type diterpenoids from a marine Micromonospora species." *Journal of natural products* 79, no. 11 (2016a): 2968-2972.

[53] Thi, QV., Van, HT., Huong, DTM., Cong, VL., Minh Le TH., Brian, TM., Van, MC., Van, CP. "Antimicrobial metabolites from a marine-derived actinomycete in Vietnam's East Sea." *Natural product communications* 11, no. 1 (2016): 49-51.

[54] Zhang, XM., Dao, FZ., Wen, JL., Chun, HL. "Pseudonocardides A–G, New γ-Butyrolactones from Marine-derived Pseudonocardia sp. YIM M13669." *Helvetica Chimica Acta* 99, no. 3 (2016b): 191-196.

[55] Ye, X., Komal, A., Tengfei, S., Wenling, W., Siran, Y., Haocai, H., Xiao, YL., Zhizhen, Z. "A new curvularin glycoside and its cytotoxic and antibacterial analogues from marine actinomycete Pseudonocardia sp. HS7." *Natural product research* 30, no. 10 (2016): 1156-1161.

[56] Huang, P., Feng, X., Biao, R., Qian, W., Jian, W., Qi, W., Wael, M AM., Miaomiao, L., Jianying, H., Ayokunmi, O., Jinzhao, S., Fuhang, S., Huanqin, D., Xueting, L., Lixin, Z. "Anti-MRSA and anti-TB metabolites from marine-derived Verrucosispora sp. MS100047." *Applied microbiology and biotechnology* 100, no. 17 (2016): 7437-7447.

[57] Fu, P., John, BM. "Spithioneines A and B, two new bohemamine derivatives possessing ergothioneine moiety from a marine-derived *Streptomyces spinoverrucosus*." *Organic letters* 17, no. 12 (2015): 3046-3049.

[58] Hassan, HM., David, D., Kyoung, HJ., Richard, HE., William, F. "Salinamide F, new depsipeptide antibiotic and inhibitor of bacterial

RNA polymerase from a marine-derived Streptomyces sp." *The Journal of antibiotics* 68, no. 3 (2015): 206.

[59] Ju, KS., Jiangtao, G., James, RD., Kwo-Kwang, AW., Christopher, JT., Steven, L., Emily, Metzger, E., Fudala, J., Su, J., Zhang, JK., Jaeheon, L., Joel PC., Bradley, SE., Ryuichi, H., David, PL., Wilfred, AD., William, WM. "Discovery of phosphonic acid natural products by mining the genomes of 10,000 actinomycetes." *Proceedings of the National Academy of Sciences* 112, no. 39 (2015): 12175-12180.

[60] Tang, X., Jie, L., Natalie, MA., Jia, JZ., Ellis, CO., Juan, AU., Paul, RJ., Simone, MM., Bradley, SM. "Identification of thiotetronic acid antibiotic biosynthetic pathways by target-directed genome mining." *ACS chemical biology* 10, no. 12 (2015): 2841-2849.

[61] Yekkour, A., Atika, M., Christian, B., Omrane, T., Rafik, E., Ahmed, L., Florence, M., Abdelghani, Z., Nasserdine, S. "A novel hydroxamic acid-containing antibiotic produced by a Saharan soil-living Streptomyces strain." *Letters in applied microbiology* 60, no. 6 (2015): 589-596.

[62] Yamanaka, K., Kirk, AR., Roland, DK., Katherine, SR., David, J G., Victor, N., Pieter, CD., Bradley, SM. "Direct cloning and refactoring of a silent lipopeptide biosynthetic gene cluster yields the antibiotic taromycin A." *Proceedings of the National Academy of Sciences* 111, no. 5 (2014): 1957-1962.

[63] Gavrish, E., Clarissa, SS., Shugeng, C., Olga, K., Amy, S., Aaron, P., Losee, L., Ashley, F., Hughes, D., Bissell, A., Torrey, H., Tatos A., Andreas, M., Slava, E., Alfred, G., Jon C., Kim, L."Lassomycin, a ribosomally synthesized cyclic peptide, kills Mycobacterium tuberculosis by targeting the ATP-dependent protease ClpC1P1P2." *Chemistry & biology* 21, no. 4 (2014): 509-518.

[64] Ellis, G., Thomas, W., Charles, F., Doug, B., Tim, B. "Solwaric acids A and B, antibacterial aromatic acids from a marine Solwaraspora sp." *Marine drugs* 12, no. 2 (2014): 1013-1022.

[65] Bonet, B., Robin, T., Max, C., Nadine, Z., Bradley, S M. "Direct capture and heterologous expression of Salinispora natural product

genes for the biosynthesis of enterocin." *Journal of natural products* 78, no. 3 (2014): 539-542.

[66] Eltamany, EE., Usama, RA., Amany, KI., Hashim, AH., Ute, H., Safwat, AA. "New antibacterial xanthone from the marine sponge-derived Micrococcus sp. EG45." *Bioorganic & medicinal chemistry letters* 24, no. 21 (2014): 4939-4942.

[67] Song, Y., Qinglian, L., Xue, L., Yuchan, C., Yun, Z., Aijun, S., Weimin, Z., Jingren, Z., Jianhua, J. "Cyclic hexapeptides from the deep South China Sea-derived *Streptomyces scopuliridis* SCSIO ZJ46 active against pathogenic Gram-positive bacteria." *Journal of natural products* 77, no. 8 (2014): 1937-1941.

[68] Jang, KH., Sang-Jip, N., Locke, JB., Kauffman, CA., Beatty, DS., Paul, LA., Fenical, W. "Corrigendum: Anthracimycin, a Potent Anthrax Antibiotic from a Marine-Derived Actinomycete." *Angewandte Chemie International Edition* 53, no. 3 (2014): 621-621.

[69] Um, S., Tae, JC., Heegyu, K., Byung, YK., Seong-Hwan K., Sang, KL., Ki-Bong, O., Jongheon, S., Dong-Chan, Oh. "Ohmyungs-amycins A and B: cytotoxic and antimicrobial cyclic peptides produced by Streptomyces sp. from a volcanic island." *The Journal of organic chemistry* 78, no. 24 (2013a): 12321-12329.

[70] Um, S., Young-Joo, K., Hyukna, K., He, W., Seong-Hwan K., Hak Cheoi, K., Sunghyouk, P., Jongheon, S., Dong-Chan, Oh. "Sungs-anpin, a lasso peptide from a deep-sea streptomycete". *Journal of Natural Product* 76, no. 5 (2013b): 873-879.

[71] Pan, HQ, Song-Ya, Z., Nan, W., Zhan-Lin, L., Hui-Ming, H., Jiang-Chun, H., Shu-Jin, W. "New spirotetronate antibiotics, lobophorins H and I, from a South China Sea-derived Streptomyces sp. 12A35." *Marine drugs*11, no. 10 (2013): 3891-3901.

[72] Niu, S., Sumei, L., Yuchan, C., Xinpeng, T., Haibo, Z., Guangtao, Z., Weimin, Z., Xiaohong Y., Si, Zhang., Changsheng, Z. "Lobophorins E and F, new spirotetronate antibiotics from a South China Sea-derived Streptomyces sp. SCSIO 01127." *The Journal of antibiotics* 64, no. 11 (2011): 711.

[73] Rateb, ME., Wael, EH., Markus, A., Mostafa, HA., Hai, D., William, TAH., Chinyere, KO., Juan, AA.,Barbara, AA., Gail, F., Alan, T B., Michael, G., Rainer, E., Marcel, J. "Chaxamycins A–D, bioactive ansamycins from a hyper-arid desert Streptomyces sp." *Journal of Natural products* 74, no. 6 (2011): 1491-1499.

[74] Van, W., Gilles, P., Kenneth, JM. "The regulation of the secondary metabolism of Streptomyces: new links and experimental advances." *Natural product reports* 28, no. 7 (2011): 1311-1333.

[75] Miguélez, EM., Carlos, H., Manuel, BM. "Hyphal death during colony development in *Streptomyces antibioticus*: morphological evidence for the existence of a process of cell deletion in a multicellular prokaryote." *The Journal of cell biology* 145, no. 3 (1999): 515-525.

[76] Rigali, S., Harald, N., Elke, EEN., Maximilian, S., Sévrine, C., Marisa, M., Bernard, J., Koerten HK., Hopwood DA., Titgemeyer, F., Van, WGP. "The sugar phosphotransferase system of *Streptomyces coelicolor* is regulated by the GntR-family regulator DasR and links N-acetylglucosamine metabolism to the control of development." *Molecular microbiology* 61, no. 5 (2006): 1237-1251.

[77] Rigali, S., Fritz, T., Sharief, B., Suzanne, M., Andreas, WT., David, AH., Gilles, PVW. "Feast or famine: the global regulator DasR links nutrient stress to antibiotic production by Streptomyces." *EMBO reports* 9, no. 7 (2008): 670-675.

[78] Thun, MJ., DeLancey, JO., Center, MM., Jemal, A., Ward, EM. “The global burden of cancer: priorities for prevention.” *Carcinogenesis*. 31, no. 1 (2010):100-110.

[79] Demain, AL., Sanchez, S. “Microbial drug discovery: 80 years of progress.” *The Journal of Antibiotics* (Tokyo) 62, no. 1(2009): 5-16.

[80] Baltz, RH. “Renaissance in antibacterial discovery from actinomycetes.” *Current Opinion in Pharmacology* 8, no.5 (2008): 557–563.

[81] Busi, S., Pattnaik, S. “Current status and applications of actinobacteria in the production of anticancerous compounds.” In: Actinobacteria:

diversity and biotechnological applications. Elsevier, Oxford. pp 137-153.

[82] Passari, AK., Mishra, VK., Saikia, R., Gupta, VK., Singh, BP. "Isolation, abundance and phylogenetic affiliation of endophytic actinomycetes associated with medicinal plants and screening for their *in vitro* antimicrobial biosynthetic potential." *Frontiers in Microbiology* 6, no. 273 (2015): 1-13.

[83] Han, Z., Xu, Y., McConnell, O., Liu, L., Li, Y., Qi, S., Huang, X., Qian, P. "Two antimycin A analogues from marine-derived actinomycete *Streptomyces lusitanus*." *Marine Drugs* 10, no. 3 (2012): 668-676.

[84] Schwartsmann, G., Brondani da Rocha, A., Berlinck, RG., Jimeno, J. "Marine organisms as a source of new anticancer agents." *The Lancet Oncology* 2, no. 4(2001):221-225.

[85] Abdelmohsen, UR., Pimentel-Elardo, SM., Hanora, A., Radwan, M., Abou-El-Ela, SH., Ahmed, S., Hentschel, U. "Isolation, phylogenetic analysis and anti-infective activity screening of marine sponge-associated actinomycetes." *Marine Drugs* 8, no.3 (2010): 399-412.

[86] Asolkar, RN., Kirkland, TN., Jensen, PR., Fenical, W. *"*Arenimycin, *an* antibiotic effective against rifampin- *and* methicillin-resistant Staphylococcus aureus *from the* marine actinomycete Salinispora Arenicola*." The Journal of Antibiotics (*Tokyo*)* 63, no. *1 (2010):*37–39.

[87] Fu, P., Wang, SX., Hong, K., Li, X., Liu, PP., Wang, Y. "Cytotoxic bipyridines from the marine-derived actinomycete *Actinoalloteichus cyanogriseus* WH1-2216-6." *Journal of Natural Products* 74, no. 8 (2011a): 1751-1756.

[88] Fu, P., Liu, PP., Li, X., Wang, Y., Wang, SX., Hong, K., Zhu, WM. "Cyclic bipyridine glycosides from the marine-derived actinomycete *Actinoalloteichus cyanogriseus* WH1-2216-6." *OrganicLetters* 13, no. 22 (2011b): 5948-5951.

[89] Williams, DE., Dalisay, DS., Patrick, BO., Matainaho, T., Andrusiak, K., Deshpande, R., Myers, CL., Piotrowski, JS., Boone, C., Yoshida, M., Andersen, RJ. "Padanamides A and B, highly modified linear

tetrapeptides produced in culture by a Streptomyces sp. isolated from a marine sediment." *Organic Letter* 13, no. 15 (2011): 3936-3939.

[90] Sato, FI., Yamada, S., Kawahara, H., Katayama, M. "Usabamycins A–C: new anthramycin-type analogues from a marine-derived actinomycete." *Bioorganic & Medicinal Chemistry Letters* 21, no. 23 (2011): 7099-7101.

[91] Gärtner, A., Ohlendorf, B., Schulz, D., Zinecker, H., Wiese, J., Imhoff, JF. "Levantilides A and B, 20-membered macrolides from a Micromonospora strain isolated from the Mediterranean deep sea sediment." *Marine Drugs* 9, no. 1 (2011): 98-108.

[92] Li, S., Tian, X., Niu, S., Zhang, W., Chen, Y., Zhang, H., et al. (2011). Pseudonocardians AC, new Diazaanthraquinone derivatives from a deep sea actinomycete Pseudonocardia sp. SCSIO 01299. *Marine Drugs* 9, no. 8 (2011): 1428–1439.

[93] Dasari, VRRK., Muthyala, MKK., Nikku, MY., Donthireddy, SRR. "Novel Pyridinium compound from marine actinomycete, *Amycolatopsis alba* var. nov., DVR D4 showing antimicrobial and cytotoxic activities in vitro." *Microbiological Research* 167, no. 6 (2012): 346-351.

[94] Xie, QY., Qu Z., Lin, HP., Li, L., Hong, K. *Micromonospora haikouensis* sp. nov., isolated from mangrove soil. *Antonie van Leeuwenhoek* 101, no. 3 (2012):649-655.

[95] Gao, X., Lu, Y., Xing, Y., Ma, Y., Lu, J., Bao, W. "A novel anticancer and antifungus phenazine derivative from a marine actinomycete BM-17." *Microbiological Research* 167, no. 10 (2012): 616-622.

[96] Fu, P., Yang, C., Wang, Y., Liu, P., Ma, Y., Xu, L. "Streptocarbazoles A and B, two novel indolocarbazoles from the marine derived actionmycete strain Streptomyces sp. FMA." *Organic Letters* 14, no.9 (2012): 2422-2425.

[97] Lu, J., Ma, Y., Liang, J., Xing, Y., Xi, T., Lu, Y. "Aureolic acids from a marine-derived Streptomyces sp. WBF16." *Microbiological Research* 167, no. 10 (2012): 590-595.

[98] Huang, HB., Yang, TT., Ren, XM., Liu, J., Song, YX., Sun, AJ., et al. "Cytotoxic Angucycline class glycosides from the deep sea

actinomycete Str*eptomyces lusitanus* SCSIO LR32." *The Journal of Natural Product* 75, no. 2 (2012): 202–208.

[99] Zhang, WJ., Liu, Z., Li, SM., Yang, TT., Zhang, QB., Ma, L., et al. Spiroindimicins A-D: new Bisindole alkaloids from a deep-sea-derived actinomycete. *Organic Letters* 14, no. 13 (2012): 3364–3367.

[100] Kornsakulkarn, J., Saepua, S., Boonruangprapa, T., Suphothina, S., Thongpanchang, C. "New ß-carboline and indole alkaloids from Actinomycete Actinomadura sp. BCC 24717." *Phytochemistry Letters*6, no. 3 (2013): 491-494.

[101] Fei, P., Chuan-Xi, W., Yang, X., Hong-Lei, J., Lu-Jie, C., Uribe, P., Bull, A T., Goodfellow, M., Hong, J., Yun-Yang, L. "A new 20-membered macrolide produced by a marine-derived Micromonospora strain." *Natural Product Research*27, no. 15 (2013): 1366-1371.

[102] Um, S., Kim, YJ., Kwon, H., Wen, H., Kim, SH.,Kwon, HC., Park S, Shin, J., Oh, DC. "Sungsanpin, a lasso peptide from a deep-sea streptomycete." *Journal of Natural Product* 76, no. 5 (2013b): 873-879.

[103] Che, Q., Zhu, T., Keyzers, RA., Liu, X., Li, J., Gu, Q., Li, D. "Polycyclic Hybrid Isoprenoids from a Reed Rhizosphere Soil Derived Streptomyces sp. CHQ-64." *Journal of Natural Product* 76, no. 4 (2013): 759-763.

[104] Zhang, Q., Li, S., Chen, Y., Tian, X., Zhang, X., Zhang, G., et al. (2013). "New diketopiperazine derivatives from a deep-sea-derived *Nocardiopsis alba* SCSIO 03039." *The Journal of Antibiotics* 66, no.1 (2013): 31–36.

[105] Trzoss, L., Fukuda, T., Costa-Lotufo, L.V., Jimenez, P., La Clair, J J., Fenical, W. "Seriniquinone, a selective anticancer agent, induces cell death by autophagocytosis, targeting the cancer-protective protein dermcidin." *Proceedings of the National Academy of Sciences of the United States of America* 111, no. 41 (2014): 14687–14692.

[106] Zhang, W., Ma, L., Li, S., Liu, Z., Chen, Y., Zhang, H., Zhang, G., Zhang, Q., Tian, X., Yuan, C. "Indimicins A-E, Bisindole Alkaloids from the Deep-Sea-Derived Streptomyces sp. SCSIO03032." *Journal of Natural Product* 77, no. 8 (2014): 1887-1892.

[107] Fu, P., MacMillan, JB. "Thiasporines A-C, thiazine and thiazole derivatives from a marine-derived Actinomycetospora chloral." *Journal of Natural Products* 78, no. 3 (2015): 548-551.

[108] Tan, L., Ser, H., Yin, W., Chan, K., Lee, L., Goh, B. "Investigation of antioxidative and anticancer potentials of Streptomyces sp. MUM256 isolated from Malaysia mangrove soil." *Frontiers in Microbiology* 6, no. 1316 (2015): 1-12.

[109] Liu, D., Lin, H., Proksch, P., Tang, X., Shao, Z., Lin, W. Microbacterins A and B, new peptaibols from the deep sea actinomycete *Microbacterium sediminis* sp. nov. YLB-01(T). *Organic Letters* 17, no. 5 (2015):1220-1223.

[110] Song, Y., Liu, G., Li, J., Huang, H., Zhang, X., Zhang, H., Ju, J. "Cytotoxic and antibacterial angucycline- and prodigiosin-analogues from the deep-sea derived Streptomyces sp. SCSIO 11594." *Marine Drugs* 13, no. 3 (2015):1304-1316.

[111] Niu, S., Zhou, TT., Xie, CL., Zhang, GY., Yang, XW. "Microindolinone A, a Novel 4,5,6,7-Tetrahydroindole, from the Deep-Sea-Derived Actinomycete *Microbacterium* sp. MCCC 1A11207." *Marine Drugs* 15, no. 7 (2017):230.

[112] Sun, M., Chen, X., Li, W., Lu, C., Shen, Y. "New diketopiperazine derivatives with cytotoxicity from Nocardiopsis sp. YIM M13066." *The Journal of Antibiotics*70, no.6 (2017): 795–797.

[113] Ahmad, MS., El-Gendy, AO., Ahmed, RR., Hassan, HM., El-Kabbany, HM., Merdash, AG. "Exploring the Antimicrobial and Antitumor Potentials of *Streptomyces* sp. AGM12-1 Isolated from Egyptian Soil." *Frontiers in Microbiology* 8, 438 (2017).

[114] Son, S., Jang, M., B., Hong, YS., Ko, SK., Jang, JH., Ahn, JS. "Ulleungdin, a Lasso Peptide with Cancer Cell Migration Inhibitory Activity Discovered by the Genome Mining Approach." *Journal of Natural Products* 81. no. 10 (2018): 2205-2211.

[115] Davies-Bolorunduro, OF., Adeleye, IA., Akinleye, MO., Wang, PG. Anticancer potential of metabolic compounds from marine actinomycetes isolated from Lagos Lagoon sediment. *Journal of Pharmaceutical Analysis* 9, no. 3 (2019): 201-208.

[116] Wadkins, RM., Vladu, B., Tung, CS. “Actinomycin D binds to metastable hairpins in single-stranded DNA.” *Biochemistry* 37, no. 34 (1998):11915–23.

[117] Olano, C., Carmen, M., José, S. "Antitumor compounds from marine actinomycetes." *Marine drugs* 7, no. 2 (2009a): 210-248.

[118] Rocha-Santos, T., Duarte, AC. “Introduction to the Analysis of Bioactive Compounds in Marine Samples.” In: Analysis of Marine Samples in Search of Bioactive Compounds, *Comprehensive Analytical Chemistry* 65, (2014), Elsevier, pp. 1–13.

[119] Olano, C., Méndez, C., Salas, JA. “Antitumor compounds from actinomycetes: from gene clusters to new derivatives by combinatorial biosynthesis.” *Natural Product Reports* 26, no. 5(2009b):628-660.

[120] Harir, M., Bendif, M., Bellahcene, M., Fortas, Z., Pogni, R. “Streptomyces Secondary Metabolites.” In: Basic Biology and Applications of Actinobacteria, (2018), IntechOpen, pp. 99-124.

[121] Gomathi, A., Gothandam, K. “Ocean Dwelling Actinobacteria as Source of Antitumor Compounds.” *Brazilian Archives of Biology and Technology* 59, (2016).

[122] Fischbach, MA., Walsh, CT. “Assembly-line enzymology for polyketide and nonribosomal peptide antibiotics: Logic, machinery, and mechanism.” *Chemical Reviews* 106, no. 8 (2006), 3468–3496.

[123] Dairi, T. “Studies on biosynthetic genes and enzymes of isoprenoids produced by actinomycetes.” *The Journal of Antibiotics* 58, no. 4 (2005): 227-243.

[124] Sánchez, C., Méndez, C., Salas, JA. “Indolocarbazole natural products: occurrence, biosynthesis, and biological activity.” Natural Product Reports 23, no. 6 (2006): 1007-1045.

[125] Karpiński, TM., Adamczak, A. “Anticancer Activity of Bacterial Proteins and Peptides.” *Pharmaceutics* 10, no. 2 (2018): pii: E54.

[126] Maskey, RP., Elisabeth, H., Oliver, K., Heinz, HF., Armin, M., Andreas, B., Hartmut, L. "Anti-cancer and antibacterial trioxacarcins with high anti-malaria activity from a marine streptomycete and their

absolute stereochemistry." *The Journal of antibiotics* 57, no. 12 (2004): 771-779.

[127] Alharbi, NS. "Novel bioactive molecules from marine actinomycetes." *Biosciences Biotechnology Research Asia* 13, no. 4 (2016): 1905-1927.

[128] Wei, Yanhong, Wei Fang, Zhongyi Wan, Kaimei Wang, Qingyu Yang, Xiaofeng Cai, Liqiao Shi, and Ziwen Yang. "Antiviral effects against EV71 of pimprinine and its derivatives isolated from Streptomyces sp." *Virology journal* 11, no. 1 (2014): 195.

[129] Wyche, TP., Jeff, SP., Yanpeng, H., Doug, B., Raamesh, D., Sean, M., Irene, MO., Chad, LM., Ilia, AG., Willam, MW., David, RA., Tim, SB. "Forazoline A: Marine-Derived Polyketide with Antifungal *In Vivo* Efficacy." *Angewandte Chemie International Edition* 53, no. 43 (2014): 11583-11586.

[130] Xie, C., Qingmei, L., Jin-Mei, X., Yuanyuan, G., Quan, Y., Zong-Ze, S., Guangming, L., Xian-Wen, Y. "Anti-allergic compounds from the deep-sea-derived actinomycete Nesterenkonia flava MCCC 1K00610." *Marine drugs* 15, no. 3 (2017): 71.

[131] Wyche, T., Miranda, S., Yanpeng, H., Doug, B., Delinda, J., Jeffrey, J., Tim, B. "Activation of the nuclear factor E2-related factor 2 pathway by novel natural products halomadurones A–D and a synthetic analogue." *Marine drugs* 11, no. 12 (2013): 5089-5099.

[132] Aoyagi, T., Hatsu, M., Imada, C., Naganawa, H., Okami, Y., Takeuchi, T. Pyrizinostat: a new inhibitor of pyroglutamyl, peptidase. *Journal of Antibiotics*, 45, no 11 (1992):1795–1796.

[133] Imada, C. "Enzyme inhibitors and other bioactive compounds from marine actinomycetes." *Antonie Van Leeuwenhoek* 87, no. 1 (2005): 59-63.

[134] Sader, HS., Gales, AC. “Emerging strategies in infectious diseases: new carbapenem and trinem antibacterial agents.” *Drugs*61, no. 5 (2001): 553-564.

[135] Zhanel, GG., Walters, M., Noreddin, A., Vercaigne, LM., Wierzbowski, A., Embil, JM., Gin, AS., Douthwaite, S., Hoban, DJ. “The ketolides: a critical review.” *Drugs* 62, no.12 (2002): 1771-1804.

[136] Perry, CM., Ibbotson, T. “Biapenem.” *Drugs* 62, no. 15 (2002):2221-2235.

[137] Choo, EU., Chamber HF. “Treatment of Methicillin-Resistant *Staphylococcus aureus* Bacteremia.” *Infection and Chemotherapy* 48, no. 4(2016): 267–273.

[138] Zhanel, GG., Homenuik, K., Nichol, K., Noreddin, A., Vercaigne, L., Embil, J., Gin, A., Karlowsky, JA., Hoban, DJ. “The glycylcyclines: a comparative review with the tetracyclines.” *Drugs* 64, no. 1 (2004): 63-88.

[139] Aggen, JB., Armstrong, ES., Goldblum, AA., Dozzo, P., Linsell, M S., Gliedt, MJ., Hildebrandt, DJ et al. “Synthesis and spectrum of the neoglycoside ACHN-490.” *Antimicrobial Agents Chemotherapy* 54, no. 11(2010): 4636-4642.

[140] Saravolatz, LD., Stein, GE., Johnson, LB. “Ceftaroline: a novel cephalosporin with activity against methicillin-resistant *Staphylococcus aureus.” Clinical Infectious Diseases* 52, no. 9 (2011):1156-1163.

[141] Genilloud, O. "Mining actinomycetes for novel antibiotics in the omics era: are we ready to exploit this new paradigm?" *Antibiotics* 7, no. 4 (2017): 85.

[142] Snydman, DR., Jacobus, NV., McDermott, LA. "Activity of a novel cyclic lipopeptide, CB-183,315, against resistant *Clostridium difficile* and other Gram-positive aerobic and anaerobic intestinal pathogens." *Antimicrobial agents and chemotherapy* 56, no. 6 (2012): 3448-3452.

[143] De Lima Procópio, RE., Da Silva, IR., Martins, MK., De Azevedo, JL., De Araújo, JM. “Antibiotics produced by *Streptomyces.” Brazilian Journal of Infectious Diseases* 16, no. 5 (2012): 466–471.

[144] Kumar, K., Chopra, S. “New drugs for methicillin-resistant *Staphylococcus aureus:* an update.” *The Journal of Antimicrobial Chemotherapy* 68, no. 7 (2013): 1465–1470.

[145] Bouza, E., and Almudena, B. "Oritavancin: a novel lipoglycopeptide active against Gram-positive pathogens including multiresistant strains." *International journal of antimicrobial agents* 36, no. 5 (2010): 401-407.

[146] Honeyman, L., Mohamed, I., Mark, LN., Beena, B., Todd, EB., Jackson, C., Rachi, M., Ohemeng, K., Verma, AK., Cannon, EP., Macone, A., Tanaka, SK., Levy, S. "Structure-activity relationship of the aminomethylcyclines and the discovery of omadacycline." *Antimicrobial agents and chemotherapy* 59, no. 11 (2015): 7044-7053.

[147] Knight, C., Victoria., Carmela, M., Laurent, C., Jared, S. "Discovery and development of surotomycin for the treatment of *Clostridium difficile*." *Journal of industrial microbiology & biotechnology* 43, no. 2-3 (2016): 195-204.

[148] Fernandes, P., Martens E. "Antibiotics in late clinical development." *Biochemical Pharmacology* 133. No. (2017): 152-163.

[149] Chapman, TM., Perry, CM. "Everolimus." *Drugs* 64, no. 8 (2004): 861-872.

[150] Pastores, GM., Barnett, NL., Kolodny, EH. "An open-label, non-comparative study of miglustat in type I Gaucher disease: efficacy and tolerability over 24 months of treatment." *Clinical Therapeutics* 27, no. 8 (2005): 1215–1227.

[151] Lee, MD., Dunne, TS., Siegel, MM., Chang, CC., Morton, GO., Borders, BD. "Calichemicins, a novel family of antitumor antibiotics. 1. Chemistry and partial structure of calichemicin." *Journal of the American Chemical Society* 109. no. 11 (1987): 3464-3466

[152] Sugiura, T., Ariyoshi, Y., Negoro, S., Nakamura, S., Ikegami, H., Takada, M., Yana, T., Fukuoka, M. "Phase I/II study of amrubicin, a novel 9-aminoanthracycline, in patients with advanced non-small-cell lung cancer." *Investigational New Drugs* 23, no. 4 (2005): 331–337.

[153] Gupta, AK., Chow, M. "Pimecrolimus: A review." *Journal of the European Academy of Dermatology and Venereology* 17, no. 5 (2003): 493-503.

[154] Li, JJ. "Synthesis of Best-Seller Drugs. By Ruben Vardanyan and Victor Hruby." Angewandte Chemie International Edition. 56. No. 10 (2017). 2541.

[155] Demain, AL., Vaishnav, P. "Natural products for cancer chemotherapy." *Microbial Biotechnology* 4, no. 6 (2010): 687-699.

[156] Russo, P., Del Bufalo, A., Fini, M. "Deep sea as a source of novel-anticancer drugs: update on discovery and preclinical/clinical evaluation in a systems medicine perspective." *Experimental and Clinical Sciences, International Online Journal* 10, no.14 (2015):228-236.

[157] Butler, MS. "Natural products to drugs: natural product-derived compounds in clinical trials." *Natural Product Reports* 25, no. 3 (2008): 475–516.

[158] Portugal, J. "Chartreusin, elsamicin A and related anti-cancer antibiotics." *Current Medicinal Chemistry - Anti-Cancer Agents* 3, no. 6 (2003): 411-420.

[159] Bisht, KS., Bradbury, M., Mattson, D., Kaushal A., Sowers, A., Markovina, S., Ortiz, KL., Sieck, LK., Isaacs, JS., Brechbiel, MW., Mitchel, JB., Neckers, LM., Gius, D. "Geldanamycin and 17-allylamino-17-demethoxygeldanamycin potentiate the *in vitro* and *in vivo* radiation response of cervical tumor cells via the heat shock protein 90-mediated intracellular signaling and cytotoxicity." *Cancer Research* 63, no. 24 (2003): 8984-8995.

[160] Broggini, M., Marchini, S., Fontana, E., Moneta, D., Fowst, C., Geroni, C. "Brostacillin: A new concept in minor groove DNA binder development." *Anticancer Drugs* 15, no. 1 (2004): 1-6.

[161] Fiedler, HP., Bruntner, C., Bull, AT., Ward, AC., Goodfellow, M., Potterat, O., Puder, C., Mihm, G. "Marine actinomycetes as a source of novel secondary metabolites." *Antonie van Leeuwenhoek* 87, no. 1 (2005): 37–42.

[162] Baltz, RH. "Antimicrobials from actinomycetes*:* Back *to the* future." *Microbe* 2, no. *3(2007):*125-131.

[163] Kekuda, TRP., Shobha, KS., Onkarappa, R. "Fascinating diversity and potent biological activities of actinomycetes metabolites." *Journal* of. *Pharmacy Research* 3, no. 2 (2010): 250-256.

[164] Xu, XN., Chen, LY., Chen, C., Tang, YJ., Bai, FW., Su, C., Zhao, XQ. "Genome Mining of the Marine Actinomycete Streptomyces sp.

DUT11 and Discovery of Tunicamycins as Anti-complement Agents." *Frontiers in Microbiology* 9, (2018):1318.

[165] Greule, A., Zhang, S., Paululat, T., and Bechthold, A. "From a natural product to its biosynthetic gene cluster: a demonstration using polyketomycin from *Streptomyces diastatochromogenes* Tü6028." *Journal of Visualized Experiments* 119, (2017), 54952.

[166] Rath, CM., Janto, B., Earl, J., Ahmed, A., Hu, FZ., Hiller, L., Dahlgren, M., Kreft, R., Yu, F. "Meta-omic characterization *of the* marine invertebrate microbial consortium *that* produces *the* chemotherapeutic natural product ET-743." *ACS Chemical Biology* 6, no. 11 (2011): 1244–1256.

In: A Closer Look at Actinomycetes ISBN: 978-1-53617-046-7
Editors: A. A. Mohamed Hatha et al.

Chapter 2

ENDOPHYTIC ACTINOMYCETES IN INDO-PAK MEDICINAL PLANTS LEADING TO NEW TRENDS IN DRUG DISCOVERY

Rabia Tanvir[1,*], Ali Ahmad Sheikh[1] and Aqeel Javeed[2]
[1]University Diagnostic Lab, Department of Microbiology,
University of Veterinary and Animal Sciences, Lahore,
Punjab, Pakistan
[2]Department of Pharmacology and Toxicology,
University of Veterinary and Animal Sciences,
Lahore, Punjab, Pakistan

ABSTRACT

The Indo-Pak region of the subcontinent that includes Pakistan and India has a long history of traditional medicines. In Pakistan, Unani-Tibb (Graeco-Arabic) medicine system has been in practice for centuries with the local population still relying on the Unani practitioners. In India, the

* Corresponding Author's Email: rabia.tanvir@uvas.edu.pk; rabiatanvir@hotmail.com.

Ayurveda system of medicine has deep historical roots. Both the systems rely on ethnobotany i.e., traditional knowledge of local plants.

With our recent understanding of endophytes that are the commensal symbionts residing within the plants, the medicinal use of such plants is connected as much as to these migrant organisms as to their biochemistry. One of such symbionts are the actinomycetes, the well-known producers of antibiotics that have been extensively isolated from soil; the researchers have now turned their attention towards those that have moved inside the plants. If the source plant has been used in traditional medicines for healing purposes then the actinomycetes residing within them are more likely candidates for novel drug sources. The most notable example of this hypothesis is the potent peptide antibiotic munumbicins produced by the *Streptomyces* NRRL-30562 resident of the endosphere of the Snake Vine plant, *Kennedia nigricans*. In this chapter, we will focus on the important medicinal plants in the Indo-Pak region and the recent studies on the diversity of their endophytic actinomycetes. Besides, we will emphasize the diverse metabolites produced by them and their bioactivities as well.

Keywords: commensals; endophytes; Indo-Pak; medicinal plants

INTRODUCTION

Natural products are secondary metabolites that are obtained from natural sources such as plants, microorganisms, and animals. They have biological activities since they are produced as a result of either adaption to the environment or a defense mechanism against predators. They help to assist and improve survival (Bernardini et al. 2018). The importance of natural products in medicines nowadays can be judged by the fact that more than one-third of the medicines approved by the Food and Drug Administration (FDA) are from natural sources. In addition, since the 1940s about half of all the anticancer drugs that have been registered are from either natural products or their derivatives. Most recently, more than 200,000 bioactive compounds derived from these natural products are in the process of becoming new drugs (Boy et al. 2018).

If we look at the history of use of such compounds then we find that it is as old as the human civilization. Every civilization has developed their own practices of using their local resources for the treatment of diseases

known as the traditional system of medicine (Bernardini et al. 2018; Prakash et al. 2018). The most prominent local resource of these natural products are the medicinal plants (Boy et al. 2018). The term medicinal plants is directed towards those plant varieties that possess medicinal properties and a variety of their parts such as leaves, roots, flowers, seeds even the whole plant can be used. In each part, certain materials are synthesized and stored referred to as active compounds (bioactive substances), which have a potential effect on the living organisms. The active compound in these parts can have an indirect or direct therapeutic effect. The constituents of the plants may also interact among themselves and this may be beneficial for the plant itself. These plant-derived compounds are so effective that they are even used for the treatment of most dangerous and hard to treat diseases such as cancer. Not only do these compounds cure but they also have the ability to prevent the development of certain diseases (Jamshidi-Kia et al. 2018).

The use of plants in the treatment of diseases go a long way back that is why they are considered as the most important source of bioactive compounds by the pharmaceutical industries. Already 25% of the drugs in the market contain one or more ingredient that is based on the plant products either natural or synthetic. With an annual growth of 6.5%, it is estimated that the global market of natural products from plants will reach 507 trillion USS by the year 2050 (Prakash et al. 2018). The main reason for such growth is the adverse side effects and toxicity of the allopathic medicines along with the population demand for herbal drugs. This has led to an increase in the number of herbal medicine manufacturers and a decrease in conventional chemical drug use (Jamshidi-Kia et al. 2018). Another reason for an increase in the use of plant-derived products may be that over three fourth of the world population cannot afford conventional medicines and therefore turn towards the traditional ones that are cheaper and affordable (Tariq et al. 2018). Because of this, currently, about 70,000 plant species are being tested for their bioactive compounds (Boy et al. 2018).

It is important to note that only those plants with a history of ethnopharmacological use have been the primary targets for drug discovery. The reason behind it is that such plants have developed a complicated defense mechanism by arming themselves with a range of bioactive

compounds. Most importantly, these compounds may be bioactive against pathogens of human diseases. The chemical structures of such compounds are quite diverse for example, terpene, alkaloids, phenolics and flavonoids. Their biological properties also vary for example they may be antimicrobial, fungicidal or even antiparasitic (Boy et al. 2018; Prakash et al. 2018).

Ever since the appearance of antibiotic resistance among pathogens, it has been spreading rapidly to the point that it is now a serious health problem. This has encouraged researchers to look for novel antimicrobial compounds from unique and unexplored habitats, and one such habitat is the plant-derived microorganisms called endophytes (Chandrakar and Gupta 2017). Since the medicinal plants are a rich source of bioactive compounds, therefore a long-term association of these endophytes with such plants may result in their participation in the plant's metabolic pathways. This may enhance their own bioactivity or may allow them to gain some genetic information to mimic the bioactive compounds of the host plant (Golinska et al. 2015). Since the Indo-Pak subcontinent is rich with ethnopharmacologically important medicinal plants, this chapter will focus on their endophytic actinobacterial population. It will also discuss the diversity of their compounds and their broad-spectrum bioactivities.

THE INDO-PAK REGION AND ITS ETHANOBOTANICAL HISTORY

The geographical boundaries of the Indo-Pak region include Pakistan, India, Bangladesh, Sri Lanka, Bhutan, Nepal, and the small islands of the Indian Ocean. Geopolitically, it is one of the largest regions of the continent of Asia (Kumar 2012) covering a 10% land surface area (Levinson and Christensen 2002) and is home to 45% of Asia's population (Levinson and Christensen 2002; Desai 2002).

Dr. John William Hershberger first described the term ethnobotany in 1895. He was an American botanist and while delivering a lecture he used this term to describe his research (Rahman et al. 2018). However, the

information on local plants and their uses is as old as the human civilization itself (Qureshi et al. 2009). Ethnobotany is defined as the interaction between local people and their plant population or more particularly the relationship between humans and plants. By understanding the word, "ethno," the study of people and "botany," the study of plants (Rahman et al. 2018), ethnobotany becomes the traditional knowledge among the indigenous people of a particular culture regarding their plant diversity and use of indigenous plants. The word traditional here also suggests a rather historical or cultural system rather than the conventional one. As the term ethnobotany includes the word botany in it, it makes it clear that the roots of ethnobotany lie in the study of plants and its interest originated from discovering medicinal plants capable of alleviating illnesses (Rupani and Chavez 2018; Qureshi et al. 2009).

Pakistan is a country blessed with unique geography; it has altitudes ranging from 0 to 8611m because of the Hindu-kush Himalayas and Karakorum ranges. This variety of climatic zones, ecological versatility, and soil conditions has given this country its rich floral diversity (Shinwari and Qaiser 2011; Rahman et al. 2018). The unique biodiversity of Pakistan comprises of more than 6,000 plant species and from them, 5700, plant species are under cultivation in the country. Approximately 400-600 species (12%) of the flora are medicinal plants that have a regular use in the traditional medicine system (Aziz et al. 2018; Shinwari and Qaiser 2011; Khan et al. 2018). Because Pakistan is a developing country, therefore traditional medicines provide a tangible and cheaper alternative for expensive primary health care system (Haq et al. 2011). An estimated 80% of the rural population is dependent on a traditional medicinal system termed as Unani system. This system is entirely dependent on the ethnobotany of the local population (Rahman et al. 2018) and was introduced by the Muslims of the sub-continent (Khan et al. 2018). Originally founded by the Greek philosophers, Hippocrates, Aristotle and Galen, it extended to the Arabian countries and was brought to the subcontinent in 1350CE. It was documented by the Muslims scholars during the Islamic civilization period and was practiced by Mughals for centuries (Qureshi et al. 2009; Rupani and Chavez 2018). Due to its foundation by the Greek philosophers, this system

is called Tibb-e-Unani or Unani-Tibb (Graeco-Arabic) with Unani meaning Greek in Urdu.

Pakistan is one of the leaders in the export of medicinal plants due to its massive crude drug market (Pansara) system. In the country, there are more than 39 thousand hakims (Unani-Tibb practitioners) and more than 400 Tibbia Dawakhana (Clinics) and dispensaries providing health care. There are more than 300 Tibb-e-Unani manufacturing companies as well (Husain et al. 2008; Shinwari and Qaiser 2011).

India is another densely populated country with a population of about 1 billion that alone corresponds to 16.5% of the entire population of the world. About 70% of its population resides in villages giving it a vast rural and multiethnic landscape (Desai 2002; Rahman et al. 2018). According to the World Health Organization (WHO), approximately 84% of its population depends on the traditional system of medicine. Since India is a large country, therefore previous studies have investigated its ethnobotany by dividing it into parts, the northern, southern and central India. Due to the diversity in its climate, altitudinal zones and varied ecological habitats, it is rich in flora and fauna. Its diversity includes different types of forests such as the tropical dry deciduous forest that occupies the largest area in Central India Madhya Pradesh (Jain et al. 2018). The country is rich in ethnobotanical knowledge with a vast biodiversity of plant species (45,000 so far) (Rupani and Chavez 2018). All India co-ordinate research project on ethnobiology showed that the rural population uses more than 9500 plant species for various purposes with over 7500 wild plant species utilized for medicine preparation only (Rahman et al. 2018).

The deeply developed traditions of healing that are practiced in India includes the Ayurveda system of healing. Ayurveda is from the Sanskrit language meaning "life knowledge' and the therapies included in this system comprise of herbal compounds, metals, and minerals. This system began in 2500 BC (Rahman et al. 2018) and have been used for several millennia. In many villages, it is the only healing practice used (Rupani and Chavez 2018). Another healing system called the Siddha medicine system is prevalent as well that came from Tamil culture. Based in South India its concept of foundation relies on diet and body-mind practices such as

meditation/yoga. It shares similarities with the Ayurveda system of medicine (Rupani and Chavez 2018).

The earliest documentation of the ethnobotanical use of indigenous plants of the Indo-Pak subcontinent was done in the literature and religious books such as Rigveda and Atharvaveda, dating back to 4000 and 400 BC. The first record of the description of medicinal plants was seen in Rig-Veda between 4500–1600 BC. It contained the description of 67 plants making it one of the earliest repository of such knowledge (Malla and Shakya 1999). Further, on, from the 16th century some foreign researchers such as Garcia da Orta took to the active study of medicinal herbs and published a book, 'Coloquios Dos Simples E Drogas E Cusas Medicinas Da India'. It explained the significance of 50 medicinal plant taxa common in Goa and Malabar. Some other earlier works included 'Materia Medica of Hindostan' and the catalog of Indian medicinal plants and drugs describing the established indigenous Indian medicine system (Rahman et al. 2018).

IMPORTANT MEDICINAL PLANTS OF THE INDO-PAK REGION

Azadirachta indica (Neem)

Azadirachta indica is a Latin binomial for neem, which means the 'Free Tree of India'. It is a member of the family Meliaceae (Rupani and Chavez 2018) and well known as 'Village dispensary' because it is the go-to cure for any domestic ailment (Shareef and Akhtar 2018). Another reason behind it being named so is its variety of uses such as its twigs are used as chew sticks or as indigenous toothbrushes. Even before toothbrushes were common in India, people used to chew on them to clean their teeth. Its leaves are a common cure for dermatological conditions such as acne, burns, and boils. They are boiled for cleaning wounds and soothing inflammations as well. The aroma of the *A. indica* flowers is described as a treatment for nausea (Jain et al. 2018; Shareef and Akhtar 2018). Millions of Asians have

used its fruits, leaves, roots, bark as well as seeds in traditional medicine for thousands of years. In traditional Ayurveda medicine, the remedies have been reported from its bark, leaves, and fruit. The *A. indica* oil (Neem oil) has been used for the treatment of leprosy, chronic syphilitic sores, skin ulcers, and pthysis. The folk medicine uses its extracts for rheumatism, intestinal helminthiasis, constipation, biliary afflictions and several respiratory disorders (Kumar et al. 2018).

A. indica is the most commonly used antiseptic nowadays with over 140 biologically active compounds present in it. The major components being terpenes, limonoids, and the primary metabolite in limonoids being azadirachtin that is found in the seeds (Kumar et al. 2018). Leaves contain a variety of bioactive compounds such as nimbanene, nimbin, nimbolide, nimbandiol, ascorbic acid, 6-desacetylnimbinene and n-hexacosanol (Rahmani et al. 2018). Other compounds extracted from fresh *A. indica* leaves include quercetin, ß-sitosterol, and polyphenolic flavonoids, all reportedly known to have antimicrobial properties (Sherma and Vaquil 2018). Other active compounds such as nimbidin, nimbolide, gedunin (from seeds), mahmoodin are reported to be significantly bioactive against pathogenic bacterial and fungal strains. Nimbidin was also observed to possess anti-inflammatory, antipyretic, antiulcer, antihistamine, spermicidal, antituberculosis and antiarthritis activity as well. Nimbolide also showed bioactivity against major pathogens such as *Staphylococcus aureus* and *S. coagulase*. It displayed antioxidant activity through prevention of oxidative DNA damage, procarcinogen activation and upregulation of carcinogen detoxification enzymes. Gedunin the bioactive compound from *A. indica* seed oil displayed significant inhibitory activity against the development of malarial parasites. Azadirachtin, the limonoid present in seeds was observed to possess anti-inflammatory activity through the inhibition of TNF-alpha activity, as well as retinoic acid-mediated biologic responses (Kumar et al. 2018, Rupani and Chavez 2018; Shareef and Akhtar 2018). For centuries, indigenous people have been using *A. indica* leaves as a mosquito repellent and the studies have shown that it is due to azadirachtin, which is the most predominant insecticidal agent in it. It displays toxicity towards insects through antifeedant effects, repellency,

antifeedancy, blocking development and growth regulation. It also causes fecundity suppression and sterilization and oviposition or attractancy. These bioactivity parameters have been investigated against arthropods such as flies, mosquitoes, fleas, and triatomines both of medical and veterinary importance (Kumar et al. 2018; Jain et al. 2018).

Ocimum tenuiflorum **(Tulsi)**

Ocimum tenuiflorum Linn. is the Latin binomial of Tulsi. It is commonly known as 'Vishnu Priya' in Sanskrit means 'the incomparable one', 'Niazbo' in Urdu and 'Kala Tulsi' in Hindi (Dubey and Pandey 2018). It is also identified as 'Holy basil' or 'Sacred basil' because of its religious importance in Hindu practices and is the main herb in Unani, Ayurvedic and Siddha systems of medicine. It belongs to the family Lamiaceae (Labiatae) (Joshi et al. 2017, Rupani and Chavez 2018) and is an erect annual herb with a sweet fragrance (Dubey and Pandey 2018) due to volatile oil with an appreciable note of clove oil. The major components of *O. tenuiflorum* include aldehydes, phenols, saponin, fats, and tannins. The major essential oils in *O. tenuiflorum* leaf extracts include eugenol (1-hydroxy-2-methoxy-4-allylbenzene) (71%), eugenol methyl ether (20%) and carvacrol (3%) (Ansari 2015; Dubey and Pandey 2018). Other phytochemical constituents are β-caryophyllene, ursolic acid, oleanolic acid, rosmarinic acid and linalool (Ansari 2015).

Traditionally *O. tenuiflorum* has been used in Siddha and Ayurveda system of medicine for the cure of common ailments such as the common cold, influenza, sore throat, fever, and cough. Because of its antiviral activity, it has been reported to be helpful in inhibiting the growth of the HIV virus (Joshi et al. 2017). *O. tenuiflorum* has been reported to possess adaptogenic properties i.e., assist and modulate the body homeostasis to cope with different kinds of stress and their effects (Rupani and Chavez 2018). For that purpose, its leaves have been used for the nerves, to sharpen memory, for migraine headaches, night blindness, and insomnia (Joshi et al. 2018). *O. tenuiflorum* was also observed to be useful in various

dermatological manifestations of stress as well such as telogen effluvium and nonscarring alopecias. Considering its useful uses for skin diseases it is commonly applied in cases of wound healing, and for limiting keloid formation (Rupani and Chavez 2018). Other traditional uses of *O. tenuiflorum* are wide ranging from uses in minor ailments such as earache, colic pain, flatulence, diarrhea to other serious disorders like hepatic diseases and arthritis (Joshi et al. 2017). Since it supports the removal of phlegm from the cartilaginous tube, it is used in bronchitis. A mixture of honey, ginger and *O. tenuiflorum* leaves are simmered for patients suffering from asthma and other respiratory disorders (Ansari 2015). It is also an antidote for scorpion or snake bites (Joshi et al. 2017).

Considering the rich ethnopharmacological background of *O. tenuiflorum*, it has been the subject of innumerable studies with more than one hundred publication during the last decade only. These *in vitro* and *in vivo* studies have confirmed that its leaves have potent pharmacological potential that includes anticancer, antioxidant, hepatoprotective, radioprotective, antimicrobial activity as well as antidiabetic effects. Its anti-stress effects are comparable to those of the antidepressant drugs resulting in protection from stress-induced cardiovascular changes. It possesses anxiolytic and anticonvulsant activities as well as improves both glucose and lipid profiles through ingestion of the leaves. The leaves also have a positive impact on the immune response. Its successful effects in animal models have led to polyherbal formulation in humans (Jamshidi and Cohen 2017).

O. tenuiflorum lives up to its reputation as, 'the mother of medicines of nature,' and 'the queen of herbs.' In the Indo-Pak region, it has been used in food, cosmetics, perfumes, and textile industries as well (Joshi et al. 2017).

***Carum copticum*, Syn: *Trachyspermum ammi* (Ajwain)**

Carum copticum Benth. is a synonym of *Trachyspermum ammi* and the Latin binomial of Ajwain. Some herbalists have also named it 'Aromaticum'. It is an aromatic annual plant that belongs to the family Apiaceae which is commonly found in Pakistan India, Iran, Egypt (Hassan

et al. 2016), Afghanistan and China (especially West Xinjiang) (Jan et al. 2015; Boskabady et al. 2014). It has different names in different languages, in Sanskrit it is known as 'Yamini'; in English, it is called 'Bishop's weed' and in Urdu, Hindi, and Baluchi it is called 'Ajowan or Ajwain' (Boskabady et al. 2014). Ajowan is derived from the Hindi name 'Ajvan' that can be traced back to Sanskrit. While the origin of the Sanskrit name came from the Greek word, 'Yavana' (Jan et al. 2015).

The essential oil of *C. copticum* contains strong antimicrobial compounds, thymol (48%), gamma-terpinen (30%) and p-cymene (15%). Its major constituents were identified as p-cymene (39.1%), gamma-terpinen (28.6%), oleic acid (10.4%) and linoleic acid (9.9%). The plant is also abundant in phytochemicals such as flavonoids, alkaloids, glycosides, terpenoids, reducing sugar and steroids. These compounds have been reported to possess bioactivities such as antimicrobial, antioxidants, antiallergic and anticancer activities. *C. copticum* is observed to contain high levels of nutrients and carbohydrates (30%), crude fats (25.3%) and proteins (17.5%). Ca was detected in the highest concentration followed by Fe and Mg whereas the amount of Cr, Mn, Zn Ni, and Cu were low. Others components included phosphorous, cobalt, iodine, thiamine, nicotinic acid, and riboflavin. The minerals work as co-factors in various metabolic processes and help in keeping water balance as well as transmission of nerve impulses (Hassan et al. 2016; Boskabady et al. 2014).

Traditional uses of *C. copticum* include the use of the essential oils from its fruit as expectorants, antispasmodic, as a carminative, a cure for flatulence and diarrhea. They are used as an antiseptic, anti-vomiting, diuretic, analgesic, antiasthma, and as an anti-dyspnea (Jan et al. 2015; Ranjan et al. 2011). The fruit itself is important for its use in food and flavor industries. They have been reported to possess antioxidant and anticholinergic effects as well as antiviral effects to relieve flu in children (Hassan et al. 2016). The *C. copticum* seeds are bitter with a pungent smell due to a brown oil called Ajwain oil (2-4.4%). The major component in it is thymol (50%) which has a strong fungicidal and overall germicide effect. It is also an antispasmodic, used for gastrointestinal ailments and for bronchial problems. Its seeds act as a galactagogue so they are used to increase milk

yield in dairy cattle. Due to their aroma, the seeds have also been used in the perfume and toothpaste industry. Because of their spicy flavor, they are added in curry powders, pickles, biscuits, confectionery, pan mixtures, beverages, snacks soups, and sauces. Particularly in the Punjab province of Pakistan and India, a bread called 'Ajwain paratha' is made with its seeds. They are either crushed before adding or added at the final stages of the recipe so to avoid the evaporation of its oils (Boskabady et al. 2014; Hassan et al. 2016; Ranjan et al. 2011; Jan et al. 2015). Other reported uses for *C. copticum* include its use as a hypolipidemic, antihypertensive, antiplatelet, abortifacient, anti-inflammatory, antitussive, anti-filarial, nematicidal, anthelmintic and antipyretic agent. It is also used for the detoxification of aflatoxins and as an ameliorative (Ranjan et al. 2011).

Nigella sativa (Kalonji)

Nigella sativa Linn. is the Latin binomial of Kalonji and belongs to the crowfoot or buttercup family (Ranunculaceae). This annual flowering plant is native to South Asia, Northern Africa and South Europe (Mukhtar et al. 2019). It is a commonly cultivated in Pakistan, India, Iran, Turkey, Syria and Saudi Arabia (Assi et al. 2016; Mukhtar et al. 2019) and is distributed widely in the Middle Eastern Mediterranean countries (Dubey et al. 2016). In Urdu and Hindi, it is known as 'Kalonji' and in Arabic, it is called 'Habatul Sauda' (seed of blessing), 'Al-Sawda', 'Habbet el-Baraka' and 'Kamounaswad' whereas in Persian, it is called 'Shonaiz'. In English, it is named as 'Black cumin', 'Black seed' (seed of capsulated plant), 'Love-in-a-mist', 'Black caraway', 'Black seed' and in German as, 'Schwarz kummel' (Gillani et al. 2004; Mukhtar et al. 2019; Ijaz et al. 2017; Assi et al. 2016).

The GC-MS qualitative analysis of the *N. sativa* seed oil has allowed the identification of more than a 100 compounds that are classified into various functional groups such as monoterpenes, carbonyl compounds, phenols, alcohols, and esters. The main bioactive component is thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone). It is reported to be antimicrobial, antiviral, antioxidant, anti-inflammatory, antitumor, antidiabetic, anti-

neurodegenerative, anti-schistosomiasis, against rheumatoid arthritis, anticonvulsant (antiepileptic), anti-asthmatic, pulmonary-protective and anti-Alzheimer. Others compounds include campesterol, stigmasterol, phytosterols, β-sitosterol, and avenasterol. *N. sativa* seeds also contain a significant amount of unsaturated fatty acids such as oleic acid, linoleic, along with small amount of saturated fatty acids, eicosanoic and arachidonic acids. Other bioactive components in plant and seeds are flavonoids, tannins, alkaloids, saponins, sesquiterpenes, sterols, diterpenes, triterpenes, cardiac glycosides, coumarins, volatile oils, volatile bases, glucosinolates, and oxygenated hydrocarbons (Dubey et al. 2016; Assi et al. 2016; Mukhtar et al. 2019). *N. sativa* also contains a high level of carbohydrates, fats, protein, and amino acids. Two anticoagulants, scopolamine, and umbelliferon are also present in it (Assi et al. 2016).

In the traditional system of medicine, especially in the Islamic world *N. sativa* seeds and their oil is extensively studied due to their traditional therapeutic value. Muslims recognize it as one of the greatest medicaments because of a saying by the Islamic Prophet Muhammad (PBUH) in which the *N. sativa* seeds are mentioned as a remedy for all diseases besides death. It is also one of the natural medicines regularly used by Prophet Muhammad (PBUH), in Tibb-e-Nabavi (Medicine of the Prophet Muhammad PBUH) (Mukhtar et al. 2019). *N. sativa* is also mentioned in the Holy Bible as 'Curative black cumin' and as a 'Glitch of Pliny' and as a 'Melancthon of Hippocrates and Dioscorides' (Ijaz et al. 2017).

The famous philosopher Avicenna (Also known as Ibn-e-Sina) in his book, 'Cannon of medicine' has described *N. sativa* seeds to be a remedy for innumerable diseases such as fatigue, depression (Assi et al. 2016), fever, headaches, toothaches, common cold, anorexia, piles, flatulence, diarrhea, constipation, conjunctivitis, skin disorders, paralysis, dyspepsia, dropsy, dysmenorrhea and amenorrhea (Mukhtar et al. 2019). In the Ayurvedic medicine, the seeds are recommended as an emmenagogue, for intermittent fever, as a diuretic, anthelmintic for jaundice and dyspepsia (Assi et al. 2016). In Unani-Tibb, *N. sativa* seeds are observed as antimicrobial, anti-oxytocic, anti-lipaemic, antiplaque, anticancer, antiulcer, anti-implantation, antihypertensive, antihistamine, anti-inflammatory, antidiabetic,

antispasmodic, for wound healing, as CNS depressant, analgesic, post-coital contraceptive, as renal protective agent, for migraine, chest congestion, asthma, hemiplegia, back pain, hypertension, rheumatism and obesity. Their use in eczema is also recognized worldwide (Gillani et al. 2004) and they are applied directly to nasal ulcers, orchitis, abscesses, and swollen joints. Because of their distinct aroma and taste, *N. sativa* seeds are a valuable spice as well. They are added to bread, pickles, savory and culinary dishes (Dubey et al. 2016).

ENDOPHYTES, THE MIGRANTS IN PLANTS

Thousands of microorganisms inhabit a single plant; they are either in the phyllospheric region on the surface or within tissues of either roots, leaves or stems. De Bary in 1866 put forward the idea of microbes residing within the plants and the term endophytes was coined for them. He defined them as, 'any organism that is growing within the plant tissues,' but as the studies progressed, this definition was modified according to the investigators prospective (Singh and Dubey 2018). Ever since the first endophyte was discovered in Germany in 1904, the prospective of their isolation and examination has led to a change in its definition. The most widely accepted definition of endophytes was later presented by Bacon and White to include a collection of microorganisms living both intra or intercellular especially those that do not cause any harmful effect on the plant. Even though they are termed as symptomless and more towards the symbiotic or mutualistic relationships, yet their biodiversity suggests they can be opportunistic. Both fungi and bacteria equally exist as endophytes (Strobel and Daisy 2003).

With the advancement in studies, more hypothesis were formed about the origin of endophytes and later a conclusion was formed that the rhizosphere is the major source of these migrants. Studies in the genome organization show that there is a certain specificity in terms of the type of plant and their mode of transmission. It was suggested that the bacteria with smaller genomes are more likely to form endophytic associations with

plants. It was later confirmed that the size of the endophytic bacteria genome was smaller from those that were free-living most likely to allow more genome stability. It has also been observed that the endophytic association between the bacteria and the plants are formed at a very early stage of plant life (Singh and Dubey 2018).

Plant endophytes include both bacteria and fungi that spend a part of their entire life cycle within the plants. Evidence of their presence in plants was found in the fossilized plant stems and leaf tissues. Nearly all the vascular plant species that have been studied till date contained endophytic fungi and/or bacteria. While being virtually present in all plant organs, some endophytes are even seed-borne (Venieraki et al. 2017).

Various studies have explained the possible entry route of these endophyte migrants into the host but all of these routes lead from the rhizosphere soil to the plant roots. One such route is through any crack or damage to the root caused by a possible wound. Microbial or nematodes predators can damage the lateral root junction making it possible for the rhizosphere bacteria to spread in the endorhizosphere. Some bacteria produce endoglucanases such as pectinases, cellulases, and exoglucanases that result in the loosening of the larger cellulose fibers and helping in the colonization of the plants. Interestingly, some strains of *Streptomyces* have been observed to produce cellulases, hemicellulases, glucanases, chitinases, and amylases that are all hydrolytic cell wall degrading even lignin degrading enzymes. In a study by Singh et al. 2017 endophytic actinomycetes of the *Streptosporangium* sp. residing in the maize plant were observed to be capable of producing glucoamylases further validating this idea. Another entry route for the endophytic bacteria is through the absorption of water through the root hair and spaces among the epidermal cells.

The endophytes that have migrated in the plant roots from the rhizosphere are greatly influenced by the plants. These microorganisms are normally stated as plant growth-promoting rhizobacteria (PGPR) because they greatly donate either directly or indirectly to the plant productivity and health. Directly, they help in the availability of nutrients through iron acquisition, phosphate mobilization, nitrogen fixation, production of plant

hormones and antimicrobial compounds. Indirectly, these endophytes are shown to enhance the immune system through induced systemic resistance (ISR) that help the plant resist pests, diseases, environmental stresses such as salinity and drought. Among the well-known plant growth promoters, numerous members of the family actinomycetes are also present (Viaene et al. 2016).

THE RATIONALE OF CHOOSING MEDICINAL PLANT FOR ENDOPHYTES

Plants with a history of ethnobotanical significance and medical use are likely candidates for the study of endophytes since the selection of the plant relates more to its endophytes population than to the plant biochemistry itself. However, there are several hypotheses that govern the strategy of choosing to isolate endophytes from such plants. One is that endophytes in plants from unique environments have unusual biology and because of which they may possess the ability to produce novel compounds. Another reason being that the medicinal plants used by the local people or documentation in the local literature for their medicinal abilities may be because of these endophytes. One of the most cited examples is the Australian Snake Vine plant, *Kennedia nigriscans*. This plant has been used for centuries by the longest standing civilizations in the world, the Australian Aborigines. The local Aborigines used its sap as bush medicine for centuries and its aqueous brew after crushing it was used for the healing of wounds and infections. Keeping in mind its ethnobotany, a study on this plant led to the isolation of a novel endophytic *Streptomyces* strain NRRL 30562, that synthesized an antimicrobial compound called munumbicin. This potent peptide antibiotic facilitated the indigenous people and the endophyte as well as the plant itself. Upon studies, it is now known that some plants produce the bioactive compounds associated with endophytes and vice versa. The most prominent example of this is the famed multimillion-dollar antitumor compound, paclitaxel from the yew tree fungal endophyte,

Taxomyces andreanae. Endophytes in plants that are endemic to a certain land mass are more likely to produce natural products than other plants. In addition, the biodiversity and uniqueness of endophytes are likely to be more in plants that are present in areas of greater biodiversity. Even endophytes of plants in unique environments are a promising reservoir of novel metabolites. One such example of this is the unique environment of the river system in southwest Venezuela where the plants are constantly subjected to injury because of rapid water waves, tumbling pebbles, rocks and falling debris. A study on its aquatic plant, *Rhyncholacis penicillata* revealed that despite the environmental pressures, the plant is healthy. Further studies on its endophytes revealed an endophyte, *Serratia marcescens* that produced a novel antifungal compound, oocydin A. It possessed the properties of a chlorinated macrocyclic compound. Possibly the damages to the plant may have led to portals of entry for the phytopathogenic oomycetes but the endophytic population possibly prevented it by producing the potent antifungal compounds. Further study on oocydin A led to its use being considered in agriculture against phytopathogens such as *Phytophthora* and *Pythium* (Strobel and daisy 2003).

ENDOPHYTIC ACTINOMYCETES AND THEIR DIVERSITY

Actinobacteria are gram-positive bacteria with a high GC content (>50%) and they appear as branching filaments that form a mycelium (Trujillo et al. 2015; Sigh and Dubey 2018). They are reported to produce an array of natural products including antitumor compounds (Doxorubicin, Bleomycin, Actinomycin D), immunosuppressive agents (FK 506/Fujimycin and Rapamycin) and most importantly antimicrobial compounds (Streptomycin, Rifamycin, Chloramphenicol, and Candicidin) (Sabu et al. 2017). They are mostly free-living and soil is their most dominant reservoir. Among the rhizospheric microbial community, they represent approximately 20–30% of the entire population. The rhizosphere is the zone of most active interactions among the bacteria and the plant root system therefore the roots of the plants are their richest source. Studies have

confirmed that the roots significantly impact the interactions in the rhizosphere through their exudates (Trujillo et al. 2015; Sigh and Dubey 2018). A study demonstrated that the plant host is specific and selective for the Streptomycete population in the soil. For Arabidopsis, the genus *Streptomyces* were dominant in the root endosphere displaying active recruitment of this genus by the roots. In contrast, within the barley plant endosphere, the Microbacteriaceae family was abundant (Viaene et al. 2016).

Studies have shown that climatic conditions also result in a greater endophytic community. One of the examples is of the tropical regions where the plants possessed a more diverse range of endophytes. Therefore, it is hypothesized that more the geographical diversity of host plants more will be physiological diversity of the endophytes (Sigh and Dubey 2018). Tropical rainforests are a good example since there the competition is high and the resources are limited allowing the selection pressure to rise. Interestingly, a statistical comparison between the bioactive natural products obtained from the endophytes of the tropical region and those of the temperate region gave a better idea of this. It was observed that not only the tropical endophytes produced more compounds than temperate endophytes, but they also produced a larger number of bioactive compounds than their temperate counterparts (Strobel and Daisy 2003).

Several studies have led to the finding that the most common taxa of endophytic actinomycetes in plants are the genus *Streptomyces* (Trujillo et al. 2015). A study by Kim et al. 2012 confirms the genus *Streptomyces* to be the most common taxon accounting for more than 50% of the total strains isolated from 11 Korean plants. The remaining strains comprised of the taxon, *Microbispora*, *Micromonospora*, *Microbacterium*, *Rhodococcus*, *Streptacidiphilus* and *Micrococcus*. In another study by Sardi et al. 1992 and de Araújo et al. 2000 other members of the family actinomycetes such as *Nocardia*, *Micromonospora*, *Microbispora*, *Streptoverticillium*, and *Streptosporangium*were were also observed to reside as endophytes but in a lesser number. A study on native Australian plant endophytes by Kaewkla and Franco (2013) also confirmed the idea that *Streptomyces* are the most abundant genera as more than 60% of the isolates were from this genus

alone. In maize plant, however, the situation was different i.e., 44% of the entire actinobacterial population comprised of the genus *Microbispora*, followed by the genus *Streptomyces* and *Streptosporangium* (Trujillo et al. 2015).

Actinomycetes hyphae have the ability to penetrate the cell walls via the lateral root hair openings and enter the intracellular regions of the roots thereby colonizing there (Meij et al. 2017). Even in previous studies, *Streptomyces* have been well documented to form interactions with plants and its first example was the isolation of the nitrogen fixation actinobacteria *Frankia* in several angiosperm plant families (Trujillo et al. 2015). *Actinosynnema*, was the first endophytic actinobacteria of plant origin isolated from grass blade (Sigh and Dubey 2018). Several reports have been published in the last decade on the isolation and variety of actinomycetes from crops and wild plants (Trujillo et al. 2015). A strain *Streptomyces lydicus* was observed to be residing in the surface layers of root nodules whereas another strain *Streptomyces griseoviridis* was found to be resident in the root hair of the plant *Brassica rapa*. In a study, GFP-tagged endophytic *Streptomyces* strain that was isolated from wheat plant was reintroduction to the same plant and it took residence in its embryos. Therefore, not only the rhizosphere but also the plant endosphere especially the roots appear to be the preferred part where *Streptomyces* like to exist intracellularly and become its substantial part (Meij et al. 2017). From wheat, Coomb and Franco 2003 demonstrated the colonization of *Streptomyces* sp. EN27 whereas Crawford et al. 1993 and Tokala et al. 2002 reported a strain of *Streptomyces* WYEC108 from the rhizosphere of linseed. It colonized the *Pisum sativum* roots and resulted in an enhancement in the assimilation of iron as well as nutrients. In response, the plant formed larger root nodules and their number increased. In terms of benefit to the plant, several actinomycetes that were isolated from wild plants in Algerian Sahara desert reportedly produced the phytohormone indole acetic acid (IAA) that resulted in root elongation and seed germination in tomato plants (Trujillo et al. 2015).

Apart from phytohormones and growth promotion benefits, these endophytic actinomycetes also act as biocontrol agents by producing

antibiotics thereby protecting the plants against diseases. The study by Cao et al. 2004 and another study by Goudjal et al. 2014 reported antifungal compounds from endophytic *Streptomyces* residing in banana and tomato plants that inhibited *Fusarium oxysporum* and *Rhizoctonia solani*. Both are important plant pathogens particularly, *Fusarium* that causes fusarium wilt.

ENDOPHYTIC ACTINOMYCETES IN INDO-PAK MEDICINAL PLANTS AND THEIR BIOACTIVITIES

Medicinal plants have been given special attention as potential reservoirs of actinobacterial communities particularly those that can be used in biotechnological applications (Trujillo et al. 2015). Since the Indo-Pak region is rich in ethnomedical plants, therefore, there have been many studies focused on them. One of them includes the study by Hassan and Imran 2015 that studied the endophytic actinomycetes population of *A. indica* (Neem) and *Melia azedarach* (Dharek), two plants of immense significance in the region. In the study, twenty actinomycetes were obtained from the roots of both the plants and four strains were identified to be *Streptomyces* sp. These strains were bioactive particularly against the clinical isolates of the famed nosocomial pathogen, *Pseudomonas aeruginosa*. The authors had found the *P. aeruginosa* strains to be multidrug-resistant (MDR) with the ability to resist more than 2 antibiotics and with MAR values higher than 0.2. The *Streptomyces* strains displayed a maximum zone of inhibition of 20 mm against these *P. aeruginosa* isolates. Interestingly, this zone of inhibition was equivalent to the zone obtained through the *A. indica* plant extract. This further confirms the idea that the endophytes and the plants have an influence on each other. The ethnobotanical use of *A. indica* by the indigenous people and it being a cure for all ailments can also be explained by such studies. It is obvious that the presence of the bioactive compounds of this endophytic *Streptomyces* in the plant extracts may be one of the reasons behind its wide usage against bacterial infections. The study further checked the cytotoxicity of the

endophytic strains by using brine shrimp cytotoxicity assay and found them to give an LC_{50} value of 5.1µg/ml^{-1}. According to Geran et al. 1972, an LC_{50} value of less than 20–30 µg/ml^{-1}is considered cytotoxic and if we compare the value in the study then it can be considered potently toxic.

Another study by Shenpagam et al. 2012 also explored the endophytic actinomycetes of *A. indica*. Interestingly, as with the study by Hassan and Imran 2015, in this study, *Streptomyces* sp. were isolated and their prominent antimicrobial activity was reported. The *Streptomyces* strain gave a zone of inhibition of 25mm against *P. aeruginosa* and it was also active against *Klebsiella pneumoniae* and *Streptococcus pyogenes* with maximum zones of inhibition of 25mm. In the case of antifungal activity, a prominent zone of 28mm was noted against the *Rhizopus* sp. A study by Gohain et al. 2015 gave contrasting results however, none of the endophytic actinomycetes isolated from *A. indica* roots, stem or leaves gave activity against *P. aeruginosa*. However, the maximum zone of inhibition was observed by the endophyte, *Microbispora rosea* against *Staphylococcus aureus* (24mm) and *Pseudomonas syringae* (23mm). The *Streptomyces* strain identified as *Streptomyces antibioticus* also gave notable activity against *S. aureus* (20mm). The percentage of isolation of *Streptomyces* sp. was high (65.78%) as compared to the other studies done before as well as the frequency of isolation from the roots (57.8%). This frequency remained unchanged in winters and in summers. The study also gave a peek into the molecular aspects of these endophytes and it was observed that 85% of the isolates contained the PKS II gene cluster. Polyketides are an important group of secondary metabolites that are a valuable source of clinically important pharmaceuticals. During the past years, the sequenced and cloned genes from this fragment has led to the understanding that actinomycetes possessing this cluster may be capable of producing novel compounds (Tanvir et al. 2013).

An endophytic actinomycete, *Rhodococcus qingshengii*, was discovered from the *A. indica* shoots (Saini et al. 2017). The phenolic compounds in the extract of this endophytic strain exhibited significant anti-diabetic activity through the inhibition of α-amylase and α-glucosidase. The inhibitors of α-amylase impede the release of glucose from complex carbohydrates as a

result the glucose absorption is delayed leading to low hyperglycemia and postprandial plasma glucose (Akshatha et al. 2014). Therefore, in a diabetic patient, the inhibitors of both these enzymes can help cause a delay in the breaking down of carbohydrate in the small intestine, which can result in a lower level of the postprandial blood glucose. With an alarming prevalence rate of 26.3% of diabetes in Pakistan (Nearly 27.4 million) (Basit et al. 2019) and nearly 1.3 billion people affected in India (Tendon et al. 2018), the endophytic actinomycetes from *A. indica* can help combat this progressive chronic disease.

The Indo-Pak region is still developing and the majority of the people belong to traditional societies. The 80% of its diabetic population consider plant-based herbal treatments as they are more accessible, reliable and with fewer side effects. Therefore, more than 1200 plants are currently being used in the traditional treatment of diabetes (Ullah et al. 2019). With the fact that endophytes mimic the natural products of their host; the endophytic actinomycetes can, therefore, provide an interesting reservoir of such medicines. In this context, Akshatha et al. 2014 conducted a study on two medicinal plants, *Leucas ciliata* (Lamiaceae), and *Rauwolfia densiflora* (Apocynaceae), that are well-documented anti-diabetics. The endophytic actinomycetes from their plant parts that were identified as *Streptomyces longisporoflavus* and *Streptomyces* sp. exhibited significant anti-diabetic activity. The strain *S. longisporoflavus* that was isolated from the stem inhibited the α-amylase enzyme with an IC_{50} 162.3 $\mu g/ml^{-1}$ as compared to acarbose standard that gave an IC_{50} value of 73.1 $\mu g/ml^{-1}$. The *Streptomyces* sp. displayed anti-diabetic activity by helping insulin to move glucose inside hemidiaphragm. The acarbose standard used in the study was a commercial α-amylase and α-glucosidase inhibitor used for the treatment of diabetes mellitus type-2. All the sources for these drugs so far had been soil actinomycetes, therefore the endophytic actinomycetes producing such compounds might become a more credible and promising source.

Interestingly, other bioactive agents such as anti-insecticidal compounds were also reported from the endophytic actinomycetes residing inside *A. indica*. One of the strains in the study by Chen et al. 2018 was observed to be producing potent bio-insecticides, particularly when tested against green

peach aphids. The strain was identified to be *Streptomyces albidoflavus* and its crude extract gave an 83.3% mortality rate after 48 hours. The insecticidal agent from *A. indica* is already known to be azadirachtin. Although the authors of the study had not identified the insecticidal agent produced by the *Streptomyces albidoflavus* strain however, it can be speculated that the presence of azadirachtin may be because of the endophytic actinomycetes residing inside it.

Apart from endophytic actinomycetes, several studies reported the isolation of bioactive endophytic fungal species as well as bacterial endophytes from *A. indica* (Yadav et al. 2015, Ananda et al. 2018). They were observed to be capable of producing industrially important extracellular enzymes such as amylases, proteases, cellulases and lipases (Patil et al. 2015) further illustrating the diversity and potential of endophytes harbored by this plant.

Ocimum sanctum is another major plant used in medicines in the region. A study on its endophytes carried out by Singh and Padmavathy 2015 showed that its plant leaves harbored endophytic *Nocardia* sp. with 99% genetic similarity to *Nocardiopsis synnemataformans* and *Nocardiopsis dassonvillei*. The strains displayed significant antimicrobial activity against *Staphylococcus aureus* (21 mm), *Enterococcus faecalis* (19mm) and *Vibrio cholera* (18mm) validating the use of the plant in cases of wound healing and other infections. The *Nocardiadiopsis* strain also displayed significant activity against important fungal pathogen, *Candida albicans* with an 18 mm zone of inhibition. A study by Gangwar et al. 2014 also explored the endophytic actinomycetes population residing within *O. sanctum*. In the study, 12 isolated were obtained from its roots, 3 from its stem and 1 from the leaves following the same isolation trend as the previous studies. Majority of the isolates were from the *Streptomyces* genus while the genus *Actinopolyspora*, *Micromonospora*, and *Saccharopolyspora* were also observed but only as single isolates. The *Streptomyces albosporus* strain exhibited the most antifungal activity with the most activity against the phytopathogenic fungus *Fusarium oxysporum* (59%). The endophytic actinomycetes belonging to the *Saccharopolyspora* sp. also displayed strong activity against the pathogenic fungi, *Alternaria brassicola* (71.4%). Anjum

and Chandra 2015 observed further diversity of the endophytes of *O. sanctum* on its endophytic bacterial population whereas Chowdhary and Kaushik 2015 studied its endophytic fungal population. Table 1 presents the summary of other endophytes isolated from medicinal plants of the Indo-Pak region and the identification of their compounds.

A study by Akshatha et al. 2016 focused on the ethnomedicinal plants such as *Cajanus lineatus* (Maesen), *Leucas ciliata*, *Rauwolfia densiflora* and *Gomphostemma heyneanum* in Western Ghats, Karnataka, India. The isolation of 135 endophytic actinomycetes from them gave an idea that these plants are a rich environment for such bacteria particularly *Streptomyces* sp. that were 68% of all the isolates. Among the majority were species such as *Streptomyces globosus*, *Streptomyces sedi*, *Streptomyces hypolithicus*, *Streptomyces longisporoflavus*, and *Streptomyces phaechromogene*. The bioactive compounds such as phenol derivatives, fatty acids such as palmitic acid, octadecanoic acid, esters such as butyl ester, di isobutyl ester, and phthalic acid were identified. Interestingly all these compounds were observed to be strong antioxidants (IC50 88.2 ± 1.03 μg/ml). Another endophytic *Streptomyces* strain, *Streptomyces parvulus* was reported to produce potent antimicrobial compounds. The strain was isolated from the roots of *Aloe vera* Brum and gave prominent bioactivity against MDR *S. aureus*, *Staphylococcus epidermidis*, *K. Pneumoniae*, *P. aeruginosa*, *Proteus vulgaris*, *Candida albicans*, and *Aspergillus niger*. On further analysis, the polypeptide nature of the produced antimicrobial was ascertained. The strains were observed to be quite versatile as they also produced the antitumor compounds, actinomycin D and actinomycin X (Chandrakar and Gupta 2018). Other endophytic actinomycetes with prominent activities were isolated from the medicinal plants, *Abrus precatorius*, *Aloe vera*, *Asparagus racemosus*, *Adhatoda vasica*, *Plumbago zeylanica* (Chandrakar and Gupta 2017), *Mentha arvensis* (Gangwar et al. 2014), *Emblica officinalis* (Gangwar et al. 2015), *Phyllanthus niruri*, *Hemidesmus indicus*, *Withania somnifera* (Priya 2012), *Catharanthus roseus* (Ranjan and Jadeja 2016), *Combretum latifolium* (Rao et al. 2015), *Zingiber officinale* (Sabu et al. 2017), *Syzygium cumini* (Saini et al. 2016) and *Pinus roxburghii* (Sharma and Banuthiyal 2018).

CONCLUSION

The plant microenvironment is an attractive niche to explore because it contains migrants that have moved inside the plant endosphere and making it their home. Since it is known that they are capable of producing new compounds under the influence of the plant environment, therefore the importance of the endosphere of medicinal plants becomes even more. Considering this, the focus of this chapter was the Indo-Pak region, their medicinal plants' diversity and the endophytes harbored by them. The reason for highlighting this region was of the already established traditional medicine systems there i.e., Tibb-e-Unani, Ayurveda and Siddha systems that are already using the indigenous medicinal plants. The studies that had been conducted on the endophytic actinomycetes residing in those plants not only validate the usage of such plants for centuries but also bring to us their diverse biosynthetic potential. However, this niche is still poorly investigated.

In this chapter, we have tried to explore the endophytic actinomycetes of the important medicinal plants of the Indo-Pak region, their diversity, and biosynthetic potential. Greater insight in their bioactive compounds may help us to explore their potential further and it may ultimately lead to the development of novel drugs. The research in the area of endophytes have peaked in the previous years so it would not be wrong to say that in the coming years the interest in them will increase for the pharmaceutical industries as well. In terms of endophytic actinomycetes research from plants of ethnopharmacological background, this is just the beginning.

Table 1. Summary of other endophytes isolated from medicinal plants of the Indo-Pak region and the identification of their compounds

Plant genus	**Plant part(s)**	**Endophyte(s)**	**Biotechnological potential**	**Active compound(s) identified**	**Reference**
Caralluma acutangula	Roots, stem and leaves	*Penicillium purpurogenum, Alterneria* sp., *Aspergillus nidulans, Peacilomyces variotii, Fusarium proliferatum, Epicoccum nigrum*	Extracellular enzymes, plant growth promotion	1-aminocyclopropane-1-carboxylate (ACC) deaminase, cellulases, phosphatases, glucosidases, Indole-3-acetic acid (IAA)	Ali et al. 2019
Dodonaea viscosa, Fagonia indica, Caralluma tuberculata, Calendula arvensis	Roots, stem and leaves	*Streptomyces alboniger, Pseudomonas taiwanensis, Pseudomonas geniculata, Enterobacter hormaechei, Bacillus tequilensis, Bacillus flexus, Pseudoarthrobacter phenanthrenivorans*	Antibacterial, antifungal	Volatile compounds	Iqrar et al. 2019
Gloriosa superba	Rhizome	Unidentified Bacteria	Nitrogen fixation, plant growth promotion	IAA	Ogale et al. 2018
Brucea mollis	NS	*Geosmithia pallida*	Antibacterial, antifungal	NS	Deka and Jha 2016
Azadirachta indica	Stem	*Xylaria sp., Phomopsis sp., Cryptococcus sp.*	Antifungal	NS	Chutulo and Chalannavar 2018
Azadirachta indica	Leaves	*Fusarium avenaceum, Trichoderma* sp., *Colletotrichum* sp., *Curvularia* sp., *Chaetomium* sp., *Alternaria alternate,*	Nematicidal activities	NS	Chutulo and Chalannavar 2018

Plant genus	Plant part(s)	Endophyte(s)	Biotechnological potential	Active compound(s) identified	Reference
		Fusarium solani, *Chaetomium globosum*, *Chaetomium globosum*, *Pestalotiopsis* sp., *Phoma* sp., *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* sp., *Penicillium* sp., *Trichoderma* sp., *Cladosporium* sp.			
Azadirachta indica	Fruit	*Humicola*, *Drechslera*, and *Colletotrichum* sp.	NS	NS	Chutulo and Chalannavar 2018
Azadirachta indica	Bark	*Cochlonema*, *Gliomastix*, *Verticillium* sp., *Trichoderma*, *Penicillium*, *Pestalotiopsis* sp., *Gliomastix*, *Curvularia*, *Phoma eupyrena*, and *Phyllosticta*, *Pestalotiopsis microspore*, *Bartalinia robillardoides*	NS	NS	Chutulo and Chalannavar 2018
Azadirachta indica	Roots	*Chloridium* sp., *Chaetomium globosum*, *Chloridium*, *Scytalidium*, *Nigrospora* and *Verticillium*, *Eupenicillium parvum*	Antibacterial	Javanicin	Chutulo and Chalannavar 2018
Azadirachta indica	Seed and twigs	*Phomopsis azadirachtae*	NS	NS	Chutulo and Chalannavar 2018
Ocimum santum and *Aloe Vera*	Roots, stem and leaves	Unidentified Bacteria	Extracellular enzymes, plant growth promotion	Urease, pectinase, cellulose, catalase, lipase, caseinase, gelatinase, chitinase, phosphate solubilisation, IAA,	Joshi et al. 2018

Table 1. (Continued)

Plant genus	Plant part(s)	Endophyte(s)	Biotechnological potential	Active compound(s) identified	Reference
				IAA, siderophores and ammonia	
Withania sominifera, Syzygium aromaticum, Ocimum bacillicum	Roots, stem and leaves	Fungus	Extracellular enzymes	Proteases, amylases, cellulases. Asparaginases	Kapoor et al. 2018
Eupatorium odoratum, Musa superba, Mirabilis jalapa, Curcuma longa, Clerodendrum colebrookianum, Alstonia scholaris, Centella asiatica	NS	*Streptomyces, Microbacterium, Actinomycete* sp., *Leifsonia, Brevibacterium*	Antifungal, plant growth promotion	IAA, phosphates, siderophores, ammonia, chitinases, hydrogen cyanide	Passari et al. 2015
Acalypha indica	Leaves	Unidentified Bacteria	Antibacterial	NS	Ramalashmi et al. 2018
Combretum latifolium	Roots, stem and leaves	*Streptomyces* sp., *Nocardiopsis* sp., *Micromonospora* sp., *Actinomadura* sp., *Glycomyces* sp.	Antibacterial	NS	Rao et al. 2015
Ailathus excelsa	Leaves	*Streptomyces* sp.	Hydrolyze tannic acid	Tannases	Roy et al. 2018
Trillium govanianum	Stem, leaves and rhizome	*Alternaria sp., Aspergillus nidulans, Aspergillus niger, Aspergillus wentii,*	Antibacterial	NS	Sagar et al. 2017

Plant genus	Plant part(s)	Endophyte(s)	Biotechnological potential	Active compound(s) identified	Reference
		Fusarium oxisporum,*Fusarium solani*, *Mucor plumbues*, *Phomas sp.*, *Pythium sp.*, *Rhizopus nigricans*, *Rhizopus oryzae*, *Stachybotrys atra*, *Trichoderma viride*			
Achillea millefolium	Roots, stem, leaves and flower	*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*	Antioxidant	Tannins, steroids, cardio glycosides, flavonoids, terpenoids, alkaloids, phenol, saponins, anthraquionines	Satari et al. 2018
Spilanthes paniculata	Roots, stem and leaves	*Colletotrichum* sp., *Fusarium* sp., *Manokwaria* sp. *Oncopodium* sp., *Pestalotopsis* sp., *Drecheslra* sp., *Cylindrocladium* sp., *Aspergillus* sp., *Nodulisporium* sp.	NS	NS	Sharma et al. 2018
Acorus calamus	Rhizome	*Ochrobactrum intermedium*	Antifungal, plant growth promotion	Chitinases, proteases, lipases, sidrophores and IAA	Singh et al. 2018
Gymnema sylvestre	Leaves and stem	*Cylindrocladium parvum*, *Cladosporium variabile*, *Cochliobolus geniculatus*, *Alternaria brassicicola*, *Colletotrichum capsici*, *Colletotrichum dematium*, *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium oxysporum*, *Alternaria alternata*	NS	NS	Vasantakumar, and Vidyasagar 2018

NS, not studied

REFERENCES

Akshatha, Jaginakere V., Harischandra S. Prakash, and Monnanda S. Nalini. "Actinomycete Endophytes from the Ethno Medicinal Plants of Southern India: Antioxidant Activity and Characterization Studies." *Journal of Biologically Active Products from Nature* 6, no. 2 (2016): 166-72. https://doi.org/10.1080/22311866.2016.1191971.

Akshatha, VJ, MS Nalini, C D'souza, and HS Prakash. "Streptomycete Endophytes from Anti-Diabetic Medicinal Plants of the Western Ghats Inhibit Alpha-Amylase and Promote Glucose Uptake." *Letters in Applied Microbiology* 58, no. 5 (2014): 433-39.

Ali, Sajid, Sumera Afzal Khan, Muhammad Hamayun, Amjad Iqbal, Abdul Latif Khan, Anwar Hussain, and Mohib Shah. "Endophytic Fungi from *Caralluma acutangula* Can Secrete Plant Growth Promoting Enzymes." *Fresenius Environmental Bulletin* 58, no. 4 (2019): 2688-96.

Ananda, Khuzyia Rizqi Triavi, Sunarno Sunarno, Muhamad Fikri Zulfikar, Hafsah Avisha, Muhamad Nastain, and Ridwan Abdullah. "Screening Endophytes of Neem Leaf That Potential AntiAnthrax through Tests of Anti *Staphylococcus aureus*." *Biosaintifika: Journal of Biology & Biology Education* 10, no. 1 (2018): 95-100.

Anjum, Naved, and Ramesh Chandra. "Endophytic Bacteria: Optimizaton of Isolation Procedure from Various Medicinal Plants and Their Preliminary Characterization." *Asian Journal of Pharmaceutical and Clinical Research* 8, no. 4 (2015): 233-38.

Ansari, Khursheed Ahmad. "Study of Plant Tulsi and Its Benefits for Human Beings." *International Journal of Advanced Research* 1, no. 3 (2015): 148-51.

Assi, Mohammed Abdulrazzaq, Mohd Hezmee Mohd Noor, Noor Farhana Bachek, Hafandi Ahmad, Abdul Wahid Haron, Md Sabri Mohd Yusoff, and Mohammed Ali Rajion. "The Various Effects of *Nigella sativa* on Multiple Body Systems in Human and Animals." *Pertanika Journal of Scholarly Research Reviews* 2, no. 3 (2016): 1-19.

Aziz, Muhammad Abdul, Muhammad Adnan, Amir Hasan Khan, Abdelaaty Abdelaziz Shahat, Mansour S Al-Said, and Riaz Ullah. "Traditional

Uses of Medicinal Plants Practiced by the Indigenous Communities at Mohmand Agency, Fata, Pakistan." *Journal of Ethnobiology and Ethnomedicine* 14, no. 1 (2018): 1-16.

Basit, Abdul, Asher Fawwad, and Kulsoom Baqa. "Pakistan and Diabetes—a Country on the Edge." *Diabetes Research and Clinical Practice* 147 (2019): 166-68.

Bernardini, S, A Tiezzi, V Laghezza Masci, and E Ovidi. "Natural Products for Human Health: An Historical Overview of the Drug Discovery Approaches." *Natural Product Research* 32, no. 16 (2018): 1926-50.

Boskabady, Mohammad Hossein, Saeed Alitaneh, and Azam Alavinezhad. "*Carum copticum* L.: A Herbal Medicine with Various Pharmacological Effects." *Bio Med Research International* 2014 (2014): 1-11.

Boy, Henry Ivanz A, Alfred Joshua H Rutilla, Kimbberly A Santos, Allister Matthew T Ty, I Yu Alicia, Tooba Mahboob, Jitbanjong Tangpoong, and Veeranoot Nissapatorn. "Recommended Medicinal Plants as Source of Natural Products: A Review." *Digital Chinese Medicine* 1, no. 2 (2018): 131-42.

Cao, L, Z Qiu, J You, H Tan, and S Zhou. "Isolation and Characterization of Endophytic *Streptomyces* Strains from Surface-Sterilized Tomato (*Lycopersicon esculentum*) Roots." *Letters in Applied Microbiology* 39, no. 5 (2004): 425-30.

Chandrakar, Sandhya, and AK Gupta. "Antibiotic Potential of Endophytic Actinomycetes of Medicinal Herbs against Human Pathogenic Bacteria." *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* 87, no. 3 (2017): 905-15.

Chandrakar, Sandhya, and Ashwini Kumar Gupta. "Actinomycin-Producing Endophytic *Streptomyces Parvulus* Associated with Root of Aloe Vera and Optimization of Conditions for Antibiotic Production." *Probiotics and Antimicrobial Proteins* (2018): 1-15.

Chen, Yan, Jamil Shafi, Maohai Li, Danni Fu, and Mingshan Ji. "Insecticidal Activity of Endophytic Actinomycetes Isolated from *Azadirachta indica* against *Myzus persicae*." *Archives of Biological Sciences* 70, no. 2 (2018): 349-57.

Chowdhary, Kanika, and Nutan Kaushik. "Fungal Endophyte Diversity and Bioactivity in the Indian Medicinal Plant *Ocimum sanctum* Linn." *PLoS One* 10, no. 11 (2015): e0141444.

Chutulo, Eyob Chukalo, and Raju Krishna Chalannavar. "Endophytic Mycoflora and Their Bioactive Compounds from *Azadirachta indica*: A Comprehensive Review." *Journal of Fungi* 4, no. 2 (2018): 42.

Coombs, J. T., and C. M. Franco. "Visualization of an Endophytic *Streptomyces* Species in Wheat Seed." [In Eng.]. Research Support, Non-U.S. Gov't. *Applied Environmental Microbiology* 69, no. 7 (Jul 2003): 4260-2.

Crawford, Don L, Jack D Doyle, Zemin Wang, Charles W Hendricks, Steven A Bentjen, Harvey Bolton, James K Fredrickson, and Bruce H Bleakley. "Effects of a Lignin Peroxidase-Expressing Recombinant, *Streptomyces lividans* Tk23. 1, on Biogeochemical Cycling and the Numbers and Activities of Microorganisms in Soil." *Applied Environmental Microbiology* 59, no. 2 (1993): 508-18.

Deka, Deepanwita, and Dhruva Kumar Jha. "Optimization of Culture Parameters for Improved Production of Bioactive Metabolite by Endophytic *Geosmithia pallida* (Ku693285) Isolated from *Brucea mollis* Wall Ex. Kurz, an Endangered Medicinal Plant." *Journal of Pure and Applied Microbiology* 12, no. 3 (2018): 1205-13.

Desai, Praful B. "Cancer Control Efforts in the Indian Subcontinent." *Japanese Journal of Clinical Oncology* 32, no. 1 (2002): S13-S16.

Dubey, PN, Balraj Singh, BK Mishra, K Kant, and RK Solanki. "*Nigella* (*Nigella sativa* L.): A High Value Seed Spice with Immense Medicinal Potential." *Indian Journal of Agricultural Sciences* 86, no. 8 (2016): 967-79.

Dubey, Rashmi, and Sudhir Kumar Pandey. "Medicinally Important Constituents of Tulsi (*Ocimum* Spp.)." In *Synthesis of Medicinal Agents from Plants*, 151-76: Elsevier, 2018.

Gangwar, Madhurama, Sonam Dogra, Urmil Phutela Gupta, and Ravindra Nath Kharwar. "Diversity and Biopotential of Endophytic Actinomycetes from Three Medicinal Plants in India." *African Journal of Microbiology Research* 8, no. 2 (2014): 184-91.

Gangwar, Mayank, C Vijay, MK Gautam, and Gopal Nath. "Isolation and Evaluation of Antimicrobial Activities of Endophytic Fungal Extract from *Mallotus philippinensis* Muell." *Applied Microbiology: Open Access* 2015 (2015): 103.

Geran, R I. "Protocols for Screening Chemical Agents and Natural Products against Animal Tumors and Other Biological Systems." *Cancer Chemotherapy Reports* 3 (1972): 51-61.

Gilani, Anwar-ul Hassan, Qaiser Jabeen, and Muhammad Asad Ullah Khan. "A Review of Medicinal Uses and Pharmacological Activities of *Nigella sativa*." *Pakistan Journal of Biological Sciences* 7, no. 4 (2004): 441-51.

Gohain, Anwesha, Animesh Gogoi, Rajal Debnath, Archana Yadav, Bhim P Singh, Vijai K Gupta, Rajeev Sharma, and Ratul Saikia. "Antimicrobial Biosynthetic Potential and Genetic Diversity of Endophytic Actinomycetes Associated with Medicinal Plants." *FEMS Microbiology Letters* 362, no. 19 (2015): fnv158.

Golinska, Patrycja, Magdalena Wypij, Gauravi Agarkar, Dnyaneshwar Rathod, Hanna Dahm, and Mahendra Rai. "Endophytic Actinobacteria of Medicinal Plants: Diversity and Bioactivity." *Antonie Van Leeuwenhoek* 108, no. 2 (2015): 267-89.

Goudjal, Y., O. Toumatia, A. Yekkour, N. Sabaou, F. Mathieu, and A. Zitouni. "Biocontrol of *Rhizoctonia solani* Damping-Off and Promotion of Tomato Plant Growth by Endophytic Actinomycetes Isolated from Native Plants of Algerian Sahara." [In Eng.]. *Microbiological Research* 169, no. 1 (2014): 59-65. https://doi.org/10.1016/j.micres.2013.06.014.

Haq, Faizul, Habib Ahmad, and Mukhtar Alam. "Traditional Uses of Medicinal Plants of Nandiar Khuwarr Catchment (District Battagram), Pakistan." *Journal of Medicinal Plants Research* 5, no. 1 (2011): 39-48.

Hassan, Ashba, and Imran Sajid. "Inhibitory Effects of Plant Extracts and Putative Endophytic Actinomycetes from the Selected Members of Meliaceae Family against MDR *Pseudomonas aeruginosa*." *European Journal of Medicinal Plants* 10, no. 4 (2015): 1-14.

Hassan, W, S Gul, S Rehman, H Noreen, Z Shah, I Mohammadzai, and B Zaman. "Chemical Composition, Essential Oil Characterization and

Antimicrobial Activity of *Carum copticum*." *Vitamins & Minerals* 5, no. 139 (2016): 2376-1318.1000139.

Husain, Syed Zahoor, Riffat Naseem Malik, Mubashera Javaid, and Sadia Bibi. "Ethonobotanical Properties and Uses of Medicinal Plants of Morgah Biodiversity Park, Rawalpindi." *Pakistan Journal of Botany* 40, no. 5 (2008): 1897-911.

Ijaz, Hira, Ume Ruqia Tulain, Junaid Qureshi, Zeeshan Danish, Samina Musayab, Muhammad Furqan Akhtar, Ammara Saleem, et al. "*Nigella sativa* (Prophetic Medicine): A Review." *Pakistan Journal of Pharmaceutical Sciences* 30, no. 1 (2017): 229-34.

Iqrar, Irum, Zabta Khan Shinwari, Ashraf El-Sayed, and Gul Shad Ali. "Bioactivity-Driven High Throughput Screening of Microbiomes of Medicinal Plants for Discovering New Biological Control Agents." *Bio Archive* (2019): 611855.

Jain, Manju, Prem Narayan Shrivastava, and Rakesh Samar. "Survey of Ethnobotanical Medicinal Plants Used by the People of District Guna, Madhya Pradesh, India." *International Journal of Life Sciences Scientific Research* 2455, no. 1716 (2018): 1880-88.

Jamshidi, Negar, and Marc M Cohen. "The Clinical Efficacy and Safety of Tulsi in Humans: A Systematic Review of the Literature." *Evidence-Based Complementary and Alternative Medicine* 2017 (2017): 1-13.

Jamshidi-Kia, Fatemeh, Zahra Lorigooini, and Hossein Amini-Khoei. "Medicinal Plants: Past History and Future Perspective." *Journal of Herbmed Pharmacology* 7, no. 1 (2018): 1-7.

Jan, Sohail Ahmad, Zabta Khan Shinwari, Amir Zeb, Ali Talha Khalil, and Sabir Hussain Shah. "Ethnobotany and Research Trends in *Trachyspermum ammi* L. (Ajowan); A Popular Folklore Remedy." *American-Eurasian Journal of Agricultural & Environmental Sciences* 15 (2015): 68-73.

Joshi, Rakesh Kumar, William N Setzer, and Joyce Kelly Da Silva. "Phytoconstituents, Traditional Medicinal Uses and Bioactivities of Tulsi (*Ocimum sanctum* Linn.): A Review." *American Journal of Essential Oils and Natural Products* 5, no. 1 (2017): 18-21.

Joshi, Samiksha, Ajay Veer Singh, and Birendra Prasad. "Enzymatic Activity and Plant Growth Promoting Potential of Endophytic Bacteria Isolated from *Ocimum sanctum* and *Aloe vera*." *International Journal of Current Microbiology and Applied Sciences* 7, no. 6 (2018): 2314-26.

Kaewkla, Onuma, and Christopher MM Franco. "Rational Approaches to Improving the Isolation of Endophytic Actinobacteria from Australian Native Trees." *Microbial Ecology* 65, no. 2 (2013): 384-93.

Kapoor, Neha, Pranchal Rajput, Md Abu Mushtaque, and Lokesh Gambhir. "Bio-Prospecting Fungal Endophytes of High Altitude Medicinal Plants for Commercially Imperative Enzymes." *Bioscience Biotechnology Research Communications* 11, no. 3 (2018): 370-75.

Khan, Kifayat Ullah, Maqarab Shah, Habib Ahmad, Shujaul Mulk Khan, Inayat Ur Rahman, Zafar Iqbal, Raees Khan, et al. "Exploration and Local Utilization of Medicinal Vegetation Naturally Grown in the Deusai Plateau of Gilgit, Pakistan." *Saudi Journal of Biological Sciences* 25, no. 2 (2018): 326-31.

Kim, Tae-Ui, Sung-Heun Cho, Ji-Hye Han, Young Min Shin, Hyang Burm Lee, and Seung Bum Kim. "Diversity and Physiological Properties of Root Endophytic Actinobacteria in Native Herbaceous Plants of Korea." *The Journal of Microbiology* 50, no. 1 (2012): 50-57.

Kumar, Dhavendra. *Genomics and Health in the Developing World.* Oxford University Press, 2012.

Kumar, Rakesh, Simpi Mehta, and Seema R Pathak. "Bioactive Constituents of Neem." In *Synthesis of Medicinal Agents from Plants*, 75-103: Elsevier, 2018.

Kumar, Ravi Ranjan, and Vasantba J Jadeja. "Isolation of Actinomycetes: A Complete Approach." *International Journal of Current Microbiology and Applied Sciences* 5 (2016): 606-18.

Levinson, D., and K. Christensen. *Encyclopedia of Modern Asia*. Charles Scribner's Sons, 2002.

Malla, SB, and PR Shakya. "Medicinal Plants in Nepal in Nepal Nature's Paradise; Eds." *Majpuria TC and Majpuria RK, M. Devi, Gwalier, India* (1999).

Mini Priya, R. "Endophytic Actinomycetes from Indian Medicinal Plants as Antagonists to Some Phytopathogenic Fungi." *Scientific Reports* 1, no. 4 (2012): 259.

Mukhtar, Hamid, Aminah Suhail Qureshi, Farooq Anwar, Muhammad Waseem Mumtaz, and Monica Marcu. "*Nigella Sativa* L. Seed and Seed Oil: Potential Sources of High-Value Components for Development of Functional Foods and Nutraceuticals/Pharmaceuticals." *Journal of Essential Oil Research* 31, no. 3 (2019): 171-83.

Ogale, Sneha, Nikhil Joshi, Aishwarya Sukhatankar, and Priyanka Manani. "Optimization of Chitinase Production by Bacteria from Novel Endophytic Niche of *Gloriosa superba* and Soil of North-Western Region of India." *World Journal of Pharmaceutical Research* 7, no. 4 (2018): 133-144.

Passari, Ajit Kumar, Vineet Kumar Mishra, Vijai Kumar Gupta, Mukesh Kumar Yadav, Ratul Saikia, and Bhim Pratap Singh. "*In Vitro* and *In Vivo* Plant Growth Promoting Activities and DNA Fingerprinting of Antagonistic Endophytic Actinomycetes Associates with Medicinal Plants." *PLoS One* 10, no. 9 (2015): e0139468.

Patil, Mohini G, Jyoti Pagare, Sucheta N Patil, and Amanpreet K Sidhu. "Extracellular Enzymatic Activities of Endophytic Fungi Isolated from Various Medicinal Plants." *International Journal of Current Microbiology and Applied Sciences* 4, no. 3 (2015): 1035-42.

Prakash, Bhanu, Anupam Kujur, and Amrita Yadav. "Drug Synthesis from Natural Products: A Historical Overview and Future Perspective." In *Synthesis of Medicinal Agents from Plants*, 25-46: Elsevier, 2018.

Qureshi, Rizwana Aleem, Muhammad Asad Ghufran, Syed Aneel Gilani, Zaheer Yousaf, Ghulam Abbas, and Aniqa Batool. "Indigenous Medicinal Plants Used by Local Women in Southern Himalayan Regions of Pakistan." *Pakistan Journal of Botany* 41, no. 1 (2009): 19-25.

Rahman, Inayat Ur, Aftab Afzal, Zafar Iqbal, Farhana Ijaz, Niaz Ali, Muzammil Shah, Sana Ullah, and Rainer W Bussmann. "Historical Perspectives of Ethnobotany" *Clinics in Dermatology* 37, no. 4 (2018): 382-88.

Rahmani, Arshad Husain, Ahmad Almatroudi, Faris Alrumaihi, and Amjad Ali Khan. "Pharmacological and Therapeutic Potential of Neem (*Azadirachta indica*)." *Pharmacognosy Reviews* 12, no. 24 (2018): 250-55.

Ramalashmi, K, K Prasanna Vengatesh, K Magesh, R Sanjana, S Siril Joe, and K Ravibalan. "A Potential Surface Sterilization Technique and Culture Media for the Isolation of Endophytic Bacteria from *Acalypha indica* and Its Antibacterial Activity." *Journal of Medicinal Plants Studies* 6, no. 1 (2018): 181-84.

Ranjan, Bairwa, Singhal Manmohan, Sodha Ravindra Singh, and Rajawat Balwant Singh. "Medicinal Uses of *Trachyspermum ammi*: A Review." *The Pharma Research* 5, no. 2 (2011): 247-58.

Rao, HC Yashavantha, Devaraju Rakshith, and Sreedharamurthy Satish. "Antimicrobial Properties of Endophytic Actinomycetes Isolated from *Combretum latifolium* Blume, a Medicinal Shrub from Western Ghats of India." *Frontiers in Biology* 10, no. 6 (2015): 528-36.

Roy, Sudipta, Rubia Parvin, Subhadeep Ghosh, Somesankar Bhattacharya, Santanu Maity, and Debdulal Banerjee. "Occurrence of a Novel Tannase (Tan B Lp) in Endophytic *Streptomyces* sp. Al1l from the Leaf of *Ailanthus Excelsa* Roxb." *3 Biotech* 8, no. 1 (2018): 33.

Rupani, Reena, and Afton Chavez. "Medicinal Plants with Traditional Use: Ethnobotany in the Indian Subcontinent." *Clinics in Dermatology* 36, no. 3 (2018): 306-09.

Sabu, Rohini, KR Soumya, and EK Radhakrishnan. "Endophytic *Nocardiopsis* Sp. From *Zingiber officinale* with Both Antiphyto-pathogenic Mechanisms and Antibiofilm Activity against Clinical Isolates." *3 Biotech* 7, no. 2 (2017): 115.

Sagar, Anand, Leena Thakur, and Joginder Singh Thakur. "Studies on Endophytes and Antibacterial Activity of *Trillium govanianum* Wall. Ex D. Don." *International Journal of Botany Studies* 2, no. 1 (2017): 63-67.

Saini, Preeti, Madhurama Gangwar, and Amrinder Kaur. "Bioactivities of the Ethyl Acetate Extract of *Rhodococcus qingshengii* Strain Bjc15-A38

an Endophyte of *Azadirachta indica* A. Juss." *International Journal of Pharmacy and Pharmaceutical Sciences* 9, no. 6 (2017): 211-14.

Saini, Preeti, Madhurama Gangwar, Anu Kalia, Narinder Singh, and Deepti Narang. "Isolation of Endophytic Actinomycetes from *Syzygium cumini* and Their Antimicrobial Activity against Human Pathogens." *Journal of Applied and Natural Science* 8, no. 1 (2016): 416-22.

Sardi, P, M Saracchi, S Quaroni, B Petrolini, GE Borgonovi, and S Merli. "Isolation of Endophytic *Streptomyces* Strains from Surface-Sterilized Roots." *Applied and Environmental Microbiology* 58, no. 8 (1992): 2691-93.

Satari, Altaf H, M Iqbal Zargar, Wajaht Amin Shah, Ranju Bansal, and Mohammad Faizan Bhat. "Isolation, Molecular Identification, Phytochemical Screening and *In Vitro* Antioxidant Activity of Endophytic Fungi from *Achilea millefolium* Linn." *Journal of Pharmacognosy and Phytochemistry* 7, no. 4 (2018): 87-92.

Shareef, Munazza, and Muhammad Sohail Akhtar. "Neem (*Azadirachta indica*) and Its Potential for Safeguarding Health, Prevention and Treatment of Diseases." *Matrix Science Medica (MSM)* 2, no. 1 (2018): 4-8.

Sharma, Pushpendra, and Mamta Baunthiyal. "Endophytic Actinobacteria from *Pinus roxburghii*: Isolation, Diversity and Antimicrobial Potential against Human Pathogens." *Journal of Pharmacognosy and Phytochemistry* 7, no. 5 (2018): 3021-27.

Sharma, Richa, Sumpam Tangjang, and Tonlong Wangpan. "First Report on Biological Evaluation and Preliminary Screening of Fungal Endophytes from *Spilanthes paniculata*, a Medicinal Herb in Arunachal Pradesh, India." *International Journal of Current Microbiology and Applied Sciences* 7, no. 11 (2018): 1346-54.

Sharma, Vikash and Vaquil. "A Review on Medicinal Properties of Neem (*Azadirachta indica*)." *The Pharma Innovation Journal* 7, no. 4 (2018): 648-50.

Shenpagam, Hema N, D Kanchana Devi, G Sinduja, and R Sandhya. "Isolation of Endophytic Actinomycetes from Medicinal Plants and Its

Mutational Effect in Biocontrol Activity." *International Journal of Pharmaceutical Sciences and Research* 3, no. 11 (2012): 4338.

Singh, Monika, Ajay Kumar, Ritu Singh, and Kapil Deo Pandey. "Endophytic Bacteria: A New Source of Bioactive Compounds." *3 Biotech* 7, no. 5 (2017): 315.

Singh, Mrinalini J, and S Padmavathy. "*Nocardiopsis* sp. 5 Endophytic to Tulsi Leaves-Isolation and Antimicrobial Activity." *British Microbiology Research Journal* 5, no. 3 (2015): 194.

Singh, Radha, and Ashok K Dubey. "Diversity and Applications of Endophytic Actinobacteria of Plants in Special and Other Ecological Niches." *Frontiers in Microbiology* 9 (2018): 1767.

Singh, YR, R Khunjamayum, A Nongthombam, TP Chanu, KM Devi, RS Asem, K Tamreihao, DS Ningthoujam, and AI Devi. "Plant Growth and Grain Yield Production of Black Rice as Influenced by *Ochrobactrum intermedium* Acrz3, an Endophyte Associated with Medicinal Plant." *Crop Research* 53, no. 3 and 4 (2018): 183-91.

Strobel, Gary, and Bryn Daisy. "Bioprospecting for Microbial Endophytes and Their Natural Products." *Microbiology and Molecular Biology Reviews* 67, no. 4 (2003): 491-502.

Tandon, Nikhil, Ranjit M Anjana, Viswanathan Mohan, Tanvir Kaur, Ashkan Afshin, Kanyin Ong, Satinath Mukhopadhyay, et al. "The Increasing Burden of Diabetes and Variations among the States of India: The Global Burden of Disease Study 1990–2016." *The Lancet Global Health* 6, no. 12 (2018): e1352-e62.

Tanvir, R, I Sajid, and S Hasnain. "Screening for Type I Polyketide Synthases Genes of Endophytic Streptomycetes Isolated from *Parthenium hysterophorus* L." *Molecular Genetics, Microbiology and Virology* 28, no. 1 (2013): 32-39.

Tariq, A, M Adnan, A Iqbal, S Sadia, Y Fan, A Nazar, S Mussarat, et al. "Ethnopharmacology and Toxicology of Pakistani Medicinal Plants Used to Treat Gynecological Complaints and Sexually Transmitted Infections." *South African Journal of Botany* 114 (2018): 132-49.

Tokala, Ranjeet K, Janice L Strap, Carina M Jung, Don L Crawford, Michelle Hamby Salove, Lee A Deobald, J Franklin Bailey, and MJ

Morra. "Novel Plant-Microbe Rhizosphere Interaction Involving *Streptomyces lydicus* Wyec108 and the Pea Plant (*Pisum sativum*)." *Applied Environmental Microbiology* 68, no. 5 (2002): 2161-71.

Trujillo, Martha E., Raúl Riesco, Patricia Benito, and Lorena Carro. "Endophytic Actinobacteria and the Interaction of *Micromonospora* and Nitrogen Fixing Plants." [In English]. Review. *Frontiers in Microbiology* 6, no. 1341 (2015). https://doi.org/10.3389/fmicb.2015.0 1341.

Ullah, Manzoor, Sultan Mehmood, Maroof Ali, Rainer W Bussmann, Ali Aldosari, Rehmat Ali Khan, Razi Ullah, Wahid Hussain, and Muhammad Abdur Rahman Shah. "An Ethnopharmacological Study of Plants Used for Treatment of Diabetes in the Southern and Tribal Regions of Khyber Pakhtunkhwa Province, Pakistan." *Ethnobotany Research and Applications* 18 (2019): 1-20.

Van der Meij, Anne, Sarah F Worsley, Matthew I Hutchings, and Gilles P van Wezel. "Chemical Ecology of Antibiotic Production by Actinomycetes." *FEMS Microbiology Reviews* 41, no. 3 (2017): 392-416.

Vasantakumar, Ambika, and GM Vidyasagar. "Isolation and Identification of Endophytic Fungi from R. Br. *Gymnema sylvestre*." *Asian Journal of Pharmacy and Pharmacology* 4, no. 4 (2018): 440-43.

Venieraki, A, M Dimou, and P Katinakis. "Endophytic Fungi Residing in Medicinal Plants Have the Ability to Produce the Same or Similar Pharmacologically Active Secondary Metabolites as Their Hosts." *Hellenic Plant Protection Journal* 10, no. 2 (2017): 51-66.

Viaene, Tom, Sarah Langendries, Stien Beirinckx, Martine Maes, and Sofie Goormachtig. "*Streptomyces* as a Plant's Best Friend?" *FEMS Microbiology Ecology* 92, no. 8 (2016): 1-9.

Yadav, Rahul, Ajay Veer Singh, Samiksha Joshi, and Manish Kumar. "Antifungal and Enzyme Activity of Endophytic Fungi Isolated from *Ocimum sanctum* and *Aloe vera*." *African Journal of Microbiology Research* 9, no. 29 (2015): 1783-88.

In: A Closer Look at Actinomycetes ISBN: 978-1-53617-046-7
Editors: A. A. Mohamed Hatha et al.

Chapter 3

TAXONOMIC DIVERSITY AND APPLICATIONS OF SECONDARY METABOLITES OF *AMYCOLATOPSIS*

Pawina Kanchanasin and Somboon Tanasupawat*
Department of Biochemistry and Microbiology,
Faculty of Pharmaceutical Sciences, Chulalongkorn University,
Bangkok, Thailand

ABSTRACT

Amycolatopsis strains are aerobic, Gram-positive, non-acid-fast, filamentous, squarish and rod-shaped fragments on the substrate and aerial mycelia. *Amycolatopsis* species are alkaliphilic, mesophilic, thermophilic and pathogenic bacteria. They were distributed in soils, plants, freshwater, salt lakes, rock, ocean sediments, clinical samples from humans and horses, sugar cane bagasse, natural caves, a mine and a catacomb, equine placenta, volcanic soil, rhizospheric soil, polluted sediment, and arid soil. *Amycolatopsis* strains are generally isolated by the serial dilutions of soil sample on actinomycetes isolation agar, humic acid-vitamin (HV) agar,

* Corresponding Author's Email: Somboon.T@chula.ac.th.

starch-casein agar, casein mineral agar, oatmeal agar, asparagine agar, ISP2 medium, brain heart infusion agar, GTY agar, medium MY10S, NY medium, SM1, SM2 and SM3 agar. They can be distinguished from other genera by using morphological and chemotaxonomic characteristics and by using genus-specific oligonucleotide primers based on 16S rRNA gene sequences. *Amycolatopsis* strains produced various bioactive compounds such as antibacterial, antifungal, antiviral, immunosuppressant and antitumor compounds, especially *A. mediterranei* and *A. rifamycinica* (ansamycin-type antibiotic rifamycin, tolypomcins), *A. orientalis* (glycopeptide antibiotic vancomycin, muraceins, orienticin, quartromicin, balhimycins, etc.), *A. azurea* (azureomycins and octacosamicins) and *A. lurida* (benzathrins, ristocetin). The ansamycin and glycopeptide classes have efficient to medicine. In addition, they have ability to degrade biopolymers such as poly (L-lactic acid) (PLA), poly(ε-caprolactone) and poly(β-hydroxybutyrate). *A. orientalis* subsp. *orientalis*, *A. thailandensis* and *A. samaneae* produced three novel PLA-degrading enzymes named PLAase I, II and III. The copper-resistant *Amycolatopsis* strain was applied in bioremediation biotechnologies. *Amycolatopsis* strains are known to have chitinase for antifungal, cellulase, and xylanase for biofuel production, in addition, has reported producing lipase and keratinase. This chapter describes the recent status of *Amycolatopsis* species on taxonomy, secondary metabolites and their other applications

INTRODUCTION

Members of the genus *Amycolatopsis* are Gram-stain-positive, filamentous bacteria belonging to the group of actinomycetes (Lechevalier et al., 1986) that mostly found in soil environments (Saintpierre-Bonaccio et al., 2005). This genus contained *meso*-2,6 diaminopimelic acid (*meso*-DAP), arabinose and galactose as the typical characteristics of the cell wall, MK-9(H_4) as the predominant menaquinone, and phosphatidylethanolamine as the diagnostic phospholipid. The fatty acid profile includes mixtures of saturated and branched-chain fatty acids but no mycolic acids. The DNA G+C content ranges from 66 to 75 mol % (Lechevalier et al., 1986). *Amycolatopsis* strains produced several relevant secondary metabolites, such as balhimycin, vancomycin, avoparcin, ristomycin, chelocardin, chloroeremomycin, ECO-0501 and rifamycin (Chen et al., 2016) and considered economically important of actinomycetes. The *Amycolatopsis*

compounds showed several biological activities: (1) antagonistic agents, including antifungal, antibacterial, antiviral, (2) pharmacological agents, including antitumoral, immunosuppressant (3) compounds with regulatory activities, such as muramyl peptide inhibitors, cytotoxic activities (4) enzyme activity, including chitinase, chitosanase, cellulase, xylanase, lipase and keratinase.

ISOLATION

Amycolatopsis strains are shown regularly to occasional fragmentation of either the substrate mycelium or the aerial mycelium or both. They are not created by sclerotia, spore vesicles, or synnemata. They formed long chains of smooth, squarish to ellipsoidal spore-like structures on substrate and aerial mycelia (Kothe et al., 1989). *Amycolatopsis* strains formed well-developed colonies on most standard media used to cultivate filamentous actinomycetes and may take abundant aerial hyphae (Mertz and Yao, 1993). They grew especially well on modified Bennett's agar supplemented with mannitol (0.5%, w/v) and soybean flour (0.5%, w/v) incubated at 28°C for 14 days (Tan et al., 2006a). *Amycolatopsis* strains grew well on standard media such as inorganic salts starch, glycerol-asparagine, peptone-yeast extract-iron, oatmeal, tyrosine, tryptone-yeast extract, and yeast extract-malt extract agar (ISP media 2-7; (Shirling and Gottlieb, 1966). They are thermophilic actinomycetes (Brock, 1986; Cross, 1968), moderate thermophiles (Goodfellow et al., 2001), mesophiles (Lee and Hah, 2001). *Amycolatopsis* strains are growing well from pH 6.0 to 9.0. *Amycolatopsis* strains will be cultivated on glucose-yeast extract agar or modified Bennett's agar at 28°C to allow abundant growth. The cultures can be maintained at 4°C or room temperature for up to 6 months. Long-term preservation can be achieved by lyophilization or in 20% glycerol frozen at −20°C or −80°C.

The members of the genus *Amycolatopsis* are broadly distributed, being isolated from diverse environments. *A. orientalis* has been isolated from cerebrospinal fluid (Gordon et al., 1978). Three species of *Amycolatopsis*, *A. kentuckyensis*, *A. lexingtonensis*, and *A. pretoriensis* are isolated from

equine placentas (Labeda et al., 2003). *A. palatopharyngis* is isolated from infected palatopharyngeal mucosa (Huang et al., 2004). *A. benzoatilytica* has been involved as an agent of submandibular mycetoma (Majumdar et al., 2006). Most species of *Amycoloatopsis* have been isolated from regionally diverse soil samples (Saintpierre-Bonaccio et al., 2005; Lee, 2006; Tan, 2006b; Tseng, 2006; Chen et al., 2010; Chomchoei et al., 2011; Nie et al., 2012; Zucchi et al., 2012b; Zucchi et al., 2012c; Camas, 2013; Everest et al., 2013; Sripreechasak et al., 2013; Everest et al., 2014; Souza et al., 2015), paddy soil (Penkhrue et al., 2018), volcanic soil (Ding et al., 2007), rice rhizosphere soil (Thawai, 2018), rhizospheric soil (Lee, 2009), arid composite soil (Zucchi et al., 2012a), scrubland soil (Kim et al., 2002), marsh soil (Al-Musallam et al., 2003), brown hypermagnesian ultramafic soil (Saintpierre-Bonaccio et al., 2005), forest soil (Huang et al., 2001; Jamjan et al., 2018) and coal mine soil (Oyuntsetseg et al., 2017). Some have been isolated from more unusual sources, such as equine placenta (Labeda et al., 2003), polluted sediment (Albarracı´n et al., 2010), marine sediment (Bian et al., 2009), marine macroalgae (Wang et al., 2018), arid Australian composite sample (Tan et al., 2006b), deep-sea sediment (Zhang et al., 2016), plants (Duangmal et al., 2011; Miao et al., 2011; Xing et al., 2013), natural caves (Lee, 2006), vegetable matter (Goodfellow et al., 2001; Lechevalier et al., 1986) salt lakes (Tang et al., 2010; Guan et al., 2013), clinical material from humans and horses (Labeda et al., 2003; Huang et al., 2004), sugar cane bagasse (Goodfellow et al., 2001), mine and catacomb (Groth et al., 2007; Carlsohn et al., 2007), filtrate substrate (Ding et al., 2007), medieval alum slate mine (Carlsohn et al., 2007) and copper-polluted sediments (Albarracı´n et al., 2010).

Amycolatopsis species have been isolated by plating dilutions of soil suspension onto nonselective media, supplemented with antifungal antibiotics, supported by colony selection and characterization. This hit and missed method, for particular, led to the isolation of *A. halotolerans* and *A. jejuensis* on starch-casein agar (Lee, 2006), *A mediterranei* on Bennett's agar (Margalith and Beretta, 1960), *A. nigrescens* on nutrient agar (Groth et al., 2007), *A. rubida* on glucose-asparagine agar (Huang et al., 2001), *A. saalfeldensis* on casein mineral agar (Carlsohn et al., 2007), and *A.*

taiwanensis on HV agar (Tseng et al., 2006), and isolation plates were incubated at either 28°C or 30°C for 14 d. *A. keratiniphila* was isolated from soil enriched with sterilized and defatted wool (Al-Musallam et al., 2003). *A. palatopharyngis* was isolated on brain-heart infusion agar at 37°C for 5 days under microaerophilic conditions (Huang et al., 2004). *A. plumensis* was isolated on yeast extract malt extract agar supplemented with streptomycin (Saintpierre-Bonaccio et al., 2005). *A. sacchari* strains from various environment samples were collected using a wind tunnel technique (Lacey, 1971). Goodfellow et al. (2001) and Tan et al. (2006a) designed a number of agar media to isolate *Amycolatopsis* strains from soil using putative selective agents drawn from phenotypic databases generated in numerical taxonomic studies on members of the family *Pseudono cardiaceae*. It has been shown that three of the following media formulations are particularly effective in separating *Amycolatopsis* strains from soil samples, namely media SM1 (Stevenson's basal medium supplemented with D-sorbitol and neomycin sulfate), SM2 (Stevenson's basal medium supplemented with D-melezitose and neomycin sulfate), and SM3 (Gauze's medium supplemented with nalidixic acid and novobiocin). There were antifungal antibiotics (cycloheximide, neomycin sulphate and nystatin) in all three media (Tan et al, 2006a). The inoculated plates have been incubated for up to 21 days at 28°C and the existence of white or whitish-yellow suspended hyphae distinguished *Amycolatopsis* strains. It has been discovered that representative isolates have morphological and chemotaxonomic characteristics compatible with their allocation to the genus *Amycolatopsis*. A set of genus-specific oligonucleotides that have been used for the rapid identify putative *Amycolatopsis* strains recovered from the selective isolation plates have generated by Tan et al., 2006a. Recently, *A. roodepoortensis* and *A. speibonae* were isolated on Modified Czapek solution (MC) agar and Middlebrook 7H9 agar (Everest et al., 2014). *A. rhabdoformis* was isolated on SM3 agar (Souza et al., 2015). *A. stemonae* was isolated from the stem of medicinal plant using starch-casein agar (Klykleung et al., 2015). *A. albispora* was isolated on modified MZ2 agar (Zhang et al., 2016). Source, medium, and antimicrobials for the isolation of *Amycolatopsis* strains are shown in Table 1.

Table 1. Source, medium, and antimicrobials for the isolation of *Amycolatopsis* strains

Source	Medium	Antibiotic	References
Soil	Starch-casein agar	-	Lee, 2006
Soil	Bennett's agar	-	Margalith and Beretta, 1960
Soil	Nutrient agar	-	Groth et al., 2007
Soil	Glucose-asparagine agar	-	Huang et al., 2001
Soil	Casein mineral agar	Cycloheximide (50 µg/ml)	Carlsohn et al., 2007
Soil	HV	Cycloheximide (50 µg/ml)	Tseng et al., 2006
Soil	Soil enriched with sterilized & defatted wool	-	Al-Musallam et al., 2003
Soil	Brain-heart infusion agar	-	Huang et al., 2004
Soil	Yeast malt extract agar	Streptomycin sulphate (10 µg/ml) & cycloheximide (100 µg/ml)	Saintpierre-Bonaccio et al., 2005
Floor dust	Half-strength nutrient agar	Actidione (50 µg/ml)	Goodfellow et al., 2001
Composite arid soil and environmental	SM1 (Stevenson's basal medium supplemented with d-sorbitol & neomycin sulfate)	Cycloheximide (50 µg/ml), neomycinsulphate (4 µg/ml) & nystatin (50 µg/ml)	Tan et al., 2006a
Composite arid soil and environmental	SM2 (Stevenson's basal medium supplemented with d-melezitose & neomycin sulfate)	Cycloheximide (50 µg/ml), neomycinsulphate (4 µg/ml) & nystatin (50 µg/ml)	Tan et al., 2006a
Composite arid soil and environmental	SM3 (Gauze's medium)	Cycloheximide (50 µg/ml), nalidixic acid (10 µg/ml), novobiocin (10 µg/ml) & nystatin (50 µg/ml)	Tan et al., 2006a
Soil	Modified Czapek solution (MC) agar & Middlebrook 7H9 agar	-	Everest et al., 2014
Deep-sea sediment	modified MZ2 agar	-	Zhang et al., 2016

TAXONOMY

The members of the genus *Amycolatopsis* representatives are aerobic, Gram-positive, non-acid-fast, non-motile actinomycetes that form branched substrate hyphae which fragment into rod-like and square-shaped elements (Lechevalier et al., 1986). Aerial hyphae can be sterile or differentiate into long chains of spore-like structures. The genus *Amycolatopsis* was first proposed as a novel genus of nocardioform actinomycetes by Lechevalier et al. (1986) and was assigned in the family *Pseudonocardiaceae*, suborder *Pseudonocardineae* by the application of the polyphasic taxonomic approach to actinomycete systematics (Stackebrandt et al., 1997; Kim and Goodfellow, 1999). Phylogenetically, this genus represents a monophyletic clade within the evolutionary radiation encompassed by the family *Pseudonocardiaceae* (Warwick et al., 1994), a taxon that closely related to the family *Actinosynnemataceae* (Labeda and Kroppenstedt, 2000). The genus *Amycolatopsis* currently consisted of 76 species and 4 subspecies with validly published names (http://www.bacterio. net/amycolatopsis. html).

The main characteristics of this genus are *meso*-diaminopimelic acid (*meso*-A2pm) in peptidoglycan, arabinose, and galactose as characteristic sugars in the whole-cell hydrolysates (wall chemotype IV *sensu* Lechevalier and Lechevalier, 1970), muramic acid in the *N*-acetylated form (Lee and Hah, 2001; Tan et al., 2006a), and absence of mycolic acids (Takahashi, 2001). Most strains have comparable qualitative fatty acid profiles in which 14-methylpentadecanoic acid (iso-$C_{16:0}$) is the major component (Carlsohn et al., 2007) though considerable amounts of hexadecenoic ($C_{16:0}$), 12-methyltridecanoic (iso-$C_{14:0}$), 13-methyltetradecanoic ($C_{15:0}$ iso), heptadecanoic ($C_{17:0}$), and octadecanoic ($C_{18:0}$) acids may be significant (Lee and Hah, 2001; Huang et al., 2004; Lee, 2006). The predominant type of menaquinone is di-, tetra-, or hexahydrogenated menaquinone with nine isoprene units [MK-9(H_2, H_4, H_6)] as the predominant isoprenologue (Yassin et al., 1991). Phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) as major polar lipids (phospholipid type II *sensu* Lechevalier et al., 1981; Lechevalier et al., 1977) with diphosphatidylglycerol (DPG), hydroxyphosphatidylethanolamine (HPE),

phosphatidylinositol (PI), phosphatidylinositol mannosides (PIMs), phosphatidylserine (PS), and phosphatidylmethylethanolamine (PME) variably present. Members of the genus have DNA G+C contents in the range from 66 to 75 mol%.

Members of the genus *Amycolatopsis* can be distinguished from the other genera classified in the family *Pseudonocardiaceae* by using genus-specific oligonucleotide primers AMY2 and ATOP based on 16S rRNA gene sequences (Tan et al., 2006b) and chemical and morphological properties (Kim and Goodfellow, 1999). Species of *Amycolatopsis* were differentiated by phenotypic properties (Chun et al., 1999), MALDI-TOF MS spectra (Groth et al., 2007) and on quantitative differences in fatty acid profiles (Ding et al., 2007; Mertz and Yao, 1993) and ribotype patterns (Mertz and Yao, 1993).

SECONDARY METABOLITES AND BIOLOGICAL ACTIVITY

Member of the genus *Amycolatopsis* is the source of commercially important antibiotics and secondary metabolites (Embley, 1992). *Amycolatopsis* strain is regarded as a significant source of various bioactive compounds including antibacterial, antifungal, antiviral, immunosuppressant and antitumor compounds (Table 2). The ansamycin and glycopeptide classes are the most important to medicine. Clinically important antibiotics produced by *Amycolatopsis* strains include balhimycin (Nadkarni et al., 1994), dethymicin (Ueno et al., 1992), rifamycin (Sensi et al., 1959), and vancomycin (Barna and Williams, 1984). The genus is known for its antibiotic-producing strains, vancomycin and rifamycin produced by *A. orientalis* (Pittenger and Brigham, 1956) and *A. mediterranei* (Sensi et al., 1959), respectively.

Vancomycin, the glycopeptide antibiotic, was isolated at the Lilly Research laboratories from *A. orientalis* strain and was presented for clinical use in 1958 for the treatment of staphylococcal infections resistant to then-available antibiotics. Increased incidence of multiple antibiotic-resistant strains of *Staphylococci, pneumococci,* and *enterococci* revived

vancomycin, and its usage has increased worldwide over the last decade. It is the drug of choice for the treatment of methicillin-resistant *Staphylococcus aureus* infections (Sorrell et al., 1982), coagulase-negative staphylococci (Gruer et al., 1984). It is also used to treat *Streptococcus pneumoniae* strain that is penicillin-resistant (Goldstein et al., 1994); Gram-positive bacilli such as *Bacillus anthracis* and *Bacillus cereus* (Weber et al., 1988); Corynebacteria such as *Corynebacterium diphtheriae* and *C. jeikeium* (Jadeja et al., 1983); and many of the clinically significance clostridial species such as *Clostridium difficile*, *C. perfringens*, *C. botulinum*, and *C. septicum* that are sensitive to vancomycin (Watanakunakorn, 1984). Intraventricular application of vancomycin is an effective therapeutic regimen for the treatment of shut-associated staphylococcal ventriculitis (Nagl et al., 1999).

A. orientalis strain produced vancomycin (Levine, 2006), a glycopeptide antibiotic of last resort against methicillin-resistant *Staphylococcus aureus*, which caused severe clinical infections (Moellering Jr, 2006). Industrial strains have been developed by random mutagenesis or optimization of culture conditions (Jung et al., 2007) from *A. orientalis* subsp. *orientalis* KCTC 9412, the type strain of this species or several decades. Rifamycin is used for the therapy of leprosy and tuberculosis, and for the control of infections in organ implants and AIDS patients. *Amycolatopsis* strains have been on trying to apply biochemical and genetic methods to the synthesis of antibiotics, as evidenced by studies on the biosynthesis of rifampin (Rifampicin) (Floss and Yu, 2005; Ghisalba and Nuesch, 1981).

The polyketide framework of rifamycin B is composed of 3-amino-5-hydroxybenzoic acid (AHBA), a component of the aminoshikimate pathway (Kim et al., 1998; Yu et al., 2001), two molecules of acetate and eight of propionate. Five multifunctional proteins (RifA-RifE) and an amide synthase (RifF) catalyze the synthesis of the core structure of rifampin (August et al., 1998). Balhimycin and vancomycin have antibiotic activity against methicillin-resistant strain of *Stapylococcus aureus*, while dethymicin has a mode of action that distinguishes it from other immunosuppresants, such as cyclosporine, FK506, and rapamycin.

Table 2. Bioactive compounds produced by *Amycolatopsis* species

Species/strain	Compounds	Feature	References
A. alba	Glycopeptide antibiotic	Inhibits peptidoglycan synthesis	Mertz and Yao, 1993
A. alba DVR D4	Pyridinium	Antimicrobial & cytotoxic	Dasari et al., 2012
A. azurea	Azureomycins A & B	Glycopeptide antibiotic	Omura et al., 1979
A azurea	Octacosamicins	Antifungal	Dobashi et al., 1988
A. balhimycina	Balhimycin	Glycopeptide antibiotic	Nadkarni et al., 1994
A. coloradensis	Avoparcin	Glycopeptide antibiotic	Kunstmann et al., 1968
A. fastidiosa	Antibiotics 41034 & 41494	Macrobicyclic peptides	Celmer et al., 1977
A. japonica	*(S,S)-N,N'*-ethylene-diaminedisuccinic acid	Inhibits phospholipase C	Nishikori et al., 1984
A. japonica, *A. mediterranei*	Dethymicin	Immunosuppressant	Ueno et al., 1992
A. keratiniphila	Nogabecin	Glycopeptide antibiotic	Shorin et al., 1957
'A. lactamdurans'	Cephamycin C	β-Lactam antibiotic	Stapley et al., 1972
	Efrotomycin	β-Isomer	Wax et al., 1976
	3-Methylpseudouridine	Polyether	Nielsen and Arison., 1989
A. lurida	Benzathrins	Quinone antibiotic with antitumor	Rasmussen et al., 1986
	Ristocetin	Glycopeptide antibiotic	Grundy et al., 1957
A. mediterranei, *A. rifamycinica*	Rifamycins	Clinically useful ansamycins, active against *Mycobacterium* spp., control infections in organ implants & AIDS patients	Lancini and Sartori., 1976
	Kanglemycin A	Ansamycin-type antibiotic	Wang et al., 1988
	Dethymicin	Immunosuppressant	Ueno et al., 1992
	3-Hydroxyrifamycin S	Ansamycin antibiotic	Traxler et al., 1981

Species/strain	Compounds	Feature	References
	Protorifamycins	Ansamycin antibiotic	Ghisalba et al., 1980
	Aromatic amino acids	Suitable for strain improvement	De Boer et al., 1990
A. orientalis	Vancomycin	Glycopeptide antibiotic, active against methicillin-resistant *Stapylococcus*	Pittenger and Brigham, 1956
	Glycopeptide compounds	Glycosyltransferase gene, *gtfA*	Baltz, 2000
	Muraceins	Muramyl peptide inhibitors	Bush et al., 1984
	N -Demethylvancomycin	Vancomycin analog	Boeck et al., 1984
	Orienticin	Glycopeptide antibiotic	Tsuji et al., 1988
	Quartromicin	Antiviral antibiotics	Tsunakawa et al., 1992
	Antibiotic UK-69753	Efrotomycin-like antibiotic	Pacey et al., 1989
A. regifaucium	Kigamicins	Antitumor antibiotics	Kunimoto et al., 2003
A. sulphurea	Cetocycline	Tetracycline derivative	Proctor et al., 1978
A. tolypomycina	Tolypomycin	Ansamycin-type antibiotic	Kishi et al., 1972
A. vancoresmycina	Homorifamycin; vancoresmycin	Ansamycin antibiotic; polyketide antibiotic	Hopmann et al., 2002
Amycolatopsis sp. K16-0194	Dipyrimicin	Antimicrobial & cytotoxic	Izuta et al., 2018
Amycolatopsis sp. MST-108494	Amycolatopsins A-C	Antimycobacterial	Khalil et al., 2017
Amycolatopsis sp. ML1-hF4	Valgamicin A, C, T	Cytotoxic	Hashizume et al., 2018
Amycolatopsis sp. MK575-fF5	Amycolamicin	Antibacterial	Sawa et al., 2012
Amycolatopsis sp.	Pargamicin A	Antibacterial	Igarashi et al., 2008

A number of non-antibiotic bioactive metabolites are produced by *Amycolatopsis* strains (Table 3), including (S,S)-N,N'-ethylenediamine disuccinic acid from *Amycolatopsis japonica* (Nishikori et al., 1984), a d-amino acid-specific peptidase from *A. orientalis* (Sugie et al., 1988), and a polylactic acid depolymerase from *Amycolatopsis* sp. strain K104-1 (Nakamura et al., 2001). *Amycolatopsis* sp. strain HR167 produced vanillic

acid from ferulic acid (Rabenhorst and Hopp, 1997), and an *Amycolatopsis* isolate for the bioconversion of lovastatin to the novel compound wuxistatin (Zhuge et al., 2008) *A. methanolica* NCIB 11946^T assimilated formaldehyde with the ribulose monophosphate shunt (Hazeu et al., 1983), a finding that made it a candidate for fermentative overproduction of aromatic amino acids (Dijkhuizen et al., 1985; Morinaga and Hirose, 1984).

BIODEGRADABLE AND OTHER APPLICATIONS

The most successful option for disposable plastic is increasingly regarded to be biodegradable plastics. Member of genus *Amycolatopsis* is capable of degrading biopolymers such as poly L-lactic acid (PLA), poly (ε-caprolactone) and poly(β-hydroxybutyrate). Poly L-lactic acid (PLA) is a biodegradable plastic that can be produced by renewable resource fermentation. It is expected that PLA will replace petrochemicals plastics (Tokiwa and Calabia, 2006; Li et al., 2008). PLA-degrading actinomycetes were first reported by (Pranamuda et al., 1997) and most of the reported PLA degrading strains belong to *Amycolatopsis* (Pranamuda and Tokiwa, 1999; Jarerat et al., 2002). *A. thailandensis* and *A. samaneae*, *A. orientalis* subsp. *orientalis* as poly L-lactic acid-degrading species produces three novel PLA-degrading enzymes named PLAase I, II and III (Li et al., 2008). *A. thailandensis*, isolated in northern Thailand from natural park soil (Chomchoei et al., 2011) and *A. samaneae* isolated from surface-sterilized roots of *Samanea saman* (Jacq.) Merr. have been reported (Duangmal et al., 2011).

A copper-resistant strain of *Amycolatopsis* has been isolated from copper-polluted sediment by Albarracín et al. (2008), which has a high bioaccumulation ability and the potential for use in bioremediation biotechnology. It was subsequently characterized and described as a novel *Amycolatopsis* species, *A. tucumanensis* (Albarracín et al., 2010). They also found that the type strain of *A. eurytherma*, a close relative of *A. tucumanensis*, has a moderate copper resistance profile.

Table 3. Non-antibiotic compounds produced by *Amycolatopsis* strains

Compound name	Species/strain	Reference
(S, S)-N,N'-Ethylenediamine disuccinic acid	*A. japonica*	Nishikori et al., 1984
D-Amino acid-specific peptidase	*A. orientalis*	Sugie et al., 1988
Polylactic acid depolymerase	*Amycolatopsis* sp. K104-1	Nakamura et al., 2001
Vanillic acid	*Amycolatopsis* sp. HR167	Rabenhorst and Hopp, 1997
Wuxistatin	*Amycolatopsis* isolate	Zhuge et al., 2008
PPi-dependent phosphofructokinase	*A. methanolica* NCIB 11946^{T}	Siebers et al., 1998
Copper resistance	*A. tucumanensis* and *A. eurytherma*	Albarracín et al., 2010
Bacterial Translocase I	*Amycolatopsis* sp. SANK 60206	Murakami et al., 2007

Chitosan-degrading actinomycete, *Amycolatopsis* sp. CsO-2, produced a 27-kD chitosanase (Okajima et al., 1994). The recombinant form of the chitosanase not only exhibited chitosan-hydrolyzing activity but also antifungal activity against Zygomycota strains. *A. orientalis* in the production of chitin hexamers produced chitinases of potential value (Tominaga and Tsujisaka, 1976; Usui et al., 1987). The organism converted tetra-N-acetylchitotelraose to a mixture of hexa-N-acetyl-chitohexaose and the corresponding dimer by a transglycosylation reaction (Usui et al., 1987).

The cellulolytic strain identified as *Amycolatopsis* sp. GDS could produce high levels of cellulase and xylanase by utilizing agricultural waste biomass and its application in the preparation of biomass feedstock and sequential ethanol fermentation (Kshirsagar et al., 2016). Among several lipase-producing actinomycete strains, *Amycolatopsis mediterranei* DSM 43304 was discovered to produce a thermostable, extracellular lipase (Dheeman et al., 2010). The presence of a novel lipase in crude extracts of a mesophilic actinomycete *A. mediterranei* DSM 43304 (Dheeman et al., 2010). Characterization of this *A. mediterranei* DSM 43304 lipase (AML)

activity indicated it had high thermostability and organic solvent stability-indicating its potential in organic synthesis. Keratinase production by actinomycetes belonging to the genera *Amycolaptosis* has also been revealed (Al-Musallam et al., 2003).

CONCLUSION

The strains of *Amycolatopsis* species are the source of commercially important antibiotics and secondary metabolites including antibacterial, antifungal, antiviral, immunosuppressant and antitumor compounds, and a number of non-antibiotic bioactive metabolites including (S, S)-N,N'-ethylenediamine disuccinic acid, a D-amino acid-specific peptidase, and a polylactic acid depolymerase. *Amycolatopsis* strain produced vanillic acid from ferulic acid, and could converse lovastatin to the novel compound wuxistatin. A copper-resistant strain has a high bioaccumulation ability and the potential for use in bioremediation biotechnology. *Amycolatopsis* strain produced chitinases of potential value and some strains produced cellulase and xylanase by utilizing agricultural waste biomass and its application in the preparation of biomass feedstock and sequential ethanol fermentation. In addition, extracellular lipase and keratinase could be found by *Amycolaptosis* strains.

REFERENCES

Albarracı´n, V. H., Alonso-Vega, P., Trujillo, M. E., Amoroso, M. J. & Abate, C. M. (2010). *Amycolatopsis tucumanensis* sp. nov., a copper-resistant actinobacterium isolated from polluted sediments. *Int. J. Syst. Evol. Microbiol., 60,* 397-401.

Albarracı´n, V. H., Winik, B., Kothe, E., Amoroso, M. J. & Abate, C. M. (2008). Copper bioaccumulation by the actinobacterium *Amycolatopsis* sp. ABO. *J. Basic. Microbiol., 48,* 323-330.

Al-Musallam, A. A., Al-Zarban, S. S., Fasasi, Y. A., Kroppenstedt, R. M. & Stackebrandt, E. (2003). *Amycolatopsis keratiniphila* sp. nov., a novel keratinolytic soil actinomycete from Kuwait. *Int. J. Syst. Evol. Microbiol., 53,* 871-874.

August, P. R., Tang, L., Yoon, Y. J., Ning, S., Muller, R., Yu, T. W., Taylor, M., Hoffmann, D., Kim, C. G., Zhang, X., Hutchinson, C. R. & Floss, H. G. (1998). Biosynthesis of the ansamycin antibiotic rifamycin: deductions from the molecular analysis of the rif biosynthetic gene cluster of *Amycolatopsis mediterranei* S699. *Chem. Biol., 5,* 69-79.

Baltz, R. (2000). Sweet home actinomycetes: The 1999 MDS Panlabs Lecture. *J. Ind. Microbiol. Biotechnol., 24,* 79-88.

Barna, J. C. & Williams, D. H. (1984). The structure and mode of action of glycopeptide antibiotics of the vancomycin group. In *Annu. Rev. Microbiol.* (Vol. *38,* pp. 339-357).

Bian, J., Li, Y., Wang, J., Song, F. H., Liu, M., Dai, H. Q., Ren, B., Gao, H., Hu, X., Liu, Z. H., Li, W. J. & Zhang, L. X. (2009). *Amycolatopsis marina* sp. nov., an actinomycete isolated from an ocean sediment. *Int. J. Syst. Evol. Microbiol., 59,* 477-481.

Boeck, L. D., Mertz, F. P., Wolter, R. K. & Higgens, C. E. (1984). N-demethylvancomycin, a novel antibiotic produced by a strain of *Nocardia orientalis*. Taxonomy and fermentation. *J. Antibiot., 37,* 446-453.

Brock, T. D. (1986). Introduction: an overview of the thermophiles. In *Thermophiles: General, Molecular and Applied Microbiology* (pp. 1-16). New York: Wiley.

Bush, K., Henry, P. R. & Slusarchyk, D. S. (1984). Muraceins-muramyl peptides produced by *Nocardia orientalis* as angiotensin-converting enzyme inhibitors. I. Taxonomy, fermentation and biological properties. *J. Antibiot., 37,* 330-335.

Camas, M., Sahin, N., Sazak, A., Spröer, C. & Klenk, H. P. (2013). *Amycolatopsis magusensis* sp. nov., isolated from soil. *Int. J. Syst. Evol. Microbiol., 63,* 1254-1260.

Carlsohn, M. R., Groth, I., Tan, G. Y., Schütze, B., Saluz, H. P., Munder, T., Yang, J., Wink, J. & Goodfellow, M. (2007). *Amycolatopsis*

saalfeldensis sp. nov., a novel actinomycete isolated from a medieval alum slate mine. *Int. J. Syst. Evol. Microbiol.*, *57*, 1640-1646.

Celmer, W. D., Cullen, W. P., Moppett, C. E., Routien, J. B., Shibakawa, R. & Tone, J. (1977). United States Patent No. 4038383.

Chen, J., Su, J. J., Wei, Y. Z., Li, Q. P., Yu, L. Y., Liu, H. Y., Zhang, Y. Q. & Zhang, Y. Q. (2010). *Amycolatopsis xylanica* sp. nov., isolated from soil. *Int. J. Syst. Evol. Microbiol.*, *60*, 2124-2128.

Chen, S., Wu, Q., Shen, Q. & Wang, H. (2016). Progress in understanding the genetic information and biosynthetic pathways behind *Amycolatopsis* antibiotics, with implications for the continued discovery of novel drugs. *ChemBioChem*., *17*, 119-128.

Chomchoei, A., Pathom-Aree, W., Yokota, A., Kanongnuch, C. & Lumyong, S. (2011). *Amycolatopsis thailandensis* sp. nov., a poly(L-lactic acid)-degrading actinomycete, isolated from soil. *Int. J. Syst. Evol. Microbiol.*, *61*, 839-843.

Chun, J. S., Kim, S. B., Oh, Y. K., Seong, C. N., Lee, D. H., Bae, K. S., Lee, K. J., Kang, S. O., Hah, Y. C. & Goodfellow, M. (1999). *Amycolatopsis thermoflava* sp. nov., a novel soil actinomycete from Hainan Island, China. *Int. J. Syst. Bacteriol.*, *49*, 1369-1373.

Cross, T. (1968). Thermophilic actinomycetes. *J. Appl. Bacteriol.*, *31*, 36-53.

Dasari, V. R., Muthyala, M. K., Nikku, M. Y. & Donthireddy, S. R. (2012). Novel Pyridinium compound from marine actinomycete, *Amycolatopsis alba* var. nov. DVRD4 showing antimicrobial and cytotoxic activities *in vitro*. *Microbiol. Res*, *167*, 346-351.

De Boer, L., Dijkhuizen, L., Grobben, G., Goodfellow, M., Stackebrandt, E., Parlett, J. H., Whitehead, D. & Witt., D. (1990). *Amycolatopsis methanolica* sp. nov., a facultatively methylotrophic actinomycete. *Int. J. Syst. Bacteriol.*, *40*, 194-204.

Dheeman, D. S., Frias, J. M. & Henehan, G. T. (2010). Influence of cultivation conditions on the production of a thermostable extracellular lipase from *Amycolatopsis mediterranei* DSM 43304. *J. Ind. Microbiol. Biotechnol.*, *37*, 1-17.

Dijkhuizen, L., Hansen, T. A. & Harder, W. (1985). Methanol, a potential feedstock for biotechnological processes. *Trends Biotechnol.*, *3*, 262-267.

Ding, L., Hirose, T. & Yokota, A. (2007). *Amycolatopsis echigonensis* sp. nov. and *Amycolatopsis niigatensis* sp. nov., novel actinomycetes isolated from a filtration substrate. *Int. J. Syst. Evol. Microbiol.*, *57*, 1747-1751.

Dobashi, K., Matsuda, N., Hamada, M., Naganawa, H., Takita, T. & Takeuchi, T. (1988). Novel antifungal antibiotics octacosamicins A and B. I. Taxonomy, fermentation and isolation, physico-chemical properties and biological activities. *J. Antibiot.*, *41*, 1525-1532.

Duangmal, K., Mingma, R., Pathom-Aree, W., Thamchaipenet, A., Inahashi, Y., Matsumoto, A. & Takahashi, Y. (2011). *Amycolatopsis samaneae* sp. nov., isolated from roots of *Samanea saman* (Jacq.) Merr. *Int. J. Syst. Evol. Microbiol.*, *61*, 951-955.

Embley, T. M. (1992). The family Pseudonocardiaceae. In *In The Prokaryotes: a Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications* (pp. 996-1027). New York: Springer.

Everest, G. J., le Roes-Hill, M., Omorogie, C., Cheung, S. K., Cook, A. E., Goodwin, C. M. & Meyers, P. R. (2013). *Amycolatopsis umgeniensis* sp. nov., isolated from soil from the banks of the Umgeni River in South Africa. *Antonie Van Leeuwenhoek.*, *103*, 673-681.

Everest, G. J., Le Roes-Hill, M., Rohland, J., Enslin, S. & Meyers, P. R. (2014). *Amycolatopsis roodepoortensis* sp. nov. and *Amycolatopsis speibonae* sp. nov.: antibiotic-producing actinobacteria isolated from South African soils. *J. Antibiot.*, *67*, 813-818.

Floss, H. G. & Yu, T. W. (2005). Rifamycin-mode of action, resistance, and biosynthesis. *Chem. Rev.*, *105*, 621-632.

Ghisalba, O. & Nuesch, J. (1981). A genetic approach to the biosynthesis of the rifamycin-chromophore in *Nocardia mediterranei*. IV. Identification of 3-amino-5-hydroxybenzoic acid as a direct precursor of the seven-carbon amino starter-unit. *J. Antibiot.*, *34*, 64-71.

Ghisalba, O., Traxler, P., Fuhrer, H. & Richter, W. J. (1980). Early intermediates in the biosynthesis of ansamycins. III. Isolation and identification of further 8-deoxyansamycins of the rifamycin-type. *J. Antibiot. Annu., 33*, 847-856.

Goldstein, F. W., Geslin, P., Acar, J. F. & Group, t. F. S. (1994). Comparative activity of teicoplanin and vancomycin against 400 penicillin susceptible and resistant *Streptococcus pneumoniae*. *Eur. J. Clin. Microbiol. Infect. Dis.*, *13*, 33-34.

Goodfellow, M., Kim, S. B., Minnikin, D. E., Whitehead, D., Zhou, Z. H. & Mattinson-Rose, A. D. (2001). *Amycolatopsis sacchari* sp. nov., a moderately thermophilic actinomycete isolated from vegetable matter. *Int. J. Syst. Evol. Microbiol.*, *51*, 187-193.

Gordon, R. E., Mishra, S. K. & Barnett, D. A. (1978). Some bits and pieces of genus *Nocardia*: *Nocardia carnea, Nocardia vaccinii, Nocardia transvalensis, Nocardia orientalis* and *Nocardia aerocolonigenes*. *J. Gen. Microbiol.*, *109*, 69-78.

Groth, I., Tan, G. Y. A., Gonzalez, J. M., Laiz, L., Carlsohn, M. R., Schütze, B., Wink J, Goodfellow, M., Gruer, L. D., Barlett, R. & Ayliffe, G. A. J. (2007). *Amycolatopsis nigrescens* sp. nov., an actinomycete isolated from a Roman catacomb. *Int. J. Syst. Evol. Microbiol.*, *57*, 513-519.

Gruer, L. D., Barlett, R. & Ayliffe, G. A. J. (1984). Species identification and antibiotic sensitivity of coagulse-negative staphylococci from CAPD peritonitis. *Antimicrob. ChemotherJ. Antimicrob. Chemother.*, *13*, 577-584.

Grundy, W. E., Sinclair, A. C., Theriault, R. J., Goldstein, A. W., Rickher, C. J., Warren, H. B. Jr, Oliver, T. J. & Sylvester, J. C. (1957). Ristocetin, microbiologic properties. *Antibiot. Annu., 1956-1957*, 687-792.

Guan, T. W., Teng, Y., Yang, L. L., Zhang, X. P. & Che, Z. M. (2013). *Isoptericola salitolerans* sp. nov., a halotolerant filamentous actinobacterium isolated from a salt lake, China. *Extremophiles.*, *17*, 471-476.

Hashizume, H., Iijima, K., Yamashita, K., Kimura, T., Wada, S. I., Sawa, R. & Igarashi, M. (2018). Valgamicin C, a novel cyclic depsipeptide containing the unusual amino acid cleonine, and related valgamicins A,

T and V produced by *Amycolatopsis* sp. ML1-hF4. *J. Antibiot.*, *71*, 129-134.

Hazeu, W., Bruyn, J. C. d. & Dijken, J. P. v. (1983). *Nocardia* sp. 239, a facultative methanol utilizer with the ribulose monophosphate pathway of formaldehyde fixation. *Arch. Microbiol.*, *135*, 205-210.

Hopmann, C., Kurz, M., Bronstrup, M., Wink, J. & LeBeller, D. (2002). Isolation and structure elucidation of vancoresmycin - a new antibiotic from *Amycolatopsis* sp. ST 101170. *Tetrahedron Lett.*, *43*, 435-438.

Huang, Y., Pas´ciak, M., Liu, Z., Xie, Q. & Gamian, A. (2004). *Amycolatopsis palatopharyngis* sp. nov., a potentially pathogenic actinomycete isolated from a human clinical source. *Int. J. Syst. Evol. Microbiol.*, *54*, 359-363.

Huang, Y., Qi, W., Lu, Z., Liu, Z. & Goodfellow, M. (2001). *Amycolatopsis rubida* sp. nov., a new *Amycolatopsis* species from soil. *Int. J. Syst. Evol. Microbiol.*, *51*, 1093-1097.

Igarashi, M., Sawa, R., Kinoshita, N., Hashizume, H., Nakagawa, N., Homma, Y., Nishimura, Y. & Akamatsu, Y. (2008). Pargamicin A, a novel cyclic peptide antibiotics from *Amycolatopsis* sp. *J. Antibiot.*, *61*, 387-393.

Izuta, S., Kosaka, S., Kawai, M., Miyano, R., Matsuo, H., Matsumoto, A., Nonaka, K., Takahashi, Y., Ōmura, S. & Nakashima, T. (2018). Dipyrimicin A and B, microbial compounds isolated from *Amycolatopsis* sp. K16-0194. *J. Antibiot.*, *71*, 535-537.

Jadeja, L., Fainstein, J., Le Blanc, B. & Bodey, G. P. (1983). Comparative *in vitro* activities of teichomycin and other antibiotics against JK diphtheroids. *Antimicrob. Agents Chemother.*, *24*, 145-146.

Jamjan, W., Suriyachadkun, C., Tanasupawat, S., Sakai, K., Tashiro, Y., Okugawa, Y. & Tongpim, S. (2018). *Amycolatopsis silviterrae* sp. nov., isolated from forest soil. *Int. J. Syst. Evol. Microbiol.*, *68*, 1455-1460.

Jarerat, A., Pranamuda, H. & Tokiwa, Y. (2002). Poly(L-lactide)-degrading activity in various actinomycetes. *Macromol. Biosci.*, *2*, 420-428.

Jung, H. M., Kim, S. Y., Moon, H. J., Oh, D. K. & Lee, J. K. (2007). Optimization of culture conditions and scale-up to pilot and plant scales

for vancomycin production by *Amycolatopsis orientalis*. *Appl. Microbiol. Biotechnol.*, *77*, 789-795.

Khalil, Z. G., Salim, A. A., Vuong, D., Crombie, A., Lacey, E., Blumenthal, A. & Capon, R. J. (2017). Amycolatopsins A-C: antimycobacterial glycosylated polyketide macrolides from the Australian soil *Amycolatopsis* sp. MST-108494. *J. Antibiot.*, *70*, 1097-1103.

Kim, B., Sahin, N., Tan, G. Y. A., Zakrzewska-Czerwinska, J. & Goodfellow, M. (2002). *Amycolatopsis eurytherma* sp. nov., a thermophilic actinomycete isolated from soil. *Int. J. Syst. Evol. Microbiol.*, *52*, 889-894.

Kim, C. G., Yu, T. W., Fryhle, C. B., Handa, S. & Floss, H. G. (1998). 3-Amino-5-hydroxybenzoic acid synthase, the terminal enzyme in the formation of the precursor of mC7N units in rifamycin and related antibiotics. *J. Biol. Chem.*, *273*, 6030-6040.

Kim, S. B. & Goodfellow, M. (1999). Reclassification of *Amycolatopsis rugosa* Lechevalier et al. 1986 as P*rauserella rugosa* gen. nov. comb. nov. *Int. J. Syst. Bacteriol.*, *49*, 507-512.

Kishi, T., Yamana, H., Muroi, M., Harada, S. & Asai, M. (1972). Tolypomycin, a new antibiotic. III. Isolation and characterization of tolypomycin Y. *J. Antibiot.*, *25*, 11-15.

Klykleung, N., Tanasupawat, S., Pittayakhajonwut, P., Ohkuma, M. & Kudo, T. (2015). *Amycolatopsis stemonae* sp. nov., isolated from a Thai medicinal plant. *Int. J. Syst. Evol. Microbiol. 65*, 3894-3899.

Kothe, H. W., Vobis, G., Kroppenstedt, R. M. & Henssen, A. (1989). A taxonomic study of mycolateless, wall chemotype IV actinomycetes. *Syst. Appl. Microbiol.*, *12*, 61-69.

Kshirsagar, S. D., Saratale, G. D., Saratale, R. G., Govindwar, S. P. & Oh, M. K. (2016). An isolated *Amycolatopsis* sp. GDS for cellulase and xylanase production using agricultural waste biomass. *J. Appl. Microbiol.*, *120*, 112-125.

Kunimoto, S., Lu, J., Esumi, H., Yamazaki, Y., Kinoshita, N., Honma, Y., Hamada, M., Ohsono, M., Ishizuka, M. & Takeuchi, T. (2003). Kigamicins, novel antitumor antibiotics. I. Taxonomy, isolation,

physico-chemical properties and biological activities. *J. Antibiot.*, *56*, 1004-1011.

Kunstmann, M. P., Mitscher, L. A., Porter, J. N., Shay, A. J. & Darken, M. A. (1968). ll-AV290, a new antibiotic. Fermentation, isolation and characterization. *Antimicrob. Agents Chemother.*, *1968*, 242-245.

Labeda, D. P. & Kroppenstedt, R. M. (2000). Phylogenetic analysis of *Saccharothrix* and related taxa: proposal for Actinosynnemataceae fam. nov. *Int. J. Syst. Evol. Microbiol.*, *50*, 331-336.

Labeda, D. P., Donahue, J. M., Williams, N. M., Sells, S. F. & Henton, M. M. (2003). *Amycolatopsis kentuckyensis* sp. nov., *Amycolatopsis lexingtonensis* sp. nov. and *Amycolatopsis pretoriensis* sp. nov., isolated from equine placentas. *Int. J. Syst. Evol. Microbiol.*, *53*, 1601-1605.

Lacey, J. (1971). The microbiology of moist barky storage in unsealed silos. *Ann. Appl. Biol.*, *69*, 187-212.

Lancini, G. & Sartori, G. (1976). Rifamycin G, a further product of *Nocardia mediterranei* metabolism. *J. Antibiot.*, *29*, 466-468.

Lechevalier, M. P. & Lechevalier, H. A. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int. J. Syst. Bacteriol.*, *20*, 435-443.

Lechevalier, M. P., De-Bievre, C. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem. Syst. Ecol.*, *5*, 249-260.

Lechevalier, M. P., Prauser, H., Labeda, D. P. & Ruan, J.-S. (1986). Two new genera of nocardioform actinomycetes: *Amycolata* gen. nov. and *Amycolatopsis* gen. nov. *Int. J. Syst. Bacteriol.*, *36*, 29-37.

Lechevalier, M. P., Stern, A. E. & Lechevalier, H. A. (1981). In *Actinomycetes*. Stuttgart, Germany: Gustav Fischer Verlag.

Lee, S. D. & Hah, Y. C. (2001). *Amycolatopsis albidoflavus* sp. nov. *Int. J. Syst. Evol. Microbiol.*, *51*, 645-650.

Lee, S. D. (2006). *Amycolatopsis jejuensis* sp. nov. and *Amycolatopsis halotolerans* sp. nov., novel actinomycetes isolated from a natural cave. *Int. J. Syst. Evol. Microbiol.*, *56*, 549-553.

Lee, S. D. (2009). *Amycolatopsis ultiminotia* sp. nov., isolated from rhizosphere soil, and emended description of the genus *Amycolatopsis*. *Int. J. Syst. Bacteriol., 59*, 1401-1404.

Levine, D. P. (2006). Vancomycin: a history. *Clin. Infect. Dis., 42*, 5-12.

Li, F., Wang, S., Liu, W. & Chen, G. (2008). Purification and characterization of b poly(L-lactic acid)-degrading enzymes from *Amycolatopsis orientalis* ssp. orientalis. *FEMS Microbiol. Lett., 282*, 52-58.

Majumdar, S., Prabhagaran, S. R., Shivaji, S. & Lal, R. (2006). Reclassification of *Amycolatopsis orientalis* DSM 43387 as *Amycolatopsis benzoatilytica* sp. nov. *Int. J. Syst. Evol. Microbiol., 56*, 199-204.

Margalith, P. & Beretta, G. (1960). Rifomycin. XI. Taxonomic study on *Streptomyces mediterranei* nov. sp. *Mycopathol. Mycol. Appl., 13*, 321-330.

Mertz, F. P. & Yao, R. C. (1993). *Amycolatopsis alba* sp. nov., isolated from soil. *Int. J. Syst. Bacteriol., 43*, 715-720.

Miao, Q., Qin, S., Bian, G. K., Yuan, B., Xing, K., Zhang, Y. J., Li, Q., Tang, S. K., Li, W. J. & Jiang, J. H. (2011). *Amycolatopsis endophytica* sp. nov., a novel endophytic actinomycete isolated from oil-seed plant Jatropha curcas L. *Antonie Van Leeuwenhoek, 100*, 333-339.

Moellering Jr, R. C. (2006). Vancomycin: a 50-year reassessment. *Clin. Infect. Dis., 42*, 3-4.

Morinaga, Y. & Hirose, Y. (1984). Production of metabolites by methylotrophs. In *Methylotrophs: Microbiology, Biochemistry, and Genetics (edited by Hou)* (pp. 107-118). Boca Raton: CRC Press.

Murakami, R., Fujita, Y., Kizuka, M., Kagawa, T., Muramatsu, Y., Miyakoshi, S., Takatsu, T. & Inukai, M. (2007). A-102395, a new inhibitor of bacterial translocase I, produced by *Amycolatopsis* sp. SANK 60206. *J. Antibiot., 60*, 690-695.

Nadkarni, S. R., Patel, M. V., Chatterjee, S., Vijayakumar, E. K., Desikan, K. R., Blumbach, J., Ganguli, B. N. & Limbert, M. (1994). Balhimycin, a new glycopeptide antibiotic produced by *Amycolatopsis* sp. Y-86,

21022. Taxonomy, production, isolation and biological activity. *J. Antibiot., 47*, 334-341.

Nagl, M., Neher, C., Hager, J., Pfausler, B., Schmutzhard, E. & Allerberger, F. (1999). Bactericidal Activity of Vancomycin in Cerebrospinal Fluid. *Antimicrob. Agents. Chemother., 43*, 1932-1934.

Nakamura, K., T., Tomita, N. A. & Kamio, Y. (2001). Purification and characterization of an extracellular poly(l-lactic acid) depolymerase from a soil isolate, *Amycolatopsis* sp. strain K104-1. *Appl. Environ. Microbiol., 67*, 345-353.

Nie, G. X., Ming, H., Li, S., Zhou, E. M., Cheng, J., Tang, X., Feng, H. G., Tang, S. K. & Li, W. J. (2012). *Amycolatopsis dongchuanensis* sp. nov., an actinobacterium isolated from soil. *Int. J. Syst. Evol. Microbiol., 62*, 2650-2656.

Nielsen, J. B. & Arison, B. H. (1989). 3-Methylpseudouridine as a fermentation product. *J. Antibiot., 42*, 1248-1252.

Nishikori, T., Okuyama, A., Naganawa, H., Takita, T., Hamada, M., Takeuchi, T., Aoyagi, T. & Umezawa, H. (1984). Production by actinomycetes of (S,S)-N,N'-ethylenediaminedisuccinic acid, an inhibitor of phospholipase C. *J. Antibiot., 37*, 426-427.

Okajima, S., Ando, A., Shinoyama, H. & Fujii, T. (1994). Purification and characterization of an extracellular chitosanase produced by *Amycolatopsis* sp. CsO-2. *J. Ferment. Bioeng., 77*, 617-620.

Omura, S., Tanaka, H., Tanaka, Y., Spiri-Nakagawa, P., Oiwa, R., Takahashi, Y., Matsuyama, K. & Iwai, Y. (1979). Studies on bacterial cell wall inhibitors. VII. Azureomycins A and B, new antibiotics produced by *Pseudonocardia azurea* nov. sp. Taxonomy of the producing organism, isolation, characterization and biological properties. *J. Antibiot., 32*, 985-994.

Oyuntsetseg, B., Cho, S. H., Jeon, S. J., Lee, H. B., Shin, K. S., Kim, I. S. & Kim, S. B. (2017). *Amycolatopsis acidiphila* sp. nov., a moderately acidophilic species isolated from coal mine soil. *Int. J. Syst. Evol. Microbiol., 67*, 3387-3392.

Pacey, M. S., Jefson, M. R., Huang, L. H., Cullen, W. P., Maeda, H., Tone, J., Nishiyama, S., Kaneda, K. & Ishiguro, M. (1989). UK-69,753, a

novel member of the efrotomycin family of antibiotics. I. Taxonomy of the producing organism, fermentation and isolation. *J. Antibiot.*, *42*, 1453-1459.

Penkhrue, W., Sujarit, K., Kudo, T., Ohkuma, M., Masaki, K., Aizawa, T., Pathom-Aree, W., Khanongnuch, C. & Lumyong, S. (2018). *Amycolatopsis oliviviridis* sp. nov., a novel polylactic acid-bioplastic-degrading actinomycete isolated from paddy soil. *Int. J. Syst. Evol. Microbiol.*, *68*, 1448-1454.

Pittenger, R. C. & Brigham, R. B. (1956). *Streptomyces orientalis*, n. sp., the source of vancomycin. *Antibiot. Chemother.*, *6*, 642-647.

Pranamuda, H. & Tokiwa, Y. (1999). Degradation of poly(L-lactide) by strains belonging to genus *Amycolatopsis*. In *Biotechnol. Lett.* (Vol. *21*, pp. 901-905).

Pranamuda, H., Tokiwa, Y. & Tanaka, H. (1997). Polylactide degradation by an *Amycolatopsis* sp. *Appl. Environ. Microbiol.*, *63*, 1637-1640.

Proctor, R., Craig, W. & Kunin, C. (1978). Cetocycline, tetracycline analog: *in vitro* studies of antimicrobial activity, serum binding, lipid solubility, and uptake by bacteria. *Antimicrob. Agents. Chemother.*, *13*, 598-604.

Rabenhorst, J. & Hopp, R. (1997). Process for the preparation of vanillin and suitable microorganisms. *European Patent Office EP*, *0761817* A2.

Rasmussen, R. R., Nuss, M. E., Scherr, M. H., Mueller, S. I., McAlpine, J. B. & Mitscher, L. A. (1986). Benzanthrin A and B, a new class of quinone antibiotics. Isolation, structure elucidation and potential antitumor activity. *J. Antibiot.*, *39*, 1516-1526.

Saintpierre-Bonaccio, D., Amir, H., Pineau, R., Tan, G. Y. & Goodfellow, M. (2005). *Amycolatopsis plumensis* sp. nov., a novel bioactive actinomycete isolated from a New-Caledonian brown hypermagnesian ultramafic soil. *Int. J. Syst. Evol. Microbiol.*, *55*, 2057-2061.

Sawa, R., Takahashi, Y., Hashizume, H., Sasaki, K., Ishizaki, Y., Umekita, M., Hatano, M., Abe, H., Watanabe, T., Kinoshita, N., Homma, Y., Hayashi, C., Inoue, K., Ohba, S., Masuda, T., Arakawa, M., Kobayashi, Y., Hamada, M., Igarashi, M., Adachi, H., Nishimura, Y. & Akamatsu, Y. (2012). Amycolamicin: a novel broad-spectrum antibiotic inhibiting bacterial topoisomerase. *Chemistry*, *18*, 15772-15781.

Sensi, P., Greco, A. M. & Ballotta, R. (1959). Rifomycin. I. Isolation and properties of rifomycin B and rifomycin complex. *Antibiot. Annu, 7*, 262-270.

Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol.*, *16*, 313-340.

Shorin, V. A., Yudinstev, S. D., Kunrat, I. A., Goldberg, L., Pevzner, N. S., Brashnikova, M. G., Lomakina, N. N. & Oparysheva, E. F. (1957). New antibiotics actinoidin. *Antibiotiki.*, *2*, 44-49.

Siebers, B., Klenk, H. P. & Hensel, R. (1998). PPi-dependent phosphofructokinase from *Thermoproteus tenax*, an archaeal descendant of an ancient line in phosphofructokinase evolution. *J Bacteriol.*, *180*, 2137-2143.

Sorrell, T. C., Packham, D. R., Shanker, S., Foldes, M. & Munro, R. (1982). Vancomycin therapy for methicillin-resistant *Staphylococcus aureus*. *Ann. Intern. Med.*, *97*, 334-350.

Souza, W. R., Silva, R. E., Goodfellow, M., Busarakam, K., Figueiro, F. S., Fereira, D., Rodrigues-Filho, E., Moraes, L. A. & Zucchi, T. D. (2015). *Amycolatopsis rhabdoformis* sp. nov., an actinomycete isolated from a tropical forest soil. *Int. J. Syst. Evol. Microbiol.*, *65*, 1786-1793.

Sripreechasak, P., Tanasupawat, S., Matsumoto, A., Inahashi, Y., Suwanborirux, K & Takahashi, Y. (2013). Identification and antimicrobial activity of actinobacteria from soils in southern Thailand. *Trop. Biomed*., *30*. 46-55.

Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997). Proposal for a new hierarchic classification system, Actinobacteria classis nov. *Int. J. Syst. Bacteriol.*, *47*, 479-491.

Stapley, E. O., Jackson, M., Hernandez, S., Zimmerman, S. B., Currie, S. A., Mochales, S., Mata, J. M., Woodruff, H. B. & Hendlin, D. (1972). Cephamycins, a new family of beta-lactam antibiotics. *Antimicrob. Agents Chemother.*, *2*, 122-131.

Sugie, M., Suzuki, H. & Tomiyaka, N. (1988). Purification and some properties of d-amino acid specific peptidase from *Nocardia orientalis*. *Rep. Ferment. Inst.*, *69*, 1-14.

Takahashi, Y. (2001). Family Pseudonocardiaceae. In *Identification Manual of Actinomycetes* (pp. 227-239). Tokyo.

Tan, G. Y. A., Ward, A. C. & Goodfellow, M. (2006a). Exploration of *Amycolatopsis* diversity in soil using genus-specific primers and novel selective media. *Syst. Appl. Microbiol.*, *29*, 557-569.

Tan, G., Robinson, S., Lacey, E. & Goodfellow, M. (2006b). *Amycolatopsis australiensis* sp. nov., an actinomycete isolated from arid soils. *Int. J. Syst. Evol. Microbiol.*, *5*, 2297-2301.

Tang, S. K., Wang, Y., Guan, T. W., Lee, J. C., Kim, C. J. & Li, W. J. (2010). *Amycolatopsis halophila* sp. nov., a halophilic actinomycete isolated from a salt lake. *Int. J. Syst. Evol. Microbiol.*, *60*, 1073-1078.

Thawai, C. (2018). *Amycolatopsis rhizosphaerae* sp. nov., isolated from rice rhizosphere soil. *Int. J. Syst. Evol. Microbiol.*, *68*, 1546-1551.

Tokiwa, Y. & Calabia, B. P. (2006). Biodegradability and biodegradation of poly(lactide). *Appl. Microbiol. Biotechnol.*, *72*, 244-251.

Tominaga, Y. & Tsujisaka, Y. (1976). Purification and properties of two chitinases from *Streptomyces orientalis* which lyse *Rhizopus* cell wall. *Agric. Biol. Chem.*, *40*, 2325-2333.

Traxler, P., Schupp, T., Fuhrer, H. & Richter, W. J. (1981). 3-Hydroxyrifamycin S and further novel ansamycins from a recombinant strain R-21 of *Nocardia mediterranei*. *J. Antibiot.*, *34*, 971-979.

Tseng, M., Yang, S. F., Li, W. J. & Jiang, C. L. (2006). *Amycolatopsis taiwanensis* sp. nov., from soil. *Int. J. Syst. Evol. Microbiol.*, *56*, 1811-1815.

Tsuji, K., Kobayashi, M., Kamigauchi, T., Yoshimura, Y. & Terui, T. (1988). New glycopeptides antibiotics. The structure of orienticins. *J. Antibiot.*, *41*, 819-822.

Tsunakawa, M., Tenmyo, O., Tomita, K., Naruse, N., Kotake, C., Miyaki, T., Konishi, M. & Oki, T. (1992). Quartromicin, a complex of novel antiviral antibiotics. I. Production, isolation, physico-chemical properties and antiviral activity. *J. Antibiot.*, *45*, 180-188.

Ueno, M., Iijima, M., Masuda, T., Kinoshita, N., Iinuma, H., Naganawa, H., Hamada, M., Ishizuka, M. & Takeuchi, T. (1992). Dethymicin, a novel

immunosuppressant isolated from an *Amycolatopsis*. *J. Antibiot.*, *45*, 1819-1826.

Usui, T., Hayashi, Y., Nanjo, F., Sakai, K. & Ishido, Y. (1987). Transglycosylation reaction of a chitinase purified from *Nocardia orientalis*. *Biochim. Biophys. Acta.*, *923*, 302-309.

Wang, J., Leiva, S., Huang, J. & Huang, Y. (2018). *Amycolatopsis antarctica* sp. nov., isolated from the surface of an Antarctic brown macroalga. *Int. J. Syst. Evol. Microbiol.*, *68*, 2348-2356.

Wang, N. J., Fu, Y., Yan, G. H., Bao, G. H., Xu, C. F. & He, C. H. (1988). Isolation and structure of a new ansamycin antibiotic kanglemycin A from a *Nocardia* sp. *J. Antibiot.*, *41*, 264-267.

Warwick, S., Bowen, T., McVeigh, H. P. & Embley, T. M. (1994). A phylogenetic analysis of the family Pseudonocardiaceae and the genera *Actinokineospora* and *Saccharothrix* with 16S rRNA sequences and a proposal to combine the genera *Amycolata* and *Pseudonocardia* in an emended genus *Pseudonocardia*. *Int. J. Syst. Bacteriol.*, *44*, 293-299.

Watanakunakorn, C. (1984). Mode of action and *in-vitro* activity of vancomycin. *Antimicrob. Agents Chemother.*, *14*, 7-18

Wax, R., Maises, W., Weston, R. & Birnbaum, J. (1976). Efrotomycin, a new antibiotic from *Streptomyces lactamdurans*. *J. Antibiot.*, *29*, 670-673.

Weber, D. J., Saviteer, S. M., Rutala, W. A. & Thoman, C. A. (1988). *In vitro* susceptibility of Bacillus spp. to selected antimicrobial agents. *Antimicrob. Agents Chemother.*, *32*, 642-645.

Xing, K., Liu, W., Zhang, Y. J., Bian, G. K., Zhang, W. D., Tamura, T., Lee, J. S, Qin, S. & Jiang, J. H. (2013). *Amycolatopsis jiangsuensis* sp. nov., a novel endophytic actinomycete isolated from a coastal plant in Jiangsu, China. *Antonie Van Leeuwenhoek*, *103*, 433-439.

Yassin, A. F., Schaal, K. P., Brzezinka, H., Goodfellow, M. & Pulverer, G. (1991). Menaquinone patterns of *Amycolatopsis* species. *Zentralbl. Bakteriol.*, *274*, 465-470.

Yu, T. W., Muller, R., Muller, M., Zhang, X., Draeger, G., Kim, C. G., Leistner, E. & Floss, H. G. (2001). Mutational analysis and reconstituted expression of the biosynthetic genes involved in the formation of 3-

amino-5-hydroxybenzoic acid, the starter unit of rifamycin biosynthesis in *Amycolatopsis mediterranei* S699. *J. Biol. Chem., 276,* 12546-12555.

Zhang, G., Wang, L., Li, J. & Zhou, Y. (2016). *Amycolatopsis albispora* sp. nov., isolated from deep-sea sediment. *Int. J. Syst. Evol. Microbiol., 66,* 3860-3864.

Zhuge, B., Fang, H. Y., Yu, H., Rao, Z. M., Shen, W., Song, J. & Zhuge, J. (2008). Bioconversion of lovastatin to a novel statin by *Amycolatopsis* sp. *Appl. Microbiol. Biotechnol., 79,* 209-216.

Zucchi, T. D., Bonda, A. N., Frank, S., Kim, B. Y., Kshetrimayum, J. D. & Goodfellow, M. (2012a). *Amycolatopsis bartoniae* sp. nov. and *Amycolatopsis bullii* sp. nov., mesophilic actinomycetes isolated from arid Australian soils *Antonie Van Leeuwenhoek, 102,* 91-98.

Zucchi, T. D., Tan, G. Y. A. & Goodfellow, M. (2012b). *Amycolatopsis thermophila* sp. nov. and *Amycolatopsis viridis* sp. nov., thermophilic actinomycetes isolated from arid soil. *Int. J. Syst. Evol. Microbiol., 62,* 168-172.

Zucchi, T. D., Tan, G. Y. A., Bonda, A. N. V., Frank, S., Kshetrimayum J. D. & Goodfellow, M. (2012c). *Amycolatopsis granulosa* sp. nov., *Amycolatopsis ruanii* sp. nov. and *Amycolatopsis thermalba* sp. nov., thermophilic actinomycetes isolated from arid soils. *Int. J. Syst. Evol. Microbiol., 62,* 1245-1251.

In: A Closer Look at Actinomycetes ISBN: 978-1-53617-046-7
Editors: A. A. Mohamed Hatha et al.

Chapter 4

MARINE ACTINOMYCETES AS RICH SOURCE OF NOVEL THERAPEUTICS FOR CANCER THERAPY

K. G. K. Deepak and Rama Rao Malla*
Department of Biochemistry and Bioinformatics, GIS,
GITAM (Deemed to be University), Visakhapatnam,
Andhra Pradesh, India

ABSTRACT

Terrestrial actinomycetes were extensively studied and screened since the 1950s to study its effect on human health and diseases. And several studies reported terrestrial actinomycetes with anti-cancer and anti-infective properties. Initially, actinomycetes in the marine ecosystems were largely neglected assuming little incentives in isolating marine strains in discovering new drugs. However, in the last two decades, the focus was shifted towards marine actinomycetes owing to their structural diversity as well as high medicinal value. Studies have paid high dividends to researchers exploring marine ecosystems for the presence of novel strains

* Corresponding Author's Email: dr.rrmalla@gmail.com

of actinomycetes. These marine actinomycetes harbored many active principles that are effective against various diseases. Marine actinomycetes are often referred to as goldmine due to the presence of diverse secondary metabolites. Marine actinomycetes research can lead to the discovery of many novel drug candidates that are effective against various deadly diseases like cancer, malaria, and several drug-resistant infections.

Keywords: actinomycetes, cancer, angiogenesis, metastasis, cytotoxicity

INTRODUCTION

Oceans are considered as treasures of biodiversity due to the presence of different marine ecosystems. According to Donia and Hamann (2003), the greatest biodiversity is seen in the oceans when compared with the terrestrial environment [1]. It is estimated that >70% of the earth surface is covered with water and oceans act as a reservoir for various bioactive natural products [2, 3]. The various marine ecosystems include coral reefs, deep seafloor habitats, high-pressure habitats, highly acidic environments, high-pressure & high-temperature regions, and habitats with below sub-zero temperatures. It is estimated that the marine ecosystems in deep-sea floors and coral reefs, the biodiversity is much higher when compared to the tropical rain forests [4]. And the existence of biodiversity is the driving force for the discovery of various natural products from marine organisms with high medicinal value [5, 6]. Mostly, these natural products are novel compounds (secondary metabolites) with high pharmaceutical importance [7, 8]. The compounds are released as a defensive response to the predators and competitors [9]. And one such marine organism with a high potential to isolate novel metabolites with high therapeutic value is the marine actinomycetes [10-12]. The existence of actinomycetes dates back to over hundreds of years.

MARINE ACTINOMYCETES AS STORE HOUSES OF NOVEL BIOACTIVE COMPOUNDS

Marine actinobacteria are store houses of potential bioactive compounds and are often referred to as a gold mine due to enormous secondary metabolites. It is estimated that a single strain of marine actinomycetes can harbor 15-25 secondary metabolites [13]. Various isolates of marine actinobacteria possess antibacterial, antifungal, antimalarial, antioxidant, anti-inflammatory, anticancer, cytotoxic, antiviral, anti-parasitic, and anti-angiogenic activities [14, 15]. Of the 23,000 antibiotics reported so far, about 10,000 of them were of actinomycetes origin. Actinomycetes are filamentous Gram-positive bacteria belonging to the phylum *Actinobacteria* with both bacterial and fungal characteristics [16, 17], Actinobacteria have diverse morphological, physiological and metabolic functions [18]. Actinomycetes exhibit branching threads or rods with non-septate hyphae, branching or non-branching sporulating mycelium, and straight or spiral shape. The presence of a rigid cell wall protects marine actinomycetes from high osmotic pressure experienced in the marine environment. The isolates from marine actinomycetes curtailed cancer cells by being selectively cytotoxic to cancer cells, by inhibiting angiogenesis, by inducing apoptosis and by preventing metastasis.

CYTOTOXIC COMPOUNDS ISOLATED FROM MARINE ACTINOMYCETES

Several researchers carried out extensive work to isolate cytotoxic compounds from marine actinomycetes (Figure 1). Abdelfattah et al. (2016) isolated different species of marine actinomycetes from the Red Sea coast of Egypt and evaluated the cytotoxic activity on triple negative breast cancer cell lines (MB-MDA-231) [19].

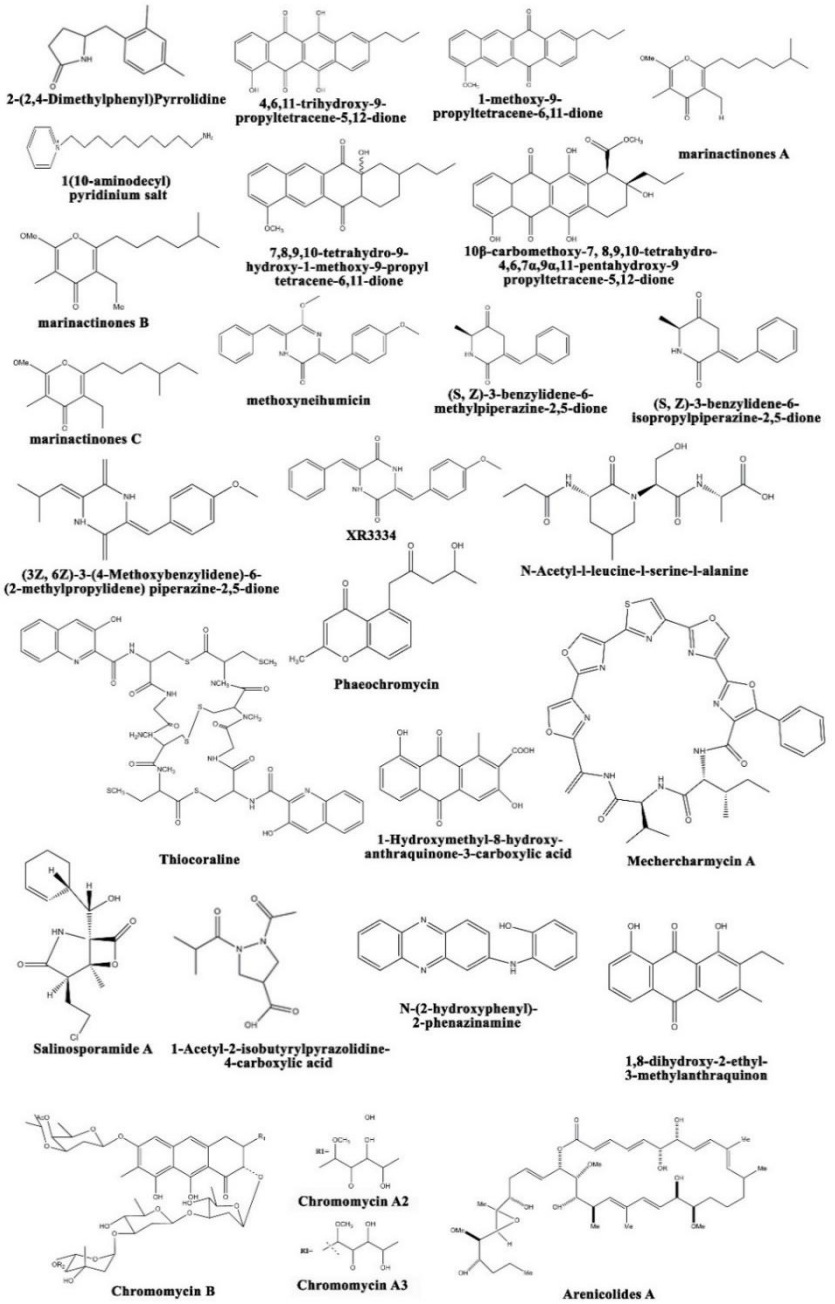


Figure 1. Structures of various cytotoxic compounds isolated from marine actinomycetes.

The 16S rRNA genes of actinomycetes strains with high cytotoxic activity were sequenced and amplified. The cytotoxic effect of different crude extracts (25-300 µg/ml) was studied by cell viability assay using methylene blue. Simultaneously, the crude extracts were also checked for antimicrobial activity using Kirby-Bauer agar disc diffusion method. The results revealed crude extracts of Actinomycetes significantly decreased cell viability in a dose-dependent manner. The microscopic studies of treated cells revealed signs of cell detachment and morphological changes signifying cell death. Condensation of nuclear heterochromatin was also observed in the apoptotic cells treated with crude extracts. Based on the 16S rRNA sequences, the few strains showing cytotoxic activity belonged to genus *Streptomyces* and *Nocardia*. The cytotoxic activity of these crude extracts could be due to the presence of cytotoxic compounds such as anthraquinones.

Zheng et al. (2000) isolated marine actinomycetes associated with marine organisms from the intertidal zone of Taiwan Strait, China to study the antibacterial and antitumor properties. The samples were ground into a paste, centrifuged and the supernatant was grown in Emerson's medium. The resulting Actinomycetes strains were identified and fermented. The culture broth was centrifuged, and the supernatant was collected and filtered. The resulting fractions were tested for cytotoxic and antimicrobial activities. The cytotoxic activity was studied on different cancer cells (KB, HLF and CNE cells) by MTT assay. Biochemical induction assay was also carried out to study the effect on DNA. Results indicated that a total of 439 actinomycetes were isolated mostly belonging to *Streptomyces* and *Micromonospora,* and randomly 360 marine actinomycetes were checked for cytotoxicity. At 1:320 dilutions, 20.6% of marine actinomycetes cultures showed cytotoxicity on P388 cells and 18.6% on KB cells. Based on BIA test, 2.96% of actinomycetes cultures interacted with DNA [20].

Saurav and Kannabiran (2011) isolated 5-(2,4-dimethylbenzyl) pyrrolidine (DMBPO) from marine actinomycetes, *Streptomyces* VITSVK5 spp. The marine sediments were collected from the coast of Marrakanam, Bay of Bengal. The strain was isolated by using starch casein agar, and the isolated strain was cultured in starch casein broth. The fermented broth was

centrifuged and the supernatant was extracted in *n*-butanol. The organic phase was evaporated and lyophilized and the suspension was loaded on silica gel column chromatography was eluted using different solvents to get DMBPO. Based on bioactive guided extraction, DMBPO was isolated and structurally elucidated. The purified compound, DMBPO was checked for the hemolytic activity to study its effect on membrane disruption, MTT assay to study the cytotoxic effect, chromosomal aberrations and antioxidant studies were carried out. The hemolytic studies revealed DMBPO has weak membrane lysis ability. The chromosomal aberration study indicated that DMBPO treated cells (200 µg/ml) showed gaps and chromatid breaks. The MTT assay was carried out on VERO, HepG2, Hep 2 normal cell lines to find out the minimum inhibitory concentration (IC_{50}) of DMBPO. The IC_{50} values were 22.6, 8.3 and 2.8 µg/ml respectively. Based on the IC_{50} values, DMBPO is less toxic to the non-cancerous cells when compared to the cancer cell lines [21].

Dasari et al. (2012) screened few marine strains of Actinomycetes from the coast of Visakhapatnam (Andhra Pradesh) to identify potent cytotoxic compounds. Using a grab sampler, sediment samples were collected and grown on starch casein agar. DVR D4 strain was successfully isolated by culturing the sediment samples on starch-casein agar and was fermented in yeast extract-malt extract. After proper growth, the fermented broth was centrifuged to get cell culture filtrate. The filtrate was extracted with ethyl acetate and was further purified in column chromatography. On purification, a cytotoxic compound 1 (10-aminodecyl) pyridinium salt was isolated and characterized. The cytotoxic activity (MTT assay) of the compound was checked on different cancer cell lines such as MCF-7, U87MG, and HeLa with tamoxifen was used as standard. Different concentrations (25-1000 µg/ml) of the compound were tested and the maximum percentage inhibition at a concentration of 1000 µg/ml showed the highest toxicity [22].

Sousa et al. (2012) isolated four novel anthracyclines viz., 4,6,11-trihydroxy-9-propyltetracene-5,12-dione; 1-methoxy-9-propyltetracene-6,11-dione; 7,8,9,10-tetrahydro-9-hydroxy-1-methoxy-9-propyltetracene-6,11-dione; and 10β-carbomethoxy-7,8,9,10-tetrahydro-4,6,7α,9α,11-pentahydroxy-9propyltetracene-5,12-dione from extract of *Micromonospora* sp.

isolated from Brazilian ascidian *Eudistoma vannamei*. Four anthracyclines were purified from the ethyl acetate fraction of *Micromonospora* sp. using silica gel column chromatography and HPLC. Anthracyclines are highly studied in cancer therapeutics and are comprised of aromatic glycosidic microbial polyketides. The compounds exhibited cytotoxic activity by damaging DNA via topoisomerases II or by the generation of free radicals. The cytotoxic activity of the isolated compounds was checked on HCT-8 human adenocarcinoma cells using MTT assay and doxorubicin was used as a positive control [23].

Wang et al. (2011) isolated marineactinones A, marineactinones B, marineactinones C, the γ-pyrones from marine actinomycetes, *Marinactinospora thermotolerans* SCSIO 00606. The marine actinomycetes strain was collected from the sea sediments of Northern South China. The strain was grown in suitable broth and was filtered. The supernatant and mycelia were concentrated and extracted with ethyl acetate and acetone-water respectively. The acetone-water fraction was concentrated and extracted using ethyl acetate. The ethyl acetate fraction was concentrated under reduced pressure and separated in a silica gel column using different solvents. Few of the fractions obtained were further separated by Sephadex LH-20 column using chloroform-methanol followed by column chromatography with silica gel to give Marineactinone A, B, and C. All the three pyrones were structurally characterized. The compounds were checked for cytotoxic effect using MTT assay on different cell lines (MSF-7, HePG2, NCI-H460, SMCC-7721, SW1990, and HeLa). The cells were treated with different concentrations of purified compounds and were also screened for DNA topoisomerase-II enzyme inhibition activity. MTT assay revealed that these compounds were moderately cytotoxic against SMCC-7721, SW1990, and HepG2 cell lines only. DNA topoisomerase-II enzyme inhibition activity studies indicated that Marineactinone B alone showed dose-dependent weak inhibition [24].

Zhang et al. (2013) isolated *Nocardiopsis alba* SCSIO 03039 from Indian Ocean using a grab sampler at a depth of 3412 m. The wet sample was aseptically air-dried and fermented in a suitable media. After suitable growth, the broth and the mycelia cakes were extracted with butanone and

methanol respectively. The solvents were removed and both the residues were mixed to get a crude extract. The crude was purified using silica gel column with different solvents to give eight different fractions. The first two fractions were combined separated in Sephadex LH-20 chromatography and eluted with suitable solvents to give six different fractions. The fractions were further subjected to different separation processes such as semi-preparative HPLC, silica gel column chromatography to yield nine different compounds. Seven are diketopiperazines viz., methoxyneihumicin; (S, Z)-3-benzylidene-6-methylpiperazine-2,5-dione; (S, Z)-3-benzylidene-6-isopropylpiperazine-2,5-dione; (3Z,6Z)-3-(4-Methoxy-benzylidene)-6-(2-methylpropylidene) piperazine-2,5-dione; XR3334)] among which two are novel compounds [(R, Z)-3-benzylpiperazine-6-(4-methoxybenzylidene)-2,5-dione and (S, Z)-3-benzylpiperazine-6-(4-methoxy-benzylidene)-2,5-dione. The isolated compounds were tested for cytotoxicity against MCF-7, NCI-H460 and SF-268 cells and the isolated compounds showed moderate cytotoxic activity [25].

Perez et al. 1997 isolated a novel antitumor compound thiocoraline (thiodepsipeptide) from marine actinomycetes, *Micromonospora marina* that collected from the coast of Mozambique [26]. Thiocoraline showed anti-proliferative activity against different cells such as breast, human non-small cell lung, renal, colon and melanoma cancer cells. *In vivo* studies carried out by Faircloth et al. (1997) showed the antitumor activity of thiocoraline against human carcinoma xenografts [27]. Erba et al. (1999) carried out several experiments such as bromodeoxyuridine incorporation (BrdU), BrdU/DNA staining, cyclin D1/DNA staining DNA unwinding assay, DNA polymerase assay and few more tests to study the action of thiocoraline. Based on the study, thiocoraline arrests the cancer cells in the G1 phase of cell cycle decreases progression of cells into G_2 or M phase and thus, blocks cell proliferation. The cell cycle in the cancer cells exposed to thiocoraline proposed that the compound interferes with the replication of DNA by inhibiting the activity of DNA polymerase α [28].

Chen et al. (2017) isolated an anticancer compound from sea-anemone (*Haliplanella lineata*) derived actinomycetes *Streptomyces* ZZ406 from Zhoushan City, Zhejiang, China. The sea anemone was homogenized and

the homogenate (10^{-1} gm/l) was further diluted and grown on Gauze's solid medium and incubated for 5 days. The single colonies were further grown as suspension cultures on Gauze's liquid medium and fermented for five days. The culture media was separated into filtrate and mycelium, the filtrate was subjected to HP-20 column and eluted with water and 100% methanol to get methanol fraction. The mycelia were also subjected to extraction with methanol to get methanol extract. Both methanol fraction and methanol extract were mixed and concentrated. The resultant concentrate was partitioned in ethyl acetate and butanol and further purified to give four new and seven already known compounds. The compounds were characterized using NMR, HRESIMS and MS-MS data. The newly isolated compounds were 1-Hydroxymethyl-8-hydroxy-anthraquinone-3-carboxylic acid; Phaeochromycin I; N-Acetyl-l-leucine-l-serine-l-alanine; 1-Acetyl-2-isobutyrylpyrazolidine-4-carboxylic acid. These compounds were checked for anti-proliferative activity using Sulforhodiamine assay on U251, SHG44, U87MG, and normal human astrocytes. 1-Hydroxymethyl-8-hydroxy-anthraquinone-3-carboxylic acid showed anti-proliferative activity, whereas, the other compounds showed either no activity or least activity.

Mincer et al. (2002) and Buchanan et al. (2005) isolated and characterized obligate marine actinomycete bacteria belonging to *Salinospora* group from the tropical and subtropical sediments of the Atlantic Ocean, Red Sea, and the Sea of Cortez [29, 30]. *Salinospora tropica* strain isolated from mangroves of Bahamas produced a highly bioactive metabolite salinosporamide A (5) and was characterized by Feling et al. (2003). Salinosporamide A has an unusual bicyclic β-lactone γ-lactone with potent cancer cell cytotoxicity and it inhibited the proteolytic activity of 20S subunit of the proteasome. Salinosporamide A interferes with the activity of transcription factor NF-*k*B that is active in malignant cells. Salinosporamide A was checked for its cytotoxic activity against HCT-116 cell lines and was found to be an effective cytotoxin and the activity could be due to the presence of β-lactone moiety. The compound is also known to show *in vitro* inhibitory action on *Plasmodium falciparum* [31].

Gao et al. (2012) isolated a marine actinomycete strain *Nocardia dassonvillei* (BM-17) from sediment sample of Arctic Ocean by culturing it

on Gause's synthetic agar medium. The purified strain was fermented in a suitable medium containing glucose, yeast extract, tyrosine, seawater, etc. for the production of secondary metabolites. Six known compounds and one new compound N-(2-hydroxyphenyl)-2-phenazinamine (NHP) were extracted from the fermented broth. The compounds were structurally elucidated and NHP was checked for its antifungal and cytotoxic activity. Different cancerous cell lines HepG2, A549, COC1, HCT116 were used for cytotoxic studies and highest cytotoxicity was seen against HCT116 cell lines with IC_{50} of 27.82 µg/ml [32].

Kanoh et al. (2005) isolated a new cytotoxic compound, mechercharmycin from marine Actinomycetes, *Thermoactinomyces* sp. YM3-251 from the North Pacific Ocean. YM3-251 was cultured in B2 medium for seven days and the fermented broth was centrifuged and the precipitated with methanol and chloroform mixture. After the further purification process, cytotoxic compound Mechercharmycin A was isolated and the structure was elucidated. The cytotoxic activity was tested on A549 and Jurkat cells and the cyclic structure of the compound contributed to high cytotoxic activity [33].

Williams et al. (2007) isolated actinomycete strain, *Salinispora arenicola* (CNR-005), from the marine sediment sample of coastal waters of Guam. The strain was fermented in Fernbach flasks containing A1DFe media to isolate three novel polyketides - Arenicolides A-C. Arenicolides A was tested for cytotoxic activity on human colon adenocarcinona cell line (HCT-116) and showed moderate cytotoxic activity [34]. Lu et al. (2012) isolated a marine Actinomycetes strain, *Streptomyces* sp. WBF16 from the sea sediments of Bijatuan, China. WBF16 was fermented in soy medium containing soluble starch, soya bean powder, potassium nitrate, and sea salt for 7 days. The fermented broth was concentrated by adding to a micro porous resin and was eluted with ethanol and water in combinations. The crude extract was separated using different solvents to give aureolic acids (a new compound, Chromomycin B along with two known compounds, Chromomycin A_2 and A_3). The three compounds were checked for its cytotoxic activity different cell lines (SGC7901, A549, HCT116, COC1, and HUVEC). All the three compounds showed strong cytotoxic activity against

HCT116, SGC7901, COC1, and A549. Whereas, Chromomycin B showed cytotoxic activity against HUVEC cells [35].

Huang et al. (2005) screened a marine plant, *Salicornia herbacea* from Shandong province of China to isolate a *Streptomyces* sp. strain FX-58. Strain FX-58 was initially cultured on seed medium in fermentation media. The strain was further fermented in broth and was centrifuged. The aqueous layer was extracted in ethyl acetate followed by drying in vacuum. The resultant extract was purified in a silica gel column using different solvent systems to isolate a novel anthraquinone, 1,8-dihydroxy-2-ethyl-3-methylanthraquinone and two know compounds octadecanoic acid and cholest-4-en-3-one. The compounds were checked for cytotoxic activity against HL-60 cell lines [36].

APOPTOSIS-INDUCING COMPOUNDS FROM MARINE ACTINOMYCETES

Active compounds isolated from marine actinomycetes are capable of inducing apoptosis when tested on cancer cells (Figure 2). Jeong et al. 2009 have isolated 23 marine actinomycetes and the cell-free supernatant of purified strain SY-103 belonging to *Streptomyces* showed cytotoxic activity against U937, HL-60, K562 and THP-1 cells. The strain was designated as *Streptomyces* sp. SP-103. The purified cytotoxic compound was separated using reverse-phase HPLC. The purified compound when tested on human leukemia cell lines inhibited cell growth and induced apoptosis by activating caspase-3 protein and down regulating Bcl-2, an anti-apoptotic protein [37].

Liu et al. 2007 isolated K252c and arcyriaflavin, the indolocarbazole alkaloids from Actinomycete $Z_2$039-2 found in the coast of Qingdao, China. The strain was fermented in liquid broth and was separated into supernatant and mycelia. The resulting supernatant was extracted thrice with ethyl acetate and was concentrated. The concentrate was subjected to column chromatography to give K252c and arcyriaflavin. These compounds were

tested on K562 cells for Annexin-V staining and they showed significant apoptosis [38].

ANGIOGENESIS INHIBITOR COMPOUNDS FROM MARINE ACTINOMYCETES

Active compounds isolated from marine actinomycetes significantly blocked tube formation in angiogenesis assay (Figure 2). Streptopyrrolidine is an angiogenic inhibitor compound isolated from marine *Streptomyces* sp. KORDI-3973 from the deep-sea sediments. The culture broth was centrifuged and separated into mycelium and supernatant. Streptopyrrolidine was isolated from the broth using reversed-phase HPLC. The purified compound, Streptopyrrolidine was checked for anti-angiogenesis activity by treating HUVEC cells for tube formation. Streptopyrrolidine dramatically inhibited tube formation with a broken tube network in Matrigel without cytotoxicity [39].

Shin et al. 2010 isolated an angiogenesis inhibitor, Cyclo-(L-Pro-L-Met) from marine actinomycetes *Nocardiopsis* sp. 03N67. The strain was isolated from seaweed *Undaria pinnatifida* from the Arctic region. The strain was fermented in broth and was centrifuged and was extracted using different solvents to obtain a purified compound Cyclo-(L-Pro-L-Met) using reversed-phase HPLC. The compound Cyclo-(L-Pro-L-Met) was checked for anti-angiogenesis activity using tube formation assay and invasion assay using HUVEC cells. The compound successfully inhibited angiogenesis with no cytotoxic effect. Cyclo-(L-Pro-L-Met) is a novel cyclic diketopiperazine with anti-angiogenic activity [40]. A small molecule, streptochlorin was isolated from *Streptomyces* sp. from the marine sediments of Korea. Streptochlorin showed potent anti-angiogenic activity in endothelial cells and also inhibited tube formation and invasion [41].

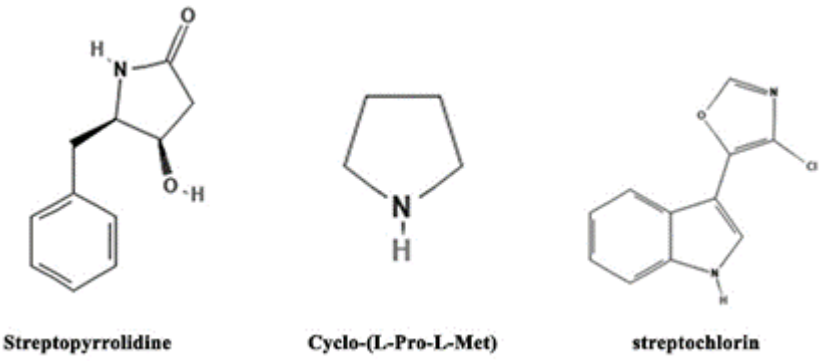


Figure 2. Structures of angiogenesis inhibitor compounds from marine actinomycetes.

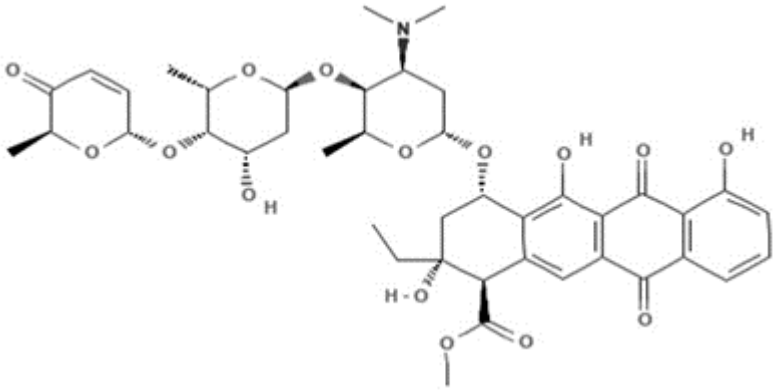


Figure 3. Structures of anti-metastatic inhibitor compounds from marine actinomycetes.

ANTI-METASTATIC COMPOUNDS FROM MARINE ACTINOMYCETES

Sunita et al. 2018 screened 54 *Streptomyces* strains for the presence of novel aromatic polyketides for targeting cancer. The *Streptomyces* strains were cultured in critically optimized medium and the fermented broth was fractionated in a silica gel column. The resulting fractions were separated using preparative TLC to obtain purified compounds and were structurally characterized [42].

CONCLUSION

Oceans harbor various novel chemicals and marine actinomycetes, in particular, are of high value due to diversity. It is understood that the exploration of marine actinomycetes as a source of novel therapeutics is still at infancy. Limited screening abilities and isolation strategies have been a hurdle in exploiting marine actinobacteria. However, enrichment techniques and development of new selective approaches help study marine actinomycetes. Efforts to carefully screen marine actinomycetes will lead to the discovery of novel drugs to target cancer, drug resistance and various diseases that are threatening mankind.

ACKNOWLEDGMENTS

We would like to thank CSIR, New Delhi, India for the project entitled "*Development of novel therapeutics from marine actinomycetes for targeting stem cells in triple negative breast cancer"* - (File No: 37(1683)/17/EMR-II) dated: 05.05.2017).

REFERENCES

[1] M. Donia, M. T. J. T. L. I. D. Hamann, Marine natural products and their potential applications as anti-infective agents, 3 (2003) 338-348.

[2] W. Fenical, P. R. J. N. C. B. Jensen, Developing a new resource for drug discovery: marine actinomycete bacteria, 2 (2006) 666.

[3] M. J. J. O. M. B. Jayaprakashvel, Therapeutically active biomolecules from marine actinomycetes, 1 (2012) 1-7.

[4] B. Haefner, Drugs from the deep: marine natural products as drug candidates, *Drug Discovery Today*, 8 (2003) 536-544.

[5] D. G. Kingston, Modern natural products drug discovery and its relevance to biodiversity conservation, *Journal of Natural Products*, 74 (2011) 496-511.

[6] C. B. Naman, C. A. Leber, W. H. Gerwick, Modern Natural Products Drug Discovery and Its Relevance to Biodiversity Conservation, *Microbial Resources*, Elsevier 2017, pp. 103-120.

[7] J. W. H. Li, J. C. J. S. Vederas, Drug discovery and natural products: end of an era or an endless frontier? 325 (2009) 161-165.

[8] C. Nikapitiya, Bioactive secondary metabolites from marine microbes for drug discovery, Advances in food and nutrition research, Elsevier 2012, pp. 363-387.

[9] P. M. Erwin, S. López-Legentil, P. W. J. E. E. Schuhmann, The pharmaceutical value of marine biodiversity for anti-cancer drug discovery, 70 (2010) 445-451.

[10] H. P. Fiedler, C. Bruntner, A. T. Bull, A. C. Ward, M. Goodfellow, O. Potterat, C. Puder, G. J. A. V. L. Mihm, Marine actinomycetes as a source of novel secondary metabolites, 87 (2005) 37-42.

[11] S. J. W. J. O. M. Dharmaraj, Biotechnology, Marine Streptomyces as a novel source of bioactive substances, 26 (2010) 2123-2139.

[12] O. Genilloud, I. González, O. Salazar, J. Martín, J. R. Tormo, F. J. J. O. I. M. Vicente, biotechnology, Current approaches to exploit actinomycetes as a source of novel natural products, 38 (2011) 375-389.

[13] K. S. Lam, Discovery of novel metabolites from marine actinomycetes, *Current Opinion in Microbiology*, 9 (2006) 245-251.

[14] P. Manivasagan, K. H. Kang, K. Sivakumar, E. C. Li-Chan, H. M. Oh, S. K. Kim, Marine actinobacteria: an important source of bioactive natural products, *Environmental Toxicology and Pharmacology*, 38 (2014) 172-188.

[15] S. Perdicaris, T. Vlachogianni, A. J. N. P. C. R. Valavanidis, Bioactive natural substances from marine sponges: new developments and prospects for future pharmaceuticals, 1 (2013) 2329-6836.

[16] M. Takizawa, R. R. Colwell, R. T. Hill, Isolation and diversity of actinomycetes in the chesapeake bay, *Applied and Environmental Microbiology*, 59 (1993) 997-1002.

[17] S. Das, L. R. Ward, C. Burke, Prospects of using marine actinobacteria as probiotics in aquaculture, *Applied Microbiology and Biotechnology*, 81 (2008) 419-429.

[18] E. A. Barka, P. Vatsa, L. Sanchez, N. Gaveau-Vaillant, C. Jacquard, H. P. Klenk, C. Clément, Y. Ouhdouch, G. P. J. M. M. B. R. van Wezel, *Taxonomy, Physiology, and Natural Products of Actinobacteria*, 80 (2016) 1-43.

[19] M. S. Abdelfattah, M. I. Y. Elmallah, U. W. Hawas, L. T. A. El-Kassema, M. A. G. J. A. P. J. O. T. B. Eid, Isolation and characterization of marine-derived actinomycetes with cytotoxic activity from the Red Sea coast, 6 (2016) 651-657.

[20] Z. Zheng, W. Zeng, Y. Huang, Z. Yang, J. Li, H. Cai, W. J. F. M. L. Su, Detection of antitumor and antimicrobial activities in marine organism associated actinomycetes isolated from the Taiwan Strait, China, 188 (2000) 87-91.

[21] K. Saurav, K. Kannabiran, Cytotoxicity and antioxidant activity of 5-(2,4-dimethylbenzyl)pyrrolidin-2-one extracted from marine Streptomyces VITSVK5 spp, *Saudi Journal of Biological Sciences*, 19 (2012) 81-86.

[22] V. R. Dasari, M. K. Muthyala, M. Y. Nikku, S. R. Donthireddy, Novel Pyridinium compound from marine actinomycete, Amycolatopsis alba var. nov. DVR D4 showing antimicrobial and cytotoxic activities *in vitro*, *Microbiological Research*, 167 (2012) 346-351.

[23] S. Sousa Tda, P. C. Jimenez, E. G. Ferreira, E. R. Silveira, R. Braz-Filho, O. D. Pessoa, L. V. Costa-Lotufo, Anthracyclinones from Micromonospora sp, *Journal of Natural Products*, 75 (2012) 489-493.

[24] F. Wang, X. Tian, C. Huang, Q. Li, S. J. T. J. O. A. Zhang, Marinactinones A-C, new γ-pyrones from marine actinomycete Marinactinospora thermotolerans SCSIO 00606, 64 (2011) 189.

[25] Q. Zhang, S. Li, Y. Chen, X. Tian, H. Zhang, G. Zhang, Y. Zhu, S. Zhang, W. Zhang, C. J. T. J. O. A. Zhang, New diketopiperazine

derivatives from a deep-sea-derived Nocardiopsis alba SCSIO 03039, 66 (2013) 31.

[26] J. Perez Baz, L. M. Canedo, J. L. Fernandez Puentes, M. V. Silva Elipe, Thiocoraline, a novel depsipeptide with antitumor activity produced by a marine Micromonospora. II. Physico-chemical properties and structure determination, *The Journal of Antibiotics*, 50 (1997) 738-741.

[27] G. Faircloth, J. Jimeno, M. J. E. J. O. C. D'Incalci, Biological activity of thiocoraline, a novel marine depsipeptide, 33 (1997) S175-S175.

[28] E. Erba, D. Bergamaschi, S. Ronzoni, M. Faretta, S. Taverna, M. Bonfanti, C. Catapano, G. Faircloth, J. Jimeno, M. J. B. J. O. C. D'incalci, Mode of action of thiocoraline, a natural marine compound with anti-tumour activity, 80 (1999) 971.

[29] T. J. Mincer, P. R. Jensen, C. A. Kauffman, W. Fenical, Widespread and persistent populations of a major new marine actinomycete taxon in ocean sediments, *Applied and Environmental Microbiology*, 68 (2002) 5005-5011.

[30] G. O. Buchanan, P. G. Williams, R. H. Feling, C. A. Kauffman, P. R. Jensen, W. Fenical, Sporolides A and B: structurally unprecedented halogenated macrolides from the marine actinomycete Salinispora tropica, *Organic Letters*, 7 (2005) 2731-2734.

[31] R. H. Feling, G. O. Buchanan, T. J. Mincer, C. A. Kauffman, P. R. Jensen, W. J. A. C. I. E. Fenical, Salinosporamide A: a highly cytotoxic proteasome inhibitor from a novel microbial source, a marine bacterium of the new genus Salinospora, 42 (2003) 355-357.

[32] X. Gao, Y. Lu, Y. Xing, Y. Ma, J. Lu, W. Bao, Y. Wang, T. J. M. R. Xi, A novel anticancer and antifungus phenazine derivative from a marine actinomycete BM-17, 167 (2012) 616-622.

[33] K. Kanoh, Y. Matsuo, K. Adachi, H. Imagawa, M. Nishizawa, Y. J. T. J. O. A. Shizuri, Mechercharmycins A and B, cytotoxic substances from marine-derived Thermoactinomyces sp. YM3-251, 58 (2005) 289.

[34] P. G. Williams, E. D. Miller, R. N. Asolkar, P. R. Jensen, W. J. T. J. O. O. C. Fenical, Arenicolides A-C, 26-Membered Ring Macrolides

from the Marine Actinomycete Salinispora arenicola, 72 (2007) 5025-5034.

[35] J. Lu, Y. Ma, J. Liang, Y. Xing, T. Xi, Y. J. M. R. Lu, Aureolic acids from a marine-derived Streptomyces sp. WBF16, 167 (2012) 590-595.

[36] Y. F. Huang, L. Tian, H. W. Fu, H. M. Hua, Y. H. J. N. P. R. Pei, One new anthraquinone from marine Streptomyces sp. FX-58, 20 (2006) 1207-1210.

[37] S. Y. Jeong, M. H. Han, C. Y. Jin, G. Y. Kim, B. T. Choi, T. J. Nam, S. K. Kim, Y. H. Choi, Apoptosis induction of human leukemia cells by Streptomyces sp. SY-103 metabolites through activation of caspase-3 and inactivation of Akt, *International Journal of Molecular Medicine*, 25 (2010) 31-40.

[38] R. Liu, T. Zhu, D. Li, J. Gu, W. Xia, Y. Fang, H. Liu, W. Zhu, Q. J. A. O. P. R. Gu, Two indolocarbazole alkaloids with apoptosis activity from a marine-derived actinomycete Z 2 039-2, 30 (2007) 270.

[39] H. J. Shin, T. S. Kim, H. S. Lee, J. Y. Park, I. K. Choi, H. J. Kwon, Streptopyrrolidine, an angiogenesis inhibitor from a marine-derived Streptomyces sp. KORDI-3973, *Phytochemistry*, 69 (2008) 2363-2366.

[40] H. J. Shin, M. M. Mondol, T. K. Yu, H. S. Lee, Y. J. Lee, H. J. Jung, J. H. Kim, H. J. J. P. L. Kwon, An angiogenesis inhibitor isolated from a marine-derived actinomycete, *Nocardiopsis* sp. 03N67, 3 (2010) 194-197.

[41] I. K. Choi, H. J. Shin, H. S. Lee, H. J. Kwon, Streptochlorin, a marine natural product, inhibits NF-kappaB activation and suppresses angiogenesis in vitro, *Journal of Microbiology and Biotechnology*, 17 (2007) 1338-1343.

[42] S. Bundale, D. Begde, D. Pillai, K. Gangwani, N. Nashikkar, T. Kadam, A. J. W. J. O. M. Upadhyay, Biotechnology, Novel aromatic polyketides from soil Streptomyces spp.: purification, characterization and bioactivity studies, 34 (2018) 67.

In: A Closer Look at Actinomycetes ISBN: 978-1-53617-046-7
Editors: A. A. Mohamed Hatha et al.

Chapter 5

STREPTOMYCES: DISTRIBUTION, BIOCONTROL AND PLANT GROWTH PROMOTING ACTIVITY

Nisachon Tedsree[1] and Somboon Tanasupawat[2,*]
[1]Department of Agricultural Technology, Faculty of Science and Arts,
Burapha University, Chanthaburi Campus, Chanthaburi, Thailand
[2]Department of Biochemistry and Microbiology,
Faculty of Pharmaceutical Sciences, Chulalongkorn University,
Bangkok, Thailand

ABSTRACT

Actinomycetes are known for their ability to produce several antibiotics. They are widely distributed in soils, compost, freshwater, marine and plants, especially *Streptomyces* strains are abundant in soils. In recent years, *Streptomyces* strains have attracted the interest of researchers as a source of biocontrol agents and use in agriculture. Several new species have been proposed, and their abilities to control plant diseases and promoted plant growth have been uncovered. In agriculture, numerous

* Corresponding Author's Email: Somboon.T@chula.ac.th.

Streptomyces strains have demonstrated the abilities to control the growth of bacterial and fungal phytopathogens, such as *Fusarium* wilt by *Fusarium oxysporum*, anthracnose by *Colletotrichum gloeosporioides*, leaf spot by *Alternaria brassicicola*. Moreover, their ability in term of plant growth promoting based on indole acetic acid (IAA) production of *Streptomyces rochei* and *Streptomyces sundarbansensis* strains have been proved in various economic crops such as in wheat. The active compounds, natamycin produced from *Streptomyces lydicus*, actinomycin D from *Streptomyces mutabilis*, chrestoxanthone A from *Streptomyces chrestomyceticus* strains have been reported. In particular, some pesticides developed for agricultural management belonged to *Streptomyces*, such as actinovate® from *S. lydicus* WYEC108 and mycostop® from *S. griseovirides* K61 were registered as commercial fungicides in Europe, Canada and USA. This chapter describes the potential of *Streptomyces* strains against plant pathogens, their isolation and cultivation methods, taxonomic information, bioactive compounds and their applications in agriculture.

Keywords: *Streptomyces*, biocontrol agents, control plant diseases

INTRODUCTION

Streptomyces strains are filamentous Gram-positive bacteria belonged to Actinobacteria that have their morphology like fungi due to their elongated cells and branch into filaments or hyphae (Ventura et al., 2007). *Actinomyces, Arthrobacter, Bifdobacterium, Cellulomonas, Clavibacter, Corynebacterium, Frankia, Microbacterium, Micrococcus, Mycobacterium, Nocardia, Propionibacterium, Pseudonocardia, Rhodococcus, Sanguibacter* and *Streptomyces* in the phylum Actinobacteria demonstrated the various activity that could be used as biocontrol agents for crop protection under the stress conditions from phytopathogenic fungi (Yadav and Yadav, 2019). Among all the microorganisms, actinobacteria showed the most significant role in plant disease management. Nowadays, *Actinomycetes* have widely attracted because of their abilities to produce numerous biologically active compounds. The most of members of Actinobacteria, especially *Streptomyces* strains are the superior candidates of the source for producing the biocontrol agents including antifungal compounds and

antibiotics (Behal, 2000). These active compounds have been exploited to control infected diseases from bacteria, fungi, pest, and insects (Solanki et al., 2016) as agrochemicals in agricultural management (Berdy, 2005). Bioactive compounds (75% commercial) are produced by *Streptomyces* strains. More than 10000 bioactive compounds from actinomycetes, 7,600 are from *Streptomyces* strains and 2,500 are from rare actinomycetes species.

Streptomyces strains are widely distributed in diverse habitats and conditions such as soils, compost, freshwater, seawater, plants, atmosphere and unfavorable environmental conditions because of their filamentous and sporulating properties of them (Vurukonda et al., 2018). However, most of the Actinobacteria were isolated from the soil environment (Prapagdee et al., 2008; Bi and Yu, 2016; Zhang et al., 2017). Lee and Hwang (2002) reported approximately 1,510 species of actinomycetes from soils. These actinomycetes lived and grew in the soil at different depths, both in cold and tropical areas (Xu et al., 2019). Dhanasekaran, Thajuddin, and Panneerselvam (2012) described *Streptomyces* strains that grew well in alkaline and neutral soils better than acid soils. In addition, they distributed at pH range of 5.1-6.5, 9.1-13.0% moisture, and 9.1-11.0% organic matter while *Micromonospora, Dactylosporangium* and *Streptosporangium* strains were at pH 4.0-5.0, 2.0-9.0% moisture, and 4.0-7.0% organic matter, *Actinomadura* and Nocardioform actinomycetes were at pH 4.0-5.0 and 13.1-20.0% moisture and with 9.1-11.0 and 4.0-7.0% organic matter, respectively (Lee and Hwang, 2002).

Actinomycetes have been interested as a source of biocontrol agents and plant growth-promoting in agricultural management because of low toxicity and ecofriendly. In addition, the identification and the mode of action of biocontrol agents are important for the development of actinomycetes based on degradation in nature while highly specific and less toxic to non-target organisms that lead to developing safer products on the environment and human health (Flores-Gallegos and Nava-Reyna, 2019). Plant diseases are one of the major problems in agricultural management. The use of agrochemicals has been the most common approach to control phytopathogens. However, long-term use of these agents was the negative

effects on both the economy and the environment. The needs for safer agrochemicals with less environmental impact have been continuously addressed. In agriculture, numerous actinobacteria have demonstrated the abilities to control the growth of bacterial and fungal phytopathogens. There are many reports that *Streptomyces* strains exhibited the ability to produce secondary metabolites such as antibiotics and antifungal compounds (Dhanasekaran et al., 2012). For example, all of the significant antifungal activity against *Fusarium oxysporum* causes of *Fusarium* wilt disease in various economic crops was found by *Streptomyces* sp.201 (Bordoloi et al., 2001), *Streptomyces* sp. TP-A0569 (Sasaki et al., 2002), *Streptomyces luozhongensis* (Zhang et al., 2017), *S. griseorubens* E44G (Al-Askar et al., 2015) and *Saccharothrix algeriensis* NRRL B-24137 (Merrouche et al., 2017). In addition, an increase in the world population by 2050, more abilities of biocontrol agents should be exposed to improve the crop yield. (Olanrewaju and Babalola, 2019). *Streptomyces* strains were used as the plant growth promoters by providing the plant hormones such as auxin, cytokinin and gibberellin (Yadav et al., 2018). They have become one of candidates to enhance the growth and yield in economic crops. *Streptomyces* strains provided a source of natural products that may have potential in agricultural management. Their activity has been evaluated against different plant pathogens for suppressing plant diseases and promote plant growth in agricultural management.

METHODS

Isolation and Cultivation of Antifungal *Streptomyces*

Sample Collection

The predominant actinomycetes in soils, especially *Streptomyces* strains exhibited the antifungal activity more than 50% of the isolated actinomycetes (Lee and Hwang, 2002). They were isolated from a high portion of rhizosphere soils in the plant roots (Yadav and Yadav, 2019). Antifungal *Streptomyces* strains were isolated from Saharan soils (10 cm in

depth) using a serial dilution method for investigation the diversity of Actinomycetes in Amenas, Algeria (Toumatia et al., 2015). Thampi and Bhai (2017) collected rhizosphere soil from healthy black pepper plants in different black pepper growing areas of Kerala and Karnataka states of India, 1 cm depth from surrounding of the root system and removed 3 cm of topsoil and kept the samples in polythene bags. Kamara and Gangwar (2015) isolated *Streptomyces* from rhizosphere soil of *Catharanthus roseus* and *Withania somnifera* plants at different locations in India. The soils were taken from the plant roots at 5 cm depth of the soil surface after remove 3 cm of soil surface. 100 actinomycetes isolates were tested the antifungal activity against 7 phytopathogenic fungi and 39 isolates showed antifungal activity. Rhizosphere soils from banana plantations in Nanbao, Meitai and Huangtong of the Hainan Province, China was collected at the depth of 10–20 cm and the broad-spectrum antifungal activity of *Streptomyces* against 11 plant pathogenic fungi was isolated. The strain exhibited antifungal activity against *Colletotrichum musae* that caused of plant disease in banana (Chen et al., 2018). Hussein et al., (2014) isolated *Streptomyces* from different soil samples at 11 locations in Sudan. Soil samples were collected at the depth 15-20 cm and 3 cm from the earth surface was removed and air-dried at room temperature for two days. One of the isolates has antifungal activity on *Drechslera halodes* and *Alternaria alternata* that caused the leaf spot on sorghum and early blight on tomato, respectively. Sharma and Parihar (2010) collected soil samples from the rhizosphere in the agricultural plantation as described by Hussein et al., (2014), and found antifungal compounds against the growth of *Alternaria* sp., *Aspergillus niger, A. flavus*, *Fusarium* sp. and *Rhizopus stolonifer*. Most endophytic actinomycetes from the roots of *Alpinia galangal* were belonged to genera *Streptomyces*, *Nocardia*, *Microbispora* and *Micromonospora* and one strain of *Streptomyces* strain was strongly inhibited *Colletotrichum musae* and *Candida albicans* (Taechowisan et al., 2006).

Selective Isolation Procedure

For the isolation, several methods can be done based on different sources of samples (Sharma et al., 2014). Various selective enrichment media and isolation techniques were reported for the isolation of actinomycetes. However, the members of the genus *Streptomyces* were isolated easier than the rare actinomycetes, which required a complication in the procedures. These techniques are involved in pre-treatment by dry heat, dry heat followed by phenol, treated with calcium carbonate, incorporation of sodium dodecyl sulphate, using a specific medium such as actinomycetes isolation agar, starch casein agar (SCA), ISP media, Humic acid vitamin medium (Patil and Chaudhari, 2011).

Sharma and Parihar (2010) isolated actinomycetes using starch casein agar medium, Actinomyces Hi Veg agar medium, actinomycete isolation agar medium and Streptomyces agar medium. Shi et al., (2018) used Gauze's medium (1 g potassium nitrate, 0.5 g dipotassium hydrogen phosphate trihydrate, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g NaCl, 0.01 g $FeSO_4 \cdot 7H_2O$, 20 g starch, 17 g agar, and 1 L distilled water) for isolation of *Streptomyces* from soils. The isolated *Streptomyces* strain had the ability to control peach brown rot caused by *Monilinia fructicola* (Ni et al., 2019) and anthracnose caused by *Colletotrichum gloeosporioide*s (Xu et al., 2015). Prapagdee, Kuekulvong, and Mongkolsuk (2008) isolated *Streptomyces* from rhizosphere soils using starch-casein-agar (soluble starch 10.0 g/l; casein 0.3 g/l; KNO_3 2.0 g/l; NaCl 2.0 g/l; $MgSO_4.7H_2O$ 0.05 g/l; $CaCO_3$ 0.02 g/l; $FeSO_4.H_2O$ 0.01 g/l; KH_2PO_4 2.0 g/l; and agar 18.0 g/l), supplemented with cycloheximide 50 µg/ml to inhibit the growth of fungal.

Abdallaha et al., (2013) screened actinomycetes from soils to control the post-harvest onion bacterial rot diseases. Starch nitrate agar (SNA) medium was used to isolate, purify and maintain the strains. Two *Streptpmyces* isolates that could inhibit *Erwinia carotovora* and *Burkholderia cepacian* caused by onion disease both in pots and field. Khamna et al., (2009) were isolated by diluting soil samples on Humic acid vitamin (HV) agar (pH 7.0), supplemented with 100 µg/ml nystatin, 100 µg/ml cycloheximide and 50 µg/ml nalidixic acid, incubation at 30ºC for 4 weeks. The strains could inhibit *Alternaria porri* on *Allium ascalonicum*. Nakaew, Rangjaroen, and

Sungthong (2015) also used Humic acid vitamin (HV) agar to isolate *Actinomycetes* from soils. *Streptomyces* sp. DH16 was isolated from the soil sample in Dalhousie (32.53°N, 75.98°E), Himachal Pradesh, India. The soil sample was pretreated with air-dried and heated at 100°C for 1 h and diluted in sterile water. Each soil dilution (0.1 ml) was spread on the starch casein nitrate agar (SCNA) medium, supplemented with cycloheximide (50 mg/ml), nystatin (25 mg/ml), and nalidixic acid (50 mg/ml), and incubated at 28°C for 7 to 21 days (Kaur and Manhas, 2014). *Streptomyces* strains were isolated from an arid soil sample of Boussaada (358100N; 4890E) in Algeria using chitin vitamin-B agar medium at pH 7.4, gentamicin (10mg/mL) and amphotericin B (50mg/ml) (Souagui et al., 2015). A novel actinomycete strain was isolated from Lop Nur desert soil from Luozhong, Xinjiang, northwest China (latitude 39350; longitude 89460) using the standard dilution plate method on Gauze's No. 1 medium at pH 7.2, incubated at 37°C (Zhang et al., 2017). Jacob et al., (2016) isolated *Actinomycetes* from the groundnut rhizospheric soil from the Patancheru fields of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). The samples were treated with 1.5% v/v phenol; dry heat at 100°C for 1 h; air drying for 24 h; water bath at 70°C for 15 min and spread onto actinomycetes isolation medium (HiMedia), Bennett's agar (composition in g/L: peptone, 10.0; CaCl2, 2.0; Tween 80, 4.7 mL; agar 20.0 g; pH 7.2) and starch casein agar (in g/L: starch, 2.0; casein, 0.3; KNO_3, 2.0; K_2HPO_4, 2.0; NaCl, 2.0; $MgSO_4.7H_2O$, 0.05; $CaCO_3$, 0.02; $FeSO_4.7H_2O$, 0.01; agar, 20.0 g; pH 7.2). Faheem et al., (2015) isolated an antagonistic microorganism from the rhizosphere soil of a healthy strawberry plant in strawberry-growing areas in Kylin, Jiangsu Province by suspended soil sample and spread on Gauze's No. 1 medium, incubated at 28°C for 5-7 days. Shen et al., (2016) collected soil samples from the rhizosphere of a healthy cucumber plant from a Fusarium wilt diseased field in Danyang, Jiangsu, China to isolate the *Streptomyces* that has the potential for controlling strawberry root rot using nutrient agar (NA).

Identification and Diversity of Antifungal *Streptomyces*

Identification Techniques

Streptomyces strains were identified based on cultural characteristics on yeast extract–malt extract agar (ISP2), oatmeal agar (ISP3) and inorganic salts-starch agar (ISP4) involved the color of aerial mycelium, substrate mycelium and the diffusible pigment. (Shirling and Gottlieb, 1966). The chemotaxonomic characteristics of the cell components including the determination of the isomeric form of diaminopimelic acid (DAP), menaquinone, polar lipid composition, mycolic acids, the DNA G+C mol% content and DNA-DNA hybridization. Whole-cell hydrolysates of *Streptomyces* strains contained the LL-isomer while other spore-forming actinomycetes contained *meso*-diaminopimelic acid. The morphological and cultural characteristics were observed on colonial appearances, color, sporulation, included spore chains and spore surface ornamentation, which were determined by scanning electron microscopy (SEM) (Abdallaha et al., 2013). The phenotypic characteristics of *Streptomyces* strains including morphological, cultural, physiological (temperature and pH tolerance) and biochemical characteristics (carbon utilization) were determined by following the standard protocol of International *Streptomyces* Project (ISP) (Shirling and Gottlieb, 1966; Williams and Cross, 1971). A molecular approach based on the amplification and sequencing of the 16S rRNA gene was used. The 16S rDNA was amplified using two primers 27F (5´-AGAGTTTGATCCTGGCTCAG-3´) and 1492R (5´-GGTTACCTTGTTA CGACTT-3´). The BLAST program (www.ncbi.nlm.nih. gov/blast) was employed in order to assess the degree of DNA similarity, and the software package MEGA 6.0 (Tamura et al., 2013) was used for multiple alignment and phylogenetic analysis. The evolutionary tree was inferred using the neighbor-joining method (Saitou and Nei, 1987), maximum parsimony (Kluge and Farris, 1969) and maximum likelihood (Felsenstein, 1981). The confidence values of nodes were evaluated by using the bootstrap resampling method with 1000 replicates (Felsenstein, 1985).

Diversity of Streptomyces Species

The members of phylum actinobacteria contained different genera such as *Streptomyces, Sanguibacter, Rhodococcus, Pseudonocardia, Propionibacterium, Nocardia, Mycobacterium, Micrococcus, Microbacterium, Frankia, Corynebacterium, Clavibacter, Cellulomonas, Bifdobacterium, Arthrobacter, Actinomyces,* and *Acidimicrobium* (Yadav and Yadav, 2019). They were isolated from various sources such as *Streptomyces hygroscopicus* OsiSh-2 from rice sheath (Xu et al., 2019(, *Streptomyces* sp. CB-75 from rhizosphere soils in banana plantations (Chen et al., 2018), *Streptomyces* spp. from herbal products (Razak, et al., 2010), *Streptomyces* sp., *Nocardia* sp., *Microbispora* sp. and *Micromonospora* sp. from root tissues of *Alpinia galangal* (Taechowisan et al., 2006). However, most of these actinobacteria were isolated from the soil environment, for example, rhizosphere soils collected from banana plantations in Nanbao, Meitai and Huangtong of the Hainan Province, China (Kamara and Gangwar, 2015). *Streptomyces* sp. strain S2, *Streptomyces* sp. strain S8, and *S. griseus* strain S4–7 were isolated from the rhizosphere of a 15-year old strawberry field (Cho et al., 2017) while the rhizosphere of sixteen medicinal plants in Lumphun Province, Thailand (Khamna et al., 2009) also be done. Acidotolerant, *Streptomyce*s sp. MBRL 10 isolated from the soil collected at Hundung limestone deposit, Ukhrul District, Manipur, showed significant antagonism against *Rhizoctonia solani* (Tamreihao et al., 2018). *S. aureofaciens* K20 is halotolerant actinobacterial from salinity soil showed antifungal activity against *Macrophomina phaseolina* causes of charcoal rot disease (Shrivastava et al., 2017). Soil samples were collected from the Qilian Mountains in China could reduce the incidence of the gray mold disease and promoted the growth of tomato and pepper seedlings in a greenhouse (Shi et al., 2018). The distribution of *Streptomyces* strains that have the ability for controlling phytopathogenic fungi is shown in Table 1.

Table 1. Distribution of Antifungal *Streptomyces* strains

Strain	**Sources**	**References**
S. spectabilis CMU-A101	Soil of medicinal plants	Khamna et al., 2009
Streptomyces sp.201	Tea garden soil	Bordoloi et al., 2001
S. hygroscopicus SRA14	Paddy fields soil	Prapagdee et al., 2008
Streptomyces sp. GS 1322	Garden soil	Kumar and Jain, 2007
Streptomyces sp. TP-0569	Leaf of leek	Sasaki et al., 2002
S. cavourensis NA4	Deep-sea sediment	Dhanasekaran et al., 2012
Streptomyces sp.	Intertidal mud flat	Bae et al., 2015
S. lydicus A01	Vegetable field soil	Li et al., 2015
S. luozhongensis	Soil in Xinjiang	Zhang et al., 2017
Streptomyces sp. SN194	Soil in Liaoning	Bi and Yu, 2016
Streptomyces sp. IA1	Soil in Saharan	Toumatia et al., 2015
S. griseus H7602	Soil on vegetable fields	Nguyen et al., 2015
S. lipmanii BG2- 53	Soil in Jeju Island	Lee et al., 2002
Streptomyces sp. S4–7	Soil in strawberry field	Cho et al., 2017
S. hygroscopicus	Alkaline soil	Alferova et al., 2018
S. chrestomyceticus BCC 24770	Soil in Srinagarindra Dam	Bunyapaiboonsri et al., 2016
S. hygroscopicus OsiSh-2	Rice sheath	Xu et al., 2019
S. lipmanii BG2- 53	Soil in Jeju Island	Lee et al., 2002
S. hygroscopicus	Soil in Serbia	Grahovac et al., 2014
S. hygroscopicus B04	soil of a healthy strawberry	Shen et al., 2016
S. rimosus MY02	Soil in China	Yu et al., 2008
Streptomyces sp. PM1 & PM3	Soil in Araucaria Forest	Dias et al., 2017
Streptomyces sp. MBRL10	Soil from limestone	Tamreihao et al., 2018
S. goshikiensis YCXU	Soil from healthy cucumber	Faheem et al., 2015
Streptomyces spp.	Native tea soils	Elango et al., 2015
S. platensis 3-10	Healthy plant of rice	Shakeel et al., 2016
Streptomyces sp. IISRBPAct1	Rhizosphere of black pepper	Thampi and Bhai, 2017
Streptomyces sp. UPMRS4	Rhizospheres of rice	Awla et al., 2017
Streptomyces sp.12-09-4, 12-09-11 and 12-08-14	Soil in China	Wang et al., 2018
Streptomyces sp. FJAT-31547	Soil in tomato plantations	Zheng et al., 2019
Streptomyces sp. HG29	Saharan soil	Khebizi et al., 2018
S. violaceolatus CMCS016	Coffee plantation soil	Sameera et al., 2018
Streptomyces sp. VSMGT1014	Rhizosphere of rice	Harikrishnan et al., 2014

Strain	Sources	References
S. malaysiensis LB35	Soil in cassava fields	Khucharoenphaisan et al., 2016
Streptomyces sp. CMU-H009	Rhizosphere soils	Khamna et al., 2010
S. nobilis WA-3	Rhizosphere soils	Anwar et al., 2016
Streptomyces sp. AUR4	Leaves of chickpea	Vijayabharathi et al.,2018
S. rochei UU07, *S. vinaceusdrappus* UU11, *Streptomyces* sp. UU15	Rotten wheat straw	Singh et al., 2019
Streptomyces sp. KLBMP 5084	Healthy halophyte	Qin et al., 2017
Streptomyces sp. KLBMP S0051	Soil from the coastal	Gong et al., 2018

Fungicidal Potential and Their Secondary Metabolites

Actinomycetes and other microbial bioagents have interacted in term of antagonism either individual or synergistic for controlling plant disease. There are many mechanisms which referred to the suppression of the plant pathogens by actinomycetes. The mechanisms of *Actinomycetes* as biocontrol agents in disease control are inhibition of pathogens by bioactive compounds (Patil and Chaudhari, 2011), competition for iron through production of siderophores, competition for colonization sites and nutrients supplied by seeds and roots, induction of plant defense mechanisms, inactivation of pathogen germination factors present in seed or root exudates, degradation of pathogenic factors of the pathogen such as toxins, parasitism that may involve in the production of extracellular cell wall degrading enzymes like chitinase and β-1, 3-glucanase that can hydrolyze cell walls (Schrempf, 2001; Karthik et al., 2015) and targeting ergosterol in the cell membrane of pathogenic fungi (Seco et al., 2005). However, different the strains of *actinomycetes* maybe show different mechanisms or combinations of mechanisms for suppression of pathogens.

Antifungal Activity of Streptomyces Strains

Streptomyces strains are commonly used as biocontrol agents against a wide variety of phytopathogens, such as *Colletotrichum* (Grahovac et al., 2014; Boukaew et al., 2018), *Alternaria* (Khamna et al., 2009; Kaur and Manhas, 2014; Phuakjaiphaeo et al., 2016), *Fusarium* (Faheem et al., 2015; Toumatia et al., 2016), *Rhizoctonia* (Ahsan et al., 2017; Ahsan et al., 2019) and *Botrytis* (Cho et al., 2017; Wang et al., 2018). *Streptomyces* has proven their ability for controlling plant diseases such as, *S. diastatochromogenes* KX852460 showed potential against the *Rhizoctonia solani* AG-3 at various stages of its life cycle including sclerotia, mycelium, basidiospores and hyphae which could be used to control disease in tobacco (Ahsan et al., 2019). Askar et al., (2015) discovered *S. griseorubens* E44G which produced the chitinase enzyme that this enzyme plays an important role as a biocontrol agent against *Fusarium oxysporum*. In the field, *S. griseorubens* E44G was applied to increase the growth and yield of tomato and decrease the disease severity of infected tomato plants (Rashad et al., 2017). *S. philanthi* RM-1-138, *S. philanthi* RL-1-178, and *S. mycarofaciens* SS-2-243 have an ability to control various strains of *Botrytis cinerea* both in vitro and in vivo (Boukaew et al., 2017). The crude culture supernatant and living cells of *S. galilaeus* CFFSUR-B12 were used as the biocontrol agents to inhibit *Mycosphaerella fijiensis* caused by Black Sigatoka Disease (BSD) of banana (Castillo et al., 2016). *Streptomyces* sp. RP1A-12 has potential biocontrol agents against stem rot pathogen, *Sclerotium rolfsii* (Jacob et al., 2016; Jacob, Sajjalaguddam, and Sudini, 2018). Among all isolated actinobacteria, TM32 exhibited against white root disease caused by the *Rigidoporus* sp. which, lead to a severe problem on latex productivity. The isolate was also able to solubilize phosphate and to produce chitinase, siderophore and indole-3-acetic acid (Nakaew, Rangjaroen, and Sungthong, 2015). Ni et al., (2019) found that fermentation broth of *S. blastmyceticus* JZB130180 had a significant inhibition on the phytopathogen, *Monilinia fructicola*. Yandigeri et al., (2016) showed antifungal activity against *Rhizoctonia solani* in a dual culture assay of *S. vinaceusdrappus* S5MW2. This isolate could produce chitinase enzyme that hydrolyzed cell wall of fungal pathogen. Xu et al., (2019) treated seedlings of rice with a fermented broth of *S. sporocinereus*

OsiSh2. The result showed OsiSh2 reduced the severity of disease 23.5% and 28.3% in the greenhouse and the field, respectively. Sadeghi et al., (2017) isolated 717 *Streptomyces* from the rhizosphere of cucumber, two of these isolates, strains C 201 and C 801, showed the potential against *Phytophthora drechsleri*c that caused of damping-off in cucumber seedling. *Streptomyces* strain BG2-53 from 400 actinomycetes were isolated from Hanla Mountain on Jeju Island, Korea. It has an antifungal activity on *Magnaporthe grisea* caused of rice blast disease (Lee and Hwang, 2002). Toumatia et al., (2016) showed that coated seeds with *Streptomyces mutabilis* strain IA1 could reduce both disease occurrence (64.7%) and decrease severity (79.6%) from *Fusarium culmorum*. The strain IA1 also produced IAA and GA3 that enhanced the growth of wheat seedlings.

Antifungal potential of *S. hygroscopicus* metabolites was tested against phytopathogenic fungi, it strongly inhibited the growth of phytopathogenic fungi, especially *Colletotrichum gloeosporioides* (Grahovac et al., 2014), *Fusarium oxysporum* (Shen et al., 2016). *Streptomyces philanthi* RM-1-138 exhibited the complete inhibition on *C. gloeosporioides* PSU-NY8 (Boukaew et al., 2018). In the greenhouse experiments, watermelon plants fresh and dry weights were significantly increased, and the incidence of *Fusarium* wilt was decreased by *S. goshikiensis* YCXU (Faheem et al., 2015). Elango et al., (2015) evaluated the potential of biocontrol agents, such as *S.griseus* and *S. lydicus* along with *Bacillus subtilis* and *Trichoderma harzianum* for controlling red root rot disease in tea plants. *S. platensis* 3-10 could inhibit spore germination of *Plasmodiophora brassicae* causes of disease in oilseed rape (Shakeel et al., 2016). *Streptomyces* sp. IISRBPAct1 showed the highest inhibition to both *Phytophthora capsici* and *Sclerotium rolfsii* cause of soil-borne pathogens of black pepper (Thampi and Bhai, 2017). Awla et al., (2017) studied *Streptomyces* sp. UPMRS4 as a potential biocontrol agent for rice blast disease, *Pyricularia oryzae* UPMPo. Strain UPMRS4 was able to reduce 67.9% of disease severity and enhance plant growth by increase shoot height (15.13%), shoot dry weight (45.75%), leaf surface area (44.6%), root length (48.93), root dry weight (63.25%), number of tillers (42.26%), yield (36.96%), panicle length (15.4%) and the number of spikelet/panicles (29.39%). Wang et al., (2018) screened actinobacteria

for their antifungal activities both in vitro and in vivo, three isolates (12-09-4, 12-09-11 and 12-08-14) showed antifungal activity against *Botrytis cinerea* and *Fusarium oxysporum*. *Streptomyces* sp. strain FJAT-31547 could be used as bioagent against *Fusarium* wilt and bacterial wilt on tomato (Zheng et al., 2019). In a greenhouse study, *S. cyaneofuscatus* ZY-153, *S. kanamyceticus* B-49, *S.rochei* X-4 and *S. flavotricini* Z-13 could against Verticillium wilt of cotton and these isolates are also increased cotton growth by producing siderophores and indole acetic acid (IAA) (Xue et al., 2013). Therefore, *Streptomyces* have a high potential for biocontrol plant diseases in an agricultural system. The antifungal activity of *Streptomyces* is shown in Table 2.

Table 2. Antifungal activity of *Streptomyces* against phytopathogen in crop

Strain	Plant pathogens	Disease control	References
S. roseoflavus NKZ-259	*B.cinerea*	Tomato gray mold	Shi et al., 2018
S. hygroscopicus SRA14	*C. gloeosporioides, S.rolfsii*	Anthracnose	Prapagdee et al., 2008
S.lavendulae HHFA1 *S.coelicolor* HHFA2	*E.carotovora, B.cepacia*	Bacterial rot of onion bulbs	Abdallaha et al., 2013
S.spectabilis CMU-A101	*A.porri*	Shallot blotch	Khamna et al., 2009
S.hydrogenans DH16	*A.brassicicola*	Black leaf spot of Raphanus sativus	Kaur and Manhas, 2014
Streptomyces sp. RP1A-12	*S.rolfsii*	Peanut stem rot	Jacob et al., 2016
Streptomyces sp. KX852460	*R.solani*	Leaf spot in tobacco	Ahsan et al., 2017
Streptomyces sp. UPMRS4	*P.oryzae*	Rice blast	Awla et al., 2016
S. lydicus A01	*B.cinerea*	Gray mold	Li et al., 2015
S. luozhongensis	*C.lunata, F.oxysporum*	Leaf spots and blight	Zhang et al., 2017
S.diastatochromogenes KX852460	*R.solani*	Tobacco target spot	Ahsan et al., 2019

Strain	Plant pathogens	Disease control	References
S. griseorubens E44G	*F.oxysporum*	Fusarium wilt	Al-Askar et al., 2015
S. philanthi RM-1-138	*B.cinerea*	Gray mold disease in tomato	Boukaew et al., 2017
S. galilaeus CFFSUR-B12	*M.fijiensis*	Black Sigatoka of banana	Castillo et al., 2016
Streptomyces sp. SN194	*B.cinerea*	Gray mold	Bi and Yu, 2016
S. sioyaensis TM32	*Rigidoporus* sp.	White root in rubber tree	Nakaew et al., 2015
S.blastmyceticus JZB130180	*M.fructicola*	Peach brown rot	Ni et al., 2019
S.sanglieri AUM 00500	*G.boninenseaq*	Basal stem rot of oil palm	Azura et al., 2016
Streptomyces sp. N2.	*C.gloeosporioides*	Anthracnose	Xu et al., 2015
S.vinaceusdrappus S5MW2	*R.solani*	Root rot of tomato	Yandigeri et al., 2016
S. sporocinereus OsiSh-2	*M.oryzae*	Rice blast	Xu et al., 2019
S.rimosus C 201 *S.monomycini* C 801	*P.drechsleri*	Damping-off in cucumber	Sadeghi et al., 2017
S.griseus H7602	*P.capsici*	Root & fruit rot	Nguyen et al., 2015
Streptomyces sp. IA1	*B.cinereal, F.oxysporum*	Spot of bean Fusarium wilt	Toumatia et al., 2015
S. mutabilis IA1	*F.culmorum*	Seedling blight	Toumatia et al., 201
S. lipmanii BG2- 53	*M.grisea*	Rice blast	Lee et al., 2002
Streptomyces sp. S4–7	*B.cinerea*	Grey mold disease	Cho et al., 2017
S. angustmyceticus NR8-2	*Colletotrichum* sp., *C.lunata*	Leaf spots of Brassica	Wonglom et al., 2019
S. chrestomyceticus BCC 24770	*C.lunata, A.brassicicola*	Leaf spots & blight disease	Bunyapaiboonsri et al., 2016
S. hygroscopicus	*C.acutatum, C.gloeosporioides*	Anthracnose in apple fruits	Grahovac et al., 2014
S.hygroscopicus B04	*F.oxysporum*	Strawberry root rot	Shen et al., 2016
Streptomyces sp. SS1, SS5 and SS8	*M.oryzae, R.solani*	Rice plant pathogen	Patel et al., 2018
S. rimosus MY02	*F.oxysporium*	Fusarium wilt	Yu et al., 2008
Streptomyces sp. PM1 and PM3	*P.carotovorum*	Soft rot disease	Dias et al., 2017

Table 2. (Continued)

Strain	Plant pathogens	Disease control	References
Streptomyces sp. MBRL 10	*R.solani*	Soft rot disease	Tamreihao et al., 2018
Streptomyces sp. PM5	*P.oryzae, R.solani*	Blast & sheath blight of rice	Prabavathy et al., 2006
S.griseus SCL1 *S.lydicus* SCL4	*P.hypolateritia*	Red root-rot in tea plants	Elango et al., 2015
S.platensis 3-10	*P.brassicae*	Clubroot of oilseed rape	Shakeel et al., 2016
Streptomyces sp. IISRBPAct1	*P.capsici, S.rolfsii*	Soil borne of black pepper	Thampi and Bhai, 2017
Streptomyces sp. UPMRS4	*P.oryzae*	Rice blast	Awla et al., 2017
Streptomyces sp.12-09-4, 12-09-11 and 12-08-14	*B.cinereal, F.oxysporum*	Tomato fruit disease	Wang et al., 2018
Streptomyces sp. FJAT-31547	*Fusarium* sp., *R.solanacearum*	Tomato Fusarium wilt	Zheng et al., 2019
S. rochei	*A.alternate, D.halodes*	Leaf spot on sorghum	Hussein et al., 2014
Streptomyces sp. Tc022	*C.musae*	Anthracnose	Taechowisan et al., 2006
Streptomyces sp. CEN26	*A.brassicicola*	Black leaf spot	Phuakjaiphaeo et al., 2016
S. malaysiensis LB35	*Phytophthora* sp.	Root rot of cassava	Khucharoenphaisan et al., 2016
S.padanus PMS-702	*R.solani*	Damping-off	Shih et al., 2003
Streptomyces sp. KNF2047	*A.mali, B.cinerea, C.cucumerinum, C.lagenarium, D.bryoniae, M.grisea*	Powdery mildew of cucumber	Kim et al., 2007
S. malaysiensis	*S.nodorum*	Blotch of wheat	Li et al., 2008
S.aureofaciens K20	*M.phaseolina*	Charcoal rot	Shrivastava et al., 2017
S.cyaneofuscatus ZY-153, *S. kanamyceticu* B-49, *S. rochei* X-4, *S. flavotricini* Z-13	*V.dahliae*	Verticillium wilt of cotton	Xue et al., 2013
S.galilaeus KPS-C004	*M.incognita*	Root knot of chili	Nimnoia et al., 2017

Bioactive Compounds of Antifungal Streptomyces

The active compounds of *Streptomyces* have been studied on antagonistic activity against fungal plant pathogens. For example, natamycin, an antibiotic was isolated from the fermentation broth of a *S.lydicus* No. AZ-55 and it could against pathogens such as *Fusarium oxysporum*, *Alternaria alternate*, and *Rhizoctonia solani* (Atta et al., 2015). Antifungal activity of *Streptomyces* strain KX852460 could inhibit *Rhizoctonia solani* AG-3 KX852461 that caused of spot disease in tobacco leaf. Twenty-seven compounds were identified and most of the compounds were the derivatives of aromatic compounds. Eicosane ($C_{20}H_{42}$) and dibutyl phthalate ($C_{16}H_{22}O_4$) were found in antifungal compounds (Ahsan et al., 2017). *S. sanglieri* AUM 00500 showed strong antifungal activity on *Ganoderma boninense*. The SEM analysis showed various modes of inhibition of the fungus. Ethyl acetate extracts of single culture and inhibition zone of cross-plug culture by HPLC indicated that strain AUM 00500 produced two different antibiotics of the glutarimide group, cycloheximide, and actiphenol. (Azura et al., 2016). Antifungalmycin N2 (3-methyl-3,5-amino-4-vinyl-2-pyrone, $C_6H_7O_2N$) was purified from *Streptomyces* sp. N2, showed a significantly effective as biocontrol agents on grapefruits anthracnose caused by *C. gloeosporioides* (Xu et al., 2015). Yang, Wu, and Li (2019) tested antifungal activity against *Rhizoctonia solani* of antifungalmycin N2 from *Streptomyces* sp. N2 and checked morphological of *R. solani* found that the hyphae became severely shriveled and flattened, irregularly folded and branched.

Nguyen et al., (2015) purified antifungal compound from the *S. griseus* H7602. The strain produced 1H-pyrrole-2-carboxylic acid (PCA) which against *Phytophthora capsici* under in vitro conditions. Toumatia et al., (2015) studied antifungal activity against the chocolate spot of field bean and Fusarium wilt of flax. Actinomycin D was isolated from *S. mutabilis* IA1 and exhibited antifungal activity against phytopathogenic fungi. Caryolan-1-ol was produced from *Streptomyces* sp. S4–7 showed antifungal activity. Results of chemical-genomics profiling assays showed that caryolan-1-ol affected the endomembrane system by disrupting sphingolipid synthesis and normal vesicle trafficking in the fungi (Cho et al., 2017). *S.*

angustmyceticus NR8-2 emitted volatile antifungal compounds, including alcohols, aldehydes, carboxylic acids, and fatty acids. Using spore suspension and cell-free culture filtrate of *S. angustmyceticus* NR8-2 on leaf spot disease reduced the severity index of disease (Wonglom et al., 2019). Three polycyclic tetrahydroxanthones, chrestoxanthones A-C, were isolated from the *S. chrestomyceticus* BCC 24770. Chrestoxanthone A was active against *Curvularia lunata* and *Alternaria brassicicola* (Bunyapaiboonsri et al., 2016). Three different strains of *Streptomyces* sp., SS1, SS5 and SS8 produced (chloromethyl)-2-cyclopropyloxirane, 2, 4- ditert-butylphenol and 1-ethylthio-3-methyl-1, 3-butadiene which suppress the growth of fungal pathogen (Patel, Madaan, and Archana, 2018). Yu et al., (2008) found that medium components (i.e., carbon and nitrogen sources) and other culture requirements (i.e., initial pH and temperature) were affected on the production of an antifungal compound of *S. rimosus* MY02. Two antifungal aliphatic compounds, SPM5C-1 with a lactone and ketone carbonyl unit, from *Streptomyces* sp. PM5 completely inhibited rice pathogens, *Pyricularia oryzae* and *Rhizoctonia solani* SPM5C-1 both in vitro and in vivo conditions (Prabavathy, Mathivanan, and Murugesan, 2006). The active compound from endophytic *Streptomyces* sp. CEN26, 2,5-bis (hydroxymethyl) furan monoacetate (BHMF-Oac), inhibits the infection of *Alternaria brassicicola* in cabbage which becomes one choice of an alternative to chemical fungicides (Phuakjaiphaeo et al., 2016). 10-(2,2-dimethyl-cyclohexyl)-6,9-dihydroxy-4,9-dimethyl-dec-2-enoic acid methyl ester (SH2) from *S. hydrogenans* DH16 has significantly control fungal pathogens than using agrochemicals. In future, this compound can be developed to be a commercial fungicide (Kaur et al., 2016). These compounds showed antifungal activity and potential for applications in future agricultural systems. The bioactive compounds of *Streptomyces* are showed in Table 3.

Table 3. Antifungal Bioactive compounds of *Streptomyces* strains

Strain	Bioactive compounds	Phytopathogenic fungi	References
S. lydicus No. AZ-55	Natamycin	*F.oxysporum, A.alternata, R.solani*	Atta et al., 2015
Streptomyces sp. X852460	Eicosane, Dibutyl phthalate	*R.solani*	Ahsan et al., 2017
S. sanglieri AUM 00500	Cycloheximide, Dctiphenol	*G.boninense*	Azura et al., 2016
Streptomyces sp. N2.	Antifungalmycin N2	*R.solani, P.grisea, F.oxysporum, P.italicum, C.gloeosporioides*	Xu et al., 2015
S. griseus H7602	1H-pyrrole-2-carboxylic acid	*P.capsici*	Nguyen et al., 2015
S. mutabilis IA1	Actinomycin D	*F.oxysporum*	Toumatia et al., 2015
Streptomyces sp. S4–7	Caryolan-1-ol	*B.cinerea*	Cho et al., 2017
S.chrestomyceticus BCC 24770	Chrestoxanthone A	*C.lunata, A.brassicicola*	Bunyapaiboonsri et al., 2016
Streptomyces sp. SS1, SS5 and SS8	2(chloromethyl)-2-cyclopropyloxirane 2, 4- ditert-butylphenol, 1-ethylthio-3-methyl-1, 3-butadiene	*M.oryzae, R.solani*	Patel et al., 2018
Streptomyces sp. CEN26	2,5-bis (hydroxymethyl) furan monoacetate	*A.brassicicola*	Phuakjaiphaeo et al., 2016
Streptomyces sp.201	2-methyl- heptyl-isonicotinate	*F.moniliforme, F.oxysporum, F.solani, F.semitectum*	Bordoloi et al., 2001
Streptomyces sp. TP-A0356	Yatakemycin	*A.fumigatus*	Igarashi et al., 2003
Streptomyces sp. TP-A0569	6-Prenylindole	*A.brassicicola, F.oxysporum*	Sasaki et al., 2002
S. cavourensis NA4	Bafilomycins B1 and C1	*Soilborne fungal pathogens*	Pan et al., 2015

Table 3. (Continued)

Strain	Bioactive compounds	Phytopathogenic fungi	References
Streptomyces sp. UPMRS4	Ergotamine, Amicomacin, Fungichromin, Rapamycin, N-Acetyl-D,L-phenylalanine	*P.oryzae*	Awla et al., 2016
Streptomyces sp. SN194	Chloroxaloterpin A and B	*B.cinerea*	Bi and Yu, 2016
Streptomyces sp. FJAT-31547	n-Hexadecanoic acid	*F.oxysporum R.solanacearum*	Zheng et al., 2019
S. gancidicus HG29	Oligomycins A & E	*Fusarium spp.*	Khebizi et al., 2018
Streptomyces sp. CB-75	1,2-Benzenedicarboxylic acid diisooctyl ester	*C.musae*	Chen et al., 2018
Streptomyces sp. No. T-7545	Validamycins A and B	*P.sasakii, R.solani*	Iwasa et al., 1970
S. humidus S5-55	Phenylacetic acid, sodium phenylacetate	*P.ultimum, P.capsici,*	Hwang et al., 2001
S.hydrogenans DH16	10-(2,2-Dimethyl-cyclohexyl)-6,9-dihydroxy-4,9-dimethyl-dec-2-enoic acid methyl ester	*A.brassicicola*	Kaur et al., 2016
S.padanus PMS-702	Fungichromin	*R.solani*	Shih et al., 2003
Streptomyces sp. KNF2047	Neopeptins	*A.mali, B.cinerea, C.cucumerinum, C.lagenarium, D.bryoniae, M.grisea.*	Kim et al., 2007
S.malaysiensis	Malayamycin	*S.nodorum*	Li et al., 2008

Plant Growth Promoting

Plant hormones are chemical compounds produced from specific tissues in the plant and transported to target tissues for plant development at very

low concentrations. Indole acetic acid (IAA) is one type of plant hormone. It stimulated cell elongation by increasing the osmotic potential in cell, increased the permeability of water into cell, decreased wall pressure, increased cell wall synthesis and protein synthesis which played a major role for inducing the root formation, callus formation and parthenocarpy (Zhao, 2010). IAA is a common product of L-tryptophan metabolism produced by several microorganisms. Tryptophan exudates from root plant and induces the IAA synthesis by microorganisms. There are many pieces of research indicated that *Streptomyces* strains could synthesize indole-3-acetic acid (IAA) through the indole-3-acetamide (IAM) pathway.

The suitable of condition enhances the potential of *Streptomyces* strains to synthesize and release IAA such as pH, temperature, incubation period, carbon source, nitrogen source and tryptophan concentration (Mohite, 2013). The maximum IAA production appeared on *Streptomyces* sp. PT2 at optimum cultural conditions (Goudjal et al., 2013). Screening IAA production from locally actinomycetes in wheat soil, 138 from 210 isolates were able to utilize tryptophan and produce the IAA and *Streptomyces* sp. ASU14 showed the highest IAA producing by using tryptophan 5 mg/ml (Abd-Alla et al., 2013). Harikrishnan, Shanmugaiah, and Balasubramanian (2014) reported the ISP-2 medium supplemented with 0.5% L - tryptophan was the best medium for IAA production of *Streptomyces* sp. VSMGT1014. This strain could enhance the growth of rice seedlings. IAA production conditions of *Streptomyces* sp. SF5 was supplemented with 2mg/ml of L-tryptophan at pH 7 at 30°C and it showed maximum IAA at 104.76±0.2µg/ml (Ameur and Mostefa, 2012). Different cultural conditions of pH and temperature and media components such as carbon and nitrogen source, tryptophan concentration used to study the ability to solubilize phosphate, produce indole-3 acetic acid (IAA), siderophore and ammonia of *Streptomyces* sp. VITMS22 (Kizhakedathil and Subathra, 2018). *S. violaceolatus* CMCS 016 produced the highest amount of IAA (109.24 µg/ml) (Sameera et al., 2018), *Streptomyces* sp. A1RT produced IAA at 26 mg ml/l (Sarwar et al., 2018), while *S. canus* produced the highest amount of IAA (10.1 mg ml/1) and GA3 (12.0 mg ml/1) (Poovarasan et al., 2013).

Streptomyces strains produced a good source of indole acetic acid to plant growth promotion which in turn increased the yield of agriculture crops (Gajendran et al., 2012; Kumar et al., 2016). For example, Khucharoenphaisan et al., (2016) showed the potential of *S. malaysiensis* LB35 to stimulate the growth of Cassava that produced IAA and also produce biological agent to inhibit *Phytophthora* sp. cause of root rot in Cassava. *Streptomyces* sp. CMU-H009 showed the maximums IAA production at 300 mg/mL and significant increase the germination and elongation of maize root and cowpea seeds (Khamna et al., 2010). *Streptomyces* sp. MBRL 10 from limestone showed significant antagonism against *Rhizoctonia solani* and showed the ability to promote the germination of rice (Tamreihao et al., 2018). Anwar, Ali, and Sajid (2016) showed the most active indole acetic acid (IAA) produced from *S. nobilis* WA-3 IAA that promoted the of growth wheat (*Triticum aestivum*) seeds, root length, fresh weight, dry weight, number of leaves and number of roots for in-vivo screening. *Streptomyces* isolate AUR4 was able to significantly enhance seed numbers, seed weight, pod numbers, pod weight and biomass in chickpea genotype JG11 (Vijayabharathi et al., 2018). Tamreihao et al., (2016) studied the potential in IAA production of *S. corchorusii* UCR3-16 for promoting the growth of rice plants. Both pot trial and field experiments showed significantly increased growth and grain yield production using talcum powder formulation. *S. rochei* UU07, *S. vinaceusdrappus* UU11 and *Streptomyces* sp. UU15 isolated from rotten wheat straw were screened and were found potential to plant growth promoting (PGP) traits (IAA, siderophore, phosphate solubilization, HCN, ammonia and AAC deaminase (Singh et al., 2019). *Streptomyces* sp. NCIM 5533 showed the potential as a true plant growth-promoting rhizobacteria by producing IAA and solubilization of ammonia and phosphate. In laboratory and greenhouse, NCIM 5533 showed ability to colonize roots and promote the growth of tomato (Puppala et al., 2019). Mohandas et al., (2013) showed the abilities of *S. canus* on IAA and GA3 production, phosphate solubilization, siderophore production and antagonistic activity against pathogens including chitinase activity and it also increase height and plant dry matter in guava. *Streptomyces* sp. DBT204 enhanced the growth of the seedling of chili and tomato by producing

phytohormones (Passari et al., 2016). *Streptomyces* sp. PM9 promoted the growth and modulate secondary metabolism of *Eucalyptus grandis* and *E. globulus*, this isolate showed the potential to use as a biological control in forestry (Salla et al., 2014).

Table 4. Plant growth promoting *Streptomyces* strains on target plants

Strain	Target plants	References
Streptomyces sp. CMU-H009	Maize Cow pea	Khamna et al., 2010
S. malaysiensis LB35	Cassava	Khucharoenphaisan et al., 2016
Streptomyces sp. VSMGT1014	Rice	Harikrishnan et al., 2014
Streptomyces sp. MBRL	Rice	Tamreihao et al., 2018
S. nobilis WA-3	Wheat	Anwar et al., 2016
S. variabilis (4NC) *S. fradiae* (8PK)	Stevia plant	Tolba et al., 2019
Streptomyces sp. AUR4	Chickpea	Vijayabharathi et al., 2018
S. corchorusii UCR3-16	Rice	Tamreihao et al., 2016
Streptomyces sp. NCIM 5533	Tomato	Puppala et al., 2019
Streptomyces sp. KLBMP 5084	Halophyte L. sinense	Qin et al., 2017
Streptomyces sp. A1RT	Potato	Sarwar et al., 2018
Streptomyces sp. KLBMP S0051	Wheat	Gong et al., 2018
S. canus	Pomegranate	Poovarasan et al., 2013
S. canus	Guava	Mohandas et al., 2013
Streptomyces sp. DBT204	Chili & tomato	Passari et al., 2016
S. ramulosus EUSKR2S82	Eucalyptus	Himaman et al., 2016
S. rochei WZS1-1 *S. sundarbansensis* WZS2-1	Wheat	Han et al., 2018
Streptomyces sp. PM9	Eucalyptus	Salla et al., 2014

Soil salinity is a worldwide environmental problem that negatively impacts crop production. *Streptomyces* sp. KLBMP 5084 enhanced both root and leaf lengths of *Limonium sinense* under both non-salt conditions and NaCl stress under glasshouse experiment (Qin et al., 2017). *Streptomyces* spp. isolated from the rhizosphere of halophytic plants have the potential to promote plant growth on stress condition at different levels of salt in Stevia plant (Tolba et al., 2019). *Streptomyces* sp. KLBMP S0019 also significantly enhanced seedling growth under NaCl stress (Gong et al.,

2018). *Streptomyces* strains are good candidates as plant growth promoter to plant adaptions in saline soils. They are used as plant growth-promoting agents (help to produce plant growth hormone, indole-3-acetic acid), antifungal compounds as a source of agrochemical compounds. It could be a promising candidate for utilization in growth improvement of plants of economic and agricultural value. *Streptomyces* as plant growth promoters on target plants is shown in Table 4.

COMMERCIAL *STREPTOMYCES* AS BIOCONTROL AGENTS

In the present day, the study of new active compounds has linked with *Streptomyces* strains both the isolation and screening of activities. There are many conditions, such as nutrients, culturing, and other factors that may affect on the metabolic pathway of *Streptomyces* strains to produce bioactive compounds (Harir et al., 2018). The application of *Streptomyces* strains both in greenhouse and in field, preparation and delivery of any formulation depend on various factors such as type of organism and site of application (seed treatment, foliar application, and soil) (Amaresan et al., 2018).

Table 5. Products of *Streptomyces* registered as pesticides to control plant diseases

Species/Strain	Commercial name	Plant Diseases	Registered in
S. colombiensis	Mycocide	Powdery mildew, gray mold, brown patch	South Korea
S. kasugaensis	Safegrow	Sheath blight, large patch	South Korea
S. albus	Bactophil	Seed germination diseases	Ukraine
S. griseoviridis K61	Mycostop	Botrytis grey mold, crown rot, damping off, Fusarium wilt,	Canada, European Union, United States
S. lydicus WYEC108	Actinovate Actino-Iron	Anthracnose, downy mildew, soilborne, damping off	Canada United States

Moreover, some of the active compounds have been used as commercial biocontrol agents in powder formulations, such as ActinovateR from *S. lydicus* WYEC108 to control root damping-off and foliar fungal pathogens and MycostopR from *S. griseoviridis* K61 to control seed-borne and soil-borne pathogens (Kabaluk et al., 2010). Research on new formula of pesticides will give upgraded the biocontrol and plant growth-promoting products for sustainable agriculture. Microbial pesticides by *Streptomyces* that was registered as active compounds in plant protection products are shown in Table 5.

CONCLUSION

Streptomyces strains represent the major actinomycete population in the soil ecosystem with the extraordinary potential of creating bioactive compounds and effectively inhibition various infected plant pathogens in economic crops. They are genuinely planned for use as biocontrol agents to control plant infection and plant growth-promoting that seem to lead to the expanded production of agriculture products beneath diverse conditions. This guarantee is based on the utilization of ecofriendly microorganisms that control disease and improve plant development. In the future, *Streptomyces* strains will be one of direction to benefit agricultural system in order to achieve agricultural sustainability.

REFERENCES

Alferova, V. A., Roman, A. N., Olga, P. B., Eugene, A. R., Maxim, V. S., Igor, A. P., Vera, S. S., Alexander, B. K., Lyubov, G. D., Elena, A. S., Mikhail, A. E., Olga, N. S., Gulnara, Kh. K., Aleksander, S. P., Pavel, N. S., Yaroslav, V. T., Galina, B. F., Larisa, P. T., Anton, P. T., Aleksey, S. T., and Vladimir, A. K. (2018). Astolides A and B, antifungal and

cytotoxic naphthoquinone-derivedpolyol macrolactones from *Streptomyces hygroscopicus*. *Tetrahedron*, 74: 7442-7449.

Abd-Alla, M. H., El-Sayed, A. E., and Abdel-Hamied, M. R. (2013). Indole-3-acetic acid (IAA) production by *Streptomyces atrovirens* isolated from rhizospheric soil in Egypt. *Journal of Biology and Earth Sciences*, 3: 182-193.

Abdallaha, M. E., Harounb, S. A., Gomahc, A. A., El-Naggard, N. E. and Badr, H. H. (2013). Application of actinomycetes as biocontrol agents in the management of onion bacterial rot diseases. *Archives of Phytopathology and Plant Protection*, 46: 1797–1808.

Ahsan, T., Jianguang, C., Xiuxiang, Z., Muhammad, I., Hina, I., and Yuanhua, W. (2019). Action mechanism of *Streptomyces diastato-chromogenes* KX852460 against *Rhizoctonia solani* AG-3 involving basidiospores suppression and oxidative damage. *Iranian Journal of Science and Technology*, 43: 2141–2147.

Ahsan, T., Jianguang, C., Xiuxiang, Z., Muhammad, I. and Yuanhua, W. (2017). Extraction and identifcation of bioactive compounds (eicosane and dibutyl phthalate) produced by *Streptomyces* strain KX852460 for the biological control of *Rhizoctonia solani* AG-3 strain KX852461 to control target spot disease in tobacco leaf. *AMB Express*, 7: 1-9.

Al-Askar, A. A., Zakaria, A. B., Younes, M. R., Khalid, M. G., Waleed, M. A., Elsayed, E. H. and Yasser, M. S. (2015). Evaluation of *Streptomyces griseorubens* E44G for the biocontrol of *Fusarium oxysporum* f. sp. lycopersici: ultrastructural and cytochemical investigations. *Annals of Microbiology*, 65: 1815–1824.

Amaresan, N., Krishna, K., Jinal, H. N., Kiran, G. B. and Raj, K. M. (2018). *Streptomyces* in plant growth promotion: mechanisms and role. In *New and Future Developments in Microbial Biotechnology and Bioengineering*, (Eds) Singh, B., Gupta, V. K., and Passari, A. K.,125-135. Retrieved from https://www.researchgate.net/publication/323631088_Streptomyces_in_plant_growth_promotion

Ameur, H. and Mostefa, G. (2012). Screening of actinomycetes antibacterial substances and indole acetic acid (IAA) and optimization of growth and

IAA production conditions in *Streptomyces* sp. SF5. *International Journal of Pharmaceutical and Biological Archives*, 3: 545-551.

Anwar, S., Ali, B. and Sajid, I. (2016). Screening of rhizospheric actinomycetes for various *In-vitro* and *In-vivo* plant growth promoting (PGP) traits and for agroactive compounds. *Frontiers in Microbiology*, 7: 1-11.

Atta, H. M., El-Sayed, A. S., El-Desoukey, M. A., Hassan, M. and El-Gazar, M. (2015). Biochemical studies on the natamycin antibiotic produced by *Streptomyces lydicus*: fermentation, extraction and biological activities. *Journal of Saudi Chemical Society*, 19: 360-371.

Awla, H. K., Jugah, K., Radziah, O., Tavga, S. R., Sathyapriya, H. and Mui-Yun, W. (2017). Plant growth-promoting abilities and biocontrol efficacy of *Streptomyces* sp. UPMRS4 against *Pyricularia oryzae*. *Biological Control*, 112: 55-63.

Awla, H. K., Jugah, K., Radziah, O., Tavga, S. R. and Mui-Yun, W. (2016). Bioactive compounds produced by *Streptomyces* sp. isolate UPMRS4 and antifungal activity against *Pyricularia oryzae*. *American Journal of Plant Sciences*, 7: 1077-1085.

Azura, A. B. Nur., Yusoff, M., Tan, G. Y. A., Jegadeesh, R., Appleton, D. R. and Vikineswary, S. (2016). *Streptomyces sanglieri* which colonised and enhanced the growth of *Elaeis guineensis* Jacq. Seedlings was antagonistic to *Ganoderma boninense* in *in vitro* studies. *Journal of Industrial Microbiology and Biotechnology*, 43: 485–493.

Bae, M., Kim, H., Moon, K., Nam, S., Shin, J., Oh, Ki-Bong and Oh, Dong-Chan. (2015). Mohangamides A and B, new dilactone-tethered pseudo-dimeric peptides inhibiting *Candida albicans* isocitrate lyase. *Organic Letters*, 17: 712-715.

Behal, V. (2000). Bioactive products from *Streptomyces*. *Advances in Applied Microbiology*, 47: 113-156.

Berdy, J. (2005). Bioactive microbial metabolites. *Journal of Antibiotics*, 58: 1–26.

Bi, Y. and Zhiguo, Y. (2016). Diterpenoids from *Streptomyces* sp. SN194 and their antifungal activity against *Botrytis cinerea*. *Journal of Agricultural and Food Chemistry*, 64: 8525−8529.

Bordoloi, G. N., Kumari, B., Guha, A., Bordoloi, M., Yadav, R. N. S., Roy, M. K. and Bora, T. C. (2001). Isolation and structure elucidation of a new antifungal and antibacterial antibiotic produced by *Streptomyces* sp. 201. *Bioscience, Biotechnology, and Biochemistry,* 65: 1856-1858.

Boukaew, S., Petlamul, W., Bunkrongcheap, R., Chookaew, T., Kabbua, T., Thippated, A. and Prasertsan, P. (2018). Fumigant activity of volatile compounds of *Streptomyces philanthi* RM-1-138 and pure chemicals (acetophenone and phenylethyl alcohol) against anthracnose pathogen in postharvest chili fruit. *Crop Protection,* 103: 1-8.

Boukaew, S., Prasertsan, P., Troulet, C., and Bardin, M. 2017. Biological control of tomato gray mold caused by *Botrytis cinerea* by using *Streptomyces* spp. *Biological Control,* 62: 793–803.

Bunyapaiboonsri, T., Lapanun, S., Supothina, S., Rachtawee, P., Chunhametha, S., Suriyachadkun, C., Boonruangprapa, T., Auncharoen, P., Chutrakul, C., and Vichai, V. (2016). Polycyclic tetrahydroxanthones from *Streptomyces chrestomyceticus* BCC 24770. *Tetrahedron,* 72: 775-778.

Castillo, B. M., Dunn, M. F., Navarro, K. G., Melendez, F. H., Ortiz, M. H., Guevara, S. E. and Palacios, G. H. (2016). Antifungal performance of extracellular chitinases and culture supernatants of *Streptomyces galilaeus* CFFSUR-B12 against *Mycosphaerella fijiensis* Morelet. *World Journal of Microbiology and Biotechnology,* 32: 1-12.

Chen, Y., Zhou, D., Qi, D., Gao, Z., Xie, J. and Luo, Y. 2018. Growth promotion and disease suppression ability of a *Streptomyces* sp. CB-75 from banana rhizosphere soil. *Frontiers in Microbiology,* 8: 1-18.

Cho, G., Kim, J., Park, C. G., Nislow, C., Weller, D. M. and Kwak, Y. S. (2017). Caryolan-1-ol, an antifungal volatile produced by *Streptomyces* spp., inhibits the endomembrane system of fungi. *Open Biology,* 7: 1-9.

Dhanasekaran, D., Thajuddin, N. and Panneerselvam A. (2012). Applications of actinobacterial fungicides in agriculture and medicine. In *Fungicides for Plant and Animal Diseases*, (Eds) Dhanasekaran, D., 29-54. Retrieved from https://www. interchopen. com/books-for-plants-and-animal-diseases/applications-of-actinobacterial-fungicides-in-agriculture-and-medicine.

Dias, M. P., Bastos, M. S., Xavier, V. B., Cassel, E., Astarita, L. V. and Santarem, E. R. (2017). Plant growth and resistance promoted by *Streptomyces* spp. in tomato. *Plant Physiology and Biochemistry*, 118: 479-493.

Elango, V., Manjukarunambika, K., Ponmurugan, P. and Marimuthu, S. (2015). Evaluation of *Streptomyces* spp. for effective management of *Poria hypolateritia* causing red root-rot disease in tea plants. *Biological Control*, 89: 75-83.

Faheem, M., Raza, W., Zhong, W., Nan, Z., Shen, Q. and Xu, Y. (2015). Evaluation of the biocontrol potential of *Streptomyces goshikiensis* YCXU against *Fusarium oxysporum* f. sp. niveum. *Biological Control*, 81: 101-110.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: A maximum likelihood approach. *Journal Molecular Evolution*, 17: 368-376.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39: 783-791.

Flores-Gallegos, A. C., and Nava-Reyna, E. (2019). Plant growth-promoting microbial enzymes in *Enzymes*. In *Food Biotechnology*, (Eds) Kuddus, M., 521-534. Retrieved from https://www.sciencedirect.com/science/article/pii/B978012813280700030X.

Gajendran, S., Rebecca, J., Sharmila, S., Dhanalakshmi, V., Dam, P. and Ranjeet. (2012). A study on the indole acetic acid production by *Streptomyces* spp. isolated from the rhizosphere soil of the five flowering plants. *International Journal of Agriculture and Food Science Technology*, 3: 21-27.

Gong, Y., Bai J. L., Yang, H. T., Zhang, W. D., Xiong, Y, W., Ding, P. and Qin, S. (2018). Phylogenetic diversity and investigation of plant growth-promoting traits of actinobacteria in coastal salt marsh plant rhizospheres from Jiangsu, China. *Systematic and Applied Microbiology*, 41: 516-527.

Goudjal, Y., Toumatia, O., Sabaou, N., Barakate, M., Mathieu, F. and Zitouni, A. (2013). Endophytic actinomycetes from spontaneous plants of Algerian Sahara: indole-3-acetic acid production and tomato plants

growth promoting activity. *World Journal of Microbiology and Biotechnology,* 29: 1821-1829.

Grahovac, J., Grahovac, M., Dodic, J., Bajic, B. and Balaz, J. (2014). Optimization of cultivation medium for enhanced production of antifungal metabolites by *Streptomyces hygroscopicus*. *Crop Protection,* 65: 143-152.

Han, D., Wang, L. and Luo, Y. (2018). Isolation, identification, and the growth promoting effects of two antagonistic actinomycete strains from the rhizosphere of *Mikania micrantha* Kunth. *Microbiological Research,* 208: 1-11.

Harikrishnan, H., Shanmugaiah, V. and Balasubramanian, N. (2014). Optimization for production of indole acetic acid (IAA) by plant growth promoting *Streptomyces* sp. VSMGT1014 isolated from rice rhizosphere. *International Journal of Current Microbiology and Applied Sciences,* 3: 158-171.

Harir, M., Bendif, H., Bellahcene, M., Fortas, Z. and Pogni, R. (2018). *Streptomyces* secondary metabolites. In *Basic Biology and Applications of Actinobacteria,* (Eds) Enany, S., 99-122. Retrieved from https://www.intechopen.com/books/basic-biology-and-applications-of-actinobacteria/streptomyces-secondary-metabolites.

Himaman, W., Thamchaipenet, A., Pathom-aree, W. and Duangmal, K. (2016). Actinomycetes from eucalyptus and their biological activities for controlling eucalyptus leaf and shoot blight. *Microbiological Research*, 188-189: 42-45.

Hussein, A. A. E., Alhasan, R. E. M., Abdelwahab, S. A. and Siddig, M. A. E. (2014). Isolation and identification of *Streptomyces rochei* strain active against phytopathogenic Fungi. *British Microbiology Research Journal*, 4: 1057-1068.

Hwang, B. K., Lim, S. W., Kim, B. S., Lee, J. Y. and Moon, S. S. (2001). Isolation and *in vivo* and *in vitro* antifungal activity of phenylacetic acid and sodium phenylacetate from *Streptomyces humidus*. *Applied and Environmental Microbiology*, 67: 3739–3745.

Igarashi, Y., Futamata, K., Fujita, T., Sekine, A., Senda, H., Naokib, H. and Furu, T. (2003). Yatakemycin, a novel antifungal antibiotic produced by *Streptomyces* sp. TP-A0356. *Journal of Antibiotics,* 56: 107-113.

Iwasa, T., Yamamoto, H. and Shibata, M. (1970). Studies on validamycins, new antibiotics. I *streptomyces hygroscopicus* var. limoneus nov. var., validamycin-producing organism. *Journal of Antibiotics*, 23: 595-602.

Jacob, S., Sajjalaguddam, R. R., Kumar, K. V. K., Varshney, R. and Sudini, H. K. (2016). Assessing the prospects of *Streptomyces* sp. RP1A-12 in managing groundnut stem rot disease caused by *Sclerotium rolfsii* Sacc. *Journal of General Plant Pathology,* 82: 96–104.

Jacob, S., Sajjalaguddam, R. R. and Sudini, H. K. (2018). *Streptomyces* sp. RP1A-12 mediated control of peanut stem rot caused by *Sclerotium rolfsii*. *Journal of Integrative Agriculture,* 17: 892–900.

Kabaluk, J. T., Svircev, A. M., Goettel, M. S. and Woo, S. G. (Eds.) (2010). The use and regulation of microbial pesticides in representative jurisdictions worldwide. *IOBC Global*, 99 pp. Retrieved from http://www.iobc-global.org/download/microbial_regulation_book_kabaluk_et_al_2010.pdf

Kamara, V., and Gangwar, M. (2015). Antifungal activity of actinomycetes from rhizospheric soil of medicinal plants against phytopathogenic fungi. *International Journal of Current Microbiology and Applied Sciences,* 4: 182-187.

Karthik, N., Binod, P. and Pandey, A. (2015). Purification and characterisation of an acidic and antifungal chitinase produced by a *Streptomyces* sp. *Bioresource Technology,* 188: 195-201.

Kaur, T., Kaur, A., Sharma, V. and Manhas, R. K. (2016). Purification and characterization of a new antifungal compound 10-(2,2-dimethyl-cyclohexyl)-6,9-dihydroxy-4,9-dimethyl-dec-2-enoic acid methyl ester from *Streptomyces hydrogenans* strain DH16. *Frontiers in Microbiology,* 7: 1-10.

Kaur, T. and Manhas, R. K. (2014). Antifungal, insecticidal, and plant growth promoting potential of *Streptomyces hydrogenans* DH16. *Journal of Basic Microbiology*, 54: 1175–1185.

Khamna, S., Yokota, A., Peberdy, J. F. and Lumyong, S. (2009). Antifungal activity of *Streptomyces* spp. isolated from rhizosphere of Thai medicinal plants. *International Journal of Integrative Biology*, 6: 143-147.

Khamna, S., Yokota, A., Peberdy, J. F. and Lumyong, S. (2010). Indole-3-acetic acid production by *Streptomyces* sp. isolated from some Thai medicinal plant rhizosphere soils. *EurAsian Journal of BioSciences*, 4: 23-32.

Khebizi, N., Boudjella, H., Bijani, C., Bouras, N., Klenk, H. P., Pont, F., Mathieu, F. and Sabaou, N. (2018). Oligomycins A and E, major bioactive secondary metabolites produced by *Streptomyces* sp. strain HG29 isolated from a Saharan soil. *Journal de Mycologie Medicale*, 28: 150-160.

Khucharoenphaisan, K., Rodbangpong, K., Saengpaen, P. and Sinma, K. (2016). Exploration on soil actinomycetes against *Phytophthora* sp. causing root rot of cassava and plant growth promoting activities. *Journal of Plant Sciences*, 11: 38-44.

Kim, Y. S., Kim, H. M., Chang, C., Hwang, I. C., Oh, H., Ahn, J. S., Kim, K. D., Hwang, B. K. and Kim, B. S. (2007). Biological evaluation of neopeptins isolated from a *Streptomyces* strain. *Pest Management Science*, 63: 1208–1214

Kizhakedathil, M. P. J. and Subathra, D. C. (2018). Rhizoshpheric bacteria isolated from the agricultural fields of Kolathur, Tamilnadu promotes plant growth in mustard plants. *Biocatalysis and Agricultural Biotechnology*, 16: 293-302.

Kluge, A. G. and Farris, J. S. (1969). Quantitative phyletics and the evolution of *Anurans*. *Systematic Zoology*, 18: 1-32.

Kumar, J. P. and Jain, P. C. (2007). Isolation, characterization and antifungal activity of *Streptomyces sampsonii* GS 1322. *Indian journal of experimental biology*, 45: 203-206.

Lee, C. H., Kim, B. J., Choi, G. J., Cho, K. Y., Yang, H., Shin, C., Min, S. and Lim, Y. (2002). *Streptomyces* with antifungal activity against rice blast causing fungus, *Magnaporthe grisea*. *Journal Microbiology and Biotechnology*, 12: 1026–1028.

Lee, J. Y. and Hwang, B. K. (2002). Diversity of antifungal Actinomycetes in various vegetative soils of Korea. *Canadian Journal of Microbiology*, 48: 407-417.

Li, J., Liu, W., Luo, L., Dong, Dan., Liu, T., Zhang, T., Lu, C., Liu, D., Zhang, D., and Wu, H. (2015). Expression of *Paeni bacillus* polymyxab-1,3-1,4-glucanase in *Streptomyces lydicus* A01 improves its biocontrol effect against *Botrytis cinerea*. *Biological Control*, 90: 140-147.

Li, W., Csukai, M., Corran, A., Crowley, P., Solomon, P. S. and Oliver, R. P. (2008). Malayamycin, a new streptomycete antifungal compound, specifically inhibits sporulation of *Stagonospora nodorum* (Berk) Castell and Germano, the cause of wheat glume blotch disease. *Pest Management Science*, 64: 1294–1302.

Merrouche, R., Yekkour, A., Lamari, L., Zitouni, A., Mathieu, F. and Sabaou, N. (2017). Efficiency of *Saccharothrix algeriensis* NRRL B-24137 and its produced antifungal dithiolopyrrolones compounds to suppress *Fusarium oxysporum* induced wilt disease occurring in some cultivated crops. *Arabian Journal for Science and Engineering*, 42: 2321–2327.

Mohandas, S., Poovarasan, S., Panneerselvam, P., Saritha, B., Upreti, K. K., Kamal, R. and Sita T. (2013). Guava (*Psidium guajava* L.) rhizosphere *Glomus mosseae* spores harbor actinomycetes with growth promoting and antifungal attributes. *Scientia Horticulturae*, 150: 371-376.

Mohite, B. (2013). Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. *Journal of Soil Science and Plant Nutrition*, 13: 638-649.

Nakaew, N., Rangjaroen, C., and Sungthong, R. (2015). Utilization of rhizospheric *Streptomyces* for biological control of *Rigidoporus* sp. causing white root disease in rubber tree. *European Journal of Plant Pathology*, 142: 93–105.

Nguyen, X. H., Naing, K. W., Lee, Y. S., Kim, Y. H., Moon, J. H. and Kim, K. Y. (2015). Antagonism of antifungal metabolites from *Streptomyces griseus* H7602 against *Phytophthora capsici*. *Journal of Basic Microbiology*, 55: 45–53.

Ni, M., Wu, Q., Wang, H., Liu, W., Hu, B., Zhang, D., Zhao, J., Liu, D., and Lu, C. (2019). Identification of a novel strain, *Streptomyces blastmyceticus* JZB130180, and evaluation of its biocontrol efficacy against *Monilinia fructicola*. *Journal of Zhejiang University-SCIENCE B (Biomedicine and Biotechnology),* 20: 84-94.

Nimnoia, P., Pongsilp, N., and Ruanpanun, P. (2017). Monitoring the efficiency of *Streptomyces galilaeus* strain KPS-C004 against root knot disease and the promotion of plant growth in the plant-parasitic nematode infested soils. *Biological Control,* 114: 158-166.

Olanrewaju, O. S. and Babalola, O. O. (2019). *Streptomyces*: implications and interactions in plant growth promotion. *Applied Microbiology and Biotechnology,* 103: 1179–1188.

Pan, H., Yu, S., Song, C., Wang, N., Hua, H., Hu, J. and Wang, S. (2015). Identification and characterization of the antifungal substances of a novel *Streptomyces cavourensis* NA4. *Journal of Microbiology and Biotechnology*, 25: 353-357.

Passari, A. K., Chandra, P., Zothanpuia, Mishra, V. K., Leo, V. V., Gupta, V. K., Kumar, B. and Singh, B. P. (2016). Detection of biosynthetic gene and phytohormone production by endophytic actinobacteria associated with *Solanum lycopersicum* and their plant growth-promoting effect. *Research in Microbiology,* 167: 692-705.

Patel, J. K., Madaan, S. and Archana, G. (2018). Antibiotic producing endophytic *Streptomyces* spp. colonize above-ground plant parts and promote shoot growth in multiple healthy and pathogen challenged cereal crops. *Microbiological Research*, 215: 36-45.

Patil, H. J., and Chaudhari, B. L. (2011). Agricultural implications of *Actinomycetes.* In *environment and biotechnology*, (Eds) Kumar, A., 60-88. Retrieved from https://www. researchgate.net/publication _agricultural_implications_of_actinomycetes

Phuakjaiphaeo, C., Chang, C. I., Ruangwong, O. and Kunasakdakul, K. (2016). Isolation and identification of an antifungal compound from endophytic *Streptomyces* sp. CEN26 active against *Alternaria brassicicola*. *Letters in Applied Microbiology,* 63: 38-44.

Poovarasan, S., Mohandas, S., Paneerselvam, P., Saritha, B. and Ajay, K. M. (2013). Mycorrhizae colonizing actinomycetes promote plant growth and control bacterial blight disease of pomegranate (*Punica granatum* L. cv Bhagwa). *Crop Protection,* 53: 175-181.

Prabavathy, V. R., Mathivanan, N. and Murugesan, K. (2006). Control of blast and sheath blight diseases of rice using antifungal metabolites produced by *Streptomyces* sp. PM5. *Biological Control,* 39: 313-319.

Prapagdee, B., Kuekulvong, C. and Mongkolsuk, S. (2008). Antifungal potential of extracellular metabolites produced by *Streptomyces hygroscopicus* against phytopathogenic fungi. *International Journal of Biological Sciences,* 4: 330-337.

Puppala, K. R., Bhavsar, K., Sonalkar, V., Khire, J. M. and Dharne, M. S. (2019). Characterization of novel acidic and thermostable phytase secreting *Streptomyces* sp. (NCIM 5533) for plant growth promoting characteristics. *Biocatalysis and Agricultural Biotechnology,* 18: 1-7.

Qin, S., Feng, W., Wang, T., Ding, P., Xing, K. and Jiang, J. (2017). Plant growth-promoting effect and genomic analysis of the beneficial endophyte *Streptomyces* sp. KLBMP 5084 isolated from halophyte *Limonium sinense*. *Plant Soil,* 416: 117–132.

Rashad, Y. M., Al-Askar, A. A., Ghoneem, K. M., Saber, W. I. A. and Hafez, E. E. (2017). Chitinolytic *Streptomyces griseorubens* E44G enhances the biocontrol efficacy against Fusarium wilt disease of tomato. *Phytoparasitica,* 45: 227–237.

Sadeghi, A., Koobaz, P., Azimi, H., Karimi, E. and Akbari, A. R. (2017). Plant growth promotion and suppression of *Phytophthora drechsleri* damping-off in cucumber by cellulase-producing *Streptomyces*. *Biological Control,* 62: 805–819.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution,* 4: 406-425.

Salla, T. D., Silva, T. R., Astarita, L. V. and Santarem, E. R. (2014). *Streptomyces rhizobacteria* modulate the secondary metabolism of eucalyptus plants. *Plant Physiology and Biochemistry,* 85: 14-20.

Sameera, B., Prakash, H. S. and Nalini, M. S. (2018). Indole acetic acid production by Actinomyces of coffee plantation soils. *International Journal of Current Research,* 10: 74482-74487.

Sarwar, A., Latif, Z., Zhang, S., Zhu, J., Zechel, David. L. and Bechthold, A. (2018). Biological control of potato common scab with rare isatropolone C compound produced by plant growth promoting *Streptomyces* A1RT. *Frontiers in Microbiology,* 9: 1-10.

Sasaki, T., Igarashi, Y., Ogawa, M. and Furumai, T. (2002). Identification of 6-prenylindole as an antifungal metabolite of *Streptomyces* sp. TP-A0595 and synthesis and bioactivity of 6-substituted indoles. *Journal of Antibiotics,* 55: 1009-1012.

Schrempf, H. (2001). Recognition and degradation of chitin by *Streptomycetes*. *Antonie van Leeuwenhoek*, 79: 285–289.

Seco, E. M., Cuesta, T., Fotso, S., Laatsch, H. and Malpartida, F. (2005). Two polyene amides produced by genetically modified *Streptomyces diastaticusvar* 108. *Chemistry & Biology,* 12: 535–543.

Shakeel, Q., Lyu, A., Zhang, J., Wu, M., Chen, S., Chen, W., Li, G. and Yang, L. (2016). Optimization of the cultural medium and conditions for production of antifungal substances by *Streptomyces platensis* 3-10 and evaluation of its efficacy in suppression of clubroot disease (*Plasmodiophora brassicae*) of oilseed rape. *Biological Control,* 101: 59-68.

Sharma, H. and Parihar, L. (2010). Antifungal activity of extracts obtained from actinomycetes. *Journal of Yeast and Fungal Research,* 1: 197-200.

Sharma, M., Dangi, P. and Choudhary, M. (2014). Actinomycetes: source, identification, and their applications. *International Journal of Current Microbiology and Applied Sciences,* 3: 801-832.

Shen, T., Wang, C., Yang, H., Deng, Z., Wang, S., Shen, B. and Shen, Q. (2016). Identification, solid-state fermentation and biocontrol effects of *Streptomyces hygroscopicus* B04 on strawberry root rot. *Applied Soil Ecology,* 103: 36-43.

Shi, L., Nwet, T. T., Ge, B., Zhao, W., Liu, B., Cui, H. and Zhang, K. (2018). Antifungal and plant growth-promoting activities of *Streptomyces roseoflavus* strain NKZ-259. *Biological Control*, 125: 57-64.

Shih, H., Liu, Y., Hsu, F., Mulabagal, V., Dodda, R. and Huang, J. (2003). Fungichromin: a substance from *Streptomyces padanus* with inhibitory effects on *Rhizoctonia solani*. *Journal of Agricultural and Food Chemistry*, 51: 95-99.

Shirling, E. B. and Gottlieb D. (1966). Method for charecterization of *Streptomyces* species. *International Journal of Systematic Bacteriology*, 16: 313-340.

Shrivastava, P., Kumar, R. and Yandigeri, M. S. (2017). In vitro biocontrol activity of halotolerant *Streptomyces aureofaciens* K20 A potent antagonist against *Macrophomina phaseolina* (Tassi) Goid. *Saudi Journal of Biological Sciences*, 24: 192-199.

Singh, R. P., Manchanda, G., Maurya, I. K., Maheshwari, N. K., Tiwari, P. K. and Rai, A. R. (2019). *Streptomyces* from rotten wheat straw endowed the high plant growth potential traits and agro-active compounds. *Biocatalysis and Agricultural Biotechnology*, 17: 507-513.

Solanki, M., Malviya, M. and Wang, Z. (2016). Actinomycetes bio-inoculants: a modern prospectus for plant disease management. In *Plant Growth Promoting Actinobacteria,* (Eds) Subramaniam, G., 63-81. Retrieved from http://www.researchgate.net/publication/3037827 01_Actinomycetes_Bio-inoculants_A_Modern_Prospectusfor_Plant_Disease_Management

Souagui, Y., Tritsch, D., Grosdemange-Billiard, C. and Kecha, M. (2015). Optimization of antifungal production by an alkaliphilic and halotolerant actinomycete, *Streptomyces* sp. SY-BS5, using response surface methodology. *Journal de Mycologie Medicale*, 25: 108-115.

Taechowisan, T., Wanbanjob, A., Tuntiwachwuttikul, P. and Taylor, W. C. (2006). Identification of *Streptomyces* sp. Tc022, an endophyte in *Alpinia galanga*, and the isolation of actinomycin D. *Annals of Microbiology*, 56: 113-117.

Tamreihao, K., Nimaichand, S., Chanu, S. B., Devi, K. A., Lynda, R., Jeeniita, N. and Ningthoujam, D. S. (2018). Acidotolerant *Streptomyces* sp. MBRL 10 from limestone quarry site showing antagonism against fungal pathogens and growth promotion in rice plants. *Journal of King Saud University –Science*, 30: 143-152.

Tamreihao, K., Ningthoujam, D. S., Nimaichand, S., Singh, E. S., Reena, P., Singh, S. H. and Nongthomba, U. (2016). Biocontrol and plant growth promoting activities of a *Streptomyces corchorusii* strain UCR3-16 and preparation of powder formulation for application as biofertilizer agents for rice plant. *Microbiological Research*, 192: 260-270.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30: 2725–2729.

Thampi, A. and Bhai, R. S. (2017). Rhizosphere actinobacteria for combating *Phytophthora capsici* and *Sclerotium rolfsii*, the major soil borne pathogens of black pepper (*Piper nigrum* L.). *Biological Control*, 109: 1-13.

Tolba, S. T., Ibrahim, M., Amer, E. A. and Ahmed, D. A. (2019). First insights into salt tolerance improvement of Stevia by plant growth-promoting *Streptomyces* species. *Archives of Microbiology*, 1-12. https://doi.org/https://doi.org/10.1007/s00203-019-01696-y.

Toumatia, O., Compant, S., Yekkour, A., Goudjal, Y., Sabaou, N., Mathieu, F., Sessitsch, A. and Zitouni, A. (2016). Biocontrol and plant growth promoting properties of *Streptomyces mutabilis* strain IA1 isolated from a Saharan soil on wheat seedlings and visualization of its niches of colonization. *South African Journal of Botany*, 105: 234-239.

Toumatia, O., Yekkour, A., Goudjal, Y., Riba, A., Coppel, Y., Mathieu, F., Sabaou, N. and Zitouni, A. (2015). Antifungal properties of an actinomycin D-producing strain, *Streptomyces* sp. IA1, isolated from a Saharan soil. *Journal of Basic Microbiology*, 55: 221–228.

Ventura, M., Canchaya C., Tauch, A., Chandra, G., Fitzgerald, GF., Chater, KF. and van Sinderen, D. (2007) Genomics of Actinobacteria: tracing the evolutionary history of an ancient phylum. *Microbiology and Molecular Biology Reviews*, 71: 495-548.

Vijayabharathi, R., Gopalakrishnan, S., Sathya, A., Srinivas, V. and Sharma, M. (2018). Deciphering the tri-dimensional effect of endophytic *Streptomyces* sp. on chickpea for plant growth promotion, helper effect with *Mesorhizobium ciceri* and host-plant resistance induction against *Botrytis cinerea*. *Microbial Pathogenesis*, 122: 98-107.

Wang, X., Zhang, M., Gao, J., Pu, T., Bilal, M., Wanga, Y. and Zhang, X. (2018). Antifungal activity screening of soil actinobacteria isolated from Inner Mongolia, China. *Biological Control,* 127: 78-84.

Williams, S. T. and Cross, T. (1971). Actinomyces. *Methods in Microbiology,* 4: 295-334.

Wonglom, P., Suwannarach, N., Lumyong, S., Ito, S., Matsui, K. and Sunpapao, A. (2019). *Streptomyces angustmyceticus* NR8-2 as a potential microorganism for the biological control of leaf spots of *Brassica rapa* subsp. pekinensis caused by *Colletotrichum* sp. and *Curvularia lunata*. *Biological Control*, 138: 1-7.

Xu, B., Chen, W., Wu, Z., Long, Y., and Li, K. (2015). A novel and effective *Streptomyces* sp. N2 against various phytopathogenic fungi. *Applied Biochemistry and Biotechnology*, 177: 1338–1347.

Xu, T., Cao, L., Zeng, J., Franco, C. M. M., Yang, Y., Hu, X., Liu, Y., Wang, X., Gao, Y., Bu, Z., Shi, L., Zhou, G., Zhou, Q., Liu, X. and Zhu, Y. (2019). The antifungal action mode of the rice endophyte *Streptomyces hygroscopicus* OsiSh-2 as a potential biocontrol agent against the rice blast pathogen. *Pesticide Biochemistry and Physiology*, 160: 58-69.

Xue, L., Xue, Q., Chen, Q., Lin, C., Shen, G. and Zhao, J. (2013). Isolation and evaluation of rhizosphere actinomycetes with potential application for biocontrol of Verticillium wilt of cotton. *Crop Protection,* 43: 231-240.

Yadav, A. N., Verma, P., Kumar, S., Kumar, V., Kumar, M., Chellammal, T., Sugitha, K., Singh, B. P., Saxena, A. K. and Dhaliwal, H. S. (2018). Actinobacteria from rhizosphere: molecular diversity, distributions, and potential biotechnological applications. In *New and Future Developments in Microbial Biotechnology and Bioengineering,* (Eds) Singh, B. P., Gupta, V. K. and Passari, A. K. 13-41. Retrieved from https://www.sciencedirect.com/science/article/pii/B9780444639943000023

Yadav, N., and Yadav, A. N. (2019). Actinobacteria for sustainable agriculture. *Journal of Applied Biotechnology and Bioengineering*, 6: 38–41.

Yandigeri, M. S., Malviya, N., Solanki, M. K., Shrivastava, P. and Sivakumar, G. (2016). Chitinolytic *Streptomyces vinaceusdrappus* S5MW2 isolated from Chilika lake, India enhances plant growth and biocontrol efficacy through chitin supplementation against *Rhizoctonia solani*. *World Journal of Microbiology and Biotechnology,* 31: 1217–1225.

Yang, Y., Wu, Z., and Li, K. (2019). The peculiar physiological responses of *Rhizoctonia solani* under the antagonistic interaction coupled by a novel antifungalmycin N2 from *Streptomyces* sp. N2. *Archives of Microbiology,* 201: 787–794.

Yu, J., Liu Q., Liu Q., Liu, X., Sun, Q., Yan, J., Qi, X., and Fan, S. (2008). Effect of liquid culture requirements on antifungal antibiotic production by *Streptomyces rimosus* MY02. *Bioresource Technology,* 99: 2087-2091.

Zeng, J., Xu, T., Cao, L., Tong, C., Zhang, X., Luo, D., Han, S., Pang, P., Fu, W., Yan, J., Liu, X. and Zhu, Y. (2018). The role of iron competition in the antagonistic action of the rice endophyte *Streptomyces sporocinereus* OsiSh-2 against the pathogen *Magnaporthe oryzae*. *Microbial Ecology,* 76: 1021–1029.

Zhang, R., Han, X., Xia, Z., Luo, X., Wan, C., and Zhang, L. (2017). *Streptomyces luozhongensis* sp. nov., a novel actinomycete with antifungal activity and antibacterial activity. *Antonie van Leeuwenhoek,* 110: 195-203.

Zhao, Y. (2010). Auxin biosynthesis and its role in plant development. *Annual Review of Plant Biology,* 61: 49–64.

Zheng, X., Wang, J., Chen, Z., Zhang, H., Wang, Z., Zhu, Y. and Liu, B. (2019). A *Streptomyces* sp. strain: isolation, identification, and potential as a biocontrol agent against soilborne diseases of tomato plants. *Biological Control,* 136: 1-10.

In: A Closer Look at Actinomycetes ISBN: 978-1-53617-046-7
Editors: A. A. Mohamed Hatha et al.

Chapter 6

DIVERSITY AND METABOLITES OF ENDOPHYTIC ACTINOMYCETES FROM PLANT ROOTS

Nattakorn Kuncharoen and Somboon Tanasupawat*
Department of Biochemistry and Microbiology,
Faculty of Pharmaceutical Sciences, Chulalongkorn University,
Bangkok, Thailand

ABSTARCT

Actinomycetes are aerobic, Gram-positive, filamentous bacteria presented true branching mycelia and high mol% guanine and cytosine (G+C) content in the genome. Over the last decade, actinomycetes which lived in unexplored habitats have obtained significant attention because of their large biodiversity and proper metabolites with pharmaceutical, agricultural and industrial values. Plant endosphere is an enormous micro-ecosystem where different niches can be resided by numerous different microorganisms, and it contributed a valuable source of actinomycetes. Endophytic actinomycetes that inhabit living root tissues of plants are a

* Corresponding Author's Email: Somboon.T@chula.ac.th.

relatively untapped source of novel species and potential bioactive metabolites. Based on the 16S rRNA gene sequences, the endophytic actinomycetes are belonged to members of *Streptomycetaceae*, *Streptosporangiaceae*, *Micromonosporaceae*, *Thermomonosporaceae*, *Pseudonocardiaceae* and *Actinosynnemataceae*. Presently, the strains associated with plant roots were *Streptomyces*, *Micromonospora*, *Microbispora*, *Nocardia*, *Nocardioides*, *Pseudonocardia* and *Streptosporangium*; and the new genera were *Plantactinospora*, *Phytohabitans*, *Actinophytocola* and *Allostreptomyces*. Furthermore, many groups of secondary metabolites were produced by plant-derived actinomycetes such as peptides, flavonoids, polyketides, macrolides, terpenes and alkaloids. These metabolites exhibited various biological activities: antibacterial, antifungal, anti-phytopathogens, immunosuppressant, anti-tumour and anti-cancer. This chapter highlights the achievement of isolation, identification, diversity and bioactive metabolites from endophytic actinomycetes associated with plant roots.

INTRODUCTION

Actinomycetes are Gram-stain-positive, aerobic, filamentous bacteria with high guanine and cytosine (G + C) content (>55 mol%) in genomic DNA which formed true branching hyphae, distinguishing from fungi by having no nucleus and smaller than fungi (3-8 μm in diameter), belonged to the phylum *Actinobacteria*. They produce monomeric spores or sporangia and mycelia with or without fragmentation. Most of them are saprophytes which played an important role in the recycling of complex organic matters consisting of dead plants, animals, algae and fungi, resulting in humus formation (Lechevalier and Lechevalier 1967), however, some are mutualistic or parasitic associated with plants and animals (Goodfellow and Williams 1983).

The phylum *Actinobacteria* is recently categorised into 6 classes, 23 orders, 50 families and 221 genera (Ludwig et al. 2012). Actinomycetes are the largest group of bacteria in class *Actinobacteria*. They are known as a key producer of more than half biologically active metabolites and are consequently interesting for the pharmaceutical industry (Bérdy 2005). Although the discovery rate of new compounds has declined, searching for new antibiotics has been continuously increased because of the rapid spread

of antibiotic-resistant pathogenic microorganisms causing life-threatening infections. More than 10,000 bioactive metabolites derived from actinomycete species, 7,600 metabolites produced by *Streptomyces* and 2,400 produced by rare actinomycetes. These actinomycete compounds represented the greatest group (45%) of microbial biological active metabolites, for instance, gentamicin from *Micromonospora purpurea*, streptomycin from *Streptomyces griseus*, salinomycin from *S. albus*, actinomycin from *S. actinomycinicus*, erythromycin from *Saccharopolyspora erythraea*, rifamycin from *Amycolatopsis mediterranei*, teicoplanin from *Actinoplanes teichomyceticus* and vancomycin from *Amycolatopsis orientalis* (Bérdy 2005, 2012). It has been reported that there are many genera of rare actinomycetes were produced antimicrobial agents composing of *Actinokineospora*, *Actinosynnema*, *Acrocarpospora*, *Actinomadura*, *Amycolatopsis*, *Actinoplane*s, *Catenuloplanes*, *Ctyptosporangium*, *Dactylosporangium*, *Kineosporia*, *Kutzneria*, *Micromonospora*, *Microtetraspora*, *Microbispora*, *Nonomuraea*, *Nocardia*, *Pseudonocardia*, *Thermomonospora*, *Thermobifida*, *Salinispora*, *Saccharomonospora*, *Saccharopolyspora*, *Spirilliplanes*, *Streptosporan-gium* and *Virgosporangium* (Lazzarini et al. 2000).

Actinomycetes are widely distributed in terrestrial soils. Recently, numerous novel species have been discovered in other habitats such as plant tissues (Taechowisan and Lumyong 2003), root nodules of Leguminosae (Trujillo et al. 2006), roots of medicinal plants (Kuncharoen et al. 2019a), marine sponges (Supong et al. 2013) and near-shore sediment (Phongsopitanun et al. 2015). The actinomycetes living as endosymbiont inside various part of plant tissues, roots, stems, and leaves, without having negative effects on host plants are called "endophytic actinomycetes" (Hallmann et al. 1997, Schulz and Boyle 2006). Since the genus, *Frankia* was firstly isolated from non-legume root nodules in early 1886 (Okazaki 2003). Endophytic actinomycetes have been increasingly attended and plant species were accepted for the sources of actinomycetes. Additionally, many reports showed the discovery of actinomycetes in various types of plants including crops, woody, mangrove, indigenous, medicinal, Leguminosae and actinorhizal plants (Trujillo et al. 2006, Supong et al. 2016).

Streptomyces strains were generally found as the major endophytic actinomycetes whilst *Micromonospora*, *Microbispora*, *Nocardia*, *Nocardioides*, *Pseudonocardia* and *Streptosporangium* were considered as common strains (Qin et al. 2009a). However, it has been reported that the strains in rare actinomycete genera, *Actinomadura*, *Actinomycetospora*, *Actinopolymorpha*, *Amycolatopsis*, *Nocardiopsis*, *Nonomuraea*, *Polymorphospora*, *Promicromonospora* (Kaewkla and Franco 2013), *Plantactinospora* (Zhu et al. 2012), *Sphaerisporangium* (Xing et al. 2015b), *Phytomonospora* (Li et al. 2012), *Streptacidiphilus*, *Herbidospora*, *Tsukamurella* and *Herbiconiux* (Kim et al. 2012), were isolated from herbaceous plants, medicinal plants, tropical plants, ferns, clubmosses, grasses, palms and rice. Moreover, some interesting compounds have been isolated from the endophytic actinomycetes, for example, polyketides (Portugal 2003), terpenes (Ding et al. 2015), macrolides (Inahashi et al. 2015) and alkaloids (Inahashi et al. 2011) which showed various biological activities including antifungal, antibacterial, anti-phytopathogen, anti-cancer, antioxidant and immunosuppressant (Strobel et al. 2004). In this chapter, the presence of remarkably diverse endophytic actinomycetes consisting of their isolation methods, taxonomic studies, secondary metabolites and biological activities are described.

2. SAMPLE COLLECTION AND SURFACE STERILISATION

Each plant sample for the isolation of endophytic actinomycetes were commonly chosen based on its local ethnobotanical properties, comprising its antibacterial, antifungal, anticancer, insecticidal, wound-healing and plant growth promotion properties (Strobel et al. 2004). No history emphasised that the plants had ever been previously used the studies of endophytic actinomycetes. Roots, stems, and leaves of each healthy host plant dug out and placed in sterile plastic bags, taken to the laboratory, and subjected to the isolation methods within 96 hours (Qin et al. 2009a).

Table 1. Surface sterilisation protocols for plant samples

Sterilisation techniques and reagents	Incubation time	Reference
1. a) Wash with running tap water		Qin et al. (2009a)
b) Soak in 5% (v/v) sodium hypochlorite (NaOCl)	4-10 min	
c) Wash in 2.5% (w/v) thiosulfate ($Na_2S_2O_3$)	10 min	
d) Wash in 75% (v/v) ethanol	5 min	
e) Rinse with sterile water three times	1 min/time	
f) Soak in 10% (w/v) sodium bicarbonate ($NaHCO_3$)	10 min	
g) Dry at 100°C	15 min	
2. a) Wash with running tap water		Zhao et al. (2011)
b) Sonicate at 150 w	10 min	
c) Rinse with 0.1% (v/v) Tween 20	30 sec	
d) Wash in 75% (v/v) ethanol	5 min	
e) Soak in 2% (v/v) NaOCl	5 min	
f) Rinse with 10% (w/v) $NaHCO_3$	10 min	
g) Rinse with sterile distilled water three times	1 min/time	
3. a) Wash with sterile distilled water		Trujillo et al. (2006)
b) Wash in 2.5% (w/v) Mercuric chloride ($HgCl_2$)	2 min	
c) Rinse with sterile distilled water several times	1-3 min/time	
d) Air-dry in a sterile condition		
4. a) Wash with running tap water	1-2 min	Bunyoo et al. (2009)
b) Wash in 70% (v/v) ethanol	10 min	
c) 1% (w/v) NaClO	15 min	
d) Air-dry in laminar air flow		
5. a) Wash with running tap water		Coombs and Franco (2003)
b) Sonicate at 150 w	10-15 min	
c) Soak in 99% (v/v) ethanol	1 min	
d) Wash in 3.125% (v/v) NaOCl	6 min	
e) Rinse with 99% (v/v) ethanol	0.5 min	
f) Rinse with sterile reverse osmosis (RO) water	3 min	
6. a) Wash with running tap water		Kuncharoen et al. (2018)
b) Wash in 75% (v/v) ethanol	3 min	
c) Soak in 3% (v/v) NaOCl	10 min	
d) Rinse with sterile distilled water three times	1 min/time	
e) Wash in 10% (w/v) $NaHCO_3$	10 min	
f) Air-dry in laminar flow		

Root, stem and leave samples do not require exclusive pre-treatment because their surfaces do not include hydrophobic substances such waxes of leaves. Washing with running tap water and brushing is enough; nonetheless, sonification may use for elimination of soil particle and organic matter from the plant samples before various surface sterilisation (Coombs and Franco 2003) as described in Table 1.

After the surface sterilisation, it has a further check for testing the effect of the sterilising agents on the surfaced fungi or bacteria by imprinting the surface sterilised plant samples on nutrient agar or spreading aliquots of the last water of rinsing onto nutrient media (Schulz et al. 2002). If no microbial growth occurs on the medium, the surface sterilisation method is considered complete (Coombs and Franco 2003).

3. Isolation Methods and Cultivation

There are a lot of different procedures have been used to isolate the endophytic actinomycetes. The isolation methods, various types of isolation media, host plants, types of tissues, sampling season, geography and habitat, surface sterilising agents and culture conditions are important because they affect the number and the genera of endophytic actinomycetes which can be isolated from the plant tissues (Takahashi and Omura 2003, Golinska et al. 2015).

The isolation procedures usually included the collection of plant samples, surface sterilisation and culture on the growth media. The plant tissues were firstly washed with tap water, brushed, sonicated to remove soils and organic matters, cut into 2-5 cm and surface sterilised with different surface sterilising agents, common disinfectants or strong oxidants, such as 70-90% (v/v) ethanol, 2-6% (w/v) NaOCl, 2.5% (w/v) sodium thiosulfate ($Na_2S_2O_3$), 5% (w/v) sodium chlorate ($NaClO_3$) and 10% (w/v) sodium bicarbonate ($NaHCO_3$) to suppress the growth of endophytic fungi. The plant samples were finally rinsed three times with sterile distilled water to eliminate the sterilising agents, pestle in sterile mortars with appropriated reagents, e.g., 0.85% (w/v) NaCl, 4% (w/v) sucrose and phosphate buffer,

to obtain the plant suspensions. The suspensions were serially diluted as 10^{-1} to 10^{-3} and spread onto the isolation media (Table 2) supplemented with 25 μg nalidixic acid mL^{-1} and 50 μg cycloheximide mL^{-1} to inhibit Gram-negative bacteria and fungi, respectively (Coombs and Franco 2003, Qin et al. 2009a, Kuncharoen et al. 2018). The isolated plates were incubated at 28±2 °C for 28 days. After the plant-derived actinomycete colonies grown on the incubated plates, they were purified on yeast extract-malt extract agar medium (International *Streptomyces* Project No. 2, ISP 2) (Shirling and Gottlieb 1966) by streak plate technique. After growth was observed, the endophytic actinomycete colonies were maintained in ISP 2 agar slants for further study and lyophilised for long-term preservation.

Table 2. Isolation media and compositions

Media	Composition	Reference
Tap water-Yeast extract agar (TWYE)	0.25 g yeast extract, 0.5 g of K_2HPO_4, 18 g agar per litter of tap water, pH 7.0-7.4 and supplemented with 50 μg·mL^{-1} of each nalidixic acid and benomyl	Coombs and Franco (2003)
Flour-Yeast extract-Sucrose-Casein hydrolysate agar	6 g plain flour, 0.3 g yeast extract, 0.3 g sucrose, 0.3 g $CaCO_3$, 18 g of agar per litter of RO water, pH 7.0-7.2 and supplemented with 50 μg·mL^{-1} of each nalidixic acid and benomyl	Coombs and Franco (2003)
Flour-Calcium Carbonate agar	4 g plain flour, 0.4 g $CaCO_3$, 16 g agar per litter of RO water, pH 7.0-7.2 and supplemented with 50 μg·mL^{-1} of each nalidixic acid and benomyl	Coombs and Franco (2003)
Yeast extract-Casein hydrolysate agar (YECD)	0.3 g yeast extract, 0.3 g D-glucose, 2 g K_2HPO_4, and 18 g of agar per litter of RO water, pH 7.0-7.4 and supplemented with 50 μg·mL^{-1} of each nalidixic acid and benomyl	Coombs and Franco (2003)
2.5% Water agar (WA)	25 g agar per litter of tap water, pH 7.0-7.2 and supplemented with 50 μg cycloheximide mL^{-1} and 25 μg nalidixic acid mL^{-1}	Okazaki (2003)
Starch Casein Nitrate agar (SCN)	10 g soluble starch, 0.3 g casein, 2 g KNO_3, 2 g NaCl, 2 g K_2HPO4, 0.05 g $MgSO_4·7H_2O$, 0.02 g $CaCO_3$, 0.01 g $FeSO_4·7H_2O$, 15 g agar per litter of distilled water, pH 7.0-7.2 and supplemented with 50 μg cycloheximide mL^{-1} and 25 μg nalidixic acid mL^{-1}	Küster and Williams (1964)
Glycerol Asparagine agar	12.5 g glycerol, 1 g L-arginine, 1 g K_2HPO_4, 1 g NaCl, 0.05 g $MgSO_4·7H_2O$, 18 g agar per litter of tap water, pH 7.0-7.2 and supplemented with 50 μg cycloheximide mL^{-1} and 25 μg nalidixic acid mL^{-1}	Arai (1975)
Gauze mineral medium no. 1 agar	20 g soluble starch, 0.5 g K_2HPO_4, 0.5 g $MgSO_4·7H_2O$, 1 g KNO_3, 0.4 g NaCl, 0.01 g $FeSO_4·7H_2O$, 15 g agar per litter of	Gauze et al. (1983)

Table 2. (Continued)

Media	Composition	Reference
	distilled water, pH 7.2-7.4 and supplemented with 50 μg cycloheximide mL^{-1} and 25 μg nalidixic acid mL^{-1}	
Sodium propionate agar	1 g sodium propionate, 0.2 g L-asparagine, 0.9 g KH_2PO_4, 0.6 g K_2HPO_4, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.2 g $CaCl_2 \cdot 2H_2O$, 15.0 g agar per litter of distilled water, pH 7.2-7.4 and supplemented with 50 μg nalidixic acid mL^{-1} and 100 μg nystatin mL^{-1}	Qin et al. (2009a)
Cellulose-proline agar	2.5 g cellulose, 2 g sodium pyruvate, 0.25 g KNO_3, 1.0 g proline, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.2 g K_2HPO_4, 0.5 g $CaCl_2$, 10 mg $FeSO_4 \cdot 7H_2O$, 15.0 g agar per litter of distilled water, pH 7.0-7.4 and supplemented with 50 μg nalidixic acid mL_{-1} and 100 μg nystatin mL_{-1}	Qin et al. (2009a)
Trehalose-proline agar	5 g fucose, 1 g proline, 1 g $(NH_4)_2SO_4$, 1 g NaCl, 2.0 g $CaCl_2$, 1 g K_2HPO_4, 1 g $MgSO_4 \cdot 7H_2O$, 15.0 g agar per litter of distilled water, pH 7.0-7.4 and supplemented with 50 μg nalidixic acid mL^{-1} and 100 μg nystatin mL^{-1}	Qin et al. (2009a)
Xylan-arginine agar	2.5 g xylan, 1 g L-arginine, 1 g $(NH_4)_2SO_4$, 2 g $CaCl_2$, 1 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 10 mg $FeSO_4 \cdot 7H_2O$, 15.0 g agar per litter of distilled water, pH 7.0-7.4 and supplemented with 50 μg nalidixic acid mL^{-1} and 100 μg nystatin mL^{-1}	Qin et al. (2009a)

4. IDENTIFICATION METHODS

Endophytic actinomycete strains were identified based on polyphasic taxonomic approaches comprising phenotypic, chemotaxonomic and genotypic characteristics. The phenotypic characteristics were determined to employ considerable standard methods as earlier described by Williams and Cross (1971) and Arai (1975). Cultural characteristics of the plant-derived actinomycete isolates were assessed by culturing on various media, yeast extract-malt extract agar (ISP No. 2), oatmeal agar (ISP No. 3), inorganic salt starch agar (ISP No. 4), glycerol asparagine agar (ISP No. 5), peptone yeast extract iron agar (ISP No. 6) and tyrosine agar (ISP No. 7), at 28 ± 2°C for 14 days (Shirling and Gottlieb 1966). The colour systems such as *Colour Harmony Manual* (Jacobson et al. 1958) and ISCC-NBS (Kelly 1964) were used to name and design the colour of colonies and diffusible pigments. Cell morphology was observed using scanning electron microscopy (SEM) after

cultivation on proper agar media at 28 ± 2°C for 7-14 days. Ranges of temperature, pH and NaCl concentration for growth of strains were evaluated on ISP 2 agar at 28 ± 2°C for 14 days. The ISP 9 medium supplemented with 1% (w/v) of different carbohydrates was used to determine the utilisation of sole carbon sources. Other biochemical properties including starch hydrolysis, nitrate reduction, gelatin liquefaction, coagulation and peptonisation of milk, and H_2S production were examined on ISP 4 agar, ISP 8 broth (0.5% peptone, 0.3% beef extract, 0.1% KNO_3, pH 7.0), glucose-peptone-gelatine medium, 10% (w/v) skimmed milk and ISP 6 agar, respectively (Shirling and Gottlieb 1966).

For chemotaxonomic properties, the isomers of diaminopimelic acid in cell-wall peptidoglycan were prepared and analysed using the thin-layer chromatography (TLC) method of Staneck and Roberts (1974). Reducing sugars of whole-cell hydrolysates were extracted following the method as described previously (Staneck and Roberts 1974) and analysed by the HPLC method of Mikami and Ishida (1983). The acyl type of muramic acid in peptidoglycan was determined by the colourimetric method as previously described by Uchida and Aida (1984). Polar lipids in cells were extracted and identified by 2-dimensional TLC following the method of Minnikin et al. (1977). Methyl esters of fatty acids were prepared according to the method of Sasser (1990) and analysed by gas chromatography (MIDI, Sherlock Microbial Identification System, TSBA6 Sherlock Version 6.2B, USA). Isoprenoid quinones were extracted according to the method of Collins et al. (1977) and analysed by HPLC and mass spectrometer equipped with a Pegasil ODS column (Senshu, Tokyo, Japan) as previously described by Tamaoka et al. (1983). The presence of mycolic acids was extracted and monitored by TLC as described earlier by Tomiyasu (1982).

For the genotypic characteristics, the 16S rRNA gene sequence was amplified using the primers, 27F and 1492R, under the condition of initial incubation 1 min at 95°C, followed by 30 cycles of 1 min at 95°C, 1 min at 50°C, 1.5 min at 72°C, followed by 2 min final extension at 72°C (Suriyachadkun et al. 2009) and sequenced using the universal primers as previously proposed by Lane (1991). The 16S rRNA gene sequence was manually edited using BioEdit software (Hall 1999) and determined the

similarity values among the most related neighbours on EzBiocloud server (Yoon et al. 2017). The 16S rRNA sequence was multiple aligned with selected reference sequences obtained from GenBank/EMBL/DDBJ databases using CLUSTAL W version 2.0 (Larkin et al. 2007). The aligned sequences were manually edited and used to construct the phylogenetic tree based on various tree-making algorithms, neighbour-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Fitch 1971) by the molecular evolutionary genetic analysis (MEGA) version 7.0 (Kumar et al. 2016). The phylogenetic distance matrix was calculated with the Kimura-2-parameter model (Kimura 1980). The confidence limits of the phylogeny were evaluated using bootstrap analysis based on 1,000 replications (Felsenstein 1985).

Currently, the whole-genome sequence analysis is publicly available. It has become a new method which provided reproducible, trustworthy, and highly informative for the identification of actinomycetes (Nouioui et al. 2018). The G+C content of the DNA was analysed and obtained from the whole-genome sequence using JSpeciesWS server (Ritcher et al. 2016). Average nucleotide identity (ANI) which is the similarity values between the genomes of the isolate and its closely related neighbours was pairwise calculated using ANI-Blast (ANIb) and ANI-MUMmer (ANIm) algorithms (Ritcher et al. 2009) on the server of JSpeciesWS (Ritcher et al. 2016). The digital DNA-DNA hybridisation (dDDH) was calculated on the Genome-to-Genome Distance Calculator (GGDC 2.1) using the BLAST+ method (Meier-Kolthoff et al. 2013).

5. Diversity of Endophytic Actinomycetes

There are over 300,000 different plant species in the kingdom Plantae (Strobel and Daisy 2003). Diverse endophytic actinomycetes have been explored in aquatic and terrestrial plant species. Actinomycetes colonised in various parts of healthy plant tissues of an extensive range of plants, e.g., crop plants, medicinal plants, tropical plants, bryophytes, aquatic plants and sea grasses (Qin et al. 2011). Recently, the diversity of endophytic actinomycetes derived from these plants is continuously under investigation

by many researchers around the world (Golinska et al. 2015). Reports of the diversity of plant-derived actinomycetes are continuing due to the significance of bioactive metabolites production and novel actinomycetes population. Wide ranges of highly common and rare genera of actinomycetes have been documented as endophytes from different plants.

Endophytic actinomycetes can associate with host plants at an early stage of the plant development (Hasegawa et al. 2006). Minamiyama et al. (2003) used scanning electron microscopic technique to observe the hyphae of *Streptomyces galbus* that spread on the surface of the tissue-culture medium which *Rhododendron* (Ericaceae) seedlings were growing, grew on the surface of leaves and infested leaf tissues via stroma. Further, the authors noticed that the mycelia of *S. galbus* were present separately or in colonies in intercellular spaces but not inside in epidermal or mesophyll cell within host leaves.

Most of the endophytic actinomycetes have been isolated from roots, stems and leaves, respectively (Qin et al. 2009a). Taechowisan and Lumyong (2003) also summarised that plant roots were the greatest sources of endophytic actinomycetes. The authors isolated the actinomycete strains from all parts of plant tissues of 36 plant species in Thailand and found that most isolates (64%) were recovered from roots sequential by leaves (29%) and stems (6%). The occurrence rate of actinomycetes is highly in roots as compared to other tissues is very general. Hence, this indicated that actinomycetes are natural inhabitants of soil which easily contact with the plant roots and may form the symbiosis in their host by entering plant tissues.

The findings of Nimnoi et al. (2010) recommended that the diversity of endophytic actinomycetes differs in different plant locations; moreover, Strobel and Daisy (2003) also reported that the larger diverse of endophytes usually occurs in the tropical and temperate zone. Du et al. (2013) isolated the endophytic actinomycetes from 37 medicinal plants and reported 600 isolates belonging to 34 genera. The researchers illustrated that there was no direct relationship between the actinomycetes flora and host plants regarding the carbon sources utilisation, enzymatic activity and acids production,

rather the physiological properties of the isolates were associated with the geographical distribution of their host plants.

Table 3. Novel actinomycete species derived from plant tissues

Species	Host plant	References
Actinoallomurus oryzae	Roots of *Oryza sativa* L. KDML 105	Indananda et al. (2011)
Actinoallomurus acaciae	Roots of *Acacia auriculiformis*	Thamchaipenet et al. (2010)
Actinomadura syzygii	Roots of *Syzygium cumini* L. Skeels	Rachniyom et al. (2015a)
Actinophytocola oryzae	Roots of *Oryza sativa* L. RD6	Indananda et al. (2010)
Amycolatopsis samaneae	Roots of *Samanea saman* (Jacq.) Merr	Duangmal et al. (2011)
Amycolatopsis stemonae	Stems of *Stemona* sp.	Klykleung et al. (2015)
Actinomycetospora endophytica	Roots of *Podochilus microphyllus* Lindl.	Sakdapetsiri et al. (2018)
Asanoa endophytica	Rhizomes of *Boesenbergia rotunda*	Niemhom et al. (2016a)
Jiangella endophytica	Rhizomes of *Kaempferia elegans*	Niemhom et al. (2019)
Micromonospora costi	Leaves of *Costus speciosus*	Thawai (2015)
Micromonospora endophytica	Leaves of *Oryza sativa* L.	Thanaboripat et al. (2015)
Micromonospora oryzae	Roots of *Oryza sativa* L.	Kittiwongwattana et al. (2015)
Nonomuraea syzygii	Roots of *Syzygium cumini* L. Skeels	Rachniyom et al. (2015b)
Phytohabitans kaempferiae	Leaves of *Kaempferia larsenii*	Niemhom et al. (2016b)
Pseudonocardia acaciae	Roots of *Acacia auriculiformis*	Duangmal et al. (2009)
Sphaerisporangium rufum	Roots of *Oryza sativa* L.	Mingma et al. (2014)
Streptomyces oryzae	Stems of *Oryza sativa* L.	Mingma et al. (2015)
Streptomyces phyllanthi	Stems of *Phyllanthus amarus*	Klykleung et al. (2016)
Actinoallomurus bryophytorum	Moss	Li et al. (2015)
Actinomadura flavalba	Leaf of *Maytenus austroyunnanensis*	Qin et al. (2009b)
Allonocardiopsis opalescens	Fruit of *Lonicera maackii* (Rupr.) Maxinm	Du et al. (2013)
Amycolatopsis jiangsuensis	Stem of *Dendranthema indicum*	Xing et al. (2013)
Glycomyces endophyticus	Root of *Carex baccans* Nees	Qin et al. (2008)
Herbidospora osyris	*Osyris wightiana* Wall. ex Wight	Li et al. (2009)
Jiangella alba	Stem of *Maytenus austroyunnanensis*	Qin et al. (2009c)
Microbispora bryophytorum	Moss	Li et al. (2015)
Micromonospora coriariae	Root nodules of *Coriaria myrtifolia*	Trujillo et al. (2006)
Micromonospora lupini *Micromonospora saelicesensis*	Root nodules of *Lupinus angustifolius*	Trujillo et al. (2007)
Micromonospora pisi	Root nodules of *Pisum sativum*	Garcia et al. (2010)
Micromonospora globbae	Root of *Globba winitii* C. H. Wright	Kuncharoen et al. (2018)

Species	**Host plant**	**References**
Micromonospora azadirachtae	Root of *Azadirachta indica* var. *siamensis*	Kuncharoen et al. (2019a)
Micromonospora radicis	Root of *Azadirachta indica* var. *siamensis*	Kuncharoen et al. (2019c)
Micromonospora radicis	Root of *Azadirachta indica* var. *siamensis*	Kuncharoen et al. (2019c)
Nocardia endophytica	Stem of *Jatropha curcas* L.	Xing et al. (2011)
Nocardioides panzhihuaensis	Stem of *Jatropha curcas* L.	Qin et al. (2012a)
Phytoactinopolyspora endophytica	Root of *Glycyrrhiza uralensis* F.	Li et al. (2015)
Phytohabitans suffuscus	Root of unknown orchid from Okinawa, Japan	Inahashi et al. (2010)
Phytomonospora endophytica	Root of *Artemisia annua* L.	Li et al. (2011)
Plantactinospora endophytica	Leaf of *Campotheca acuminata*	Zhu et al. (2012)
Promicromonospora xylanilytica	Leaf of *Maytenus austroyunnanensis*	Qin et al. (2012b)
Pseudonocardia endophytica	*Lobelia clavata*	Chen et al. (2009)
Saccharopolyspora dendranthemae	Stem of *Dendranthema indicum*	Zhang et al. (2013)
Sphaerisporangium dianthi	Root of *Dianthus chinensis* L.	Xing et al. (2015)
Tamaricihabitans halophyticus	Root of *Tamarix chinensis* Lour	Qin et al. (2015)
Wangella harbinensis	Root of *Glycine max* (L.) Merr	Jia et al. (2013)

Functional biodiversity measures might be more credible and dynamic than taxonomic measures conducive to understand the mechanism of diversity and its effects on the plant-endophyte interactions (Parrent et al. 2010). The plant species can be divided into three clusters including high, moderate and low diversity of endophytic actinomycetes relied on the generic diversity analysis. The actinomycetes associated with plants show high functional diversity based on 44 different features consisting of catabolic and plant-growth promotion features which may characterise as a key criterion for prosperous habitation of endophytes within plant-endosphere. In addition, the stress-tolerance features were a more predicting measure of the functional diversity of endophytic actinomycetes (El-Shatoury et al. 2013).

Janso and Carter (2010) studied the diversity of endophytic actinomycetes from native tropical plants collected from Papua New Guinea (Solomon Island and Mborokua Island) using the ribotyping technique. The authors reported that isolates were prevalently found in roots followed by leaves and were classified into 6 families and 17 genera. *Streptomyces* was accounted for the major genus which commonly found in plant-endosphere (Qin et al. 2009a, Zhao et al. 2011, Shutsrirung et al. 2013) whilst others comprised genera such as *Micromonospora*, *Saccharopolyspora*, *Nocardia*, *Oerskovia*, *Actinopolyspora*, *Nonomuraea*, *Streptoverticillium*, *Micro-bispora*, *Streptosporangium*, *Promicromonospora* and *Rhodococcus* (Zhao et al. 2011). Some rare genera of actinomycetes, i.e., *Dietzia*, *Herbiconiux*, *Rathayibacter*, *Tsukamurella*, *Plantactinospora*, *Actinoallomurus*, *Kribbella*, *Promicromonospora*, *Phytomonospora*, *Polymorpho-spora*, *Actinomycetospora*, *Actinomadura* and *Dactylosporangium* were also reported as endophytes (Bunyoo et al. 2009, Kim et al. 2012, Zhu et al. 2012, Li et al. 2012, Kaewkla and Franco 2013). However, many recent studies reported that the genus *Micromonospora* was distributed in various plants comprising leguminous, actinorhizal, medicinal and tropical plant species as same as the genus *Streptomyces* (Valdes et al. 2005, Trujillo et al. 2010, Kuncharoen et al. 2019b).

As mentioned above, plant endosphere is an invaluable residence of diverse actinomycetes. Presently, the endophytic actinomycetes from different tissues of various plant species across the globe were affiliated to a wide range of genera and numerous novel species have been discovered. The most recent new plant-derived actinomycete species were described in Table 3. These exploration open new windows for searching known and novel actinomycetes which can be a further study for biotechnological applications such as drug discovery and plant-growth promotion.

6. SECONDARY METABOLITES AND BIOLOGICAL ACTIVITIES

Endophytic actinomycetes are relatively untapped potential sources of new biological active compounds and continued as the attention for researchers around the world. Metabolites derived from them have been justified to be beneficial in the area of pharmaceutics and agriculture. Most bioactive metabolites were designed into various classes such as polyketides, terpenes, macrolides, aminoglycosides and peptides (Genilloud 2017). The bioactive secondary metabolites which have been isolated from the endophytic actinomycetes from 2003 until now were concluded in Table 4.

Plant endosphere is a myriad of endophytic microorganisms that formed a complex of micro-ecosystem. In recent years, actinomycetes reside in plant tissues are an invaluable repertoire of metabolic potential products (El-Shatoury et al. 2013). *Streptomyces* sp. DSM 11575, isolated from root nodules of *Alnus glutinosa*, produced alnumycin, a novel naphthoquinone antibiotic, which was active against Gram-positive bacteria such as *Bacillus subtilis*, *Arthrobacter crystallopoites*, *Kocuria rhizophila* (*Micrococcus luteus*) and *Rhodococcus* but did not show antimicrobial activity against Gram-negative bacteria including *Escherichia coli*, *Proteus rettgeri* and *Agrobacterium tumefaciens* (Bieber et al. 1998).

Castillo et al. (2002) reported that munumbicins A-D obtaining from *Streptomyces* NRRL 30562, which was isolated from *Kennedia nigriscans*, were represented as new antibiotics with broad-spectrum activity against a lot of human pathogenic bacteria and fungi. Munumbicins generally showed antimicrobial activity against *B. anthracis*, multidrug-resistant *Mycobacterium tuberculosis* and multidrug-resistant *Staphylococcus aureus* (MRSA) while munumbicin A was not active against all tested organisms. Moreover, munumbicin D exhibited the most impressive biological activity against *Plasmodium falciparum* with IC_{50} of 4.5 ± 0.07 ng·mL^{-1}.

Table 4. Bioactive metabolites from endophytic actinomycetes

Metabolite	Chemical class	Species	Activity	Reference
Actinoallolides A-E	Macrolide	*Actinoallomurus flavus*	Anti-trypanosomal	Inahashi et al. (2015)
Antimycin A18	Macrolide	*Streptomyces albidoflavus*	Antifungal	Yan et al. (2010)
Bacaryolanes A-C	Sesquiterpene	*Streptomyces* sp.	Antibacterial	Ding et al. (2015)
Bafilomycins	Macrolide	*Streptomyces* sp.	Cytotoxic	Yu et al. (2011)
Coronamycins	Peptide	*Streptomyces* sp.	Antifungal	Ezra et al. (2014)
Kakadumycin A	Peptide	*Streptomyces* sp.	Antibacterial	Castillo et al. (2003)
Kandenols A-E	Sesquiterpene	*Streptomyces* sp.	Antibacterial, cytotoxic	Ding et al. (2012)
Lupinacidins A-B	Anthraquinone	*Micromonospora lupini*	antitumor	Igarashi et al. (2007)
Pterocidin	Polyketide	*S. hygroscopicus*	Anticancer	Igarashi et al. (2006)
Trehangelins A-C	Polyketide	*Polymorphospora rubra*	Photo-oxidative hemolysis inhibitor	Nakashima et al. (2013)

Maklamicin, a new spirotetronate polyketide, isolated from endophytic *Micromonospora* sp.GMKU 236 was strongly active against Gram-positive bacteria, *K. rhizophila*, *B. subtilus*, *B. cereus*, *S. aureus* and *Enterococcus faecalis* with MIC of 0.2, 1.7, 6.5, 13, and 13 $\mu g \cdot mL^{-1}$, respectively, whilst was slightly active against *C. albicans* with the MIC of 50 $\mu g \cdot mL^{-1}$. Furthermore, maklamicin exhibited moderate cell cytotoxicity against human cervical cancer cells (HeLa) and human breast cancer cells (MCF7) with the IC_{50} of 17 and 34 μM (Igarashi et al. 2011).

Micromonospora lupini Lupac 08, isolated from root nodules of *Lupinus angustifolius*, produced three novel anthraquinones. Lupinacidins A, B and C showed significant inhibitory effects on the invasion of murine colon (26-L5) carcinoma cells without inhibiting cell growth (Igarashi et al. 2007, Igarashi et al. 2011).

Human African trypanosomiasis (HAT), known as sleeping sickness, is caused by infection with parasites, *Trypanosoma brucei rhodesiense* or *T. b. gambiense*. Recently, there are four drugs including pentamidine, suramin, melarsoprol and eflornithine for the treatment of HAT (Otoguro et al. 2008).

However, the resistant parasites have been increased; therefore, endophytic actinomycetes have continuously been for the production of novel anti-trypanosomal medicine. *Streptomyces* sp. SUK 17, isolated from *Cinnamomum zrylanicum* in Malaysia, showed anti-trypanosomal activity against *T. b. brucei* strain BS221 with the IC_{50} of 4.48 $\mu g \cdot mL^{-1}$ (Zin et al. 2011).

Streptosporangium oxazolinicum K07-0460, isolated from unknown orchids in Japan, produced three new alkaloid antibiotics, Spoxazomicins A, B and C (Inahashi et al. 2011). Spoxazomicin A exhibited the highest anti-trypanosomal activity against *T. b. brucei* strain GUTat 3.1 (causative agent of Nagana disease in animals) with IC_{50} of 0.11 $\mu g \cdot mL^{-1}$, which more potent than those of clinically used for anti-trypanosomal drugs14 to 21-fold. Spoxazomicin B also showed anti-trypanosomal activity with an IC_{50} value of 0.55 $\mu g \cdot mL^{-1}$ while spoxazomicin C was weakly active against *T. b. brucei* strain GUTat 3.1 with IC_{50} value around 3 $\mu g \cdot mL^{-1}$.

Five novel anti-trypanosomal macrolides, actinoallolides A, B, C, D and E, were isolated from fermented broth of endophytic *Actinoallomurus fulvus* MK10-036. Actinoallolide A exhibited the most potent anti-trypanosomal activity against *T. b. brucei* strain GUTat 3.1 and *T. cruzi* Tulahuen C4C8 strain (causative agent of Chagas disease)with the IC_{50} of 0.0049 and 0.226 $\mu g \cdot mL^{-1}$, respectively, without cytotoxicity against MRC-5 cell (IC_{50}> 100 $\mu g \cdot mL^{-1}$). This suggested that actinoallolide A can be further developed for drugs used for the treatment of sleeping sickness and Chagas disease (Inahashi et al. 2015).

Four compounds, lumichrome, perlolyrine, 1-hydroxy-β-carboline and 1*H*-indole-3-carboxaldehyde, discovered from cultured broth of *Micromonospora endophytica* 161111 (isolated from roots of *Xylocarpus granatum*) were active against the influenza A virus subtype H1N1 with the IC_{50} of 39.7, 38.3, 25.0 and 45.9 $\mu g \cdot mL^{-1}$, respectively (Wang et al. 2014). The compound, 1-hydroxy-β-carboline exhibited strong antiviral activity against H1N1 and thus further developed for the anti-H1N1 drug in the future.

Indananda et al. (2013) discovered a new polyketide, linfuranone A, from a cultured broth of endophytic *Microbispora* sp. GMKU 363. The compound displayed antiatherogenic activity but did not show antimicrobial and cytotoxic activity.

Endophytic actinomycetes have been used to promote plant growth by implement with a range of compounds consisting of nutrients, growth regulators, transporters and biocontrol substances. Shutsrirung et al. (2013) isolated the endophytic actinomycetes from *Citrus reticulata* L. and found that they can promote shoot height, fresh shoot weight and fresh root weight of the seedlings with the values ranged from 20.2-49.1, 14.9-53.6 and 1.6-102%, respectively. Likewise, two isolates of endophytic *Streptomyces* sp. strains CA10 and CA26 from *Centella asiatica* could enhance seed germination and seedling growth of *Phaseolus vulgaris* by producing a higher concentration of indole-3-acetic acid (IAA) from 71 to 197 $\mu g \cdot mL^{-1}$ (Dochhil et al. 2013).

The dumping off of the seedlings and roots and crown rots of *Cucumis sativus* (cucumber) usually caused by *Pythium aphanidermatum* and affected the commercial production of cucumber in the United Arab Emirates (UAE). El-Tarabily et al. (2010) found that three endophytic actinomycetes, *Streptomyces spiralis*, *Micromonospora chalcea* and *Actinoplanes campanulatus*, and have shown to reduce that causes, so these endophytes can be further developed and used instead of fungicide, metalaxyl which widely used to control the Pythium disease in UAE. Furthermore, to solve the fungal diseases in cucumber, Li et al. (2014) isolated the endophytic *Streptomyces* sp. CNS-42 from *Alisma orientale*. This strain produced staurosporine, an indolocarbazole alkaloid, which used as a potential biocontrol agents for inhibiting the pathogenic fungi, *Fusarium oxysporum* f. sp. *cucumerinum*.

CONCLUSION

In conclusion, the plant endosphere has biodiversity and has been considered as a valuable reservoir of the greater of actinomycetes containing

Streptomyces and rare actinomycetes which provided bioactive compounds with various biological activities. In order to develop the future advantage of bioactive metabolites from endophytic actinomycetes, there are many factors to attend, and these consist of the use of suitable isolation procedures (surface sterilisation and culture media), plant species and geographic and habitat of plants. Whole-genome sequences and the identification, annotation and analysis of secondary metabolite biosynthetic gene clusters are required. Moreover, the preliminary screening of the crude extracts using classical method to determine the compounds combined with LC-MS/MS analysis comparison to the databases of secondary metabolites, the information of chemical structures through spectroscopic methods including mass spectrometry, ultraviolet-visible spectroscopy, Fourier-transform infrared spectroscopy (IR), nuclear magnetic resonance (NMR) and 2-dimensional NMR spectra, are also advantageous foe drug discovery and development in the future.

REFERNCES

Arai, T. 1975. *Culture media for actinomycetes*. Tokyo: The Society for Actinomycetes, Japan.

Bérdy, J. (2005). Bioactive microbial metabolites. *J Antibiot*, 58 (1):1-26.

Bérdy, J. (2012). Thoughts and facts about antibiotics: where we are now and where we are heading. *J Antibiot*, 65 (8):385-395.

Bieber, B., Nuske, J., Ritzau, M., and Grafe, U. (1998). Alnumycin a new naphthoquinone antibiotic produced by an endophytic *Streptomyces* sp. *J Antibiot*, 51 (3):381-382.

Bunyoo, C., Duangmal, K., Nuntagij, A., and Thamchaipenet, A. (2009). Characterisation of endophytic actinomycetes isolated from wattle trees (*Acacia auriculiformis* A. Cunn. ex. Benth.) in Thailand. *Thai J Genet*, 2:155-163.

Castillo, U. F., Strobel, G. A., Ford, E. J., Hess, W. M., Porter, H., Jensen, J. B., Albert, H., Robison, R., Condron, M. A., Teplow, D. B., Stevens, D., and Yaver, D. (2002). Munumbicins, wide-spectrum antibiotics

produced by *Streptomyces* NRRL 30562, endophytic on *Kennedia nigriscans*. *Microbiol*, 148:2675-2685.

Castillo, U., Harper, J. K., Strobel, G. A., Sears, J., Alesi, K., and Teplow, D. (2003). Kakadumycins, novel antibiotics from *Streptomyces* sp. NRRL 30566, an endophyte of *Grevillea pteridifolia*. *FEMS Microbiol Lett*, 224:183-190.

Chen, H. H., Qin, S., Li, J., Zhang, Y. Q., Xu, L. H., Jiang, C. L., Kim, C. J., and Li, W. J. (2009). *Pseudonocardia endophytica* sp. nov., isolated from the pharmaceutical plant Lobelia clavata. *Int J Syst Evol Microbiol*, 59:559-563.

Collins, M. D., Pirouz, T., Goodfellow, M., and Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol*, 100:221-230.

Coombs, J. T., and Franco, C. M. (2003). Isolation and identification of actinobacteria from surface-sterilized wheat roots. *Appl Environ Microbiol*, 69 (9):5603-8.

Ding, L., Goerls, H., Dornblut, K., Lin, W., Maier, A., Fiebig, H.-H., and Hertweck, C. (2015). Bacaryolanes A-C, rare bacterial caryolanes from a mangrove endophyte. *J Nat Prod*, 78:2963-2967.

Ding, L., Maier, A., Fiebig, H. H., Lin, W. H., Peschel, G., and Hertweck, C. (2012). Kandenols A-E, Eudesmenes from an endophytic *Streptomyces* sp. of the mangrove tree *Kandelia candel*. *J Nat Prod*, 75 2223-2227.

Dochhil, H., Dkhar, M. S., and Barman, D. (2013). Seed germination enhancing activity of endophytic *Streptomyces* isolated from indigenous ethno-medicinal plant *Centella asiatica*. *Int J Pharm Biol Sci*, 41:256-262.

Du, H., Su, J., Yu, L., and Zhang, Y. (2013). Isolation and physiological characteristics of endophytic actinobacteria from medicinal plants. *Wei Sheng Wu Xue Bao*, 53:15-23.

Du, H. J., Zhang, Y. Q., Liu, H. Y., Su, J., Wei, Y. Z., Ma, B. P., Guo, B. L., and Yu, L. Y. (2013). *Allonocardiopsis opalescens* gen. nov., sp. nov., a new member of the suborder *Streptosporangineae*, from the surface-

sterilized fruit of a medicinal plant. *Int J Syst Evol Microbiol*, 63:900-904.

Duangmal, K., Mingma, R., Pathom-Aree, W., Thamchaipenet, A., Inahashi, Y., Matsumoto, A., and Takahashi, Y. (2011). *Amycolatopsis samaneae* sp. nov., isolated from roots of *Samanea saman* (Jacq.) Merr. *Int J Syst Evol Microbiol*, 61 (Pt 4):951-955.

Duangmal, K., Thamchaipenet, A., Matsumoto, A., and Takahashi, Y. (2009). *Pseudonocardia acaciae* sp. nov., isolated from roots of *Acacia auriculiformis* A. Cunn. ex Benth. *Int J Syst Evol Microbiol*, 59 (6):1487-1491.

El-Shatoury, S. A., El-Kraly, O. A., Trujillo, M. E., El-Kazzaz, W. M., El-Din, E. S. G., and Dewedar, A. (2013). Generic and functional diversity in endophytic actinomycetes from wild Compositae plant species at South Sinai - Egypt. *Res Microbiol*, 164 (7):761-769.

El-Tarabily, K. A., Hardy, G. E. S. J., and Sivasithamparam, K. (2010). Performance of three endophytic actinomycetes in relation to plant growth promotion and biological control of *Pythium aphanidermatum*, a pathogen of cucumber under commercial field production conditions in the United Arab Emirates. *Eur J Plant Pathol*, 128:527-539.

Ezra, D., Castillo, U. F., Strobel, G. A., Hess, W. M., Porter, H., and Yaver, D. (2014). Coronamycins, peptide antibiotics produced by a verticillate *Streptomyces* sp. (MSU-2110) endophytic on *Monstera* sp. *Microbiol*, 150 785-793.

Felsenstein, J (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39:783-791.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol*, 17:368-376.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for specific tree topology. *Syst Zool*, 20:406-416.

Garcia, L. C., Martinez-Molina, E., and Trujillo, M. E. (2010). *Micromonospora pisi* sp. nov., isolated from root nodules of *Pisum sativum*. *Int J Syst Evol Microbiol*, 60:331-337.

Gauze, G. F., Preobrazhenskaya, T. P., Sveshnikova, M. A., Terekhova, L. P., and Maximova, T. S. (1983). A guide to actinomycetes: genera

Streptomyces, *Streptoverticillum*, *Chainia*. "In *Opredelitel' aktinomitsetov* Moscow: Nauka.

Genilloud, O. (2017). Actinomycetes: still a source of novel antibiotics. *Nat Prod Rep*, 34 (10):1203-1232.

Golinska, P., Wypij, M., Agarkar, G., Rathod, D., Dahm, H., and Rai, M. (2015). Endophytic actinobacteria of medicinal plants: diversity and bioactivity. *Anton Leeuw Int J G*, 108:267-289.

Goodfellow, M., and Williams, S. T. (1983). Ecology of actinomycetes. *Annu Rev Microbiol*, 37 189-216.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98 NT. *Nucleic acids Symp Ser*, 41:95-98.

Hallmann, J., Quadt-Hallmann, A., Mahaffee, W. F., and J. W., Kloepper. (1997). Bacterial endophytes in agricultural crops. *Can J Microbiol*, 43 895-914.

Hasegawa, S., Meguro, A., Shimizu, M., Nishimura, T., and Kunoh, H. (2006). Endophytic actinomycetes and their interactions with host plants. *Actinomycetologica*, 20:72-81.

Igarashi, Y., Miura, S. S., Fujita, T., and Furumai, T. (2006). Pterocidin, a cytotoxic compound from endophytic *Streptomyces hygroscopicus*. *J Antibiot*, 59:193-195.

Igarashi, Y., Ogura, H., Furihata, K., Oku, N., Indananda, C., and Thamchaipenet, A. (2011). Maklamicin, an antibacterial polyketide from an endophytic *Micromonospora* sp. *J Nat Prod*, 74 (4):670-674.

Igarashi, Y., Trujillo, M. E., Martinez-Molina, E., Yanase, S., Miyanaga, S., Obata, T., Sakurai, H., Saiki, I., Fujita, T., and Furumai, T. (2007). Antitumor anthraquinones from an endophytic actinomycete *Micromonospora lupini* sp. nov. *Bioorg Med Chem Lett*, 17 (13):3702-3705.

Igarashi, Y., Yanase, S., Sugimoto, K., Enomoto, M., Miyanaga, S., Trujillo, M. E., Saiki, I., and Kuwahara, S. (2011). Lupinacidin C, an inhibitor of tumor cell invasion from *Micromonospora lupini*. *J Nat Prod*, 74 (4):862-5.

Inahashi, Y., Iwatsuki, M., Ishiyama, A., Matsumoto, A., Hirose, T., Oshita, J., Sunazuka, T., Panbangred, W., Takahashi, Y., Kaiser, M., Otoguro, K., and Omura, S. (2015). Actinoallolides A-E, new anti-trypanosomal macrolides, produced by an endophytic actinomycete, *Actinoallomurus fulvus* MK10-036. *Org Lett*, 17 (4):864-7.

Inahashi, Y., Iwatsuki, M., Ishiyama, A., Namatame, M., Nishihara-Tsukashima, A., Matsumoto, A., Hirose, T., Sunazuka, T., Yamada, H., Otoguro, K., Takahashi, Y., Omura, S., and Shiomi, K. (2011). Spoxazomicins A-C, novel antitrypanosomal alkaloids produced by an endophytic actinomycete, *Streptosporangium oxazolinicum* K07-0460^{T}. *J Antibiot*, 64 (4):303-7.

Inahashi, Y., Matsumoto, A., Danbara, H., Omura, S., and Takahashi, Y. (2010). *Phytohabitans suffuscus* gen. nov., sp. nov., an actinomycete of the family *Micromonosporaceae* isolated from plant roots. *Int J Syst Evol Microbiol*, 60:2652-2658.

Indananda, C., Igarashi, Y., Ikeda, M., Oikawa, T., and Thamchaipenet, A. (2013). Linfuranone A, a new polyketide from plant-derived *Microbispora* sp. GMKU 363. *J Antibiot*, 66 (11):675-677.

Indananda, C., Matsumoto, A., Inahashi, Y., Takahashi, Y., Duangmal, K., and Thamchaipenet, A. (2010). *Actinophytocola oryzae* gen. nov., sp. nov., isolated from the roots of Thai glutinous rice plants, a new member of the family *Pseudonocardiaceae*. *Int J Syst Evol Microbiol*, 60 (5):1141-1146.

Indananda, C., Thamchaipenet, A., Matsumoto, A., Inahashi, Y., Duangmal, K., and Takahashi, Y. (2011). *Actinoallomurus oryzae* sp. nov., an endophytic actinomycete isolated from roots of a Thai jasmine rice plant. *Int J Syst Evol Microbiol*, 61 (Pt 4):737-41.

Jacobson, E., Grauville, W. C., and Fogs, C. E. (1958). *Colour Harmony Manual*. 4th (ed). Chicago: Container Corporation of America.

Janso, J. E., and Carter, G. T. (2010). Biosynthetic potential of phylogenetically unique endophytic actinomycetes from tropical plants. *Appl Environ Microbiol*, 76:4377-4386.

Jia, F. Y., Liu, C. X., Wang, X. J., Zhao, J. W., Liu, Q. F., Zhang, J., Gao, R. X., and Xiang, W. S. (2013). *Wangella harbinensis* gen. nov., sp.

nov., a new member of the family *Micromonosporaceae*. *Anton Leeuw Int J G*, 103 (2):399-408.

Kaewkla, O., and Franco, C. M. M. (2013). Rational approaches to improving the isolation of endophytic actinobacteria from Australian native trees. *Microb Ecol*, 65:384-393.

Kelly, K. L. (1964). Inter-society color council - national bureau of standards color name charts illustrated with centroid colors. Washington DC: US Government Printing Office.

Kim, T. U., Cho, S. H., Han, J. H., Shin, Y. M., Lee, H. B., and Kim, S. B. (2012). Diversity and physiological properties of root endophytic actinobacteria in native herbaceous plants of Korea. *J Microbiol*, 50 (1):50-57.

Kimura, M. A. (1980). Simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol*, 16:111-120.

Kittiwongwattana, C., Thanaboripat, D., Laosinwattana, C., Koohakan, P., Parinthawong, N., and Thawai, C. (2015). *Micromonospora oryzae* sp. nov., isolated from roots of upland rice. *Int J Syst Evol Microbiol*, 65 (11):3818-3823.

Klykleung, N., Phongsopitanun, W., Pittayakhajonwut, P., Ohkuma, M., Kudo, T., and Tanasupawat, S. (2016). *Streptomyces phyllanthi* sp. nov., isolated from the stem of *Phyllanthus amarus*. *Int J Syst Evol Microbiol*, 66 (10):3923-3928.

Klykleung, N., Tanasupawat, S., Pittayakhajonwut, P., Ohkuma, M., and Kudo, T. (2015). *Amycolatopsis stemonae* sp. nov., isolated from a Thai medicinal plant. *Int J Syst Evol Microbiol*, 65 (11):3894-3899.

Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol*, 33 (7):1870-1874.

Kuncharoen, N., Pittayakhajonwut, P., and Tanasupawat, S. (2018). *Micromonospora globbae* sp nov., an endophytic actinomycete isolated from roots of *Globba winitii* C. H. Wright. *Int J Syst Evol Microbiol*, 68 (4):1073-1077.

Kuncharoen, N., Kudo, T., Ohkuma, M., and Tanasupawat, S. (2019a). *Micromonospora azadirachtae* sp. nov., isolated from roots of *Azadirachta indica* A. Juss. var. *siamensis* Valeton. *Anton Leeuw Int J G*, 112 (2):253-262.

Kuncharoen, N., Fukasawa, W., Mori, M., Shiomi, K., and Tanasupawat, S. (2019b). Diversity and antimicrobial activity of endophytic actinomycetes isolated from plant roots in Thailand. *Microbiol*, 88 (4):479-488.

Kuncharoen, N., Kudo, T., Yuki, M., Okuma, M., Pittayakhajonwut, P., and Tanasupawat, S. (2019c). *Micromonospora radicis* sp. nov., isolated from roots of *Azadirachta indica* var. siamensis Valenton, and reclassification of *Jishengella zingiberis* as *Micromonospora zingiberis* comb. nov. *Int J Syst Evol Microbiol*, 69 (9):2884-2891.

Küster, E., and Williams, S. T. (1964). Selection of Media for Isolation of *Streptomycetes*. *Nature*, 202:928-9.

Lane, D. J. (1991). *16S/23S rRNA sequencing*. (Eds). M., Goodfellow, and E., Stackebrandt, *Nucleic Acid Techniques in Bacterial Systematics*. Chichester: Wiley.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., and Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics*, 23 (21):2947-8.

Lazzarini, A., Cavaletti, L., Toppo, G., and Marinelli, F. (2000). Rare genera of actinomycetes as potential producers of new antibiotics. *Anton Leeuw Int J G*, 78 (3-4):399-405.

Lechevalier, H. A., and Lechevalier, M. P. (1967). Biology of actinomycetes. *Annu Rev Microbiol*, 21:71-100.

Li, C., Wang, H. Y., Jin, P. J., Zheng, W. J., Chu, L. Y., Liu, C. X., Li, J. S., Xiang, W. S., and Wang, X. J. (2015). *Actinoallomurus bryophytorum* sp. nov., an endophytic actinomycete isolated from moss (Bryophyta). *Anton Leeuw Int J G*, 108 (2):453-459.

Li, C., Zhang, Y. J., Liu, C. X., Wang, H. Y., Zhao, J. W., Li, L. J., Zhang, Z. W., Wang, X. J., and Xiang, W. S. (2015). *Microbispora*

bryophytorum sp nov., an actinomycete isolated from moss (Bryophyta). *Int J Syst Evol Microbiol*, 65:1274-1279.

Li, J., Zhao, G. Z., Qin, S., Zhu, W. Y., Xu, L. H., and Li, W. J. (2009). *Herbidospora osyris* sp. nov., isolated from surface-sterilized tissue of *Osyris wightiana* Wall. ex Wight. *Int J Syst Evol Microbiol*, 59:3123-3127.

Li, J., Zhao, G. Z., Zhu, W. Y., Huang, H. Y., Xu, L. H., Zhang, S., and Li, W. J. (2011). *Phytomonospora endophytica* gen. nov., sp nov., isolated from the roots of *Artemisia annua* L. *Int J Syst Evol Microbiol*, 61:2967-2973.

Li, J., Zhao, G. Z., Huang, H. Y., Qin, S., Zhu, W. Y., Zhao, L. X., Xu, L. H., Zhang, S., Li, W. J., and Strobel, G. A. (2012). Isolation and characterization of culturable endophytic actinobacteria associated with *Artemisia annua* L. *Anton Leeuw Int J G*, 101:515-527.

Li, L., Ma, J. B., Mohamad, O. A., Li, S. H., Osman, G., Li, Y. Q., Guo, J. W., Hozzein, W. N., and Li, W. J. (2015). *Phytoactinopolyspora endophytica* gen. nov., sp nov., a halotolerant filamentous actinomycete isolated from the roots of *Glycyrrhiza uralensis* F. *Int J Syst Evol Microbiol*, 65:2671-2677.

Li, X., Huang, P., Wang, Q., Xiao, L., Liu, M., Bolla, K., Zhang, B., Zheng, L., Gan, B., Liu, X., Zhang, L., and Zhang, X. (2014). Staurosporine from the endophytic *Streptomyces* sp. strain CNS-42 acts as a potential biocontrol agent and growth elicitor in cucumber. *Anton Leeuw Int J G*, 106 (3):515-25.

Ludwig, W., Euzeby, J., Schumann, P., Busse, H. J., Trujillo, M. E., Kampfer, P., and Whitman, W. B. (2012). Road map of the phylum *Actinobacteria*. In *Bergey's Manual of Systematic Bacteriology* (Eds.) M., Goodfellow, P., Kampfer, M. J., Busse, M. E., Trujillo, K., Suzuki, W., Ludwig and W. B., Whitman, 1-28. New York: Springer.

Meier-Kolthoff, J. P., Auch, A. F., Klenk, H. P., and Göker, M. (2013). Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics*, 14:1-14.

Mikami, H., and Ishida, Y. (1983). Post-column fluorometric detection of reducing sugar in high-performance liquid chromatography using arginine. *Bunseki Kagaku*, (32):E207-E210.

Minamiyama, H., Shimizu, M., Kunoh, H., Furumai, T., Igarashi, Y., Onaka, H., and Yoshida, R. (2003). Multiplication of isolate R-5 of *Streptomyces galbus* on rhododendron leaves and its production of cell wall-degrading enzyme. *J Gen Plant Pathol*, 69:65-70.

Mingma, R., Duangmal, K., Thamchaipenet, A., Trakulnaleamsai, S., Matsumoto, A., and Takahashi, Y. (2015). *Streptomyces oryzae* sp. nov., an endophytic actinomycete isolated from stems of rice plant. *J Antibiot*, 68 (6):368-372.

Mingma, R., Duangmal, K., Trakulnaleamsai, S., Thamchaipenet, A., Matsumoto, A., and Takahashi, Y. (2014). *Sphaerisporangium rufum* sp. nov., an endophytic actinomycete from roots of *Oryza sativa* L. *Int J Syst Evol Microbiol*, 64 (4):1077-1082.

Minnikin, D. E., Patel, P. V., Alshamoany, L., and Goodfellow, M. (1977). Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol*, 27:104-117.

Nakashima, T., Okuyama, R., Kamiya, Y., Matsumoto, A., Iwatsuki, M., Inahashi, Y., Yamaji, K., Takahashi, Y., and Omura, S. (2013). Trehangelins A, B and C, novel photo-oxidative hemolysis inhibitors produced by an endophytic actinomycete, *Polymorphospora rubra* K07-0510. *J Antibiot*, 66:311-317.

Niemhom, N., Chutrakul, C., Suriyachadkun, C., Tadtong, S., and Thawai, C. (2019). *Jiangella endophytica* sp. nov., an endophytic actinomycete isolated from the rhizome of *Kaempferia elegans*. *Int J Syst Evol Microbiol*, 69 (2):454-459.

Niemhom, N., Chutrakul, C., Suriyachadkun, C., and Thawai, C. (2016a). *Asanoa endophytica* sp. nov., an endophytic actinomycete isolated from the rhizome of *Boesenbergia rotunda*. *Int J Syst Evol Microbiol*, 66 (3):1377-1382.

Niemhom, N., Chutrakul, C., Suriyachadkun, C., and Thawai, C. (2016b). *Phytohabitans kaempferiae* sp. nov., an endophytic actinomycete

isolated from the leaf of *Kaempferia larsenii*. *Int J Syst Evol Microbiol*, 66 (8):2917-22.

Nimnoi, P., Pongsilp, N., and Lumyong, S. (2010). Genetic diversity and community of endophytic actinomycetes within the roots of Aquilaria crassna Pierre ex Lec assessed by Actinomycetes-specific PCR and PCR-DGGE of 16S rRNA gene. *Biochem Syst Ecol*, 38 (4):595-601.

Nouioui, I., Carro, L., Garcia-Lopez, M., Meier-Kolthoff, J. P., Woyke, T., Kyrpides, N. C., Pukall, R., Klenk, H. P., Goodfellow, M., and Goker, M. (2018). Genome-Based Taxonomic Classification of the Phylum *Actinobacteria*. *Front Microbiol*, 9:2007.

Okazaki, T. 2003. Studies on actinomycetes isolated from plant leaves. In *Selective Isolation of Actinomycetes*, (Eds) I., Kurtboke, M., Hayakawa, L., Terekhova, and T., Okazaki, 102-122. Queensland: National Library of Australia.

Otoguro, K., Ishiyama, A., Namatame, M., Nishihara, A., Furusawa, T., Masuma, R., Shiomi, K., Takahashi, Y., Yamada, H., and Omura, S. (2008). Selective and potent in vitro antitrypanosomal activities of ten microbial metabolites. *J Antibiot*, 61 (6):372-8.

Parrent, J. L., Peay, K., Arnold, A. E., Comas, L., Avis, P., and Tuininga, A. (2010). Moving from pattern to process in fungal symbioses: linking functional traits, community ecology, and phylogenetics. *New Phytologist*, 185:882-886.

Phongsopitanun, P., Kudo, T., Mori, M., Shiomi, K., Pittayakhajonwut, P., Suwanborirux, K., and Tanasupawat, S. (2015). *Micromonospora fluostatini* sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol*, 65:4417-4423.

Portugal, J. (2003). Chartreusin, elsamicin A and related anti-cancer antibiotics. *Curr Med Chem Anticancer Agents*, 3 (6):411-420.

Qin, S., Wang, H. B., Chen, H. H., Zhang, Y. Q., Jiang, C. L., Xu, L. H., and Li, W. J. (2008). *Glycomyces endophyticus* sp. nov., an endophytic actinomycete isolated from the root of *Carex baccans* Nees. *Int J Syst Evol Microbiol*, 58:2525-2528.

Qin, S., Li, J., Chen, H. H., Zhao, G. Z., Zhu, W. Y., Jiang, C. L., Xu, L. H., and Li, W. J. (2009a). Isolation, diversity, and antimicrobial activity of

rare actinobacteria from medicinal plants of tropical rain forests in Xishuangbanna, China. *Appl Environ Microbiol*, 75 (19):6176-86.

Qin, S., Zhao, G. Z., Li, J., Zhu, W. Y., Xu, L. H., and Li, W. J. (2009b). *Actinomadura flavalba* sp. nov., an endophytic actinomycete isolated from leaves of *Maytenus austroyunnanensis*. *Int J Syst Evol Microbiol*, 59:2453-2457.

Qin, S., Zhao, G. Z., Li, J., Zhu, W. Y., Xu, L. H., and Li, W. J. (2009c). *Jiangella alba* sp. nov., an endophytic actinomycete isolated from the stem of *Maytenus austroyunnanensis*. *Int J Syst Evol Microbiol*, 59:2162-2165.

Qin, S., Xing, K., Jiang, J. H., Xu, L. H., and Li, W. J. (2011). Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. *Appl Microbiol Biotechnol*, 89 (3):457-473.

Qin, S., Yuan, B., Zhang, Y. J., Bian, G. K., Tamura, T., Sun, B. Z., Li, W. J., and Jiang, J. H. (2012a). *Nocardioides panzhihuaensis* sp. nov., a novel endophytic actinomycete isolated from medicinal plant *Jatropha curcas* L. *Anton Leeuw Int J G*, 102 (2):353-360.

Qin, S., Jiang, J. H., Klenk, H. P., Zhu, W. Y., Zhao, G. Z., Zhao, L. X., Tang, S. K., Xu, L. H., and Li, W. J. (2012b). *Promicromonospora xylanilytica* sp. nov., an endophytic actinomycete isolated from surface-sterilized leaves of the medicinal plant *Maytenus austroyunnanensis*. *Int J Syst Evol Microbiol*, 62:84-89.

Qin, S., Bai, J. L., Wang, Y., Feng, W. W., Yuan, B., Sun, Y., Cao, C. L., Ju, X. Y., Huang, Y., and Jiang, J. H. (2015). *Tamaricihabitans halophyticus* gen. nov., sp nov., an endophytic actinomycete of the family *Pseudonocardiaceae*. *Int J Syst Evol Microbiol*, 65:4662-4668.

Rachniyom, H., Matsumoto, A., Indananda, C., Duangmal, K., Takahashi, Y., and Thamchaipenet, A. (2015a). *Actinomadura syzygii* sp. nov., an endophytic actinomycete isolated from the roots of a jambolan plum tree (*Syzygium cumini* L. Skeels). *Int J Syst Evol Microbiol*, 65:1946-1949.

Rachniyom, H., Matsumoto, A., Indananda, C., Duangmal, K., Takahashi, Y., and Thamchaipenet, A. (2015b). *Nonomuraea syzygii* sp. nov., an endophytic actinomycete isolated from the roots of a jambolan plum tree

(*Syzygium cumini* L. Skeels). *Int J Syst Evol Microbiol*, 65 (4):1234-1240.

Ritcher, M., and Rosselló-Móra, R. (2009). Shifting the genomics gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA*, 106:19126-19131.

Ritcher, M., Rosselló-Móra, R., Oliver Glöckner, F., and Peplies, J. . (2016). JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics*, 32:929-931.

Saitou, N., and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol*, 4 (4):406-25.

Sakdapetsiri, C., Ngaemthao, W., Suriyachadkun, C., Duangmal, K., and Kitpreechavanich, V. (2018). *Actinomycetospora endophytica* sp. nov., isolated from wild orchid (*Podochilus microphyllus* Lindl.) in Thailand. *Int J Syst Evol Microbiol*, 68 (9):3017-3021.

Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *Technical* Note, 101.

Schulz, B., and Boyle, C. (2006). What are endophytes?. In *Microbial root endophytes*, (Eds). T. N., Sieber, 1-13. Berlin: Springer.

Schulz, B., Boyle, C., Draeger, S., Rommert, A. K., and Krohn, K. (2002). Endophytic fungi: a source of novel biologically active secondary metabolites. *Mycol Res*, 106:996-1004.

Shirling, E. B., and Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol*, 16:313-340.

Shutsrirung, A., Chromkaew, Y., Pathom-Aree, W., Choonluchanon, S., and Boonkerd, N. (2013). Diversity of endophytic actinomycetes in mandarin grown in northern Thailand, their phytohormone production potential and plant growth promoting activity. *Soil Sci Plant Nutr*, 59 (3):322-330.

Staneck, J. L., and Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol*, 28 (2):226-31.

Strobel, G. A., and Daisy, B. (2003). Bioprospecting for microbial endophytes and their natural products. *Microbiol Mol Biol Rev*, 67 (4):492-502.

Strobel, G. A., Daisy, B., Castillo, U., and Harper, J. (2004). Natural products from endophytic microorganisms. *J Nat Prod*, 67 (2):257-68.

Supong, K., Suriyachadkun, C., Pittayakhajonwut, P., Suwanborirux, K., and Thawai, C. (2013). *Micromonospora spongicola* sp. nov., an actinomycete isolated from a marine sponge in the gulf of Thailand. *J Antibiot*, 66:505-509.

Supong, K., Thawai, C., Choowong, W., Kittiwongwattana, C., Thanaboripat, D., Laosinwattana, C., Koohakan, P., Parinthawong, N., and Pittayakhajonwut, P. (2016). Antimicrobial compounds from endophytic *Streptomyces* sp. BCC72023 isolated from rice (*Oryza sativa* L.). *Res Microbiol*, 167 (4):290-298.

Suriyachadkun, C., Chunhametha, S., Thawai, C., Tamura, T., Potacharoen, W., Kirtikara, K., and Sanglier, J. J. (2009). *Planotetraspora thailandica* sp. nov., isolated from soil in Thailand. *Int J Syst Evol Microbiol*, 59 (Pt 5):992-7.

Taechowisan, T., and Lumyong, S. (2003). Activity of endophytic actinomycetes from roots of *Zingiber officinale* and *Alpinia galanga* against phytopathogenic fungi. *Ann Microbiol*, 53 (3):291-298.

Takahashi, Y., and Omura, S. (2003). Isolation of new actinomycete strains for the screening of new bioactive compounds. *J Gen Appl Microbiol*, 49 (3):141-154.

Tamaoka, J., Katayama-Fujimura, Y., and Kuraishi, H. (1983). Analysis of bacterial menaquinone mixtures by high performance liquid chromatography. *J Appl Bacteriol*, 54:31-36.

Thamchaipenet, A., Indananda, C., Bunyoo, C., Duangmal, K., Matsumoto, A., and Takahashi, Y. (2010). *Actinoallomurus acaciae* sp. nov., an endophytic actinomycete isolated from *Acacia auriculiformis* A. Cunn. ex. Benth. *Int J Syst Evol Microbiol*, 60:554-559.

Thanaboripat, D., Thawai, C., Kittiwongwattana, C., Laosinwattana, C., Koohakan, P., and Parinthawong, N. (2015). *Micromonospora endophytica* sp. nov., an endophytic actinobacteria of Thai upland rice (*Oryza sativa*). *J Antibiot*, 68 (11):680-684.

Thawai, C. (2015). *Micromonospora costi* sp. nov., isolated from a leaf of *Costus speciosus*. *Int J Syst Evol Microbiol*, 65 (Pt 5):1456-1461.

Tomiyasu, I. (1982). Mycolic acid composition and thermally adaptative changes in *Nocardia asteroids*. *J Bacteriol*, 151:828-837.

Trujillo, M. E., Alonso-Vega, P., Rodriguez, R., Carro, L., Cerda, E., Alonso, P., and Martinez-Molina, E. (2010). The genus *Micromonospora* is widespread in legume root nodules: the example of *Lupinus angustifolius*. *ISME Journal*, 4 (10):1265-1281.

Trujillo, M. E., Kroppenstedt, R. M., Fernandez-Molinero, C., Schumann, P., and Martinez-Molina, E. (2007). *Micromonospora lupini* sp. nov. and *Micromonospora saelicesensis* sp. nov., isolated from root nodules of Lupinus angustifolius. *Int J Syst Evol Microbiol*, 57:2799-2804.

Trujillo, M. E., Kroppenstedt, R. M., Schumann, P., Carro, L., and Martinez-Molina, E. (2006). *Micromonospora coriariae* sp nov., isolated from root nodules of *Coriaria myrtifolia*. *Int J Syst Evol Microbiol*, 56:2381-2385.

Uchida, K., and Aida, K. (1984). An improved method for the glycolate test for simple identification of the acyl type of bacterial cell walls. *J Gen Appl Microbiol*, 30:131-134.

Valdes, M., Perez, N. O., Estrada de los Santos, P., Caballero-Mellado, J., Pena-Cabriales, J. J., Normand, P., and Hirsch, A. M. (2005). Non-Frankia actinomycetes isolated from surface-sterilized roots of *Casuarina equisetifolia* fix nitrogen. *Appl Environ Microbiol*, 71 (1):460-466.

Wang, P., Kong, F., Wei, J., Wang, Y., Wang, W., Hong, K., and Zhu, W. (2014). Alkaloids from the mangrove-derived actinomycete *Jishengella endophytica* 161111. *Mar Drugs*, 12 (1):477-90.

Williams, S. T., and Cross, T. (1971). Chapter XI: Actinomycetes. *Method Microbiol*, 4:295-334.

Xing, J., Liu, C. X., Zhang, Y. J., He, H. R., Zhou, Y., Li, L. J., Zhao, J. W., Liu, S. H., Wang, X. J., and Xiang, W. S. (2015a). *Sphaerisporangium dianthi* sp. nov., an endophytic actinomycete isolated from a root of *Dianthus chinensis* L. *Anton Leeuw Int J G*, 107 (1):9-14.

Xing, J., Liu, C., Zhang, Y., He, H., Zhou, Y., Li, L., Zhao, J., Liu, S., Wang, X., and Xiang, W. (2015b). *Sphaerisporangium dianthi* sp. nov., an

endophytic actinomycete isolated from a root of *Dianthus chinensis* L. *Anton Leeuw Int J G*, 107:9-14.

Xing, K., Liu, W., Zhang, Y. J., Bian, G. K., Zhang, W. D., Tamura, T., Lee, J. S., Qin, S., and Jiang, J. H. (2013). *Amycolatopsis jiangsuensis* sp. nov., a novel endophytic actinomycete isolated from a coastal plant in Jiangsu, China. *Anton Leeuw Int J G*, 103 (2):433-439.

Xing, K., Qin, S., Fei, S. M., Lin, Q., Bian, G. K., Miao, Q., Wang, Y., Cao, C. L., Tang, S. K., Jiang, J. H., and Li, W. J. (2011). *Nocardia endophytica* sp. nov., an endophytic actinomycete isolated from the oil-seed plant *Jatropha curcas* L. *Int J Syst Evol Microbiol*, 61:1854-1858.

Yan, L. L., Han, N. N., Zhang, Y. Q., Yu, L. Y., Chen, J., and Sun, C. H. (2010). Antimycin A_{18} produced by an endophytic *Streptomyces albidoflavus* isolated from a mangrove plant. *J Antibiot*, 63:259-261.

Yoon, S. H., Ha, S. M., Kwon, S., Lim, J., Kim, Y., Seo, H., and Chun, J. (2017). Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol*, 67 (5):1613-1617.

Yu, Z., Zhao, L. X., Jiang, C. L., Duan, Y., Wong, L., Carver, K. C., Schuler, L. A., and Shen, B. (2011). Bafilomycins, produced by an endophytic actinomycete *Streptomyces* sp. YIM 56209. *J Antibiot*, 64:159-162.

Zhang, Y. J., Zhang, W. D., Qin, S., Bian, G. K., Xing, K., Li, Y. F., Cao, C. L., and Jiang, J. H. (2013). *Saccharopolyspora dendranthemae* sp. nov., a halotolerant endophytic actinomycete isolated from a coastal salt marsh plant in Jiangsu, China. *Anton Leeuw Int J G*, 103 (6):1369-1376.

Zhao, K., Penttinen, P., Guan, T., Xiao, J., Chen, Q., Xu, J., Lindstrom, K., Zhang, L., Zhang, X., and Strobel, G. A. (2011). The diversity and anti-microbial activity of endophytic actinomycetes isolated from medicinal plants in Panxi plateau, China. *Curr Microbiol*, 62 (1):182-90.

Zhu, W. Y., Zhao, L. X., Zhao, G. Z., Duan, X. W., Qin, S., Li, J., Xu, L. H., and Li, W. J. (2012). *Plantactinospora endophytica* sp. nov., an actinomycete isolated from *Camptotheca acuminata* Decne., reclassification of *Actinaurispora siamensis* as *Plantactinospora siamensis* comb. nov. and emended descriptions of the genus

Plantactinospora and *Plantactinospora mayteni*. *Int J Syst Evol Microbiol*, 62:2435-2442.

Zin, N. M., Ng, K. T., Sarmin, N. M., Getha, K., and Tan, G. Y. (2011). Anti-trypanosomal activity of endophytic streptomycete. *Curr Res Bacteriol*, 4:1-8.

In: A Closer Look at Actinomycetes ISBN: 978-1-53617-046-7
Editors: A. A. Mohamed Hatha et al.

Chapter 7

METABOLIC PROFILING OF *STREPTOMYCES* SP. STRAIN 51 FOR DETECTION OF BIOACTIVE COMPOUNDS

Prateek Kumar[1], Aditi Kundu[2], Renu Solanki[3], Munendra Kumar[1] and Monisha Khanna Kapur[1,*]
[1]Microbial Technology Lab, Acharya Narendra Dev College,
University of Delhi, New Delhi, India
[2]Division of Agricultural Chemicals,
ICAR - Indian Agricultural Research Institute, New Delhi, India
[3]Deen Dayal Upadhyaya College,
University of Delhi, New Delhi, India

ABSTRACT

Actinomycetes are Gram- positive bacteria having high GC content in their genome. They are crucial from industrial perspective as they have great ability for production of bioactive secondary metabolites. Compounds produced by them possess diverse biological activities such as

* Corresponding Author's Email: monishaandc@gmail.com.

anticancer, antifungal, antibacterial, antiviral and belong to distinct chemical classes. Members of genus *Streptomyces* are well known producers of bioactive compounds. Due to emergence of drug resistant pathogens, there is a dire need for the discovery of new compounds having unique modes of action. During isolation and screening programme of actinomycetes carried out in our laboratory, a biologically active strain was isolated from agricultural soil, Dhanaura, Uttar Pradesh, India and designated as Strain 51. Morphological and biochemical studies revealed that Strain 51 belongs to genus *Streptomyces* and it showed 100% 16S rRNA gene sequence homology with *Streptomyces griseochromogenes*. Present investigation was undertaken as an effort to extract and characterize potent compounds from Strain 51 which are responsible for higher bioactivity. Extraction of bioactive metabolites was performed using cold extraction method taking ethyl acetate as solvent. Minimum inhibitory concentration (MIC) of compounds against *Bacillus cereus* was determined by microdilution method taking industrial antibiotic-chloramphenicol as positive control. Crude extract of Strain 51 showed inhibition of *Bacillus cereus* growth at 0.0050 mg/ml while chloramphenicol suppressed growth at 0.0075 mg/ml. Metabolomic studies were carried out for identification and structural elucidation of bioactive molecules using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS/MS) techniques. GC-MS analysis of strain 51 extract showed the presence of thirty three volatile organic constituents, out of which some are reported in literature to have diverse biological activities. Extract of Strain 51 was also subjected to LC-MS analysis which gave several sharp peaks in the spectrum. Metabolites identified in LC-MS data showed molecular ion peaks at m/z 228, 758, 548, 784 and 803. The structure was elucidated and confirmed for each peak through their mass fragmentation patterns; as a result structure of compounds were confirmed as thiolutin, streptothricin D, antimycin A, rifaximin and fujimycin. PCR was performed for detection of biosynthetic gene clusters responsible for production of bioactive compounds in Strain 51. Amplification of polyketide synthase-I (PKS-I) and non- ribosomal peptide synthetases (NRPS) was observed, the amplicons were purified and sequenced. The gene sequences were submitted in NCBI-GenBank database under accession numbers MK355718 and MK355717. In future studies, our aim is to produce a novel compound by introducing genetic manipulations in these genes.

Keywords: metabolic profiling, *Streptomyces* sp., minimum inhibitory concentration (MIC), bioactive compounds, GC-MS and LC-MS/MS, structural elucidation, PKS-I and NRPS

INTRODUCTION

Actinomycetes are Gram- positive, soil-dwelling microorganisms that produce diverse biologically active secondary metabolites with industrial value. *Streptomyces* is the largest genus of Actinobacteria, which is known for the production of ~75% of all reported bioactive compounds (Lim et al., 2018). Many notable therapeutic agents such as tetracycline, amphotericin, adriamycin and tarcolimus are reported from *Streptomyces* (Hopwood, 2007). Development of resistance among pathogenic microbes is a serious challenge to public health globally. Hence there is requirement of bioactive compounds having unique modes of action to act against resistant pathogens. Actinomycetes continue to be important sources of secondary metabolites having wide range of bioactivities such as anticancer, antibacterial, antifungal and anti-infectives (Bibb, 2005; Dewi et al., 2017). Actino-bacterial strains from diverse ecological habitats can serve as sources of pharmaceutically important molecules. Exploration of distinct habitats in search of pharmaceutically potent strains in modern science era is in great interest to isolate bioactive molecules which can acts against resistant pathogens. Therefore, screening for potential bioactive compounds from this bacterial group can provide with the pharmaceutically important molecules with unique modes of action. During isolation and screening programme in our laboratory, a potent strain (Strain 51) was isolated from agricultural soil sample. Morphological and biochemical studies revealed that Strain 51 belongs to *Streptomyces* genus and based on 16S rRNA gene sequence homology it showed 100% similarity with *Streptomyces griseochromogenes* (Das, 2017; Kapur et al., 2018). Present study is undertaken as an effort to extract and characterize potential bioactive compounds of pharmaceutical value from *Streptomyces* sp. Strain 51. Cold extraction method was applied for extraction of bioactive compounds using ethyl acetate as solvent. Minimum inhibitory concentration (MIC) of Strain 51 crude extract against *Bacillus cereus* was determined by applying microdilution method using chloramphenicol as positive control. Identification and structural elucidation of bioactive compounds present in crude extract/residue of Strain 51 was performed using gas chromatography-mass spectrometry (GC-MS) and

liquid chromatography-mass spectrometry (LC-MS/MS) techniques. Detection of biosynthetic gene clusters (i.e., PKS and NRPS) responsible for production of bioactive compounds in Strain 51 was carried out using degenerate PCR primers. Amplified products were purified, sequenced and submitted at NCBI- GenBank database under accession numbers MK355718 and MK355717.

METHODS

Actinomycete Strain

Actinomycete strain under study (Strain 51) was collected from agricultural soil, Dhanaura, Uttar Pradesh, India during our earlier studies. The isolate was purified and maintained on yeast extract-malt extract (YM) medium and stored in 20% glycerol at -20ºC. During molecular taxonomy using 16S rRNA gene sequencing approach it was concluded that bioactively potent Strain 51 belongs to the genus *Streptomyces* (Das, 2017; Kapur et al., 2018).

This chapter is focused on extraction, minimum inhibitory concentration (MIC), determination and characterization of bioactive compounds from this strain. Also, we detected the bioactive metabolites encoding biosynthetic gene clusters present in the strain under study.

Extraction of Bioactive Secondary Metabolites

For extraction of bioactive metabolites from actinomycete culture plates cold extraction method was used (Schimana et al., 2000; Solanki et al., 2015). *Streptomyces* sp. Strain 51 was inoculated on YM medium plates for 15 days at 28ºC for production of bioactive secondary metabolites. The well grown culture plates were chopped down in small pieces and the pieces were transferred in flasks. Ethyl acetate was added as solvent in each flask and incubated at incubator shaker (New Brunswick Scientific *Excella* E24, Germany) for 4-5 hours at 200 rpm and 28ºC. The resulted extract was

filtered through whatman No. 1 filter paper. Rotary evaporator (Buchi R-200, Switzerland) was used for evaporation of solvent, which was resulted in light yellow colored semisolid residue.

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) determination of extracted metabolites against *Bacillus cereus* MTCC 430 was performed using microdilution method (Andrews, 2001; Solanki, 2013). A commercial drug chloramphenicol (Abbott Healthcare Pvt. Ltd., India) was used as positive control. Culture of pathogenic strain *Bacillus cereus* in log phase was diluted in sterile growth medium so as to have a final concentration of 10^5cfu/ml. Stock solutions of dried extract of Strain 51 were prepared in fraction amount of DMSO + maximum amount of autoclaved MQ water whereas stocks from chloramphenicol were prepared in autoclaved MQ water only. Different dilutions of these stocks were added to the wells of micro-titer plate and 100 µl from log phase culture of pathogen having 10^5 cfu/ml concentration was added to each well in the micro-titer plate and incubated at optimum temperature. After interval of 48h, the plate was examined for inhibition of growth of pathogenic strain.

Characterization of Bioactive Metabolites

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis of extract from Strain 51 was carried out using Shimadzu QP-2010 Plus with Thermal Desorption System TD 20. Conditions used during GC-MS analysis are described as follows. The column oven temperature was kept 60°C, injection temperature 280°C, mode of injection was- split, mode of flow control- linear velocity, pressure was 73.3 kPa, total flow 16.3 mL/min, flow of column was 1.21 mL/min, linear velocity was 40.1 cm/sec, purge flow was 3 mL/min, split ration 10 and equilibrium time 1 min. The MS acquisition parameters were: ion source temperature 230°C, interface temperature 290°C, time of solvent cut 3.50

min, mode of detector gain - relative, detector gain 0.00 kV, threshold 1000, time of start 4 min, time of end 55.74 min, ACQ mode- scan, time of event 0.50 sec, speed of scan 1428, start m/z 40 and end m/z 700. Strain 51 GC-MS spectra was matched with Wiley and NIST (National Institute of Standards and Technologies) taken as reference mass spectra libraries, which was resulted in the identification of volatile organic compounds.

Liquid Chromatography-Mass Spectrometry (LC-MS/MS)

Crude extract of Strain 51 was subjected to LC-MS/MS analysis using Waters SYNAPT G2 with 2D nano ACQUITY System with following working conditions: Polarity ES, analyser- resolution mode, voltage of capillary 2.7200 kV, temperature of source 110°C, sampling cone 40.0000, extraction cone 4.0000, gas flow of source 0.00 mL/min, temperature of desolvation was 450°C, gas flow of cone 52.0 L/Hr, gas flow of desolvation 647.0 L/Hr, resolution of LM 4.7, resolution of HM 15.0, aperture 10.0, pre-filter 2.0, ion energy 1.8, energy of manual trap collision- TRUE, energy of trap collision 6.0, energy of manual transfer collision- FALSE, energy of transfer collision 0.0, manual gas control- FALSE, trap gas flow 2.00 mL/min, helium cell gas flow 0.00, IMS gas flow 0.00 mL/min, detector 2750, backing 1.74e0, source 1.23e-3, sample plate 1.00e-6, trap 6.85e-3, helium cell 1.09e-4, IMS 1.52e-4, transfer 6.52e-3, range of acquisition mass- start mass 100.000 end mass 1000.000, range of calibration mass- start mass 126.784 end mass 1926.553.

Polymerase Chain Reaction (PCR) and Sequencing of Bioactive Metabolites Encoding Genes of *Streptomyces* sp. Strain 51

Genomic DNA Isolation

Streptomyces sp. Strain 51genomic DNA was isolated using small scale method standardized for actinomycetes (Hopwood et al., 1985; Khanna and Solanki, 2012).

PCR Amplification

PCR primers K1F (5'-TSAAGTCSAACATCGGBCA-3') and M6R (5'- CGCAGGTTSCSGTACCAGTA-3') were used to amplify conserved ketosynthase and methyl-malonyltransferase domain sequences of PKS-I while primer pair A3F (5'- GCSTACSYSATSTACACSTCSGG-3') and A7R (5'- SASGTCVCCSGTSCGGTAS -3') was used for NRPS gene conserved adenylation domain sequences amplification (Ayuso-Sacido and Genilloud, 2005). PCR was performed in *Mastercycler* gradient (eppendorf, Germany). Reaction mixture was prepared in volume of 100 µl which contains: 5 µl of genomic DNA, 1 µl of each primer (Sigma, USA) having 100 mM concentration, 2 µl of dNTP mix (Qiagen, Germany) having concentration 10 mM each, 1.3 µl of *Taq*polymerase (Qiagen, Germany) having concentration 5U/µl, 10 µl of recommended 10X *Taq*polymerase buffer containing 15mM $MgCl_2$, 2 µl of DMSO (Hi-media) and final volume was made up by 77.7 µl of sterile MQ water. Following reaction conditions were used for PCR: 5 min at 95ºC and 35 cycles of 30 sec at 95ºC, 2 min at 58ºC, 4 min at 72ºC and 10 min at 72ºC. Amplified products were observed using 1.2% agarose gel stained with ethidium bromide. PCR products of desired sizes were purified using Gel Extraction Kit (Qiagen, Germany). Purified products were sequenced using highly advanced DNA sequencer (Applied Biosystems, USA) with specific pair of PKS-I and NRPS primers. Gene sequences were submitted at NCBI-GenBank database using Bankit tool under the accession numbers MK355718 and MK355717.

RESULTS AND DISCUSSION

Actinomycete Strain

Bioactively potent *Streptomyces* sp. Strain 51 cultured on yeast extract-malt extract (YM) medium at 28ºC showed white color aerial spores on the culture plate. This well grown culture was further utilized for the extraction of bioactive metabolites.

Extraction of Bioactive Secondary Metabolites

Extraction of bioactive secondary metabolites was performed using cold extraction method taking ethyl acetate as solvent. Evaporation of solvent in rotavapour yields a light yellow colored semi-solid residue. This residue was used for further studies of MIC determination, GC-MS and LC-MS/MS. The extraction protocol followed in the present investigation was previously adopted by many researchers for extraction of bioactive secondary metabolites from actinobacteria (Schimana et al., 2000; Solanki et al., 2015; Kapur et al., 2018).

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of *Streptomyces* sp. Strain 51 extract using commercial drug chloramphenicol as positive control was determined against pathogenic bacteria *Bacillus cereus*. The inhibition of pathogen growth by Strain 51 extract was started at 0.0050 mg/ml whereas chloramphenicol suppressed growth at 0.0075 mg/ml. The comparison of MIC values for both Strain 51 extract and industrial drug chloramphenicol revealed that strain under study is more active than the commercial drug.

Characterization of Bioactive Metabolites

Gas Chromatography-Mass Spectrometry (GC-MS)

Ethyl acetate soluble fraction of *Streptomyces* sp. Strain 51 extract was subjected to GC-MS analysis for identification of its volatile organic constituents. Thirty three volatile compounds, representing 47.37% of total volatile organic compounds present in ethyl acetate extract (Table 1). Total ion chromatography (TIC) of GC-MS analysis showed several peaks (Figure 1) corresponding to hydrocarbons, alcohols, ketones and acidic compounds (Table 2). Hydrocarbons were identified as the major fraction of total volatile organic constituents (Table 2). Among these hexadecane (7.73%) was most abundant (Table 1). Hexatricontane (4.81%), pyrrolopyrazine (3.16%), heneicosane (3.15%), eicosane (2.16%), tricontane (1.66%)

and methylheptadecane (1.20%) were identified as major constituents.

Table 1. Chemical composition of metabolites in GC-MS analysis of ethyl acetate extract of *Streptomyces* sp. Strain 51

Peak	RT (min.)	Compound name	Area %
1	7.034	Trimethyl octane	0.34
2	11.104	Dodecane	0.75
3	14.648	Tetradecane	0.82
4	16.461	Dimethyl ethyl phenol	0.40
5	16.542	Dodecanamine	0.38
6	17.768	Hexadecane	7.73
7	19.891	Heneicosane	3.15
8	20.475	Pyrrolopyrazine 1,4-dione	3.16
9	21.092	Octadecenoic acid	1.85
10	21.777	Ethyl heptadecanedione	0.35
11	22.258	Octadecanoic acid	0.61
12	23.309	Cyclohexylnonadecane	0.72
13	23.632	Eicosanoic acid	0.34
14	24.143	Hexadecanol	1.55
15	24.287	Octadecenoic acid methyl ester	0.32
16	24.850	Eicosane	2.16
17	25.516	Eicosyl acetate	1.78
18	26.468	Tetradecanol	0.75
19	26.592	Tetracontane	2.23
20	26.881	Tricontane	0.88
21	26.940	Tetramethyl hexadecane	0.41
22	27.282	Docosane	0.92
23	27.714	Octacosyl acetate	1.40
24	29.568	Methyl heptadecane	1.20
25	29.862	Tetracosane	0.43
26	30.560	Tridecane	0.86
27	31.113	Octacosanol	1.75
28	31.412	Dodecadienol	1.58
29	31.638	Hexatriacontane	4.81
30	31.811	Methylhexacosane	0.80
31	32.347	Triacontane	1.66
32	32.493	Squalene	1.01
33	35.693	Hexadecatetraenol	0.27
			Total = 47.37%

Besides, squalene (1.01%), docosane (0.92%), tricontane (0.88%), tridecane (0.86%), tetradecane (0.82%), methylhexacosane (0.80%), dodecane

(0.75%) and tetracosane (0.43%) have been identified as minor components. Despite the major content of hydrocarbons, long chain acids namely octadecenoic acid (1.85%), octadecanoic acid (0.61%), eicosanoic acid (0.34%) and long chain alcohols octacosanol (1.75%), dodecadienol (1.58%), hexadecanol (1.55%), tetradecanol (0.75%) and hexadecatetraenol (0.27%) were identified (Table 1). Eicosyl acetate (1.78%), octacosoil acetate (1.40%) and octadecanoic acid methyl ester was contributed or identified as ester components (Table 1). In the detected major constituents hexadecane is known for antibacterial, antioxidant, antianginal activities and for application in cognition disorders treatment (Plaza et al., 2010; Kumaresan et al., 2015; Prasad, et al., 2017). Heneicosaneis reported antineoplastic activity (Kumaresan et al., 2015), eicosane used for phobic disorder treatment (Kumaresan et al., 2015), tetradecane and dodecane known for antineoplastic activity (Kumaresan et al., 2015). Long chain octadecenoicacid reported for application in treatment of type-2 diabetes, high blood pressure disorders as well as it generates myelin and has antioxidant activity (Sharma et al., 2018). Octadecanoic acid contains anticancer, antibacterial and antifungal activities (Sharma et al., 2018).

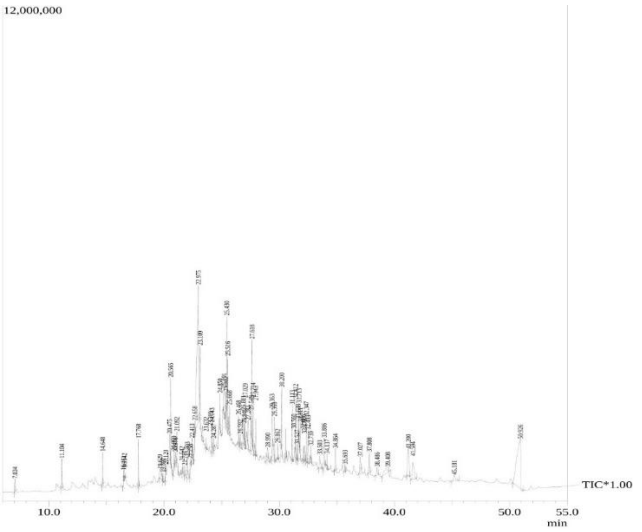


Figure 1. Total ion chromatography (TIC) of ethyl acetate extract of *Streptomyces* sp. Strain 51.

Table 2. Classes of compounds detected in GC-MS analysis of ethyl acetate extract of *Streptomyces* sp. strain 51

S. No.	Compounds	Area %
1.	Hydrocarbons	30.88
2.	Long chain alcohols	6.30
3.	Long chain acids	3.12
4.	Esters	3.18
5.	Ketones	3.51

Liquid Chromatography-Mass Spectrometry (LC-MS/MS)

Ethyl acetate extract/mixture of strain 51 containing bioactive compounds was subjected to LC-MS/MS analysis which gave several sharp peaks within 20min run time (Table 3).

Table 3. Chemical composition of metabolites in LC-MS/MS analysis of *Streptomyces* sp. Strain 51 extract

RT (min.)	Compound name	Molecular weight peak
3.11	Thiolutin	228.16
9.54	Streptothricin D	758.63
13.61	Antimycin A	548.51
15.16	Rifaximin	784.64
18.08	Fujimycin, FK 506	803.61

A sharp peak was observed at 3.11 min interval which indicated molecular ion peak $[M+H]^+$m/z 228.1695, corresponding to thiolutin (Jimenez et al., 1973; Jia et al., 2010). The structure was further confirmed through its fragmentation which upon losing a proton gave peak with $[M^+]$ m/z 227.1662 with 100% abundance (Figure 2).

Another peak of LC-MS/MS was observed at 9.55 min interval corresponding to parent molecular ion peak m/z 758.6326. The parent compound upon fragmentation with energy gave another two daughter ions at m/z 315.2084 and m/z 443.0000. From the literature data and fragmentation pattern of the molecular ion in LC-MS/MS analysis the compound was identified as streptothricin D (Figure 3) (Miyashiro et al., 1983).

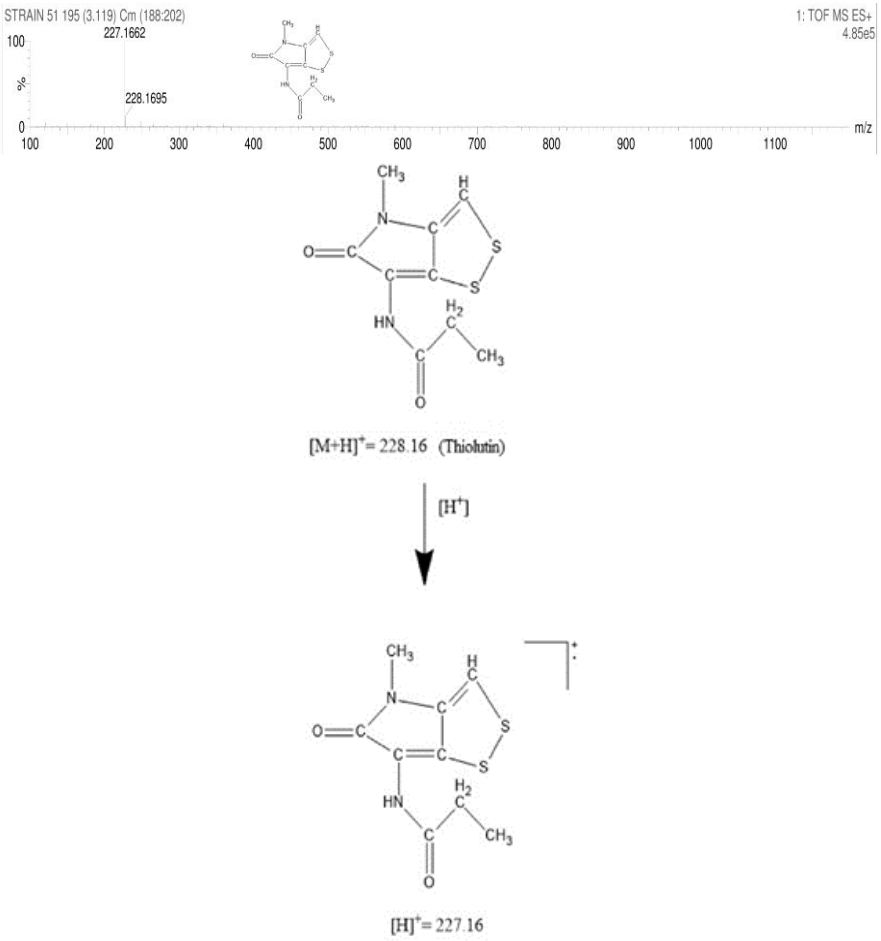


Figure 2. ESI-MS and fragmentation pattern of compound identified as thiolutin during LC-MS/MS analysis of *Streptomyces* sp. Strain 51 crude extract/mixture.

Another major peak was observed at 13.61 min. in LC profiling of ethyl acetate extract. The mass chromatogram indicated molecular ion peak at m/z 548.5180, which suggested cyclic macromolecular structure of antimycin A (m/z 549) gave daughter ion peaks at m/z 395.3289 after losing hydrocarbon long chain $(CH_2)_5CH_3$ and carbonyl group associated with long chain hydrocarbon, $(CH_3)_2CHCH_2CO$. The daughter ion (m/z 395.33) again losses its proton to give major fragment peak at m/z 394.3256. So by the

fragmentation pattern and literature reports the compound was identified as antimycin A (Figure 4) (Tener et al., 1953; Walter and Lardy, 1964).

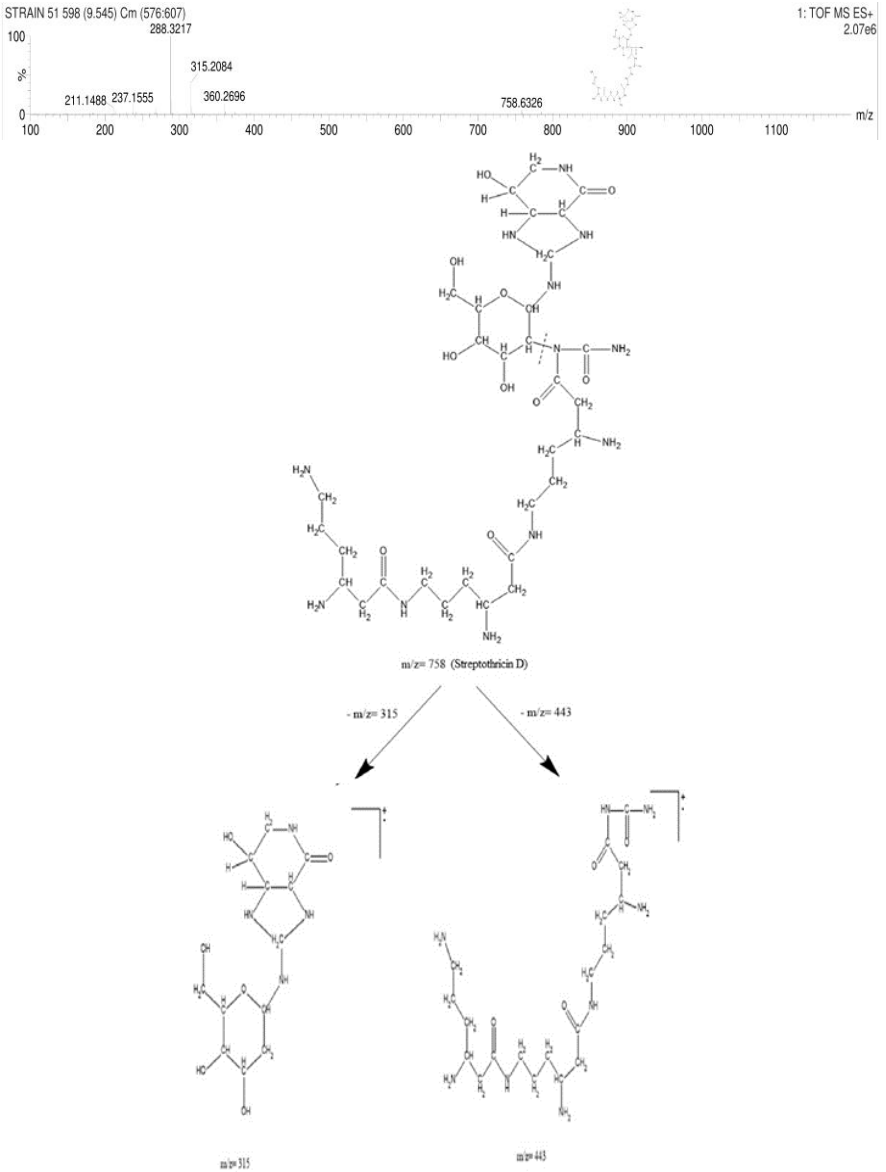


Figure 3. ESI-MS and fragmentation pattern of compound identified as streptothricin D during LC-MS/MS analysis of *Streptomyces* sp. Strain 51 crude extract/mixture.

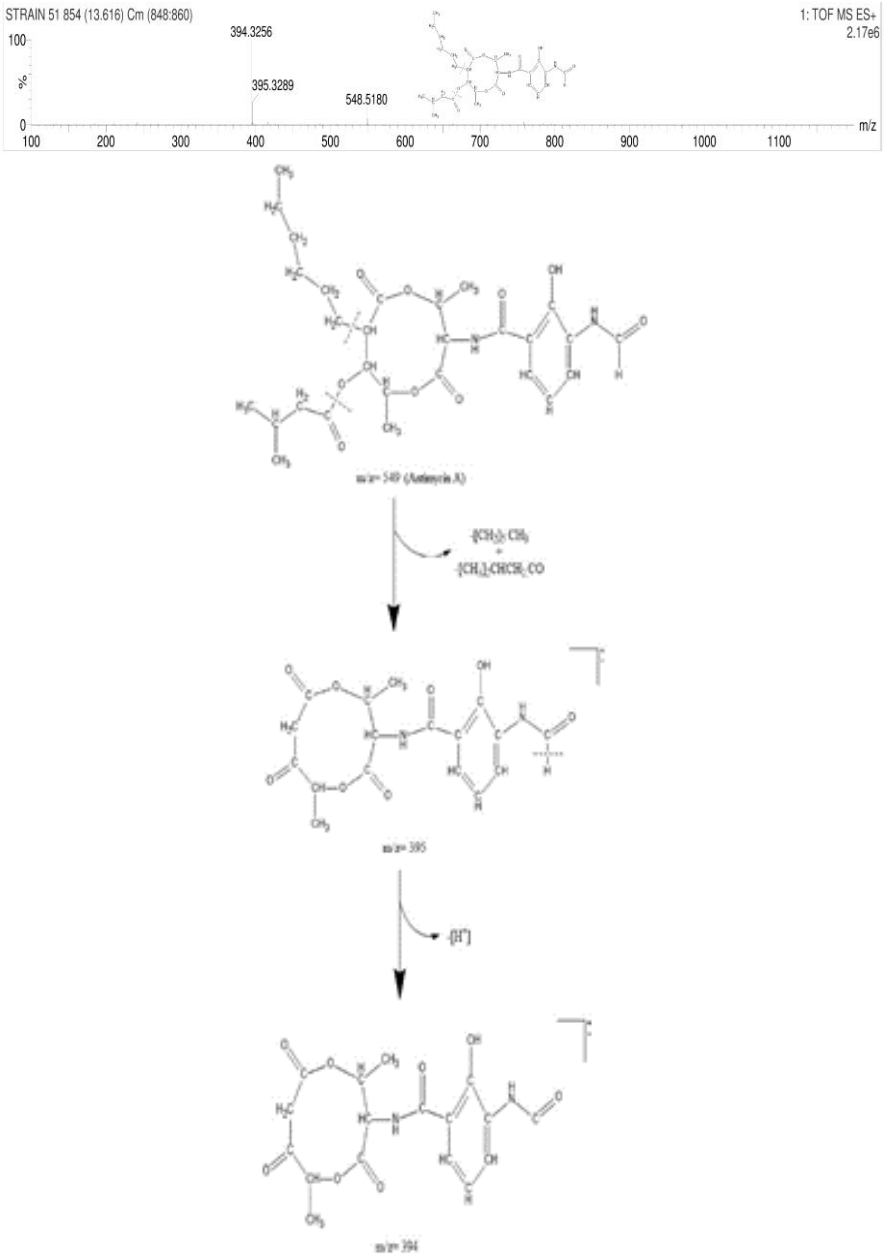


Figure 4. ESI-MS and fragmentation pattern of compound identified as antimycin A during LC-MS/MS analysis of *Streptomyces* sp. Strain 51 crude extract/mixture.

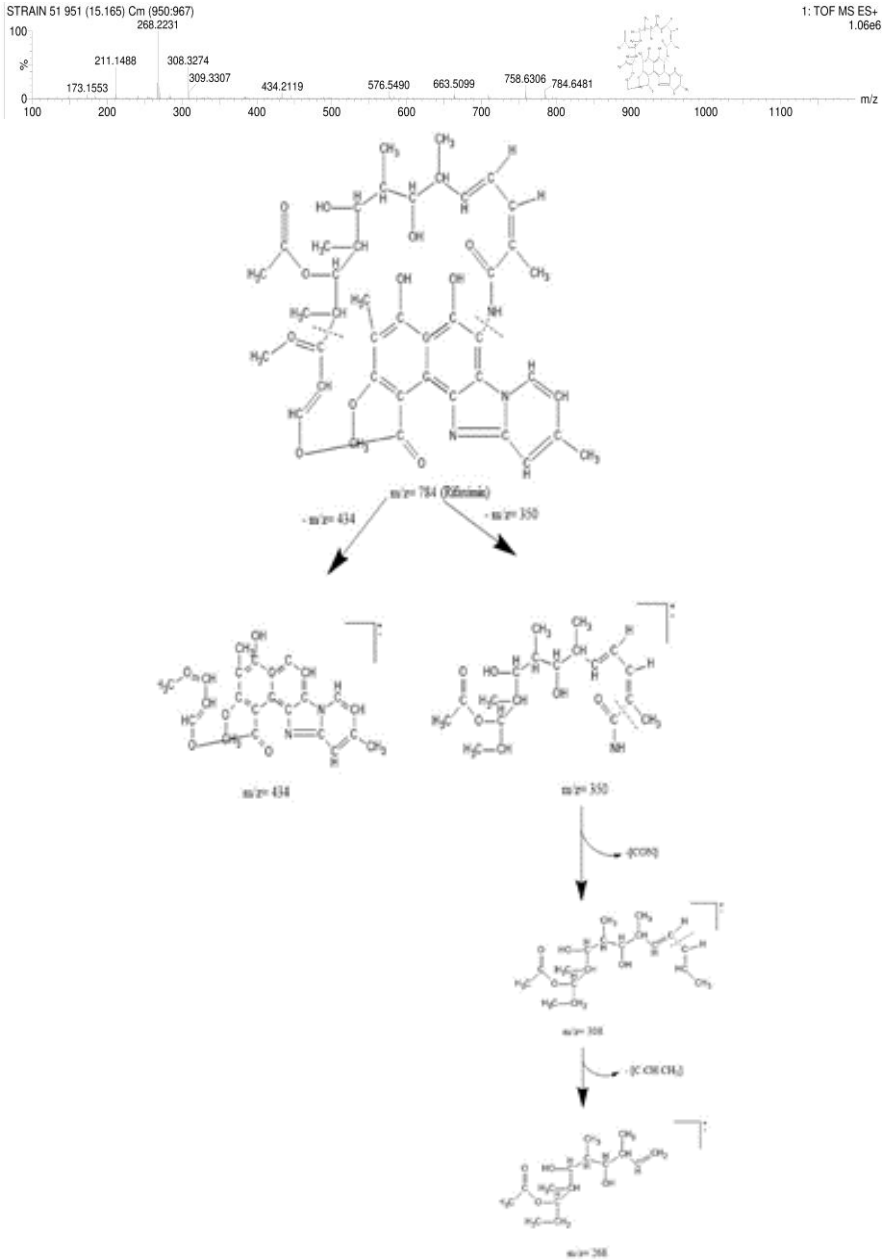


Figure 5. ESI-MS and fragmentation pattern of compound identified as rifaximin during LC-MS/MS analysis of *Streptomyces* sp. Strain 51 crude extract/mixture.

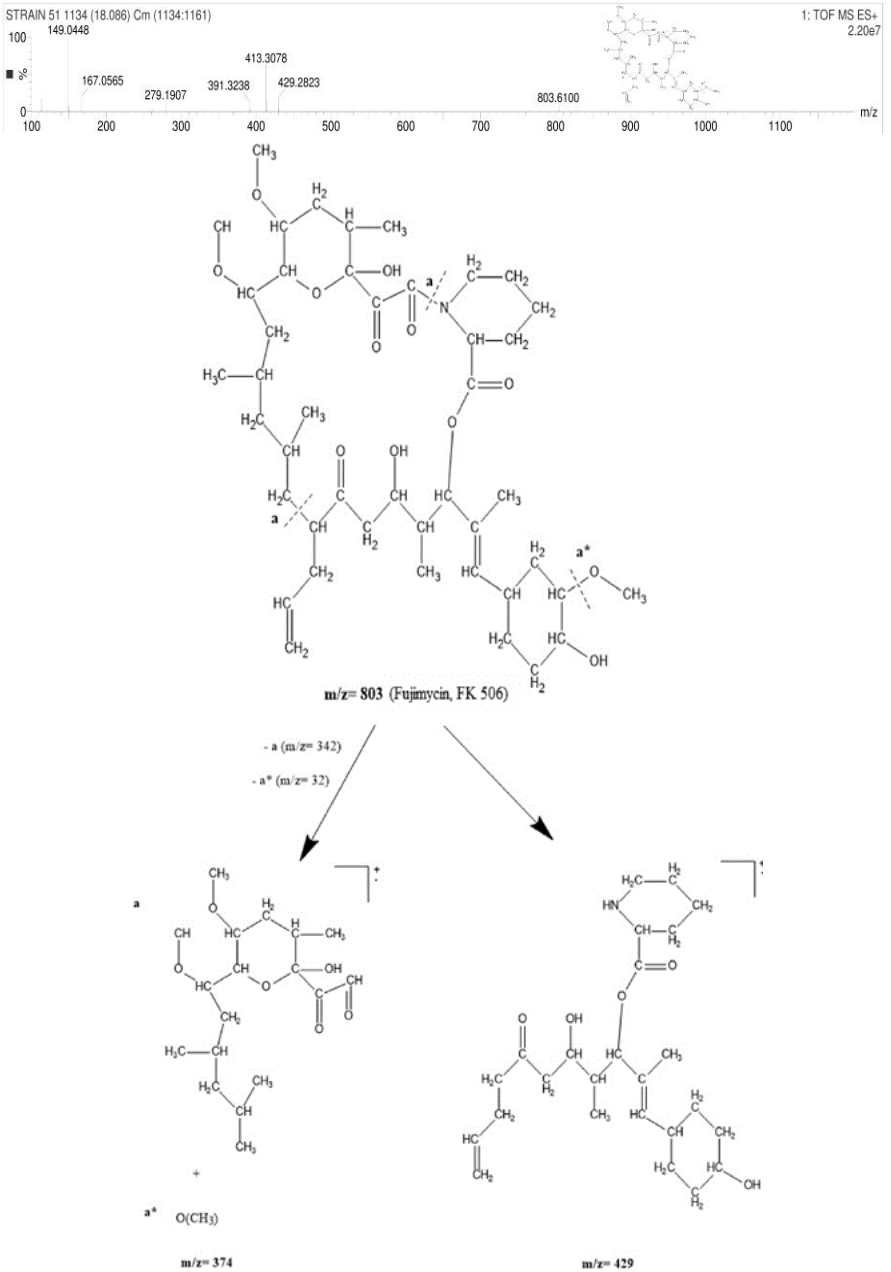


Figure 6. ESI-MS and fragmentation pattern of compound identified as fujimycin during LC-MS/MS analysis of *Streptomyces* sp. Strain 51 crude extract/mixture.

The fourth compound was identified as rifaximin from its LC-MS/MS spectrum; mass fragmentation pattern and literature report (Calanni et al., 2014). The complex molecular structure was elucidated from its LC-MS/MS chromatogram which showed a sharp peak at 15.16 min interval, corresponding to its molecular ion peak at m/z 784.6481. Further fragmentation of the molecular ion peak [784] gave daughter ion peaks at m/z 434.2119 and at 350 due to breaking of C-CH and C-NH bond, respectively. The daughter ion at m/z 350 again fragmented upon energy to produce subsequent fragment ions at m/z 308.3274 and m/z 268.2231 due to loss of amide (-CONH) and hydrocarbon [C-CH-CH_3] (Figure 5).

The structure of the fifth compound was elucidated as fujimycin from its LC-MS/MS profile and literature report (Pourtier-Manzanedo et al., 1991). The macrocyclic antibiotic showed distinct peak at 18.08 min interval in LC. The molecular ion peak of this compound was observed at m/z 803.61. The fragmentation of the molecular ion peak (803.61) showed corresponding peaks at m/z 429.2823, the remaining fragment ion was observed at m/z 374, which took OH^- group from the system to satisfy its charge and gave a peak at m/z 391.3238 (Figure 6).

Polymerase Chain Reaction (PCR) and Sequencing of Bioactive Metabolites Encoding Genes of *Streptomyces* sp. Strain 51

Genomic DNA Isolation

A clear prominent band of genomic DNA was observed on 0.8% agarose gel electrophoresis. This good quality genomic DNA was used as template for the polymerase chain reaction (PCR) detection of polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) biosynthetic gene clusters.

PCR Amplification

PKS and NRPS genes are known for the production of diverse bioactive metabolites in actinobacteria (Sharma et al., 2016). Bioactive compounds yielded from this bacterial group have a wide range of applications in drug

development/pharmaceutical industries (Cane and Walsh, 1999). During detection of biosynthetic gene clusters in strain 51 using degenerate primers, it was observed that strain 51 possess both PKS-I and NRPS genes (Figure 7A, 7B). PCR product of PKS-I gene of strain 51 yielded a band of size ~1200 bp with K1F/M6R primers which specifically target methyl-malonyl-CoA transferase modules and ketosynthase (KS) sequences of PKS-I (Figure 7 A). The amplified product of NRPS gene of strain 51 was found to be ~700bp using A3F/A7R primers which target adenylation domain of NRPS (Figure 7B). The sequences obtained after sequencing were submitted in NCBI-GenBank database using Bankit tool under accession numbers MK355718 and MK355717. Detection of both PKS-I and NRPS gene clusters indicates that these may be responsible for production of bioactive compounds in Strain 51. The results are comparable with the investigations of researchers (Passari et al., 2015, Sharma et al., 2016, Samak et al., 2018), where it was noticed that actinomycetes showing bioactivity were found positive for the presence of one/both of these gene clusters.

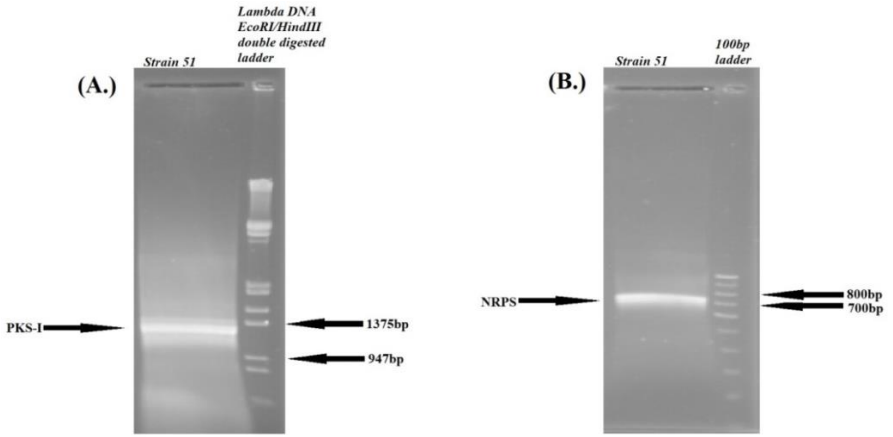


Figure 7.1. 2% Agarose gel electrophoresis of PCR amplicons of *Streptomyces* sp. Strain 51; (A) Amplification of Strain 51 PKS-I gene sequence using K1F/M6R primers, (B) Strain 51 NRPS gene sequence amplification using A3F/A7R primers.

CONCLUSION

Actinomycetes from diverse terrestrial habitats have exceptional physiological and metabolic abilities due to variations in soil conditions such as pH, temperature and moisture etc. These metabolic and physiological capabilities help these microbes to survive in extreme conditions and led them to produce various kinds of metabolites having pharmaceutical/ industrial values. Extensive studies on soil natural products over the past four decades has revealed that soil derived actinomycetes are the most prolific sources of diverse metabolites. In present study, soil derived actinomycete, *Streptomyces* sp. Strain 51 was found to produce secondary metabolites having higher bioactivity than the industrial antibiotic chloramphenicol. GC-MS and LC-MS/MS analysis revealed that the higher bioactivity of strain 51 secondary metabolites is due to the presence of bioactive compounds. During GC-MS analysis thirty three volatile compounds were detected in Strain 51 which contains hydrocarbons, long chain alcohols, long chain acids, esters, ketones and some of them are reported to have diverse bioactivities. Non-volatile bioactive compounds present in extract were detected using LC-MS/MS analysis. Total five compounds thiolutin, streptothricin D, antimycin A, rifaximin and fujimycin FK 506 were detected. These compounds are reported for having broad range of biological activities such as antibacterial, antifungal, antitumor, insecticidal and clinical applications like treatment of gastrointestinal tract related diseases, traveler's diarrhea, applications in allogenic organ transplant etc. The results of present investigation provide information that actinomycete Strain 51 is a rich source of bioactive compounds. Furthermore, these compounds can be exploited for the drug development by pharmaceutical industries. Strain 51 contains both PKS-I and NRPS gene clusters, in future studies introduction of modifications in these genes may results into the production of new bioactive compounds having unique modes of action against the pathogens.

ACKNOWLEDGMENTS

Authors PK and MK thank Council of Scientific and Industrial Research (CSIR), Government of India for providing Research Fellowships. Authors are also thankful to Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University (JNU), India for providing GC-MS and LC-MS/MS instrumentation facilities. Central Instrumentation Facility (CIF), Biotech Centre, University of Delhi South Campus, India is acknowledged for providing DNA sequencing facility. We also thank Acharya Narendra Dev College, University of Delhi, India for providing infrastructural and other necessary facilities for research.

REFERENCES

Ayuso-sacido, A. and Genilloud, O. 2005. New PCR primers for the screening of NRPS and PKS-I systems in actinomycetes: Detection and distribution of these biosynthetic gene sequences in major taxonomic groups." *Microbial Ecology* 49 (1): 24-10. https://doi.org/10.1007/ s002 48-004-0249-6.

Andrews, J. A. 2001. Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy* 48: 5-16. https://doi.org/10.10 93/jac/48.suppl_1.5.

Bibb, M. J. 2005. Regulation of secondary metabolism in Streptomycetes. *Current Opinion in Microbiology* 8(2): 215-208. https://doi.org/10.10 16/j.mib.2005.02.016.

Calanni, F., Renzulli, C., Barbanti, M. and Viscomi, G. C. 2014. Rifaximin: Beyond the traditional antibiotic activity. *Journal of Antibiotics* 67 (9): 667–70. https://doi.org/10.1038/ja.2014.106.

Cane, D. E., and Walsh, C. T. 1999. The parallel and convergent universes of polyketide synthases and nonribosomal peptide synthetases. *Chemistry & Biology* 6 (12): 319-325. https://doi.org/10.1016/S1074-55 21(00)80001-0.

Dewi, T. K., Dwi, A. and Sarjiya, A. 2017. Secondary metabolites production by Actinomycetes and their antifungal activity. *KnE Life Sciences* 3 (4): 256. https://doi.org/10.18502/kls.v3i4.713.

Das, P. 2017. Microbial Extracellular Enzymes: Purification, Molecular Characterization and Applications. PhD Thesis, University of Delhi.

Hopwood, D. A. 2007. Therapeutic treasures from the deep. N*ature Chemical Biology* 3 (8): 458-457. https://doi.org/10.1038/nchembio0807-457.

Hopwood, D. A., Bibb, M. J., Chater, K. F., Kieser, T., Bruton, C. J., Kiesser, H. M., Lydiate, D. J., Smith, C. P., Ward, J. M. and Schrempf, H. 1985. Genetic manipulation of *Streptomyces*: A laboratory manual. United Kingdom (UK): John Innes Foundation, 1985.

Jia, Y., Shiaw, L. W., Jeff, S. I., Shujia Dai, John M. S., Lyndsay, F., Bixi, Z., Russel, W. B., Lisa, A. R., David, A. W., Ramani, R., Barry, L. K. and David, D. R. et al., 2010. Thiolutin inhibits endothelial cell adhesion by perturbing Hsp27 interactions with components of the actin and intermediate filament cytoskeleton. *Cell Stress and Chaperones* 15 (2): 165–81. https://doi.org/10.1007/s12192-009-0130-0.

Jimenez, A., Tipper, D. J. and Davies. J. 1973. Mode of action of thiolutin, an inhibitor of macromolecular synthesis in *Saccharomyces cerevisiae*. *Antimicrobial Agents and Chemotherapy* 3 (6): 729–38. https://doi.org/10.1128/AAC.3.6.729.

Kapur, M. K., Solanki, R., Das, P. and M. Kumar. 2018. Antimicrobial activity analysis of bioactive compounds from soil actinomycetes. *Journal of Pharmaceutical, Chemical and Biological Sciences* 6(3): 187-178.

Kumaresan, S., Senthilkumar, V., Stephen, A. and. Balakumar, B. S. 2015. GC-MS analysis and pass-assisted prediction of biological activity spectra of extract of *Phomopsis* sp. isolated from *Andrographis paniculata*. *World Journal of Pharmaceutical Research* 4(1): 1053-1035.

Khanna, M. and Solanki, R. 2012. *Streptomyces antibioticalis*, a novel species from a sanitary landfill soil. *Indian Journal of Microbiology* 52: 611-605. https://doi.org/10.1007/s12088-012-0309-4.

Lim, Y., Jung, E. S., Lee, J. H., Kim, E. J., Hong, S. J., Lee, Y. H. and Lee, C. H.. 2018. Non-targeted metabolomics unravels a media-dependent prodiginines production pathway in *Streptomyces coelicolor* A3(2). *PLoS ONE* 13 (11): 1–17. https://doi.org/10.1371/journal.pone.0207541.

Miyashiro, S., Ando, T., Hirayama, K., Kida, T., Shibai, H., Murai, A., Shiio, T. and Shigzeo Udaka. S. 1983. New streptothricin–group antibiotics, AN-201 I and II. Screening, fermentation, isolation, structure and biological activity. *The Journal of Antibiotics* (Tokyo) 36 (12) : 1638 -1643.

Prasad, L., Aditi Kundu and D. Bahukhandi. 2017. Comparative analysis of volatile fractions of *Fomes fomentarius and F. Rhabarbarinus*. *Indian Phytopathology* 71 (1): 25–31. https://doi.org/10.1007/s42360-018-0003-5.

Passari, A. K., Mishra, V. K., Saikia, R., Gupta, V. K. and Singh, B. P.. 2015. Isolation, abundance and phylogenetic affiliation of endophytic actinomycetes associated with medicinal plants and screening for their *in vitro* antimicrobial biosynthetic potential. *Frontiers in Microbiology* 6: Article 273. https://doi.org/10.3389/fmicb.2015.00273.

Plaza, M., Santoyo, S., Jaime, L., Garcia-Blairsy Reina, G., Herrero, M., Senorans, F. J. and Ibanez. E. 2010. Screening of bioactive compounds from algae. *Journal of Pharmaceutical and Biomedical Analysis* 51 (2): 450-455. https://doi.org/10.1016/j.jpba.2009.03.016.

Pourtier-Manzanedo, A., Boesch, D. and Loor, F. 1991. FK-506 (fujimycin) reverses the multidrug resistance of tumor cells *in vitro*. *Anti-Cancer Drugs* 2 (3): 2719- 283.

Samak, M. El., Samar M. S. and Hanora. A. 2018. Antimicrobial activity of bacteria isolated from Red Sea marine invertebrates. *Biotechnology Reports* 19: e00275. https://doi.org/10.1016/j.btre.2018.e00275.

Sharma, A., Rai, P. K. and Prasad, S. 2018. GC–MS detection and determination of major volatile compounds in *Brassica juncea* L. leaves and seeds. *Microchemical Journal* 138 (2018) : 488–493. https://doi.org/10.1016/j.microc.2018.01.015.

Sharma, P., Kalita, M. C. and Thakur. D. 2016. Broad spectrum antimicrobial activity of forest-derived soil actinomycete, *Nocardia* sp. PB-52. *Frontiers in Microbiology* 7: 347. https://doi.org/10.3389/fmicb.2016.00347.

Solanki, R., AditiKundu, Das, P. and Khanna, M. 2015. Characterization of antimicrobial compounds from *Streptomyces sp*. *World Journal of Pharmaceutical Research* 4(7): 1626 -1641.

Solanki, R. 2013. Isolation and characterization of actinomycetes and analyses of their antimicrobial potential. PhD, thesis, University of Delhi. 153p.

Schimana, J., Fiedler, H-P., Groth, I., Submuth, R., Beil, W., Walker, M. and Zeeck, A. 2000. Simocyclinones, novel cytostatic angucyclinone antibiotics produced by *Streptomyces antibioticus* Tü6040. *The Journal of Antibiotics* 53(8): 787-779. https://doi.org/10.7164/antibiotics.53.779.

Tener, G. M., Van Tamelen, E. E. and Strong. F. M. 1953. The chemistry of antimycin A. III. The structure of antimycic acid. *Journal of the American Chemical Society* 75 (15): 3623–3625. https://doi.org/10.1021/ja01111a005.

Walter, P. and Lardy, H. A. 1964. Effect of antimycin A on oxidative phosphorylation with ferricyanide as electron acceptor. *Biochemistry* 3 (6): 812–16. https://doi.org/10.1021/bi00894a015.

In: A Closer Look at Actinomycetes ISBN: 978-1-53617-046-7
Editors: A. A. Mohamed Hatha et al.

Chapter 8

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF BIOACTIVE ACTINOMYCETES (ACTINOMYCETALES) FROM TROPICAL WETLAND ECOSYSTEM

George Maya[1,*], Azis Anas Abdul [2], C. Jasmin[2,**], K. M. Mujeeb Rahiman[3,€] and A. A. Mohamed Hatha[4,€€]

[1]Department of Zoology, Alphonsa College Pala,
Kottayam, India
[2]National Institute of Oceanography, Cochin, India
[3]Department of Aquaculture and Fishery Microbiology,
M.E.S. Ponnani College, Ponnani, India
[4]Department of Marine Biology,
Microbiology and Biochemistry,
Cochin University of Science and Technology,
Cochin, India

[*] Corresponding Author's Email: mayarosegeorge@gmail.com.
[**] Email: cjasmina78@gmail.com.
[€] Email: rkmmujeeb@gmail.com.
[€€] Email: mohamedhatha@gmail.com.

ABSTRACT

As emergence of multi drug resistant bacteria has become more frequent, the search is on for novel antibiotics. Under explored and unique habitats are being prospected for novel strains of Actinomycetes which have the potential to produce new antibiotics. Accordingly, the present study has been taken up with an objective to explore the diversity of actinomycetes from Vembandu estuary, the biggest and one of the 3 Ramsar sites in Kerala. Sediment samples were collected seasonally, using van veen grab from selected stations for a period of one year. Actinomycetes were isolated using Kusters agar and characterized by polyphasic taxonomy. Physiological capabilities of isolates were evaluated and the antibacterial activities against a range of pathogens were studied using standard methods. Various genera of Actinomycetes such as *Actinobispora*, *Actinokineospora*, *Actinosynnema*, *Catellospora*, *Kibdelosporangium*, *Micromonospora*, *Nocardiopsis*, *Rhodococcus*, *Saccharopolyspora*, *Streptoalloteichus*, *Streptomyces*, *Streptosporangium*, *Thermoactinomyces* and atypical *Nocardia asteroids* were encountered in the lake sediments. More than 90% of the actinomycete isolates revealed good antibacterial activity against pathogenic bacteria. While 81% of them were able to produce protease, 56% of them decomposed hypoxanthine and tyrosine. Ability to decompose xanthine was relatively low (11%). Molecular identification of potential Actinomycete strains were carried out based on 16s rRNA gene, which revealed their identity as *Streptomyces olivaceus*, *Streptomyces costaricanus*, *Nocardiopsis flavescens* and *Nocardiopsis alkaliphila*. This study revealed that lake sediments could be good source of potential actinomycetes and reconfirms the need for exploring under explored and unexplored habitats for diverse actinomycetes which could probably yield novel antibiotics to fight the emerging threat of multidrug resistant pathogens.

Keywords: Vembanadu lake, wetland sediment, actinomycetes, antibiotics, bioactive compounds

INTRODUCTION

Actinomycetes belonging to the phylum Actinobacteria are widely sought after for various bioactive compounds especially antibiotics [1]. They play key roles in various biogeochemical cycles, production of humus and

have the enzymatic machinery to breakdown recalcitrant organic polymers keratin, ligonocellulose and chitin [2]. As per Bergey's manual there are eight diverse groups and comprise sixty three genera.

Class Actinobacteria are capable of producing secondary metabolites that have a wide range of bioactivity such as antibacterial, antifungal, antioxidant, antitumor and antiviral properties. More than eighty percent of antibiotics that are in use today have come from actinomycetes, with a lion's share of it coming from the genera such as *Streptomyces* and *Micromonospora* [3]. This has resulted in an active search for actinobacteria and most of the easily accessible habitats such as terrestrial and near shore areas were widely explored in the hunt for novel strains. However, the frequent and inappropriate use of antibiotics leads to the emergence of multi drug resistant pathogens, which outpaced the discovery of new antibiotics. Hence the search is being extended to under explored, unexplored and extreme habitats for unearthing potential strains of actinomycetes.

Vembanadu lake the largest Ramsar site in Kerala is an extensive spread of water body which receives terrogenous sediments through rivers draining into it as well as modified by tidal influx of sea water. This makes it a unique habitat which could probably sustain novel microorganisms, including actinomycetes. Accordingly this study has been conceived with an objective to explore the diversity of actinomycetes in the sediment from Vembanadu lake and to characterize their potential to produce various bioactive substances, especially antibiotics.

METHODS

Study Area: Sediment samples were collected from five different stations (S1, S2, S3, S4 and S5) at Kumarakom region of Vembanadu lake (9º37'33"- 9º38'21'' N and 76º23'11"- 76º25'06" E). Samples were collected by using Van Veen grab. Using a sterile spatula nearly 100g of sample was transferred into sterile Whirlpack polythene bags and transported to the laboratory in an ice chest. Samples are collected on a seasonal basis such as pre-monsoon, monsoon and post monsoon. Fifteen

samples were collected over period of one year ranging from July 2007 to June 2008.

Pretreatment of samples and isolation of actinomycetes: The samples upon arrival in the laboratory were air dried at room temperature in a sterile chamber for about one week. The air dried samples were heated aseptically at 50^{o}C for one hour [4]. Samples were streaked over Kusters Agar [5], and are incubated at room temperature for two weeks. The colonies are enumerated and typical actinomycetes colonies were isolated based on colony morphology, restreaked on Kusters agar.

Characterization of Actinomycetes: As described in the Bergey's Manual of Determinative Bacteriology [6] actinomycetes isolates were characterized by polyphasic approach [7].

Determination of antibacterial activity by well diffusion method: Antibacterial activity of actinomycetes was determined against a range of test microorganisms by well diffusion method. The twenty four hour old broth cultures of test pathogens (*Salmonella bovis, S. typhimurium, S. senftenberg, S. typhi, S. paratyphi, S. mgulani, S. enteritidis, S. welteverden, S. bareilly, Vibrio cholerae, Bacillus subtilis, B. cereus,* Enterotoxigenic *Escherichia coli* and Enteropathogenic *Escherichia coli*) were used for preparing a lawn culture over Glycerol-Yeast Extract Agar. Using a sterile agar puncher, an agar well of three mm diameter were punched into the seeded Glycerol-Yeast Extract Agar. Thirty microliters of four day old broth culture of actinomycetes was dispensed into each well. After twenty four hour of incubation at room temperature, zone of inhibition (in mm) around each well was measured.

Molecular Characterization

Sample preparation: The actinomycete isolates were grown in Kuster's broth and incubated at room temperature for five days on a shaking incubator. After incubation, biomass was harvested by centrifugation at 5000rpm for ten min.

DNA isolation: DNA was extracted from the actinomycetes as per standard methods [8].

PCR amplification and sequencing: PCR amplification of the 16 S rDNA of the isolates was performed using two primers *viz.* '16S rRNA 27F: AGAGTTTGATCCTGGCTCAG' and '16S rRNA 1492R: TACGGY TACCTTGTTACGACTT [9].

Briefly, PCR reactions in 50 µl volumes, containing 50 ng of template DNA, 2µl each of the primers (10 pmol/µl), 3 U Taq DNA polymerase (SIGMA India), 3µl 10x Taq polymerase buffer (SIGMA India), 200µM each deoxynucleotide (SIGMA India) and 2.5mM $MgCl_2$. The amplification was performed on an Eppendorf Mastercycler (Eppendorf, Germany), according to the following conditions: an initial denaturation step at 95°C for 5min, followed by 30 cycles of denaturation at 94°C for 50s, annealing at 60°C for 50 s, and elongation at 72°C for 1.30 min and a final extension step of 72°C for 10 min. The PCR product was detected by agarose gel electrophoresis and was visualized after staining with ethidium bromide in a Gel documentation system (Biorad, USA). The sequencing of the PCR products was carried out on an ABI sequencer (Scigenom Labs Pvt. Ltd. Kochi, India).

Phylogenetic analysis: The obtained sequences were compared with the reference species of microorganisms contained in genomic database banks, using 'NCBI Blast' (http://www.ncbi.nlm.nih.gov/) and assigned phylogenetically according to their best matches to sequences in the NCBI database/ RDP-ribosomal database project: http://rdp.cme.msu.edu/ .Neighbour-joining algorithm was used to construct phylogenetic tree using software MEGA 5.05.

RESULTS AND DISCUSSION

Load of Actinomycetes in Vembanadu lake sediment samples: Wetlands are commonly referred to as nature's kidneys as they filter out most of the pollutants before they reach the aquatic environment. By doing this they also act as a repository for wide range of organic nutrients that can support

diverse microorganisms, including bioactive actinomycetes [10]. In this investigation actinomycetes were isolated from Kumarakom lake sediment samples by using Kuster's agar [11]. Different sampling seasons of the year revealed differences in population density of actinomycetes.

Highest population density was observed during monsoon (1.5 x 10^4cfu/g) followed by pre-monsoon (1.2 x 10^4cfu/g) (Figure 1). A total of 27 actinomycetes strains were isolated from the sediment samples collected over three seasons of the year (A1 to A27).

Diversity of Actinomycetes: Very few reports are available about diversity and distribution of actinomycetes in the southern coastal parts of Indian peninsula.

Sivakumar et al. [12], in their study reported fourty one species of actinobacteria coming under eight genera. In the present study fourteen generic groups of actinomycetes were identified in sediment of Kumarakom lake (Figure 2).

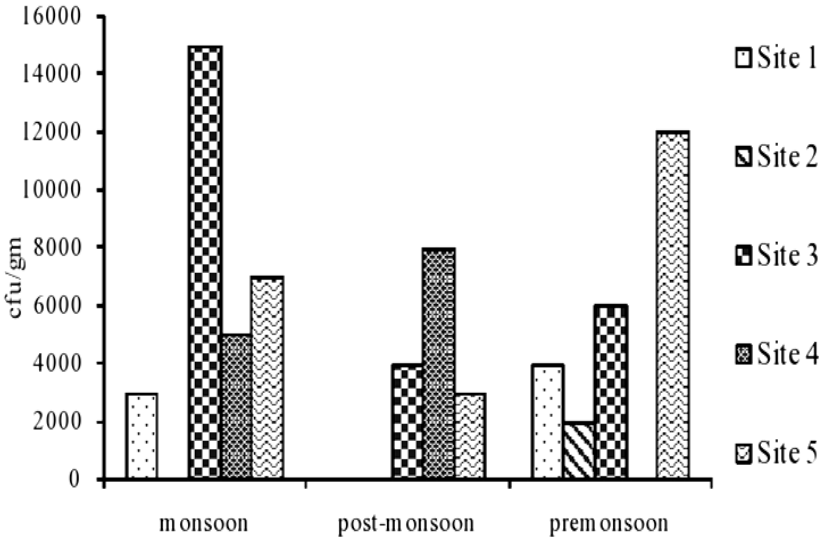


Figure 1. Seasonal variation in the load of actinomycetes from the sediment samples.

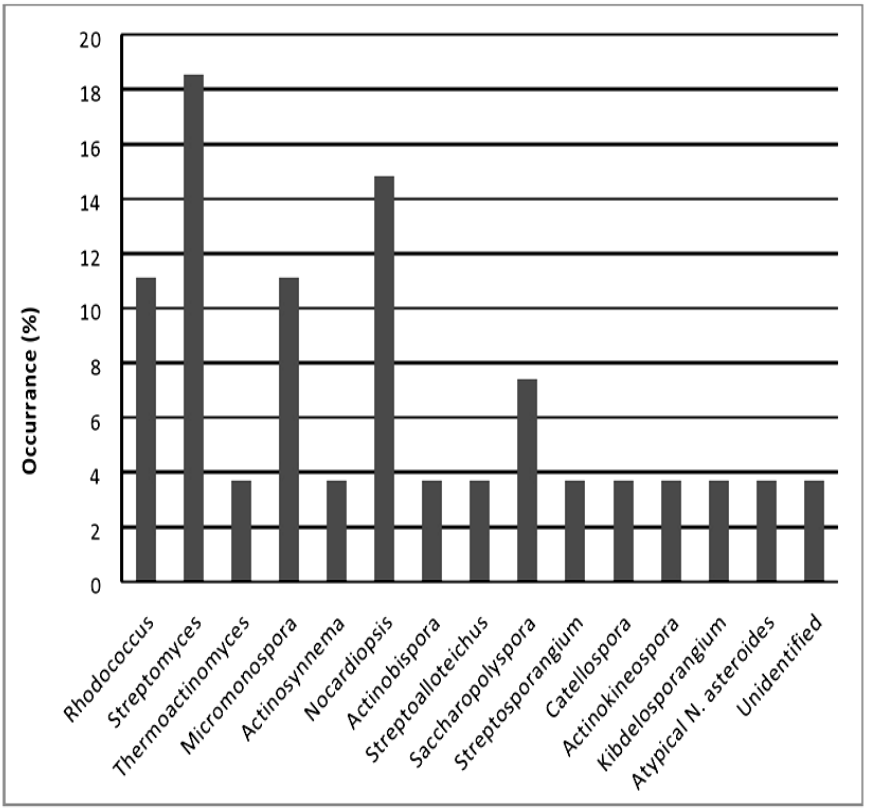


Figure 2. Diversity of actinomycetes in the Kumarakom lake sediments.

Marine and lake ecosystems were reported to have the dominance of actinomycete genera *Streptomyces*, *Micromonospora* and *Salinispora* [13, 14].

The isolated *Streptomyces* preferred arabinose as the carbon source and they do not have urease activity. In the present study *Rhodococcus* were identified which are devoid of aerial mycelia and diffusible exo-pigment. The morphological characteristics of producing aerial and substrate mycelia have led to some confusion in the classification of the genus *Thermoactinomyces* [15].

Physiological and biochemical characteristics of isolates: Actinomycetes are the most economical and biotechnologically valuable class of prokaryotes producing bioactive secondary metabolites [16]. In the

present investigation protease activity was observed among 81% of isolates, and all the isolates were resistant to lysozyme (Table 1).

Table 1. Physiological characteristics of Actinomycetes isolates

Characeristics	**% postives**
Decomposition of	
Casein	81.48
Xanthine	11.11
Hypoxanthine	55.56
Tyrosine	55.56
Enzymatic activity	
Urease	33.33
Esculinase	29.62
Lysozyme resistance	100
Utilization of carbohydrates	
Arabinose	81.48
Fructose	44.44
Inositol	55.56
Lactose	70.37
Mannitol	81.48
Rhamnose	64
Sorbitol	78.26
Trehalose	66.67
Xylose	70.37

Prevalence of these properties among our isolates was much higher than previously reported activities of actinomycetes from lake environment [14].

Antibacterial activity of sediment derived actinomycetes: It was found that considerable number of actinomycete isolates from Kumarakom lake sediment samples have an inhibitory effect on test pathogens used. About 30% of sediment derived actinomycetes showed antibiosis towards enterotoxigenic *E. coli*. Elbendary et al. [17] reported that soil derived actinomycetes could not inhibit *E. coli*.

It was observed that *Streptomyces costaricanus* strain (A22) revealed good zone of inhibition (18mm) against *V. cholerae*. Four actinomycete isolates out of 27 (14.82%) from Kumarakom lake showed activity against *Bacillus subtilis* and 44% showed antagonism towards *S. typhi* (Table 2). The actinomycete isolate A19 has shown to be capable of inhibiting 12 pathogens studied.

Molecular characterization of actinomycete strains: Those actinomycete isolates which revealed relatively high antibacterial activity (A12, A15, A18, A19 and A22) (Table 3) were characterized completely by molecular methods.

The sequences were submitted to GenBank and results revealed that isolate A12 and A15 are *Streptomyces olivaceus* (accession no. KM386046, KM386047 respectively), A18 is *Nocardiopsis flavescens*strain (accession no. KM386048), A19 is *Nocardiopsis alkaliphila* strain (accession no. KM386049), and A22 is identified as *Streptomyces costaricanus* (accession no. KM386050). Phylogenetic tree (Figure 3) revealed 98% 16S rDNA gene sequences similarity between isolates A15 and A12. Strain A19 formed an independent separate phyletic line within the clade.

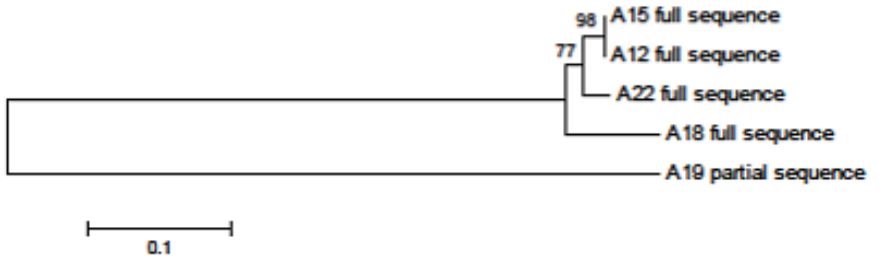


Figure 3. Phylogenetic tree derived from 16S rDNA gene sequences of pure cultures of 5 bioactive actinomycetes. Numbers on branches are confidence limits as percentages estimated from a bootstrap analysis with 1000 replicates.

Table 2. Antibacterial activity of actinomycetes isolated from lake sediments

Actinomycete strains	**Diameter of inhibition zone (mm) against pathogenic bacteria**													
	S.bareilly	E.c.12	S.s	S.m	S.e	S.b	S.p	S.t.	S.w	*S.typhi*	V.c	E.c.78	B.c	B.s
A1	0	14	0	0	0	20	0	27	0	0	0	0	0	0
A2	0	0	0	0	0	0	0	24	0	0	0	0	0	0
A3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A4	0	0	0	0	0	22	0	17	0	0	0	0	0	0
A6	0	0	28	0	0	0	12	30	28	0	16	0	0	0
A7	0	0	0	0	0	0	0	22	0	0	0	0	0	0
A8	0	0	0	0	0	20	0	0	0	0	14	0	0	0
A9	0	0	0	0	0	15	12	0	0	0	12	0	10	0
A10	0	0	0	0	18	15	20	16	0	12	0	0	10	0
A11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A12	0	0	0	25	20	18	16	0	0	18	0	15	18	0
A13	0	0	0	12	0	30	0	0	0	0	0	0	0	0
A14	0	0	0	0	20	25	15	0	0	17	0	15	0	0
A15	0	0	0	10	0	26	14	0	14	12	12	15	0	0
A16	0	0	0	13	0	14	0	0	14	16	0	0	0	0
A17	0	12	0	20	22	14	0	0	18	14	0	0	12	0
A18	0	17	0	15	25	17	0	13	16	15	0	0	0	0

Actinomycete strains	**Diameter of inhibition zone (mm) against pathogenic bacteria**													
	S. bareilly	E.c.12	S.s	S.m	S.e	S.b	S.p	S.t.	S.w	*S.typhi*	V.c	E.c.78	B.c	B.s
A19	0	17	23	20	28	12	20	15	12	22	0	15	12	13
A20	0	0	0	20	0	16	0	0	13	12	14	0	0	13
A21	0	0	0	19	23	22	0	0	0	22	0	0	12	0
A22	0	20	0	20	22	21	13	0	14	0	18	12	0	0
A23	0	16	0	0	21	0	22	0	0	25	0	12	0	30
A24	0	12	0	0	0	0	0	14	0	0	0	13	0	0
A25	0	13	0	0	0	0	0	12	0	0	12	0	0	0
A26	0	12	0	0	0	0	0	12	12	0	0	0	12	0
A27	0	15	0	0	22	0	30	0	0	14	0	12	0	20
A28	0	0	0	0	0	0	14	0	0	0	0	0	0	0

S.b = Salmonella bovis, E.c = Escherichia coli, S.t = Salmonella typhimurium,

S.s = Salmonella senftenberg, S.m = Salmonella mgulani, V.c = Vibrio cholerae,

S.e = Salmonella enteritidis, B.s = Bacillus subtilis,

S.w = Salmonella welteverden, S.p = Salmonella paratyphi,

B.c = Bacillus cereus.

Table 3. Antibacterial activity of the selected isolates of Actinomycetes against pathogenic bacteria

Actinomycete species	*S.bareilly*	E.c.12	S.s	S.m	S.e	S.b	S.p	S.t.	S.w	S.typhi	V.c	E.c-78	B.c	B.s
	(Diameter of inhibition zone (mm)													
Streptomyces olivaceus (A12)*	0	0	0	25	20	18	16	0	0	18	0	15	18	0
Streptomyces olivaceus (A15)	0	0	0	10	0	26	14	0	14	12	12	15	0	0
Nocardiopsis flavescens (A18)	0	17	0	15	25	17	0	13	16	15	0	0	0	0
Nocardiopsis alkaliphila (A19)	0	17	23	20	28	12	20	15	12	22	0	15	12	13
Streptomyces costaricanus (A22)	0	20	0	20	22	21	13	0	14	0	18	12	0	0

*Figure in the parenthesis indicate isolate number.

CONCLUSION

This chapter describes about generic diversity of bioactive actinomycetes at Kumarakom lake. The genera *Streptomyces* was found to be dominant in the Kumarakom lake sediments, followed by *Nocardiopsis*. The number of all other genera in the sediment samples of the lake were small. Protease enzyme was observed among 81% of actinomycetes, and 56% had tyrosinase activity. These actinomycetes might play a significant role in the biogeochemical cycles that operates at Kumarakom lake. Aquatic actinomycetes found in this research might also have potential for antibiotic production. Thus it is concluded that, Vembanadu lake especially Kumarakom region, is an eminently suitable ecosystem for the isolation of bioactive actinomycetes.

REFERENCES

[1] Charousova, Ivana, Juraj Medo, Eva Halenarova and Sona Javorekova. "Antimicrobial and enzymatic activity of actinomycetes isolated from soils of coastal islands". *Journal of advanced pharmaceutical technology and research*, 8, no. 2 (2017). 46 - 51.

[2] Stach, James E. M., Alan T. Bull. "Estimatiing and comparing the diversity of marine actinobacteria". *Antonie van Leeuwenhoek*, 87, no. 1 (2005). 3 - 9.

[3] Kumar, Narendra, Ravi Kant Singh, Mishra, S. K., Singh, A. K. and Pachouri, U. C. "Isolation and screening of soil Actinomycetes as source of antibiotics active against bacteria". *International Journal of Microbiology Research*, 2, no. 2 (2010). 12 - 16.

[4] Rana, Sandeep, Menaka Devi Salam. "Antimicrobial potential of Actinomycetes isolated from soil samples of Punjab, India". *Journal of Microbiology and Experimentation*, 1, no. 2 (2014). 63 - 68.

[5] George, Maya, Anjumol, A., Gisha George and Mohamed Hatha, A. A. "Distribution and bioactive potential of soil actinomycetes from different

ecological habitats". *African Journal of Microbiology Research,* 6, no. 10 (2012). 2265 - 2271.

[6] Holt, G. J., Krieg, R. N., Sneath, A. H. P., Staley, T. J. and Williams, T. S. "Bergey's Manual of Determinative Bacteriology". Ninth Edition. Lippincott Williams and Wilkins:Philadelphia, 2000.

[7] Gordon, Ruth E. "Some Strains in Search of a Genus-Corynebacterium, Mycobacterium, Nocardia or What? *Journal of General Microbiology*, 43, (1966). 329 - 343.

[8] Liu, Don, Sue Coloe, Rob Baird and John Pedersen. "Rapid Mini-Preparation of fungal DNA for PCR". *Journal of Clinical Microbiology*, 38, no. 1 (2000). 471.

[9] Lane, D. J. "16S/23S rRNA sequencing". Edited by E. Stackebrandt and M. Goodfellow. John Wiley & Sons: New York, 1991.

[10] Karthik, L., Gaurav Kumar and Bhaskara Rao K. V. "Diversity of Marine Actinomycetes from Nicobar marine sediments and its antifungal activity". *International Journal of Pharmacy and Pharmaceutical Sciences,* 2, no. 1 (2010). 199 - 203.

[11] George, Maya, Neethu Cyriac, Aswathy Nair and Hatha, A. A. M. "Diversity of *Bacillus* and Actinomycetes in the water and sediment samples from Kumarakom region of Vembanadu lake". *Indian Journal of Geo-marine Sciences,* 40, no. 3 (2011). 430 - 437.

[12] Sivakumar, K., Maloy Kumar Sahu, Thangaradjou, T., Kannan, L. "Research on marine actinobacteria in India". *Indian Journal of Microbiology,* 47 (2007). 186 - 196.

[13] Solano, Godofredo, Keilor Rojas Jimenez, Marcel Jaspars and Giselle Tamayo. "Study of the diversity of culturable actinomycetes in the North Pacific and Caribbean coasts of Costa Rica". *Antonie Van Leeuwenhoek*, 96, no. 1 (2009). 71 - 78.

[14] Jiang, Cheng Lin and Li Hua Xu. "Diversity of Aquatic Actinomycetes in Lakes of the Middle Plateau, Yunnan, China". *Applied and Environmental Microbiology,* 62 (1996). 249 - 253.

[15] Yoon, Jung Hoon and Young Ha Park. "Phylogenetic analysis of the genus Thermoactinomyces based on 16S rDNA sequences". *International Journal of Systematic and Evolutionary Microbiology,* 50 (2000). 1081 - 1086.

[16] Berdy, Janos. "Bioactive microbial metabolites". *Journal of Antibiotics,* 58 (2005). 1 - 26.

[17] Elbendary, Afaf Ahmed, Ashgan Mohamed Hessain, Mahmoud Darderi El-Hariri, Ahmed Adel Seida, Ihab Mohamed Moussa, Ayman Salem Mubarak, Saleh A. Kabli, Hassan A. Hemeg and Jakeen Kamal El Jakee. "Isolation of antimicrobial producing Actinobacteria from soil samples". *Saudi Journal of Biological Sciences,* 25 (2018). 44 - 46.

In: A Closer Look at Actinomycetes ISBN: 978-1-53617-046-7
Editors: A. A. Mohamed Hatha et al.

Chapter 9

ACTINOMYCETES: TAXONOMY, GENOMIC APPROACH AND APPLICATIONS

Nattakorn Kuncharoen, Wongsakorn Phongsopitanun and Somboon Tanasupawat*

Department of Biochemistry and Microbiology,
Faculty of Pharmaceutical Sciences, Chulalongkorn University,
Bangkok, Thailand

ABSTRACT

Actinomycetes are Gram-positive, filamentous bacteria that formed true branching mycelia and high mol% guanine and cytosine (G+C) content in chromosomal DNA. They are widely distributed in terrestrial, aquatic and marine ecosystems and played important role in recycling of complex organic matters resulting in humus formation. Actinomycetes are normally isolated by the standard dilution plate technique using different media, for instance, humic acid-vitamin (HV) agar, starch casein nitrate (SCN) agar, glycerol arginine agar, glucose asparagine agar, Gauze mineral medium no. 1, arginine-vitamin (AV) agar, soil extract agar, water agar (WA), Küster's agar and glycerol yeast extract agar. The identification

* Corresponding Author's Email: somboon.T@chula.ac.th.

of actinomycetes are based on phenotypic and chemotaxonomic characteristics: isomers of diaminopimelic acid, whole-cell sugars, fatty acid profiles, polar lipid patterns and isoprenoid quinones, involving 16SrRNA gene sequences analysis and DNA-DNA hybridization. Presently, next-generation sequencing (NGS) has given a rapid and cost-effective approach to obtain whole-genome sequences (WGS) of actinomycete strains. Whole genome sequence analyses of the actinomycetes were based on average nucleotide identity (ANI), and *in silico* genomic similarity with the optimum threshold ranges appropriate for species delineation. The genomic analysis not only provides reliable taxonomic position, but also gives invaluable insights into the biology of actinomycetes.

INTRODUCTION

Actinomycetes are well-known as Gram-positive filamentous bacteria with high G+C content (>55 mol%) in their chromosomal DNA which belonged to the phylum *Actinobacteria* (Ludwig et al. 2012). They have a hyphal lifestyle with complex morphological differentiation such as fragmenting mycelia, permanent and extensively hyphae, monomeric spores and sporangia. Actinomycetes are saprophytic microorganisms that played a key role as decomposers in both terrestrial and aquatic environments by recycling complex organic material including dead animals and plants into humus (Goodfellow and Williams 1983). In addition, actinomycetes also represent as symbionts and pathogens in plants and animals (Lechevalier and Lechevalier 1967). Moreover, they are known as the important producer of antibiotics, for instance, avermectin, ivermectin, gentamicin, streptomycin, actinomycin, erythromycin, rifamycin and vancomycin (Bérdy 2005).

Classification of actinomycetes, which are presently known as actinobacteria, was relied on morphological, phenotypic, chemotaxonomic and genotypic characteristics. These known as polyphasic taxonomy which has become the principal of bacterial systematics, including the delineation of novel species (Colwell 1970, Vandamme et al. 1996). The use of polyphasic taxonomy was widespread and led to sweeping changes in the

recent classification of the phylum *Actinobacteria* which divided into 6 classes, 23 orders, 50 families and 221 genera (Ludwig et al. 2012).

Although the 16S rRNA gene sequences successfully provided the phylogenetic backbone for the classification of bacteria and archaea (Ludwig and Klenk 2005), it seems to be problems in delineating actinobacterial taxa, especially at the genus levels. The phylogenetic analysis based on the 16S rRNA gene sequences lacked the resolution to discriminate between closely related genera which are problematic to distinguish when proposing novel species to either genus (Labeda et al. 2001). For instance, the genera *Verrucosispora* (Rheims et al. 1998), *Salinispora* (Maldonado et al. 2005), *Jiangella* (Song et al. 2005), *Plantactinospora* (Qin et al. 2009), and *Polymorphospora* (Tamura et al. 2006) were generally interspersed within the 16S rRNA gene tree of *Micromonospora* (Trujillo et al. 2014).

Therefore, it is impelling need to improve the framework for the classification of actinomycetes, not only for its own right in taxonomic position but also driven for biotechnological and ecological research (Klenk and Göker 2010). According to the rapid progress in sequencing technologies (Mavromatis et al. 2012), whole-genome sequences and associated bioinformatic tools were integrated with the polyphasic taxonomic data and used for the classification of actinomycetes. These strategies provided a step-change in credibility, as significantly evidence by high bootstrap values in the phylogenomic trees (Meier-Kolthoff et al. 2014). Furthermore, the phylogenomic methods have already been used to exemplify the classification of complex actinobacterial taxa, for example, the genera *Micromonospora*, *Salinispora* and *Amycolatopsis* and in some cases have marked for reclassification such as the genera *Jishengella*, *Verrucosispora* and *Xiangella* were reclassified into to the genus *Micromonospora* (Nouioui et al. 2018). This chapter aimed to describe the identification of actinomycete species based on the polyphasic taxonomic approach including phenotypic, chemotaxonomic and genotypic characteristics as well as truly the genome-scale methods.

1. TAXONOMY

Actinomycetes, recently known as actinobacteria, are ubiquitous mycelial Gram-positive bacteria with high G+C content in their genomic DNA and classified into the phylum *Actinobacteria* (Lechevalier and Lechevalier 1967). As in Bergey's Manual of Systematic Bacteriology Volume 5 (Goodfellow et al. 2012), the phylum *Actinobacteria* includes 6 classes, 23 orders. Class *Actinobacteria* is the largest of this phylum which comprised 16 orders consisting of *Actinomycetales*, *Actinopolysporales*, *Bifidobacteriales*, *Catenulisporales*, *Corynebacteriales*, *Frankiales*, *Glycomycetales*, *Jiangelales*, *Kineosporiales*, *Micrococcales*, *Micromonosporales*, *Propionibacteriales*, *Pseudonocardiales*, *Strepto-mycetales*, *Streptosporangiales*, and *Insertaesedis*. Class *Nitriliruptoria* contains 2 orders, *Nitriliruptorales* and *Euzebyales*. Class *Thermoleophilia* encompasses 2 orders, *Thermoleophilales* and *Solirubrobacterales*. Each of the class *Acidimibrobiia*, *Coriobacteriia* and *Rubrobacteria* include 1 order such as *Acidimicrobiales*, *Coriobacteriales* and *Rubrobacterales*, respectively (Ludwig et al. 2012).

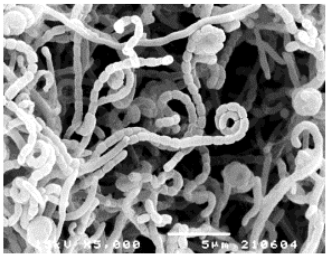
Actinomycetes are currently divided into two groups, streptomycete and non-streptomycete. The non-streptomycete actinomycetes are called rare actinomycetes which up to date contains approximately 220 genera. Some genera of rare actinomycetes are *Actinoplanes*, *Actinokineospora*, *Actinosynnema*, *Catenuloplanes*, *Dactylosporangium*, *Jiangella*, *Kineosporia*, *Kutzneria*, *Intrasporangium*, *Microbiospora*, *Micromonospora*, *Microtetraspora*, *Nonomuraea*, *Plantactinospora*, *Planomonospora*, *Polymorphospora*, *Pseudonocardia*, *Saccharomonospora*, *Streptosporangium*, *Salinispora*, *Thermomonospora*, and *Thermobifida* (Tiwari and Gupta 2012).

2. IDENTIFICATION

Polyphasic taxonomy is the combination of different information consisting of phenotypes, chemotypes, genotypes and phylogenies on bacteria which essentially used for the delineation of taxa at all levels (Vandamme et al. 1996). To identify the species of actinomycetes, the data of phenotypic, chemotaxonomic and genotypic characteristics were considered.

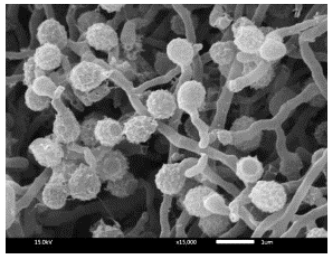
2.1. Phenotypic Characteristics

Actinomycetes exhibited a significant variety of morphologies from other bacteria. Most of them, *Streptomyces*, *Amycolatopsis*, *Kitasatospora* and *Actinomadura*, produce true branched aerial and substrate mycelia, fragmenting hyphae and/or permanent extensively differentiated branched mycelia. Nonetheless, some genera such as *Micromonospora*, *Salinispora*, *Actinoplanes*, *Catellatospora* and *Catenuloplanes* lack of aerial mycelia (Goodfellow et al. 2012). The spore formation including its position, surface, and arrangement is a key morphological criterion to identify the actinomycetes at the genus level (Locci and Sharples 1984). Spores are commonly produced on the substrate and/or the aerial hyphae as monomeric or in chains of different lengths with various surfaces consisting of smooth, warty, spiny, hairy, or rugose. In some cases, the spores were found to be sheltered in sporangia and endowed with flagella (Barka et al. 2016). For example, in the genus *Streptomyces*, spore chains grew out from the aerial mycelia whilst in the genus *Micromonospora*, spores borne directly on the substrate hyphae. In addition, some genera produce sporangia such as *Dactylosporangium*, *Actinoplanes*, *Planomonospora* and *Streptosporangium* (Cross and Goodfellow 1973). Cell morphology and spore formation were observed by using scanning electron microscopy (SEM) after cultivation on suitable agar media at 28±2 °C for 7-14 days. The spore morphologies of some actinomycete genera are presented in Figure 1.



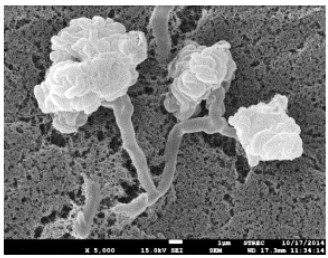
Sreptomyces tendae KC-075

(Sripreechasak et al., 2014)

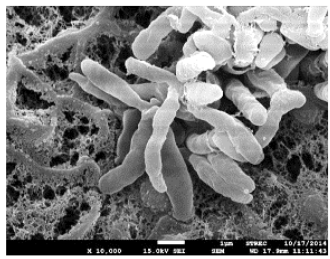


Micromonospora azadirachtae AZ1-19^{T}

(Kuncharoen et al., 2019)



*Actinoplanes lichenis*LDG1-22^{T}
(Phongsopitanun et al., 2016)



Dactylosporangium sucinum RY35-23^{T}
(Phongsopitanun et al., 2015)

Figure 1. Scanning electron micrographs of the actinomycete strains.

The actinomycetes were cultured on various media to determine the growth, the colonial appearance and the formation of soluble pigments according to the standard method of Shirling and Gottlieb (1966). Actinomycetes formed the colours of the colonies ranged from white, cream, yellow, vivid orange, olive, grey to black (Cross and Goodfellow 1973) which can be designed by using the colour systems, *Colour Harmony Manual* (Jacobson et al. 1958) or ISCC-NBS (Kelly 1964). Moreover, they also produced melanoid, brown-black colour observing on peptone-iron agar (ISP 6), and other soluble pigments such as red, yellow, orange, brownish, greenish-brown, blue, or black, depending on the isolates, media, and age of the cultures (Barka et al. 2016).

In addition, the actinomycetes were tested for the physiological and biochemical properties following the standard procedure (Shirling and

Gottlieb 1966). Starch hydrolysis, nitrate reduction, gelatin liquefaction and coagulation and peptonisation of milk were examined on ISP 4 agar, ISP 8 broth, glucose-peptone-gelatin medium and 10% (w/v) skimmed milk, respectively. Carbon utilisation was evaluated on the ISP 9 medium supplemented with 1% (w/v) of different sugars. Ranges of temperature, pH and NaCl concentration for growth were also determined on ISP 2 agar at 30°C for 2 weeks.

2.2. Chemotaxonomic Characteristics

Chemotaxonomic properties are the investigation of chemical components to group the actinomycetes at the genus level relied on the similarities of their cellular chemical profiles. The key chemical compositions that used for the delineation of novel taxa contained isomers of diaminopimelic acid and *N*-acyl types of muramic acid in cell-wall peptidoglycan, whole-cell sugars, polar lipids, cellular fatty acids and isoprenoid quinones (Goodfellow and Minnikin 1985).

The isomers of diaminopimelic acid (DAP) present in the cell-wall peptidoglycan are one of the keys to separate the members of the genus *Streptomyces* and other genera of actinomycetes. LL-diaminopimelic acid isomer is generally found in all *Streptomyces* species whereas others comprised *meso*-diaminopimelic acid, 3-OH-diaminopimelic acid, 3,4-dihydroxydiaminopimelic acid and/or the combination of various isomers (Staneck and Roberts 1974, Matsumoto et al. 2014). Furthermore, the *N*-acyl type of muramic acid, acetyl or glycolyl type, also used for the classification of actinomycetes (Uchida & Aida, 1984). The isomers of diaminopimelic acid and *N*-acyl type of muramic acid in cell wall peptidoglycan were prepared and analysed according to the procedures of Staneck and Roberts (1974) and Uchida and Aida (1984), respectively. The cell-wall types of actinomycetes are shown in Table 1.

Table 1. Cell-wall types and whole-cell sugar patterns containing in actinomycetes

Cell-wall		Whole-cell		Genera
Type	Characteristic amino acids and sugars	Sugar pattern	Diagnostic sugars	
I	LL-DAP, Glycine	C	None	*Streptomyces*, *Actinosporangium*, *Intrasporangium*
II	*meso*-DAP*, Glycine	D	Arabinose, Xylose	*Actinoplanes*, *Dactylosporangium*, *Micromonospora*,
III	*meso*-DAP	B	Madurose†	*Actinomadura*, *Microbispora*, *Streptosporangium*
		C	None	*Geodermatophilus*, *Saccharothrix*, *Thermoactinomyces*
IV	*meso*-DAP	A	Arabinose, Galactose	*Nocardia*, *Pseudonocardia*, *Thermomonospora*

Modified from Lechevalier and Lechevalier (1970).
*and/or 3-OH DAP, †3-*O*-Methyl-*D*-galactose.

Table 2. Polar lipid types of actinomycetes

Type	Polar lipid								Genera
	PE	PME	PC	GlcNU	PG	DPG	PIM	PI	
PI	-	-	-	-	v	v	+	+	*Actinomadura*, *Actinopolymorpha*, *Glycomyces*, *Nocardioides*
PII	+	-	-	-	v	+	+	+	*Actinoplanes*, *Amycolatopsis*, *Micromonospora*, *Streptomyces*
PIII	v	v	+	-	v	+	v	+	*Catenuloplanes*, *Nocardiopsis*, *Pseudonocardia*, *Saccharopolyspora*
PIV	v	v	-	+	-	+	ND	+	*Herbidospora*, *Microbispora*, *Microtetraspora*, *Streptosporangium*
PV	v	-	-	+	v	+	ND	+	*Oerskovia*, *Promicromonospora*

Modified from Lechevalier et al. (1977).
ND, no data; v, variable; -, absent

Sugar compositions in whole-cell hydrolysates are necessary for the identification of actinomycetes which have *meso*-DAP in the cell-wall

peptidoglycan. Based on the presence of diagnostic sugars, the actinomycetes can be categorised into four groups consisting of group A contains arabinose and galactose; group B includes madurose; group C has no diagnostic sugars; group D encompasses arabinose and xylose (Lechevalier and Lechevalier 1970, Lechevalier et al. 1971). To determine the sugar components, whole-cell hydrolysates were extracted following the method as described by Staneck and Roberts (1974) and identified using the high-performance liquid chromatography (HPLC) as described previously (Mikami and Ishida 1983). The whole-cell sugar patterns of actinomycetes are shown in Table 1.

Polar lipids or phospholipids are significant chemical compositions of bacterial plasma membranes which can be used to separate the actinomycetes into five patterns (Table 2) based on the presence and absence of nitrogenous phospholipids. Phospholipid type PI has no nitrogenous phospholipids; type PII comprises one nitrogenous phospholipid; type PIII includes phosphatidylcholine; type PIV contains an unidentified phospholipid containing glucosamine (GluNU), and type PV encompasses phosphatidylglycerol as well as GluNU (Lechevalier et al. 1977). The cellular polar lipids were prepared and analysed using 2-dimensional TLC following the protocol of Minnikin et al. (1977).

Fatty acids are also presented in cell membranes of actinomycetes. They are varied in the number of carbon atoms in the molecule. The occurrence of saturated and unsaturated (iso- and anteiso-) fatty acids, methyl groups fatty acid, cyclopropane fatty acids hydroxyl-fatty acids is properly used to identify the actinomycetes at the genus level. Moreover, the presence of mycolic acids and 2-alkyl-3-hydroxy long-chain fatty acids is a key characteristic for identifying the members in the families *Corynebacteriaceae*, *Mycobacteriaceae* and *Nocardiaceae* (Lechevalier et al. 1971). At present, the analysis of the cellular fatty acid components was performed using gas chromatography which provided the reproducible and trustworthy results (Sasser 1990). Mycolic acids were analysed using various methods such as TLC (Tomiyasu 1982) and HPLC (Butler and Guthertz 2001).

Table 3. Distribution of menaquinones in actinomycetes

Menaquinone	Family	Genera
MK-9(H_6) and MK-9(H_8)	*Streptomycetaceae*	*Streptomyces*, *Kitasatospora*
	Thermomonosporaceae	*Actinomadura*, *Thermomonospora*
MK-9(H_2) and MK-9(H_4)	*Streptosporangiaceae*	*Streptosporangium*, *Microbispora*, *Nonomuraea*
MK-10(H_4)		*Herbidospora*
MK-8(H_4)	*Pseudonocardiaceae*	*Pseudonocardia*
MK-9(H_4)	*Pseudonocardiaceae*	*Amycolatopsis*, *Saccharomonospora*
	Actinosynnemataceae	*Actinosynnema*, *Actinokineospora*
MK-9(H_4) and MK-10(H_4)	*Actinosynnemataceae*	*Saccharothrix*
MK-9(H_4)/MK-10(H_4, $_6$)/MK-12($H_{4,\ 6,\ 8}$)	*Micromonosporaceae*	*Micromonospora*
MK-9($H_{4,\ 6}$)/ MK-10(H_4)/MK-10($H_{6,\ 8}$)		*Catellatospora*
MK-9($H_{8,\ 6}$)		*Dactylosporangium*

Modified from Kroppenstedt (1985).

Table 4. Method for chemotaxonomic and genotypic characteristics

Characteristic	Method	Reference
Chemotype		
Isomers of DAP	TLC	Staneck and Roberts (1974)
Whole-cell sugars	HPLC	Mikami and Ishida (1983)
Polar lipids	2D TLC	Minnikin et al. (1977)
Cellular Fatty acids	GC	Sasser (1990)
Mycolic acids	TLC/HPLC	Tomiyasu (1982)/Butler and Guthertz (2001)
Menaquinones	LC-MS	Tamaoka et al. (1983)
Genotype		
G+C content	HPLC	Tamaoka (1994)
16S rRNA gene	Sanger sequencing	Sanger et al. (1977)
In vitro DDH	Fluorometry	Ezaki et al. (1989)

Several types of isoprenoid quinones, especially menaquinones, are normally found in the cytoplasmic membrane of actinomycetes. They play an important role in the electron transport system. The different number of the isoprene units and the degree of hydrogenation provided the invaluable characteristics for classifying actinomycetes at the genus level (Table 3). Recently, the menaquinones were prepared following the procedure of

Collins et al. (1977) and identified using HPLC and mass spectrometer implemented with a Pegasil ODS column (Senshu, Tokyo, Japan) as described earlier by Tamaoka et al. (1983). The chemotaxonomic analysis techniques were summarised in Table 4.

2.3. Genotypic Characteristics

The molecular systematic methods, 16S rRNA gene sequences analysis, phylogenetic analysis, DNA base composition and DNA-DNA hybridisation, have been effected on modern microbial taxonomy (Tindall et al. 2010) and commonly used for identifying the actinomycetes.

The 16S rRNA gene is a molecular taxonomic marker showing highly conserved regions and poorly subject to the horizontal gene transfer in archaea and bacteria. The sequencing and phylogenetic analysis of 16S rRNA gene has consequently been considered as a proper method for identifying the prokaryotic organisms, including actinomycetes, at all taxonomic levels (Ramasamy et al. 2014). There are many useful web-based tools for the identification of actinomycetes relied on Basic Local Alignment Search Tool (BLAST) searches and pairwise sequence alignments of the 16S rRNA gene sequences, resulting in the percentage of similarity values (Altschul et al. 1990, Mount 2007), for instance, EzBiocloud server (Yoon et al. 2017), DDBJ Blast (http://blast.ddbj.nig.ac.jp/), NCBI Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and EMBL-EBI Blast (https://www.ebi.ac.uk/Tools/sss/ncbiblast/nucleotide.html). The isolates which showed the 16S rRNA gene sequence similarity values ranging from 99-97% should be mandatory for the candidates of new species (Stackebrandt and Ebers 2006).

The phylogenetic analysis was also used to confirm the evolution and taxonomic position of actinomycetes. The phylogeny was mainly constructed based on three tree-making methods containing neighbour-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Fitch 1971) using the molecular evolutionary genetics analysis (MEGA) version 7.0 (Kumar et al. 2016). Kimura-2-

parameter model (Kimura 1980) was generally used for the calculation of the distance matrix. The confidence limits of the phylogenetic tree were determined using the analysis of bootstrap values based on 1,000 replications (Felsenstein 1985).

Guanine plus cytosine (G+C) content in the bacterial chromosomal DNA varies significantly in archaea and bacteria ranging from 25-80 mol%. Actinomycetes are high G+C content bacteria; hence, this character can distinguish them from other bacteria (Goodfellow and Minnikin 1985). Furthermore, the variation of G+C content (mol%) in bacterial genomes is adequately used to differentiate bacterial taxa at the genus and maybe species levels, observing from the range of mol% G+C content which is not more than 3% within a species and not rather than 10% within a genus (Vandamme et al., 1996). Early identification of actinomycetes, the G+C content was prepared and analysed according to the methods as previously reported by Tamaoka (1994).

In vitro DNA-DNA hybridisation is one of the techniques which attempted to compare whole bacterial genomes between different species to calculate their genomic similarities. The values of less than 70% DNA-DNA relatedness values have been accepted for the thresholds for proposing the novel species (Wayne et al. 1987). The fluorometric method was used for detecting the *in vitro* DNA-DNA hybridisation as previously described by Ezaki et al. (1989). The genotypic characteristics procedures were concluded in Table 4.

3. GENOME ANALYSIS

Currently, there are many problems with using the 16S rRNA gene sequence for identifying the taxonomic position of actinomycetes because it cannot separate between closely related genera. Thus, it is difficult to propose the new genus and species (Labeda et al. 2001). This situation now occurs in the phylum *Actinobacteria* at family, genus and species levels (Nouioui et al. 2018). The genera *Gordonia*, *Millisia*, *Skermania* and *Williamsia* which formerly in the family *Nocardiaceae* were recently

transferred into the family *Gordoniaceae* (Goodfellow et al. 2012). Some genera, *Verrucosispora* (Rheims et al. 1998), *Jishengella* (Xie et al. 2011) and *Plantactinospora* (Qin et al. 2009), were commonly fall within the 16S rRNA gene tree of *Micromonospora* (Trujillo et al. 2014). Additionally, the members of the genus *Amycolatopsis* were also revisited for their taxonomic status (Sangal et al. 2018). To solve these issues, the next-generation sequencing (NGS) technologies are significantly useful for the species delineation of actinomycetes.

3.1. NGS Technologies for Genome Sequencing

Over ten years ago, the use of genome sequencing for describing the actinobacterial taxa was limited because of the high-cost, time-consuming and labour-intensive (Margulies et al. 2005). Presently, there are many NGS platforms (Table 5) including Roche 454 sequencing system (Roche), Illumina Hiseq and Miseq (Illumina), PacBio (Pacific Biosciences), Ion Torrents (Life Technologies) and Nanopore (Oxford Nanopore Technologies) which can yield massive quantities of DNA sequence data with more accurate over 99% and cheaper than the conventional Sanger method (Chun and Rainey 2014). Therefore, the whole-genome sequencing should be integrated into the polyphasic taxonomy for providing reproducibility and reliability for the taxonomic status of actinomycetes.

3.2. Applying Genomics to the Actinobacterial Taxonomy

Advances in DNA sequencing technology are up to date introduced the use of whole-genome sequences (WGS) in various microbiological fields. The data obtaining from genome sequences proved to provide purposes and credible means in the taxonomy of prokaryotes including actinomycetes. The application of whole-genome sequencing technologies and relevant bioinformatic tools is not only granting trusty to the polyphasic taxonomic approach but are also providing knowledge of developmental and

evolutionary progress, as well as the ecological, physiological and biotechnological potential of actinomycetes (Carro et al. 2018).

Now, complete and/or draft genome sequences are publicly available and already required as minimal standards for using in the taxonomy of prokaryotes (Chun et al. 2018). Base on the genome sequence, it is focused on the comparison between two genomes resulting in similarity or distance values. These values were designed as the overall genome-related index (OGRI). The OGRI, average nucleotide identity (ANI) and digital DNA-DNA hybridisation (dDDH), can be used to identify whether the actinomycete isolates belong to known or new species by calculating the relatedness values between genome sequences of the isolates and type strains (Chun and Rainey 2014).

Table 5. Sequencing platforms used in bacterial genome sequencing

Platform	**Manufacturer**	**Run time†**	**Read length**	**Yield per run**
Illumina Hiseq	Illumina	40 h	2×150 bp	75-90 Gb
Illumina Miseq	Illumina	39 h	2×250 bp	7.5-8.5 Gb
454 GS Junior	Roche	10 ~ 12 h	400-500 bp	50 Mb
454 FLX Titanium XL+	Roche	23 ~ 24 h	700-1000 bp	1 Gb
PacBio RS II	Pacific Biosciences	2 h	3-5 kbp	250 Mb
Ion PGM System	Life Technologies	5 h	400 bp	600-1000 Mb
Ion Proton PGM System	Life Technologies	2 ~ 4 h	200 bp	10 Gb
Flongle	Oxford Nanopore Technologies	1 min – 16 h	>2 Mb	1-2 Gb
MinION Mk1B	Oxford Nanopore Technologies	1 min – 48 h		15-30 Gb
MinION Mk1C	Oxford Nanopore Technologies	1 min – 48 h		15-30 Gb
GridION Mk1	Oxford Nanopore Technologies	1 min – 48 h		75-150 Gb
PromethION 24	Oxford Nanopore Technologies	1 min – 72 h		2.4-4.3 Tb
PromethION 48	Oxford Nanopore Technologies	1 min – 72 h		4.8-8.6 Tb

†Run time is the time for the reaction of DNA sequencing after the library preparation.

Average nucleotide identity (ANI) is a basic, effective, and overall description of genetic relatedness, more than 1,000 genes in total, which are better than the measurement of a single gene such as the 16S rRNA gene. Moreover, the ANI values are not affected by the variation of evolutionary rates and horizontal gene transfer (Arahal 2014). Presently, the ANI values are usually determined using BLAST, called ANIb, (Goris et al. 2007), MUMmer, called ANIm, (Kurtz et al. 2004) and EzBiocloud, OrthoANIu, (Yoon et al. 2017) software. The ANI value boundary of 95~96 is generally recommended for taxonomically delineating actinomycete species (Ritcher et al. 2009). Although the genome data of type strains with validly published type strains have already available, over 50% still has only the nearest complete 16S rRNA gene sequences. Consequently, integration of the 16S rRNA gene sequence similarity and ANI values can be used in the systematic process for identifying and proposing a novel actinomycete species (Kim et al. 2014). In this combination method, the actinomycete strains exhibiting the 16S similarity in ranges of 97-99% (Stackebrandt and Ebers 2006) and the ANI below 95~96% (Ritcher et al. 2009) were should be mandatory for testing of new species.

DNA-DNA hybridisation (DDH) is a key technique which continuously used as the taxonomic gold standard for circumscribing the species in *Archaea* and *Bacteria*, involving actinomycetes. If the genomes of two organisms show a DDH value of less than 70%, this means to consider them as distinct species and vice versa (Wayne et al. 1987, Stackebrandt and Goebel 1994). Previously, the DDH values were obtained from the wet-lab method, but it was widely regarded as tedious, labour-intensive, time-consuming and seemingly rather error-prone (Klenk and Göker 2010). Hence, *in silico* or digital method which developed based on the computational algorithm as now used to replace the wet-lab method. The values obtained from this method called *in silico* DDH or digital DDH (dDDH) which provided much higher reliability and reproducibility for species delineation (Goris et al. 2007).

The dDDH values were calculated based on the Genome Blast Distance Phylogeny approach (GBDP). This approach has two steps as follows: 1) two genomes A and B were regionally aligned using BLAST (Altschul et al.

1990), which provided a set of high-scoring segment pairs (HSPs) (Auch et al. 2010) between the genome sequences; 2) these HSPs data were transformed into a single genome-to-genome distance value implemented with a specific distance formula. The actinobacterial taxonomists can calculate the dDDH values based on the GBDP with the appropriate model-based DDH estimated formula by submitting the draft or complete genomes to the Genome-to-Genome Distance Calculator (GGDC) version 2.1 (Meier-Kolthoff et al. 2013) on a free web service at http://ggdc.dsmz.de.

Table 6. Bioinformatic web-based tools for genome-to-genome comparison for taxonomic purposes

Tools	Function	Threshold for species distinction	URL	References
JSpeciesWS	ANIb ANIm	<95~96%	http://jspecies.ribohost.com/jspeciesws/	Ritcher et al. (2016)
OrthoANI with usearch	OrthoANIu	<95~96%	https://www.ezbioclou d.net/tools/ani	Yoon et al. (2017)
GGDC 2.1	dDDH	<70%	https://ggdc.dsmz.de/g gdc.php	Meier-Kolthoff et al. (2013)
TYGS	Phylogenomic tree	-	https://tygs.dsmz.de/	Meier-Kolthoff and Göker (2019)

Furthermore, TYGS, the Type (Strain) Genome Server, has now launched and used for classification and identification of the prokaryotic strains, including actinomycetes, without overestimating phylogenetic confidence. In addition, it provides fast, trustworthy and easily interpretable analyses. After the submitted genomes were calculated on the TYGS server (https://tygs.dsmz.de/), the phylogenetic trees based on the whole-genome and the 16S rRNA gene sequences, as well as the dDDH values were obtained. The truly genome-based phylogenies can be used to confirm the taxonomic status of the candidates of novel species by integrating with the phylogenetic trees based on the 16S rRNA gene and other important house-

keeping gene sequences (Meier-Kolthoff and Göker 2019). The relevant bioinformatics web services are shown in Table 6.

4. APPLICATIONS OF ACTINOMYCETES

Actinomycetes are well recognised as a key organism for producing economic primary and secondary metabolites such as antibiotics, enzymes, biocontrol agents and growth-promoting substances for plants. Among 10,000 bioactive metabolites, *Streptomyces* species are the greatest producer providing approximately 7,600 metabolites while 2,400 derived from rare actinomycete species. The actinomycete-derived substances recently represented as the largest group (45%) of microbial bioactive metabolites, for instance,streptomycin from *Streptomyces griseus*, roseoflavin from *S. davawensis*, gentamicin from *Micromonospora echinospora*, rifamycin from *Amycolatopsis mediterranei* and vancomycin from *A. orientalis* (Bérdy 2005, 2012). In addition, some novel antibiotics were discovered from actinomycetes such as munumbicins A-D from *Streptomyces* sp. NRRL 30562, kakadumycins from *Streptomyces* sp. NRRL 30566, coronamycins from *Streptomyces* sp. MSU-2110, antimycin A_{18} from *S. albidoflavus*, lajollamycin from *S. nodosus*, lincomycin from *S. lincolnensis* and pacificanones A-B from *Salinispora pacifica* (Lam 2006, Qin et al. 2011).

It has been reported that actinomycetes are an important source of various such as amylases, cellulases, xylanases and chitinases. These enzymes are employed in many industries such as foods and beverages, pharmaceutics, biomedicines, detergents, textiles, agriculture and waste management (Mukhtar et al. 2017). Amylases are a significant enzyme for hydrolysing starch into reducing sugars, mainly maltose. Some actinomycetes generated amylases and industrially used, for example, *Streptomyces erumpens* (Kar and Ray 2008), *Thermobifida fusca* (Yang and Liu 2004) and *Nocardiopsis* sp. (Stamford et al. 2001). Cellulases are a key enzyme for converting celluloses into fermentable sugar in the process of biofuel production. Several actinomycetes produced high yields of these enzymes, which were properly for commercial used, such as *Streptomyces*

ruber, *S. lividans*, *S. rutgersensis* (Mukhtar et al. 2017), *T. fusca* (Li et al. 2007), *T. halotolerans* 90462^T (Zhang et al. 2011) and *Thermomonospora curvata* (Stutzenberger 1971). Xylanases are an important enzyme which responsible for cleaving the β-1, 4 backbone of the complex polysaccharides of plant cell wall, xylan, before using in the industrial process, especially paper production. Xylanases can be produced by many actinomycete species: *Actinomadura* sp. Cpt20 (Taibi et al. 2012), *Streptomyces cyaneus* SN32 (Ninawe et al. 2008) and *T. halotolerans* 90462^T (Zhang et al. 2012). Chitinases are the hydrolase enzyme for cleaving the β-1,4 glycosidic linkages of *N*-acetylglucosamine in the molecule of chitin. These enzymes are importantly used in preparation of protoplast from fungi and biopharmaceutical products. Some actinomycetes produced chitinases and used commercially such as *Nocardiopsis prasina* (Horikoshi 1999), *Streptomyces thermoviolaceus* (Bhattacharya et al. 2007) and *Microbiospora* sp. V2 (Nawani et al. 2002).

Currently, soil-borne diseases are a critical problem in plants around the world. The use of toxic chemicals to eradicate soil pathogens causes environmental hazards, so the biocontrol agents have been considered and more desirable than chemical uses. Many actinomycetes have been reported as the biocontrol agents for soil-borne plant pathogens (Shimizu 2011). Cao et al. (2004) reported that *Rhizoctonia solani*, which causes dumping-off of tomato seedlings, was suppressed by *Streptomyces* sp. S30 observing from 90% germination in tomato seeds. Lee et al. (2008) found that *Microbispora rosea* subsp. *rosea* and *Streptomyces olivochromogenes* inhibited *Plasmodiophora brassicae* which causes clubroot in cabbages by reducing its sensitivity of 33-58%. Moreover, El-Tarabily et al. (2009) studied the mechanisms of action of *Actinoplanes campanulatus*, *Micromonospora chalcea* and *Streptomyces spiralis* on the inhibition of *Pythium aphanidermatum*, causing dumping-off, crown- and root-rot in cucumber. The authors found that these actinomycetes highly produced cell-wall degrading enzymes for disrupting the fungal cell-wall synthesis and antibiotics for inhibiting the fungal growth. Therefore, these let the opportunity to find more actinomycete strains with biocontrol effects and use in plant protection.

Actinomycetes, particularly habitat in rhizosphere and plant-tissue, exhibited plant-growth-promoting (PGP) activity. The possible mechanisms of this activity included 1) the ability to produce plant hormone, indole-3-acetic acid (IAA); 2) nitrogen fixation; 3) siderophore production; 4) solubilisation of phosphates and other minerals (Cattelan et al. 1999). Many recent reports described that the main mechanisms of PGP in actinomycetes are the production of IAA (Shimizu 2011). For instance, wheat plants inoculated with endophytic *Streptomyces rochei* IDWR19 (IAA 17.8 $mg{\cdot}L^{-1}$) and *S. thermolilacinus* IDWR81 (IAA 11.5 $mg{\cdot}L^{-1}$) had higher biomass (2 and 2.5 times for *S. rochei* IDWR19 and *S. thermolilacinus* IDWR81, respectively) and increase of shoot length (12.2 and 24.5% for *S. rochei* IDWR19 and *S. thermolilacinus* IDWR81, respectively) when compared to the control (Jog et al. 2012). Dochhil et al. (2013) revealed that the percentages of seed germination and seedling growth of *Phaseolus vulgaris*were increased because of the synthesis of higher concentrations (71-197 $\mu g{\cdot}mL^{-1}$) of IAA produced by the endophytic *Streptomyces* strains CA10 and CA26. Meguro et al. (2006) also reported that *Streptomyces* sp. MBR-52 could colonise and increase the elongation of *Rhododendron* roots. Besides *Streptomyces* sp., it was found that other actinomycete genera *Actinomadura, Micromonospora, Nonomuraea, Pseudonocardia, Nocardia* and *Streptosporangium* have been reported to produce IAA and increase dry-weight of cucumbers, tomatoes and carrots (El-Tarabily et al. 1997, Nimnoi et al. 2010). These suggested that actinomycetes are promised as helper organisms leading to an increased sustainable agricultural production.

CONCLUSION

The polyphasic taxonomic approach is useful in the systematics of actinomycetes while the use of 16S rRNA gene sequences has changed our understanding of actinomycetes and led toa rapid increase in the number of descriptions of novel taxa, particularly at the species level. Although the phenotypic and chemotaxonomic properties, as well as the 16S rRNA gene sequences, were combined and used for the species delineation, those cannot

distinguish some actinomycetes into the distinct species, especially the members in the families *Micromonosporaceae*, *Nocardiaceae*, *Dermacoccaceae*, *Intrasporangiaceae*, and *Nocardioidaceae*. Nowadays, the sequencing technologies of DNA have provided a fast and cost-effective approach to obtaining whole-genome sequences of actinomycete strains and integrative used with the polyphasic taxonomic techniques. This integration provided credibility and reproducibility for circumscribing actinomycete species and now proposed as the minimal standards for taxonomic descriptions. Additionally, some actinomycete genera have been reclassified based on the whole-genome sequencing data, for instance, *Jishengella* and *Verrucosispora* were transferred into the genus *Micromonospora*. In the future, the use of genomic data not only provides greater impact on taxonomic purposes, but it also handles on the ecological, pharmaceutical, medical and biotechnological research.

REFERENCES

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol*, 215 (3):403-10.

Arahal, R. D. (2014). Whole-Genome Analyses: Average Nucleotide Identity. *Method Microbiol*, 41:103-122.

Auch, A. F., Klenk, H. P., and Goker, M. (2010). Standard operating procedure for calculating genome-to-genome distances based on high-scoring segment pairs. *Stand Genomic Sci*, 2 (1):142-148.

Barka, E. A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Meier-Kolthoff, J. P., Klenk, H. P., Clement, C., Ouhdouch, Y., and van Wezel, G. P. (2016). Taxonomy, physiology, and natural products of actinobacteria. *Microbiol Mol Biol Rev*, 80 (1):1-43.

Bérdy, J. (2005). Bioactive microbial metabolites. *J Antibiot*, 58 (1):1-26.

Bérdy, J. (2012). Thoughts and facts about antibiotics: where we are now and where we are heading. *J Antibiot*, 65 (8):385-395.

Bhattacharya, D., Nagpure, A., and Gupta, R. K. (2007). Bacterial chitinases: properties and potential. *Crit Rev Biotechnol*, 27 (1):21-8.

Butler, W. R., and Guthertz, L. S. (2001). Mycolic acid analysis by high-performance liquid chromatography for identification of *Mycobacterium* species. *Clin Microbiol Rev*, 14 (4):704-26, table of contents.

Cao, L., Qiu, Z., You, J., Tan, H., and Zhou, S. (2004). Isolation and characterization of endophytic *Streptomyces* strains from surface-sterilized tomato (*Lycopersicon esculentum*) roots. *Lett Appl Microbiol*, 39 (5):425-30.

Carro, L., Nouioui, I., Sangal, V., Meier-Kolthoff, J. P., Trujillo, M. E., Montero-Calasanz, M. C., Sahin, N., Smith, D. L., Kim, K. E., Peluso, Paul, D., Shweta, W., Tanja, S., Nicole, K., Nikos C., Klenk, H.-P., Göker, M., and Goodfellow, M.. (2018). Genome-based classification of micromonosporae with a focus on their biotechnological and ecological potential. *Sci Rep*, 8 (1):525.

Cattelan, A. J., Hartel, P. G., and Fuhrmann, J. J. (1999). Screening for plant growth-promoting rhizobacteria to promote early soybean growth. *Soil Sc Soc Am J*, 63 (6):1670-1680.

Chun, J., Oren, A., Ventosa, A., Christensen, H., Arahal, D. R., da Costa, M. S., Rooney, A. P., Yi, H., Xu, X. W., De Meyer, S., and Trujillo, M. E. (2018). Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *Int J Syst Evol Microbiol*, 68 (1):461-466.

Chun, J., and Rainey, F. A. (2014). Integrating genomics into the taxonomy and systematics of the Bacteria and Archaea. *Int J Syst Evol Microbiol*, 64:316-324.

Collins, M. D., Pirouz, T., Goodfellow, M., and Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol*, 100:221-230.

Colwell, R. R. (1970). Polyphasic taxonomy of the genus vibrio: numerical taxonomy of *Vibrio cholerae*, *Vibrio parahaemolyticus*, and related *Vibrio* species. *J Bacteriol*, 104 (1):410-33.

Cross, T., and Goodfellow, M. (1973). Taxonomy and classification of the actinomycetes. *Soc Appl Bacteriol Symp Ser*, 2:11-112.

Dochhil, H., Dkhar, M. S., and Barman, D. (2013). Seed germination enhancing activity of endophytic *Streptomyces* isolated from indigenous

ethno-medicinal plant *Centella asiatica*. *Inter J Pharm Biol Sci*, 41:256-262.

El-Tarabily, K. A., Nassar, A. H., Hardy, G. E., and Sivasithamparam, K. (2009). Plant growth promotion and biological control of *Pythium aphanidermatum*, a pathogen of cucumber, by endophytic actinomycetes. *J Appl Microbiol*, 106 (1):13-26.

El-Tarabily, K. A., Hardy, G. E. St. J., Sivasithamparam, K., Hussein, A. M., and KurtbÖke, I. D. (1997). The potential for the biological control of cavity spot disease of carrots caused by *Pythium coloratum* by streptomycete and non-streptomycete actinomycetes in Western Australia. *New Phytol*, 137:495-507.

Ezaki, T., Hashimoto, Y., and Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol*, (39):224-229.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39:783-791.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol*, 17:368-376.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for specific tree topology. *Syst Zool*, 20:406-416.

Goodfellow, M., Kämpfer, P., Busse, H. J., Trujillo, M. E., Suzuki, K., and Ludwig, W. (2012). *Bergey's Manual of Systematic Bacteriology*. Vol. 5, *The Actinobacteria*. New York: Springer.

Goodfellow, M., and Minnikin, D. E. (1985). *Chemical methods in bacterial systematics*. London: Academic Press.

Goodfellow, M., and Williams, S. T. (1983). Ecology of actinomycetes. *Annu Rev Microbiol*, 37:189-216.

Goris, J., Konstantinidis, K., Klappenbach, J., Coenye, T., Vandamme, P., and Tiedje, J. (2007). DNA-DNA hybridization values and their relationship to whole genome sequence similarities. *Int J Syst Evol Microbiol*, 57:81-91.

Horikoshi, K. (1999). Alkaliphiles: Some applications of their products for biotechnology. *Microbiol Mol Biol R*, 63 (4):735-750.

Jacobson, E., Grauville, W. C., and Fogs, C. E. (1958). *Colour Harmony Manual*. (Eds) 4th. Chicago: Container Corporation of America.

Jog, R., Nareshkumar, G., and Rajkumar, S. (2012). Plant growth promoting potential and soil enzyme production of the most abundant *Streptomyces* spp. from wheat rhizosphere. *J Appl Microbiol*, 113 (5):1154-1164.

Kar, S., and Ray, R. C. (2008). Statistical optimization of α-amylase production by *Streptomyces erumpens* MTCC 7317 cells in calcium alginate beads using response surface methodology. *Pol J Microbiol*, 57 (1):49-57.

Kelly, K. L. (1964). Intersociety color council - national bureau of standards color name charts illustrated with centroid colors. Washington DC: US Government Printing Office.

Kim, M., Oh, H. S., Park, S. C., and Chun, J. (2014). Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int J Syst Evol Microbiol*, 64 (Pt 2):346-51.

Kimura, M. A. (1980). Simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol*, 16:111-120.

Klenk, H.-P., and Göker, M. (2010). En route to a genome-based classification of archaea and bacteria?. *Syst Appl Microbiol*, 33 (4):175-82.

Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics*, (Eds) M. Goodfellow, and D. E. Minnikin, 173-199. New York: Academic Press.

Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Bio and Evol*, 33 (7):1870-1874.

Kuncharoen, N., Kudo, T., Ohkuma, M., and Tanasupawat, S. (2019). *Micromonospora azadirachtae* sp. nov., isolated from roots of

Azadirachta indica A. Juss. var. *siamensis* Valeton. *Anton Leeuw Int J G*, 112 (2):253-262.

Kurtz, S., Phillippy, A., Delcher, A. L., Smoot, M., Shumway, M., Antonescu, C., and Salzberg, S. L. (2004). Versatile and open software for comparing large genomes. *Genome Biol*, 5 (2):R12.

Labeda, D. P., Hatano, K., Kroppenstedt, R. M., and Tamura, T. (2001). Revival of the genus *Lentzea* and proposal for *Lechevalieria* gen. nov. *Int J Syst Evol Microbiol*, 51 (Pt 3):1045-50.

Lam, K. S. (2006). Discovery of novel metabolites from marine actinomycetes. *Curr Opin Microbiol*, 9 (3):245-51.

Lechevalier, H. A., and Lechevalier, M. P. (1970). A critical evaluation of the genera of aerobic actinomycetes. In *The Actinomycetales*, (Eds) Prauser, 393-405. Jena: Gustav Fischer Verlag.

Lechevalier, H. A., and Lechevalier, M. P. (1967). Biology of actinomycetes. *Annu Rev Microbiol*, 21:71-100.

Lechevalier, H. A., Lechevalier, M. P., and Gerber, N. N. (1971). Chemical composition as a criterion in the classification of actinomycetes. *Adv Appl Microbiol*, 14:47-72.

Lechevalier, M. P., De Bièvre, C., and Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol*, 5:249-260.

Lee, S. O., Choi, G. J., Choi, Y. H., Jang, K. S., Park, D. J., Kim, C. J., and Kim, J. C. (2008). Isolation and Characterization of Endophytic Actinomycetes from Chinese Cabbage Roots as Antagonists to *Plasmodiophora brassicae*. *J Microbiol Biotechnol*, 18 (11):1741-1746.

Li, Y., Irwin, D. C., and Wilson, D. B. (2007). Processivity, substrate binding, and mechanism of cellulose hydrolysis by *Thermobifida fusca* Cel9A. *Appl Environ Microbiol*, 73 (10):3165-72.

Locci, R., and Sharples, G. (1984). *The biology of Actinomycetes*, (Eds) M. Goodfellow, M. Mordarski and S. T. Williams, 165-199. London: Academic Press.

Ludwig, W., Euzeby, J., Schumann, P., Busse, H. J., Trujillo, M. E., Kampfer, P., and Whitman, W. B. (2012). Road map of the phylum *Actinobacteria*. In *Bergey's Manual of Systematic Bacteriology* (Eds)

M. Goodfellow, P. Kampfer, M. J. Busse, M. E. Trujillo, K. Suzuki, W. Ludwig and W. B. Whitman, 1-28. New York: Springer.

Ludwig, W., and Klenk, H. P. (2005). Overview: a phylogenetic backbone and taxonomic framework for procaryotic systematics. In *Bergey's Manual of Systematic Bacteriology*, (Eds) D. J. Brenner, N. R. Krieg, J. T. Staley and G. M. Garrity, 49-66. New York: Springer.

Maldonado, L. A., Fenical, W., Jensen, P. R., Kauffman, C. A., Mincer, T. J., Ward, A. C., Bull, A. T., and Goodfellow, M. (2005). *Salinispora arenicola* gen. nov., sp. nov. and *Salinispora tropica* sp. nov., obligate marine actinomycetes belonging to the family *Micromonosporaceae*. *Int J Syst Evol Microbiol*, 55 (Pt 5):1759-66.

Margulies, M., Egholm, M., Altman, W. E., Attiya, S., Bader, J. S., Bemben, L. A., Berka, J., Braverman, M. S., Chen, Y. J., Chen, Z. T., Dewell, S. B., Du, L., Fierro, J. M., Gomes, X. V., Godwin, B. C., He, W., Helgesen, S., Ho, C. H., Irzyk, G. P., Jando, S. C., Alenquer, M. L. I., Jarvie, T. P., Jirage, K. B., Kim, J. B., Knight, J. R., Lanza, J. R., Leamon, J. H., Lefkowitz, S. M., Lei, M., Li, J., Lohman, K. L., Lu, H., Makhijani, V. B., McDade, K. E., McKenna, M. P., Myers, E. W., Nickerson, E., Nobile, J. R., Plant, R., Puc, B. P., Ronan, M. T., Roth, G. T., Sarkis, G. J., Simons, J. F., Simpson, J. W., Srinivasan, M., Tartaro, K. R., Tomasz, A., Vogt, K. A., Volkmer, G. A., Wang, S. H., Wang, Y., Weiner, M. P., Yu, P. G., Begley, R. F., and Rothberg, J. M. (2005). Genome sequencing in microfabricated high-density picolitre reactors. *Nature*, 437 (7057):376-380.

Matsumoto, A., Kawaguchi, Y., Nakashima, T., Iwatsuki, M., Omura, S., and Takahashi, Y. (2014). *Rhizocola hellebori* gen. nov., sp. nov., an actinomycete of the family *Micromonosporaceae* containing 3,4-dihydroxydiaminopimelic acid in the cell-wall peptidoglycan. *Int J Syst Evol Microbiol*, 64 (8):2706-2711.

Mavromatis, K., Land, M. L., Brettin, T. S., Quest, D. J., Copeland, A., Clum, A., Goodwin, L., Woyke, T., Lapidus, A., Klenk, H. P., Cottingham, R. W., and Kyrpides, N. C. (2012). The fast changing landscape of sequencing technologies and their impact on microbial genome assemblies and annotation. *PLoS One*, 7 (12):e48837.

Meguro, A., Ohmura, Y., Hasegawa, S., Shimizu, M., Nishimura, T., and Kunoh, H. . (2006). An endophytic actinomycete, *Streptomyces* sp. MBR-52, that accelerates emergence and elongation of plant adventitious roots. *Actinomycetologica* 20:1-9.

Meier-Kolthoff, J. P., and Göker, M. (2019). TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nature Communications*, 10.

Meier-Kolthoff, J. P., Auch, A. F., Klenk, H. P., and Göker, M. (2014). Highly parallelized inference of large genome-based phylogenies. *Concurr Comp-Prac E*, 26:1715-1729.

Meier-Kolthoff, J. P., Auch, A. F., Klenk, H. P., and Göker, M. (2013). Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 14:1-14.

Mikami, H., and Ishida, Y. (1983). Post-column fluorometric detection of reducing sugar in high-performance liquid chromatography using arginine. *Bunseki Kagaku*, (32):E207-E210.

Minnikin, D. E., Patel, P. V., Alshamoany, L., and Goodfellow, M. (1977). Polar lipid composition in the classification of *Nocardia* and related bacteria, *Int J Syst Bacteriol*, 27:104-117.

Mount, D. W. (2007). Using the basic local alignment search tool (BLAST). *CSH Protoc*, 17.

Mukhtar, S., Zaheer, A., Aiysha, D., Malik, K. A., and Mehnaz, S. (2017). Actinomycetes: A Source of Industrially Important Enzymes. *J Proteomics Bioinform*, 10 (12):316-319.

Nawani, N. N., Kapadnis, B. P., Das, A. D., Rao, A. S., and Mahajan, S. K. (2002). Purification and characterization of a thermophilic and acidophilic chitinase from *Microbispora* sp. V2. *J Appl Microbiol*, 93 (6):965-75.

Nimnoi, P., Pongsilp, N., and Lumyong, S. (2010). Endophytic actinomycetes isolated from *Aquilaria crassna* Pierre ex. Lec and screening of plant growth promoters production. *World J Microbiol Biotechnol*, 26 (2):193-203.

Ninawe, S., Kapoor, M., and Kuhad, R. C. (2008). Purification and characterization of extracellular xylanase from *Streptomyces cyaneus* SN32. *Bioresour Technol*, 99 (5):1252-8.

Nouioui, I., Carro, L., Garcia-Lopez, M., Meier-Kolthoff, J. P., Woyke, T., Kyrpides, N. C., Pukall, R., Klenk, H. P., Goodfellow, M., and Goker, M. (2018). Genome-based taxonomic classification of the phylum *Actinobacteria*. *Front Microbiol*, 9:2007.

Phongsopitanun, W., Kudo, T., Ohkuma, M., Suwanborirux, K., and Tanasupawat, S. (2015). *Dactylosporangium sucinum* sp. nov., isolated from Thai peat swamp forest soil. *J Antibiot*, 68 (6):379-84.

Phongsopitanun, W., Matsumoto, A., Inahashi, Y., Kudo, T., Mori, M., Shiomi, K., Takahashi, Y., and Tanasupawat, S. (2016). *Actinoplanes lichenis* sp. nov., isolated from lichen. *Int J Syst Evol Microbiol*, 66 (1):468-73.

Qin, S., Li, J., Zhang, Y. Q., Zhu, W. Y., Zhao, G. Z., Xu, L. H., and Li, W. J. (2009). *Plantactinospora mayteni* gen. nov., sp. nov., a member of the family *Micromonosporaceae*. *Int J Syst Evol Microbiol*, 59 (Pt 10):2527-33.

Qin, S., Xing, K., Jiang, J. H., Xu, L. H., and Li, W. J. (2011). Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. *Appl Microbiol Biotechnol*, 89 (3):457-473.

Ramasamy, D., Mishra, A. K., Lagier, J. C., Padhmanabhan, R., Rossi, M., Sentausa, E., Raoult, D., and Fournier, P. E. (2014). A polyphasic strategy incorporating genomic data for the taxonomic description of novel bacterial species. *Int J Syst Evol Microbiol*, 64 (Pt 2):384-91.

Rheims, H., Schumann, P., Rohde, M., and Stackebrandt, E. (1998). *Verrucosispora gifhornensis* gen. nov., sp. nov., a new member of the actinobacterial family *Micromonosporaceae*. *Int J Syst Bacteriol*, 48 (Pt 4):1119-1127.

Ritcher, M., and Rosselló-Móra, R. (2009). Shifting the genomics gold standard for the prokaryotic species definition. *Proc Natl Acad Sci* USA, 106:19126-19131.

Ritcher, M., Rosselló-Móra, R., Oliver Glöckner, F., and Peplies, J. (2016). JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics*, 32:929-931.

Saitou, N., and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol*, 4 (4):406-25.

Sangal, V., Goodfellow, M., Blom, J., Tan, G. Y. A., Klenk, H. P., and Sutcliffe, I. C. (2018). Revisiting the taxonomic status of the biomedically and industrially important genus *Amycolatopsis*, using a phylogenomic approach. *Front Microbiol*, 9.

Sanger, F., Nicklen, S., and Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U S A*, 74 (12):5463-5467.

Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *Technical Note*, 101.

Shimizu, M. 2011. Endophytic Actinomycetes: biocontrol agents and growth promoters. In *Bacteria in Agrobiology: Plant Growth Responses*, (Eds) D. Maheshwari. Berlin, Heidelberg: Springer.

Shirling, E. B., and Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol*, 16:313-340.

Song, L., Li, W. J., Wang, Q. L., Chen, G. Z., Zhang, Y. S., and Xu, L. H. (2005). *Jiangella gansuensis* gen. nov., sp. nov., a novel actinomycete from a desert soil in north-west China. *Int J Syst Evol Microbiol*, 55 (Pt 2):881-884.

Sripreechasak, P., Suwanborirux, K., and Tanasupawat, S. (2014). Characterization and antimicrobial activity of *Streptomyces* strains from soils in southern Thailand. *J Appl Pharm Sci*, 4 (10):24-31.

Stackebrandt, E., and Ebers, J. (2006). Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today*, 33 (15):152-155.

Stackebrandt, E., and Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol*, 44 (4):846-849.

Stamford, T. L., Stamford, N. P., Coelho, L. C., and Araujo, J. M. (2001). Production and characterization of a thermostable alpha-amylase from

Nocardiopsis sp. endophyte of yam bean. *Bioresour Technol*, 76 (2):137-41.

Staneck, J. L., and Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol*, 28 (2):226-31.

Stutzenberger, F. J. (1971). Cellulase production by *Thermomonospora curvata* isolated from municipal solid waste compost. *Appl Microbiol*, 22 (2):147-52.

Taibi, Z., Saoudi, B., Boudelaa, M., Trigui, H., Belghith, H., Gargouri, A., and Ladjama, A. (2012). Purification and biochemical characterization of a highly thermostable xylanase from *Actinomadura* sp. strain Cpt20 isolated from poultry compost. *Appl Biochem Biotechnol*, 166 (3):663-79.

Tamaoka, J. (1994). Determination of DNA base composition. In *Chemical Methods in Prokaryotic Systematics*, (Eds) M. Goodfellow, and A. G. O'Donnell, 463-470. Chichester: John Wiley and Sons.

Tamaoka, J., Katayama-Fujimura, Y., and Kuraishi, H. (1983). Analysis of bacterial menaquinone mixtures by high performance liquid chromatography. *J Appl Bacteriol*, 54:31-36.

Tamura, T., Hatano, K., and Suzuki, K. (2006). A new genus of the family *Micromonosporaceae, Polymorphospora* gen. nov., with description of *Polymorphospora rubra* sp. nov. *Int J Syst Evol Microbiol*, 56 (Pt 8):1959-64.

Tindall, B. J., Rossello-Mora, R., Busse, H. J., Ludwig, W., and Kampfer, P. (2010). Notes on the characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol*, 60 (Pt 1):249-66.

Tiwari, K., and Gupta, R. K. (2012). Rare actinomycetes: a potential storehouse for novel antibiotics. *Crit Rev Biotechnol*, 32 (2):108-32.

Tomiyasu, I. (1982). Mycolic acid composition and thermally adaptative changes in *Nocardia asteroids*. *J Bacteriol*, 151:828-837.

Trujillo, M. E., Bacigalupe, R., Pujic, P., Igarashi, Y., Benito, P., Riesco, R., Medigue, C., and Normand, P. (2014). Genome features of the endophytic actinobacterium *Micromonospora lupini* strain Lupac 08: on the process of adaptation to an endophytic life style?. *Plos One*, 9 (9).

Uchida, K., and Aida, K. (1984). An improved method for the glycolate test for simple identification of the acyl type of bacterial cell walls. *J Gen Appl Microbiol*, 30:131-134.

Vandamme, P., Pot, B., Gillis, M., de Vos, P., Kersters, K., and Swings, J. (1996). Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microbiol Rev*, 60 (2):407-438.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., and Murray, R. G. E. (1987). International committee on systematic bacteriology, report of the ad hoc committee on the reconciliation of approaches to bacterial systematic. *Int J Syst Bacteriol*, 37:463-464.

Xie, Q. Y., Wang, C., Wang, R., Qu, Z., Lin, H. P., Goodfellow, M., and Hong, K. (2011). *Jishengella endophytica* gen. nov., sp. nov., a new member of the family *Micromonosporaceae*. *Int J Syst Evol Microbiol*, 61 (Pt 5):1153-1159.

Yang, C. H., and Liu, W. H. (2004). Purification and properties of a maltotriose-producing alpha-amylase from *Thermobifida fusca*. *Enzyme Microb Technol*, 35:254-260.

Yoon, S. H., Ha, S. M., Kwon, S., Lim, J., Kim, Y., Seo, H., and Chun, J. (2017). Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol*, 67 (5):1613-1617.

Yoon, S. H., Ha, S. M., Lim, J., Kwon, S., and Chun, J. (2017). A large-scale evaluation of algorithms to calculate average nucleotide identity. *Anton Leeuw Int J G*, 110 (10):1281-1286.

Zhang, F., Chen, J. J., Ren, W. Z., Nie, G. X., Ming, H., Tang, S. K., and Li, W. J. (2011). Cloning, expression and characterization of an alkaline thermostable GH9 endoglucanase from *Thermobifida halotolerans* YIM 90462^{T}. *Bioresour Technol*, 102 (21):10143-6.

Zhang, F., Hu, S. N., Chen, J. J., Lin, L. B., Wei, Y. L., Tang, S. K., Xu, L. H., and Li, W. J. (2012). Purification and partial characterisation of a thermostable xylanase from salt-tolerant *Thermobifida halotolerans* YIM 90462^{T}. *Process Biochem*, 47 (2):225-228.

ABOUT THE EDITORS

A. A. Mohamed Hatha
Fulbright Scholar Department of Marine Biology,
Microbiology and Biochemistry School of Marine Science
Cochin University of Science and Technology Cochin, Kerala, India
mohamedhatha@gmail.com

Dr. Hatha is passionate about microbial diversity in the environment and has explored many unique habitats such as ocean depths, thermal springs and arctic fjords and tundra for diverse microbes and their unique capabilities. Had carried out considerable research on antimicrobial

resistance among food and water borne pathogens and published more than 200 papers in international journals of repute. So far guided 20 students for Ph.D. Currently Professor in the Department of Marine Biology, Microbiology and Biochemistry, CUSAT.

P. Lakshmanaperumalsamy
Department of Environmental Sciences
Bharathiar University Coimbatore, Tamil Nadu, India
drplpsamy@gmail.com

With more than 45 years of teaching and research experience, Prof. Lakshmanaperumalsamy is an authority on environmental microbiology. He had completed his PhD research on Actinomycetes and has published nearly 200 research papers in peer reviewed international journals of repute. He had successfully implemented several funded research projects and guided 29 students for PhD.

INDEX

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

T

U

V

W

X

Y