SELECTIVE ISOLATION, IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF *STREPTOMYCES* SPECIES FROM SOIL OF SULAIMANI PROVINCE, NORTH OF IRAQ

Miran Hussein QADIR
Master Thesis

Biology Department

Supervisor: Prof. Dr. Ekrem ATALAN
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REPUBLIC OF TURKEY
BİNGOL UNIVERSITY
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This thesis was approved unanimously by the following jury in the date of 23/01/2017

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PREFACE

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DEDICATION

This research paper is dedicated to:

- My beloved parents, especially my most merciful and generous mother.
- My lovely siblings.
- All teachers and doctors who taught me throughout my studying.
- All students who can get benefit from this study in the future.
- My dear friends.

CONTENTS

LIST OF ABBRIVATIONS

%	: Percentage
ºC	: Degree Celsius
µl	: Microliter
µg	: Microgram
CFU	: Colony forming unit
cm	: Centimeter
EDTA	: Ethylenediaminetetraacetic acid
g	: Gram
L	: Liter
Min	: Minute
m	: Meter
ml	: Milliliter
mm	: Millimeter
PCR	: Polymarase Chain Reaction
RHA	: Rafinnose histidine agar
SCA	: Starch casein agar
TLC	: Thin layer chromatography
TE	: Tris EDTA
TBE	: Tris Borate EDTA

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SÜLEYMANIYE (IRAK) TOPRAKLARINDAN *STREPTOMYCES* BAKTERILERININ IZOLASYONU, TEŞHISI VE MOLECÜLER KARAKTERISAZYONU

ÖZET

Bu çalışmada, Irak'ın Süleymaniye vilayetinin Pshdar ve Ranye bölgesinde toplanan 32 toprak numunesinden actinomycetes cinsleri ve *Streptomyces* cinsine ait bakteriler izole etmek amaçlandı. Başlangıçta toprak numunelerinin fizikokimyasal parametreleri ölçüldü ve ondan sonra *Streptomyces* suşlarını izole etmek için içerisine cycloheximide ve nystatine içeren nişasta-kazein agar ve yine içerisine cycloheximide, nystatine ve novobiocin antibiyotikleri eklenmiş raffinoz-histidin agar içeren petri kutularına ekim yapıldı. Toprak numunelerinde izole edilen toplam 164 *Streptomyces* suşu oatmeal agar besiyerinde havasal ve substrat miselyum spor renkleri, diffüziye pigment rengi ve ayrıca pepton-yeast agar besi ortamında melanın pigment üretimi dikate alınarak gruplandırdı.

Renk gruplarını temsilen seçilen 20 test suşu 40 diagnostik teste tabi tutuldu ve sonuçlar numerik analizi yapıldı ve test suşları 10 gruba ayrıldı. Renk grupları ile numerik analiz gruplarının içerdiği suşlar genelde uygunluk gösterdi. Bilgisayara dayalı renk gruplandırma analizleri hem ekolojik hemde biyolojik ürün araştırmalarında ön çalışma olarak önemlidir.

Test suşlarının filogenetik analizleri için 20'inin 16S rDNA genleri DNA ekstraksiyonundan sonra evrensel primerler kullanılarak 16S rDNA genleri çoğaltıldı ve 18'inin nükleotid zinciri belirlendi. Bu 18 suşun 16S rDNA genleri filogenetik analizi yapıldı ve 11'i *Streptomyces* türü olaral teşhis edilirken 3'ü *Amycolatopsis umgeniensis* UM16 ve *Amycolatopsis xuchangensis* CFH S0322. türü olarak teşhis edildi. 3'ü ise *Nocardia ignorata* DSM 44496 ve *Nocardia rhamnosiphila* NRRL B-24637 ve 1'i *Lentzea flaviverrucosa* AS4.0578 türü olarak teşhis edildi. 11 *Streptomyces* türlerinden 4'ü *S. Anulatus*, 4'ü *S. fulvissimus*, 2'si *S. lateritius* LMG 19372 ve 1'i *S. atrovirens* türü olarak teşhis edildi. 16S rDNA genlerinin analiz sonucu oluşan dendogramdaki gruplar ile renk gruplandırması arasında korelasyon görüldü.

Yine filogenetik analiz sonucu oluşan dendogramdan seçilen 4 suşun tüm hücre şeker analizi ve diaminopimelik asit (DAP) gibi kemotaksonomik sonuçları numerik analiz ve filogenetik analiz sonuçları uygunluk gösterdi ve önceki sonuçları destekledi. Bu veriler K02008 (13K) and K20134 (4K) suşunun *Amycolatopsis xuchangensis* CFH S0322

%99,15 oranında benzerlik gösterdiğinden yeni tür olarak kabul edimesi gerekir. Fakat DNA-DNA hibdridisazyon çalışması en yakın tip suşla yapılması gerekir.

Yine kemotaksonomik çalışmalar için filogenetik analiz sonucu 4 test suşu seçildi, şeker analizi ve diaminopimelik asit (DAP) analizi yapıldı. Sonuçlar numerik ve filogenetik analiz sonuçlari ile uyum gösterdi ve önceki çalışmalar ile desteklendi. Verilerimiz K02008 (13K) ve K20134 (4K) *Amycolatopsis xuchangensis* CFH S0322. türüne % 99.15 benzerliğinden dolayı yeni tür olabilir. Yine K18126 (6K) ve K21147 (16K) suşlarıda *Lentzea flaviverrucosa* AS40578 ile %99.15 benzerliğinden dolayı yeni tür olması muhtemeldir. Bunun ile birlikte bütün izole edilen türlerin yeni tür olduğunu ispatlamak için en yakın tip suşu ile DNA-DNA hibridizasyon çalışması yapılması gerekir.

Anahtar Kelimeler: *Streptomyces,* 16S rDNA, Süleymaniye.

SELECTIVE ISOLATION, IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF *STREPTOMYCES* SPECIES FROM SOIL OF SULAIMANI PROVINCE, NORTH OF IRAQ

ABSTRACT

In this study, a total 32 soil samples collected from Pshdar and Ranya sites which are districts in Sulaimani governorate, Kurdistan Region of Iraq. The samples were subject to isolation of actinomycetes genera and *streptomyces* bacteria. Initially, physicochemical parameters of soil samples were measured and then selective media raffinose-histidine agar supplemented with cycloheximide and nystatin, and starch-casein agar plates supplemented with same antifungal antibiotics and novobiocin, were used to isolate *Streptomyces* strains from soil samples. In total, 164 streptomycetes were isolated from the soil samples and were de-replicated manually based on: aerial spore mass, colony reverse and diffusible pigment colours formed on oatmeal agar, and their capacity to produce melanin pigments on peptone yeast extract iron agar.

Using numerical analysis method, 40 diagnostic tests were carried out on 20 representative test strains of the colour groups; the samples were assigned into 10 clusters. In general, the clusters of isolates delineated in the dendrogram generated using the distances were found to match those obtained by manual colour-grouping of the isolates. The implications of the computer-assisted colour-grouping method for ecological studies are important.

For phylogenetic analysis, 16S rDNA genes of 20 test strains were amplified with 2 universal primers after extraction of genomic DNA and then sequenced; however, only 18 of them were successfully sequenced. These representative 18 test strains were subject to phylogenetic analysis of DNA sequence of 16S rDNA and 11 out of 18 test strains were identified as *Streptomyces* species while 3 of them identified as *Amycolatopsis* species; they were *Amycolatopsis umgeniensis* UM16 and *Amycolatopsis xuchangensis* CFH S0322. Two test strains were identified as *Nocardia* species and they were *Nocardia ignorata* DSM 44496 and *Nocardia rhamnosiphila* NRRL B-24637. The other two test strains were identified as *Lentzea flaviverrucosa* AS4.0578 species. Out of the 11 *strptomyces*; 4 were *S. anulatus*, 4 were *S. fulvissimus*, 2 were *S. lateritius*, and 1 was *S. atrovirens*. A reasonable linear correlation was found between the colour-group, and corresponding dendogram that was generated after analysis of base sequence of 16S rDNA genes.

Also, chemotaxonomic studies, sugar analysis and diaminopimelic acid (DAP) analysis, on 4 test strains of representatives from phylogenetic dendogram were more or less in agreement with the result of phylogenetic analysis and numerical analysis of test strains, and these are supported by previous studies. The data indicates that the K02008 (13K) and K20134 (4K) which were isolated should be recognized as new species in the genus *Amycolatopsis*, because they are 99,15% similar to *Amycolatopsis xuchangensis* CFH S0322(T). Also, K18126 (6K), and K21147 (16K) that were isolated should be recognized as new species in the genus *Lentzea*, since they are 99,15% similar to *Lentzea flaviverrucosa* AS4.0578(T). However, all these new isolated species mentioned should be further studies using DNA-DNA hybridization of the strains with nearest type strain.

Keywords: *Streptomyces*, 16S rDNA, Sulaimani.

1. INTRODUCTION

Actinobacteria are one of the most widely distributed groups of microorganisms in world, particularly in the soil. The Actinobacteria are a group of Gram-positive bacteria that plays an important role in degradation of organic matter and in the formation humus type soil. At the time of their discovery, they were assigned as fungi due to producing mycelia but later classified as Actinomycetes because of having prokaryotic cell type. Actinobacteria is one of biggest phylum that contains the 6-major classes; including 23 orders in the domain Bacteria. The phylum is gram-positive bacteria that has a high G+C content in its DNA which ranges between 51% up to 70%.

Streptomyces species, belonging to the Actinobacteria calss, are aerobic and Gram-positive soil bacteria that show filamentous growth from a single spore. As their filaments grow through tip extension, and branching; they ultimately form a network of branched filaments called a substrate mycelium (Dyson 2011). *Streptomyces* species have been an important source of medicines, especially antibiotics. Many antibiotics were isolated from various *Streptomyces* species from the late 1940s to 1960s, and entered clinical use (Berdy 2005). The percentage of recently discovered antibiotics that is isolated from *Streptomyces* species has declined to about 20–30%. The medical uses of these secondary metabolites are not just confined to antibiotics, but also include immunosuppressants, antifungals, anticancers, and antiparasitics (Newman and Cragg 2007: Goodfellow and Fiedler 2010: Mao et al. 2011).

Consequently, isolation of *Streptomyces* species is becoming more important given that they are already a proven source of medically-useful compounds with diverse structures and with the potential to produce even more secondary metabolites (Baltz 2011: Craney et al. 2013). The *Streptomyces* species also stand competitive in comparison with plant cells as extracting and characterizing bioactive compounds from plants are extremely difficult in addition to their long cultivation time (Xu 2011)

Here, we isolated *streptomyces* and some other related genera of actinobacteria strains from soils of Pshdar and Ranya district in Sulaimani, North of Iraq. These kinds habitats which are similar to desert and marine ecosystems, have not been studied in detail, and are likely to contain rich sources of novel filamentous actinomycetes that could produce new compounds. The isolates were characterized using colour grouping, and numerical analysis methods. The identification and analysis of the base sequence of 16S rDNA genes, and diaminopimelic acid (DAP) involved observing sugar content of the cell wall of these strains. The present study was designed to observe biodiversity of actinomycetes but only species of *Streptomyces*, *Nocardia*, *Lentzea* and *Amycolatopsis* genera were discovered. Briefly; the aim of the present study is to:

1. Study *Streptomyces* strain in soil samples collected from different location of Pshdar and Ranya districts, northern Iraq.
2. To understand the association between physico-chemical properties of the soil and their *Streptomyces* population.
3. Isolation and purification of potential *Streptomyces* preliminarily according to their morphological properties through various in vitro bioassays.
4. To characterize the selected strains using morphological, cultural, biochemical, physiological, molecular characteristics, sugar and DAP analysis, including; colour grouping, numerical analysis, chemotaxonomic and 16S rRNA gene sequence.
5. Identification of the 18 strains of isolates by phylogenetic analysis of 16S rRNA gene sequencing at species level.

2. LITERATURE REVIEW

2.1. Actinobacteria

The domain Bacteria contains many phyla that Actinobacteria is the major Gram positive phyla (Ludwig and Klenk 2005). The phylum actinobacteria include six classes: Actinobacteria, Acidimicrobiia, Coriobacteriia, Nitriliruptoria, Rubrobacteria, and Thermoleophilia, 23 orders, 53 families, and 252 genera (Goodfellow et al. 2012). Most genera of Actinobacteria phylum are chemo-organotrophs, and they grow at neutral pH. While some of them are acidophiles, alkalophiles or halophiles and thermophiles (Stach and Bull 2005) their DNA G+C content is of a high percentage (Verma et al. 2013). Their DNA G+C content is in the range of 50 mol% to over 70 mol% (Zaburannyi et al. 2014; Amin et al. 2016). Many species of actinobacteria do live freely in nature and are found abundantly in soil, water, and saprophytic. Some species of the bacteria are pathogenic for plants and animals (Jensen and Lauro 2008).

The production of substrate and aerial mycelium on solid media is similar to the mycelium generated by filamentous fungi, and it is one of its most essential characteristics (Madigan et al. 2009). They live in different habitats such as soil, water, plants, sediments, animals and human bodies (Nithya 2013). Actinobacteria plays an important role in the decomposing of organic matter in the soil, and or other environments, (Goodfellow and Williams 1983) and they produce different biologically active substances such as, antibiotics, anti-tumor, antiviral, antifungal, antiparasite and immuno-suppressant properties. Importantly, Streptomyces are the source of many bioactive compounds, and produce about 75% of all known antibiotics that are used in human therapy (Demain 2014: Cheng et al. 2016).

2.2. Streptomycetaceae family

Many different types of *Streptomyces* strains are described by Waksman and Henrici 1943, 107AL. The family members produce branched substrate mycelium, gram positive, and aerobic actinomycetes. The aerial mycelium produce chains of three to many spores at the late age of their life cycle while short spore chains on their substrate mycelium can be produced by some species. Also, various pigments are produced which are responsible for the color of the substrate and aerial mycelium. They grow within different pH and chemoorganotrophic organisms having an oxidative type of metabolism. Their cell walls of the substrate mycelium made of either LL- or meso-diaminopimelic acid while aerial or submerged spores contain LL-diaminopimelic acid. On the other hand, lipid content of cell walls contains complex mixtures of saturated iso- and anteiso-fatty acids, isoprenologues, diphosphatidylglycerol, phosphatidylethanol amine, phosphatidylinositol, and phosphatidylinositol mannosides (major polar lipids hexa- and octa-hydrogenated menaquinones with nine isoprene units). No mycolic acids are present, and one of the galactose or galactose and rhamnose are sugar profiles which contain high quantities of G+C rate of the DNA change in the range of 66 % and 74 %.

Waksman and Henrici (1943) established the family Streptomycetaceae to accommodate actinomycetes that produce mycelia and spores on aerial hyphae and over 600 species have been published (Zhang et al. 2016). The hyphae are sometimes septate fragment into oidia. Micoorganisms were describing only according to their morphological characteristics at that time. Pridham and Tresner (1974a) listed the genera *Streptomyces*, *Streptoverticillium*, *Sporichthya*, and *Microellobospora* as Streptomycetaceae family. Taxonomic statues of *Kitasatospora* genus and some other genera such as *Actinopycnidium*, *Actinosporangium*, *Chainia*, *Elytrosporangium*, *Kitasatoa*, and *Microellobospora* were disputed at the time of their discovery (Goodfellow 1986: Ludwig et al. 2012: Girard et al. 2013: Labeda et al. 2012). Wellington et al. (1992) proposed the unification of *Kitasatospora* with *Streptomyces* based on phenetic, biochemical and 16S rRNA gene sequence similarities. Girard et al. (2013) suggest that *Kitasatospora* should be sister genus of *Streptomyces* because of having similar phenetic character and relationships based on the phylogeny of the SsgA-like proteins data.

Stackebrandt et al. (1997) proposed the genus of *Sporichthya* within the family Sporichthyaceae. The genus *Sporichthya* was accommodated within Sporichthyaceae family of the order Frankiales. Also, the genus *Streptoverticillium* accepted as synonym of streptomycetes although members of the genus have distinguishable verticillate sporophores (Goodfellow et al. 2012). A study of *Streptomyces* was carried out using numerical phenetic data by (Williams et al. 1983a: Kampfer et al. 1991) and rRNA/DNA similarities by (Witt and Stackebrandt 1990). Kim et al. (2003) proposed *Streptacidiphilus* genus with pH growth range of 3.5 to 6.5. Within the Streptomycetaceae family, molecular studies show that it and *Kitasataspora* are separate genus (Kämpfer 2012: Labeda et al. 2012: 2010: Girard et al. 2014). Still, many techniques such as 16S rRNA gene sequence analyses, protein profiles were carried out to solve disputable genera of Streptomycetaceae in the framework for prokaryotic classification. However, the taxonomic problems within the family Streptomycetaceae were not completely solved (Anderson & wellington 2001).

2.3. Molecular Studies

Genome sequence of *Streptomyces* species increase rapidly owing to producing potential of bio-technologic compounds, and they are widely distribution in the soil. Many studies were carried out to study genes related active compounds of streptomycetes, and many research papers have been published on this topic within the last 10 years (Dyson 2010). Importantly, 16S rDNA sequences and genome sequences of *Streptomyces* species are available in some online databases. It is well known that *Streptomyces* produce many secondary metabolites, and over 60% of commercially available antibiotics. Hopwood (2007) reported that *Streptomyces* produce many different bioactive compounds such as antibacterial, antifungal, antiparasitic, and immunosuppressant compounds via secondary metabolism.

The genome size of *Streptomyces* species contains large genome and have linear topology. The chromosome size of *S. cattleya* is 6,283,062 bp while *S. bingchenggensis* contain 11,936,683 bp, and code for 10,023 genes. Pang et al. (2002a, b) has revealed by pulse-field gel electrophoresis (PFGE) studies that, *S. ambofaciens*, *S. antibioticus*, *S. lipmanii*, *S. moderatus*, *S. parvulus*, *S. rochei*, *S. griseus*, and *S. hygroscopicus* contain

linear chromosomes. Ikeda et al. (2003) reported that the size of *S. coelicolor* A3(2) chromosome is about 8,667 mega base pairs (Mbp: 7,825 genes), and two-thirds of those genes (about 4,837 genes) are conserved orthologs using BLAST analysis. Ventura et al. (2007) reported that only about 17 % of the 3,566 genes common to the four *Streptomyces* genomes are present in *E. coli* K-12 and *Bacillus subtilis*.

Comparison of genome of 3 *Streptomyces* and 1 *Kitasataspora* strains to *S. coelicolor* using DNA-DNA microarray hybridization technique revealed that, some parts of the chromosome are well conserved (central core region) while terminal regions of the linear chromosome contain low degree of gene conservation (Hsiao and Kirby 2008). The replication mechanisms of the *Streptomyces* chromosome have been explained by Ventura et al. (2007). The region of the origin (oriC) has rich A+T (64%) while the genome has the high overall G+C content (69–73 mol%) of *Streptomyces* DNA. Also, it is found that chromosomes of *Streptomyces* species contain terminal inverted repeats (TIRs) at their ends and they have a terminal protein that is covalently bound.

Replication of chromosome telomer regions initiate and have special mechanisms (Ventura et al. 2007). (Kirby and Chen 2011: Fowler-Goldsworthy et al. 2011) studied using the genome structure of Streptomycete. It is found that some redundancy of metabolic genes is probably have complex morphological and physiological differentiation. Schrempf (2006) suggested that Streptomycetes genome contain some unstable genes such as involving antibiotic resistances, A-factor formation, and synthesis of tyrosinase or arginosuccinate. It is well known that streptomycetes strains show pigmentation spontaneously, sporulation and antibiotic biosynthesis. The structure of chromosomal variation may be affected by linear interaction and circular plasmids, phages, transposons, and insertion elements.

2.3.1. *Streptomyces* Plasmids

The members of *Streptomyces* contain two types of plasmids, and those are linear and circular. Circular plasmids (covalently closed circular, ccc) generate from chromosome by site-specific recombination while linear plasmids contain replication origin gene and inverted repeats similar to the chromosome (Vogelmann et al. 2011). Plasmids can be

transfer to other organisms by their self-transmissibility and make recombination with host chromosomes. By doing this, they promote genetic exchange between organisms owing to almost 100% frequency of plasmid transfer. However; conjugation mechanism is un-similar to *E. coli*. Plasmid transfer occurs via hyphae of strains. Tra protein bridge help transferring plasmids into host by integrating plasmids, and recombination may be carried on between chromosome and recipient plasmid. Plasmids encode some features such as antibiotic production and mercury resistance (Ravel et al. 1998). Interestingly, some other genera of actinomycetales contain linear plasmids (Schrempf 2006). Today, some of *Streptomyces* plasmids are used for gene cloning Kieser et al. (2000). Also, phages are important for transduction and some of them have been isolated from soil and used for classification of *Streptomyces*.

2.3.2. DNA-DNA Hybridization

This technique is an important for identification at species level and it uses 16S rDNA gene analysis to confirm whether it is new or known species. Either whole genome or partial gene of *Streptomyces* hybridize in this technique. Witt and Stackebrandt (1990) used DNA-DNA hybridization to affirm the position of *S. albidoflavus* strains cluster generated by the numerical phenetic study of Williams et al. (1983a) and *Streptoverticillium* strains to the genus *Streptomyces*. Labeda (1998) used the technique for clusters of Williams et al. (1983a) and found little correlation between numerical phenetic clusters and DNA-DNA hybridization results (Hatano et al. 2003). It should be noted that, some regions of the chromosome are not stable, and therefore this can affect their hybridization technique (Redenbach et al. 1993).

2.3.3. Fingerprinting Techniques

Different fingerprinting techniques were applied to characterize *streptomyces* and other genera. These were randomly amplified polymorphic DNA polymerase chain reaction (RAPD-PCR), restriction fragment length polymorphisms (RFLP), and pulsed-field Gel Electrophoresis (PFGE). In RAPD PCR, a single arbitrary primer is designed to amplify many different DNA fragments, and the fragments give genetic variations (Welsh and

McClelland 1990). Polymorphisms is used to distinguish test strains; this sequence differentiates in one or both of the primer binding sites and can be observed.

The technique was applied to closely *streptomyces* strains to detect specific banding patterns (Mehling et al. 1995). When Anzai et al. (1994) used 11 primers to differentiate *Streptomyces* species he found zero to 20 bands. Then he changed each nucleotide of primer one by one and obtained 30 bands. Although results of RAPD-PCR are in good agreement with the findings of other methods such as RFLP, LFRFA, DNA-DNA hybridization and phenetic tests; the technique did not distinguish *S. lavendulae* and *S. virginiae* strains at specific level.

2.3.4. Pulsed-field gel electrophoresis (PFGE)

After extraction of DNA from microorganisms, The DNA is immobilized in the agarose and then digested with restriction enzymes at specific locations. The treated plugs are then loaded onto an agarose gel, and the DNA fragments were separated based on size using an electric field. At the end, many DNA bands were produced as fingerprints with a specific pattern and each lane represents a DNA fingerprint or pattern. PFGE technique is different from conventional DNA electrophoresis owing to generation of very large fragments a fingerprint by constantly changing the direction of the electric field. Briefly, the technique is based on digestion of whole genome with restriction and producing of many DNA bands (https://www.cdc.gov/pulsenet/pathogens/pfge.html). Some authors have applied for identification and delineation of *Streptomces* and other actinobacteria strains (Beyazova and Lechevalier 1993: Kieser et al. 1992: Pisabarro et al. 1998). Pisabarro et al. (1998) applied total DNA of a virulent strain of *Rhodococcus fascians* strains and digested with the endonucleases AseI and DraI, they identified a physical map of the organism. Rauland et al. (1995) suggested useful techniques to identify closely related species.

2.3.5. 16S rRNA and housekeeping Genes in identification

To study the diversity of bacterial communities from different environment there is need for a rapid, fast, and cheap technique. 16S ribosomal RNA (rDNA) sequencing is cheap,

reliable and fast profiling technique based on variation in the bacterial 16S rRNA gene (Janda & Abbott 2007). The most important technique is 16S rDNA sequence analysis of microorganism identification and it is used in isolating new microorganisms, Labeda (2014) reported that over 100 new species were discovered by this technique. Stackebrandt et al. (1992) used to classify *Streptomyces* species and found that variable region in 16S rDNA gene affect the variations. Chatellier et al (2014) analyzed 16S rDNA gene sequences of 1025 clinic strains and they were identified correctly at the genus level. Kampfer (2012) and Labeda et al. (2012) have analyzed all current *Streptomyces* species using 16S rRNA gene sequence and generated phylogenetic dendogram. Some author used 5S rDNA gene and 16S-23S rRNA spacer regions to identify and clarify *Streptomyces* species, they found these techniques to be helpful in species differentiation but not for delineating strains (Mehling et al. 1995: Kampfer 2012).

(Rong & Huang 2014: Cheng et al. 2015) have used MLSA technique which is include a five gene of 141 strains of *Streptomyces* isolated from different habitats, and they found relation between strains and isolated habitats. atpD, recA, gyrB, rpoD, rpoB, tryB genes are called housekeeping genes that are used to identify and characterize bacterial strains, this technique is called multilocus sequence analysis, (MLSA). The technique and DNA-DNA hybridization give valuable information to characterize bacterial strains. Meantime, whole genome gene sequencing analysis is the most crucial technique to characterize in prokaryotic species. Today, a new technique called whole-cell matrix-assisted laser desorption ionization time of flight (WC-MALDI-TOF), is used in the identification of bacteria, and it is one of most useful tools to characterize and identify strains. Many researchers use such techniques to characterize bacterial strains and identify them (Hatano et al. 2003: Kim et al. 2004: Stackebrandt et al. 2002: Tindall et al. 2010: Guo et al. 2008). Rong and Huang (2010, 2012) used MLSA technique in delineation of Streptomyces and proposed a cut-off value for DNA-DNA hybridization. Gau et al. (2000) reported that DNA-DNA hybridization results are better comparing to other analysis. Doroghazi and Buckley (2010) suggested that partial genome sequencing have potential to solve characterization of bacteria.

2.3.6. Restriction fragment length polymorphism (RFLP)

The technique is based on differences in homologous DNA sequences that can be detected by the presence of fragments of different lengths, this is observed after digestion of the DNA samples in organism with specific restriction endonucleases (https://www.ncbi.nlm.nih.gov/probe/docs/techrflp/). After DNA extraction, specific gene are amplified and digested with a restriction enzyme. Produced DNA bands are analyzed and organisms are grouped according to fragmentation size and numbers. Also, specific DNA fragments can be hybridized with RFLP probe and may be used for genotyping, forensics, paternity tests, and hereditary disease diagnostics. This method is fast since it does not require DNA sequencing. Many different endonuclease enzymes have been used to evaluate *Streptomyces* and other bacteria species (Fulton et al. 1995), Huddleston et al. (1997) used this technique in addition to sequence comparisons, PFGE, and numerical taxonomy to describe new isolates.

2.4. Description of Streptomycetes

Project *Streptomycetes* produce aerial and substrate mycelium on solid media and liquid media. Their aerial mycelium produce spores called arthrospore or sporophore (Kutzner 1981: Glazebrook et al. 1990). Arthrospore or sporophore generate after maturity of colonies by septation of aerial hyphae and by holothallic growth after which a thin sheath appears (Locci and Sharples 1984). Also, spore chain morphology is very important characteristic for description of species, and there are three types of spore chain morphology that can appear a) spirals, b) retinaculiaperti and c) helix. Also, spore surface ornamentation is used for phenotypic characterization and identification. (Pridham et al. 1958: Shirling and Gottlieb 1966) used spore chain morphology and morphology for identification and established a project called the International *Streptomyces* Project (ISP).

It is well known that chemical, physical and biologic factors are affecting the growth of bacteria and spore development. At the beginning, spore swell in presence of moisture and organic matter. Once germ tubes appear their extension starts, extension of filaments cause branching of hyphae and produce mycelium (Flardh et al. 2012: Hempel et al.

2012). As they maturity, hyphae can septate to produce spores called arthrospore owing to lack of nutrients and other negative factors. Chater et al. (2010) reported that bld gene is responsible for sporulation, while whi gene also related to septations of spores.

Another important character of *Streptomyces* is the production of pigments including diffusible pigments. In early days of *Streptomyces* discovery mass colour and pigmentation were used for characterization, the results were used to assign species groups (Pridham et al. 1958: Kutzner 1981). Pigment and spore mass colour is still used in pre-identification of *Streptomyces*. Kutzner (1981) reported that pigment and spore mass colour are influenced by chemical and physical factors such as moisture, temperature, acid, alkali conditions and age. The pigmentation is the reflection of antibiotic produced by the strain (Shirling and Gottlieb 1970).

2.5. Chemotaxonomy

2.5.1. Peptidoglycans

Diamino acids are most important component of peptidoglycan molecule in the cell wall of bacteria. Cell wall of Gram-negative bacteria contain only meso-diaminopimelic acid (meso-DAP). There is DAP type in Gram-positive bacteria that is showing variations such as meso- (DL-) and LL-DAP, L-ornithine, L-lizin and L-2,4-DAP and interpeptide bridge contain ornithine. DAP is known to be useful for Gram-positive bacteria but not for gram negative bacteria (Komagata and Suzuki 1987). *Streptomyces* bacteria is Chemotype III based on its cell wall contents (Takahashi et al. 1984).

2.5.2. Cell Wall Polysaccharides

In addition to muramic acid and glucosamine of peptidoglycan, the cell wall also contains sugars. Comparison of sugar types are used commonly for characterization of gram positive bacteria (Busse et al. 1996). Cell wall of different genera of bacteria have various types of sugars, for example, filamentous actinobacteria contain 5 different sugar (arabinose, galactose, xylose, mannose, and ribose) while some of genera has not specific sugar type. Sugar types can be detected by running hydrolysate of cell wall sample using

thin layer chromatography (TLC), Rf values and colour differences help to characterize. Unless standard marker compounds it is difficult to name an unknown sugar type (Kroppenstedt 1977).

2.6. Extracellular Enzymes

Streptomycetes, Gram-positive bacteria, has a unique capacity for the production of primary and secondary metabolites. *Streptomyces* species is one important source of producing enzymes owing to using organic matter in the habitats for their growth. Chater et al. (2010) found that *streptomyces* genes code over 800 proteins. They use various carbon source compounds such as chitin and produce chitinase to break down chitin. Also, they produce protease and inhibitors, keratinase, lipases (Hiraga et al. 2000; Sommer et al. 1997). They have 3 cell types in their life. More studies related secondary metabolisms carried out comparing to primary studies of streptomycestes. When main nutrient is abundant in habitats *Streptomyces* carry on primary metabolism. Depletion of soil nutrient levels cause bacteria to start secondary metabolism (Hodgson 2000). It is known that *Streptomyces* secret extracellular enzymes to degrade plant debris and other organic matter in the soil. Particularly glucose repression, control of amino acid catabolisms and their repression are important for metabolism of *Streptomyces*. Van Keulen et al. (2011) identified some genes related key pathway enzymes and Hesketh et al. (2002a) analyzed some of proteins of primary and secondary metabolisms. Some studies related proteomic, metabolic, transcriptomic data analyzed by (Novotna et al. 2003: Borodina et al. 2008).

Secondary metabolism starts under controlled conditions in laboratory. Functions of most secondary metabolites are unknown, but physiological stress is known to affect synthesis of metabolites in nature. Many genes involved in the control secondary metabolites and some genes are only belonging to actinomycetales bacteria (Bibb 2013). Ventura et al. (2007) reported at least 30 genes related to production of antibiotics, cytostatics, fungicides, or as modulators of immune responses in *Streptomyces*. Many studies related to genes controlling secondary metabolisms were carried out (Ikeda et al. 2003: Laureti et al. 2011: van Wezel and McDowall 2011).

2.7. Isolation of *Streptomyces*

Most of isolation methods to isolate actinobacteria genera are designed on physiological characters (Korn-Wendisch and Kutzner 1992: Kieser et al. 2000). Selective isolation media have been formulated after numerical study of Williams et al (1983a, b). Main steps of isolation of *Streptomyces* or other bacteria are collection of samples, pretreatment, incubation time and colony selection. In general plating out inoculum on selective media is a general method (Williams and Wellington 1982a, b: Williams et al. 1984).

First of all, soil samples are shaken vigorously on vortex owing to vegetative cells are associated with soil particles and serial dilutions are prepared. Sometimes, glass beads can be added into first serial dilution to make homogen suspension of spores and mycelium and a few methods are used those are the Turmix blender, Ultra-Turrax homogenizer, Ultrasonics sonicator-disrupter, Waring blender, or a mortar, pestle, the dispersion and differential centrifugation (DDC) technique (Goodfellow and Fiedler 2010). In early studies, many different methods and isolation media have been used (Williams and Robinson 1981: Williams and Mayfield 1971: Porter and Wilhelm 1961: Hsu and Lockwood 1975: Hayakawa and Nonomura 1987a, b). Meantime, different chemical inhibitor compounds have been added into dilutions to reduce or inhibit unwanted microorganisms such as fungi (Goodfellow 2010: Voelskow 1988, 1989). Also, membrane filtration method has been used to isolate *Streptomyces* and other genera of actinobacteria from water (Burman et al. 1969), sea water and mud (Okami and Okazaki 1972) this is due to resistance of arthrospores. One of the most efficient methods for *Streptomyces* is to add antibiotics such as cycloheximide, nystatin, rifampicin, novobiocin and candicidin (Polsinelli and Mazza 1984: Hanka et al. 1985). Chitin agar and Actinomyces isolation agar were both used to recover actinomycetes from habitats such as seawater, water and soil (Hsu and Lockwood 1975: Goodfellow and Haynes 1984: Okami and Okazaki 1978). Wind tunnel, Andersen sampler, and sedimentation chamber were used to increase the number of spores or hyphal fragments in the samples such as plant debris (Lacey and Dutkiewicz 1976b: Lacey and Dutkiewicz 1976: Goodfellow and Williams 1986: Archuleta and Easton 1981).

The most important character of *Streptomyces* is producing bioactive secondary metabolites such as antifungals, antivirals, antitumorals, antihypertensives, immunosuppressants, and especially antibiotics. They use specific pathway to produce antibiotics (Araújo et al. 2012). To discover a new antibiotic or other bioactive compounds, new species have to be isolated. Isolation of new species of *Streptomyces* can be done using the methods above (Nolan and Cross 1988). Once a strain is isolated and purified test strains should be tested against some pathogenic bacteria or fungi potential strains can be selected for further studies.

2.8. Selective Media for *Streptomyces*

As mentioned above, many media have been formulated for isolation of actinomycetes bacteria. Today, most of selective media such as starch casein or raffinose histidine and supplemented with antifungal and antibacterial antibiotics such as cycloheximide and rifampicin to inhibit fungi (Hayakawa and Nonomura 1987a, b), 10–50 mg/L of nystatin and pimaricin were added into selective media (Cross 1982), Nitrogen source compounds are added the media (Fischer et al. 2010: Van Keulen et al. 2003). pH is a crucial factor affecting bacterial growth and most of *Streptomyces* are grown in neutral conditions (7.0) and some can grow in acidophilic or alkali pH conditions (Antony-Babu and Goodfellow 2008). Another factor is temperature, most of *Streptomyces* are mesophilic, they grow in temperatures between 25-50°C. Psychrophilic grow under 20°C while thermophilic bacteria grow in over 50°C and extreme thermophilic bacteria can develop even over 80°C. Therefore, isolation plates should be incubated in the temperature that colonies can be grown for 1-2 weeks. After incubation, the most fragile stage is purification of isolates (Williams and Wellington 1982a: Kim et al. 2003).

2.9. Cultivation and Inoculum

To cultivate streptomycetes bacteria, media should include suitable organic carbon and nitrogen sources (Kampfer et al. 1991). Moisture content of samples support growth, but high water content may prevent the growth of spores or hyphae in selective isolation media. Trace elements also need for cultivation of *Streptomyces* in soil or in media (Williams et al. 1972). Shirling and Gottlieb (1966) has proposed 4 different media for

selection and isolation of *Streptomyces* in the International *Streptomyces* Project. After incubation of selective isolation plates, colonies are examined by eyes or microscopy to purify them. Inoculum should be spread out on the plates (Hopwood et al. 1985). Single colonies may be selected to subculture on the purification plates. Homogenous spores of isolates should be transferred into sterile tube for further studies. Once purification of isolates is completed, identification tests can be carried out using stored inoculum (Kieser et al. 2000).

Bacteria can be stored short, or long term, a number of methods have been developed for this. One of methods is preserve culture plates in 4°C fridge up to 6 months, a second method is keeping spore suspension in 20% glycerol for 2 years, and third method is to lyophilized spore suspension via freeze drier over 2 years (Korn-Wendisch and Kutzner 1992: Wellington and Williams 1978). Liquid nitrogen cryopreservation is one of simple and most reliable methods to control temperatures at or below -80°C.

2.10. Ecology

Streptomyces live in soil abundantly (Yikmis and Steinbuchel 2012: Lin et al. 2011), and can break down many different organic carbon source compounds such as animal and plant debris (Kaneko et al. 2003). Wirth and Ulrich (2002) found that this bacterium can produce cellulases and other enzymes. In addition, amylases, keratinase and lipases can also be produced by the bacterium (McKillop et al. 1986: Kornillowicz-Kowalska and Bohacz 2011). The bacterium can grow in the soil for long term as spores and remain in semi dormant life style (Ensign 1978: Morita 1985). Micro-environment is a habitat that express very small area (micrometer diameter) and a wide habitat for bacteria. They degrade organic compound present in the small habitat and hyphal growth can help them to obtained nutrients from its surroundings (Nazir et al. 2010: Yeo and Chater 2005). It has been reported that *Streptomyces* can use inorganic compounds such petrol derivatives (Bachoon et al. 2001) and grass land soils (Nacke et al. 2011). Gundlapally et al. (2006) reported that *Streptomyces* strains were abundant in a study carried on crust of Colorado Plateau. Babalola et al. (2009) found *Streptomyces* were at high number in a metagenomic study.

Spore mass colour and pigment colour of isolates are diagnostic for characterization of *Streptomyces* and Williams et al. (1969) grouped isolates according to their spore pigmentation and substrate-aerial mycelium colour. Production of melanin pigment by *Streptomyces* has importance in characterization. Atalan (2000) used colour grouping of isolates to identify and a good agreement was found between polyphasic taxonomy and colour groups. Antony-Babu et al. (2010) used colour grouping to differentiate alkalophilic bacteria and defined groups were assigned different species. Chronakova et al. (2010) reported that most of isolates were belong to *Streptomyces*. When *Streptomyces* live in a root or other habitats they secrete antibiotics to compete to other microorganisms and this trait is combination of antibiotic and antibiosis (Laskaris et al. 2010). *Streptomyces* are resistant to freezing conditions due to their arthrospore generation (Schlatter et al. 2009). In addition, soil *Streptomyces* can live inside internal organs of animals and invertebrates (Sudakaran et al. 2012; Seipke et al. 2012).

Biologic control is one of popular technique to overcome infection in plants. Some metabolites secreted by *Streptomyces* can prevent fungal and other bacterial disease (Strap and Crawford 2006: Schrey et al. 2012: Kinkel et al. 2012). Seipke et al. (2012) reported endophytic *Streptomyces* can be used as biologic control organisms. Also, representatives of many genera of actinobacteria live in aquatic habitats such as river, lake and sea. Mostly, these isolates from water come from soil which leak into the rivers and lakes after rainfall. Terkina et al. (2002) found most of isolates from Baikal lake were *Streptomyces* species. Nakade (2012) reported that a quarter of isolates from Rankala Lake of Kolhapur City, India were *Streptomyces* species. Weyland and Helmke (1988) reported the presence of Streptomycetes bacteria in littoral and inshore zone and Deep-sea level. Pathomaree et al. (2006) have isolated even barophilic streptomycetes bacteria in the Pacific ocean, the bacteria live in symbiotic relationship with different organisms such as plant, sponges and snail (Peraud et al. 2009: Lin et al. 2010). A few pathogenic *Streptomyces* bacteria have been found in plants such as potatoes, beets, carrot, peanut, and radish (Loria et al. 2008). *Streptomyces scabies* which is causing a disease in potatoes has been isolated (Park et al. 2003). Out of it 7 different species also cause infection on potatoes plant. Also, Trujillo and Goodfellow (2003) reported that a few species of *Actinomadura* and *Nocardia* were pathogen for humans and cause actinomycetoma, particularly in Sudan and subtropical regions (Develoux et al. 1999).

3. MATERIALS AND METHOD

3.1. Description of sampling sites

A total of 32 different locations such as hills, valleys, mountains and lake sides were chosen to obtain soil samples. 27 soil samples are collected in different sites from Pshdar and 5 soil samples are taken in Ranya (Table 3.1). Location of samples taken from Pshdar; (latitude 36° 11’ 00” N and longitude 45° 07’ 40” E), and Ranya; (latitude 35° 33’ 0” N and longitude 45° 26’ 0” E). The two sub-districts mentioned are located within the district of the Sulaimani Governorate that is located in the Kurdistan Region of Iraq. (Figure. 3.1).

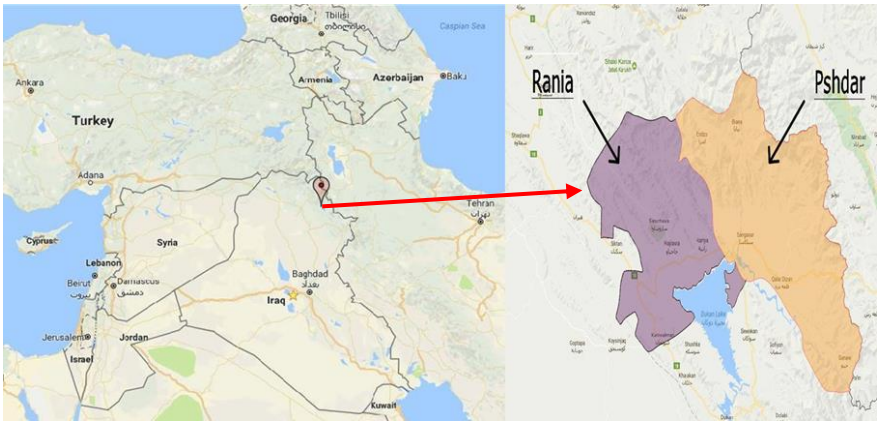


Figure 3.1. Sampling locations of Pshdar and Ranya sub-districts, district of the Sulaimani Governorate in the Kurdistan Region of Iraq (Google map)

3.2. Soil sampling

Total 32 soil samples were collected from different sites of Pshdar and Ranya sub-districts, (Table 3.1). 29 of the samples were taken in winter and 3 of them were taken in summer season. Soil samples were taken using a clean tubular soil sampler from a depth

of 10 cm after removing nearly 3 cm of the surface soil. Once the soil samples were collected, they were dried for 7 days at 30°C. Each soil sample was a mixture of 15 spots, and stored in sterile plastic bags before being transported to the laboratory where they were kept in refrigerator at 4°C until microbial assays performed.

3.3. Physiochemical Characteristics of soil samples

3.3.1. Measurement of Moisture content

To measure the moisture content of each soil sample, 1 g of a sample was weighted accurately in a crucible, and then the crucible plus the soil were weighted. Then, the crucibles that contain soil samples were put into the oven for 24 hours at 105°C. The weight of each crucible was measured everyday. During measurment, the soil samples taken out of the oven and once they are naturally cooled; they were weighted again. This process was carried out until no more weight change was observable. Finally, moisture content was found based on the original sample weight and final sample weight.

3.3.2. Measurment of organic matter content

The samples which had no moisture were placed in a muffle furnace and heated at 700°C for 90 minutes, and then their organic contents were measured. The average percentages of the organic matter were recorded once the crucible had cooled down.

3.3.3. Measurement of pH

Reed and Cummings (1945) method was used for measuring the pH of soil samples. 20-25 g of soil samples were placed in beakers and distilled water was slowly added while the sample was shaken, this is continued until the sample was wet. On the surface of the soil sample a flimsy layer appeared. A glass electrode pH meter was using to measure the pH of each of the soil samples.

3.4. Selective isolation of streptomycetes

One gram of soil sample was accurately weighted and aseptically taken in 9 ml of sterile Ringers solution ¼ strength, and agitated forcefully and the test tube that contain 10^{-1} were pretreated by putting in a water bath at 55°C for 10 minutes. After pretreatment, the dilution reagitated by vortex for a few seconds. Tenfold of serial dilution were prepared by pipette 1ml of pretreated sample aseptically up to 10^{-4} dilution. And the test tube that containing 9 ml of Ringers solution ¼ strength and 1 ml of diluted sample were mixed by using a vortex mixer for each dilution.

Generally, 0.1 ml of dilution 10^{-3}& 10^{-4} aseptically by using a sterile pipette that fitted with sterile tips was inoculated onto the selective medium containing starch casein agar supplemented with nystatin (50 µg ml^{-1}), cycloheximide (50 µg ml^{-1}) and novobiocin (25 µg ml^{-1}). Also, it inoculated on raffinose histidine agar supplemented with nystatin (50 µg ml^{-1}) and cycloheximide (50 µg ml^{-1}; Table 3.2). Two plates were inoculated for dilution and the diluted samples were spread on to the surface of the agar plates using L-shape spreader. Inoculated plates in the laminar flow cabinet were allowed to dry and then plates in an inverted position were incubated at 28°C for nearly two weeks.

3.5. Selection and Purification of isolates

The colonies of *Streptomyces* were examined by both eye and binocular microscope objective at 100X and 400X magnifications after 14 days incubation. The desired colonies were distinguished and separated from other bacteria on the basis of colony morphology, pigmentation and ability to produce different color of aerial hyphae and substrate mycelium on starch-casein agar and raffinose-histidine agar plates. Representatives of *Streptomyces* were taken from selective isolation medium were transferred and streaked onto Modified Bennett's agar (Jones 1949) in order to get pure colonies.

3.6. Culturing and stocking of isolates

164 strains were isolated according to locality. To purify *Streptomyces* isolates, strains sub-cultured on Modified Bennett's agar, and this was repeated until yielding pure culture. Pure isolates grown on modified Bennett's agar plates which were scraped and transferred in sterile 2 ml Eppendorf tube containing 20% glycerol, and stock at -80ºC. The suspensions of glycerol were prepared by scrapping aerial and substrate mycelium from Modified Bennett's agar plates. The frozen glycerol culture served as a mean of long term storage and also uses as a convenient source of the test strains. Inoculate was taken by thawing in the room temperature for about 10 minutes and inoculated onto test plates. The glycerol suspensions were stored again at -80ºC after use.

3.7. Color grouping of test microorganisms

Morphological observation is the important character for classification of *Streptomyces*, but it is not adequate for differentiate between many genera. Color grouping is to assign unknown isolates. Total 164 isolates *Streptomyces* were inoculated on Oatmeal agar (International Streptomyces Project medium 3 or ISP 3; Küster 1959) and Peptone yeast extract iron agar plates (International Streptomyces Project medium 6 or ISP 6; Shirling & Gottlieb 1966) and incubated at 28°C for 2 weeks. After incubation, colonies growing on plates were examined by eye to detect spore aerial hyphae, substrate mycelium color and pigmentation of the diffusible pigments. Color were determined by direct matching of the strains examined against color charts reference tables from the ISSC-NBS Color-Name Charts Illustrated with Centroid color. The peptone yeast extract iron agar (ISP6) was checked to detect ability of test strain to produce dark colored melanin pigmentation.

3.8. Numerical analysis of test microorganisms

40 diagnostic tests were carried out for 20 test strains representatives of color groups for numerical analysis (Williams et al. 1983a). Tests and strains used in the study are given on Table 3.3 and Table 3.4.

Table 3.1. List of soil samples collected from (Pshdar and Ranya), North of Iraq

Soil samples	Place	Location	Date of isolation
K01	Pshdar	Delo	5-Feb-16
K02	Pshdar	Delo	5-Feb-16
K03	Pshdar	Kashwna	4-Feb-16
K04	Pshdar	Said Ahmedan	5-Feb-16
K05	Pshdar	Esewa	3-Feb-16
K06	Ranya	Darband	5-Feb-16
K07	Pshdar	Kawya	4-Feb-16
K08	Pshdar	Bnawshan	4-Feb-16
K09	Ranya	Darband	5-Feb-16
K10	Pshdar	Sndolan	5-Feb-16
K11	Pshdar	Shex mahmud	4-Feb-16
K12	Pshdar	Dawzhan	6-Feb-16
K13	Pshdar	Piran	4-Feb-16
K14	Pshdar	Shex mahmud	4-Feb-16
K15	Pshdar	Xwaran	5-Feb-16
K16	Pshdar	Mamanda	5-Feb-16
K17	Pshdar	Nuraddin	4-Feb-16
K18	Pshdar	Kuratawl	4-Feb-16
K19	Pshdar	Darwina	4-Feb-16
K20	Pshdar	Badawa	5-Feb-16
K21	Ranya	Qamtaran	6-Feb-16
K22	Pshdar	Kuratawl	4-Feb-16
K23	Pshdar	Sndolan	5-Feb-16
K24	Ranya	Kewarash	5-Feb-16
K25	Pshdar	Sangasar	5-Feb-16
K26	Pshdar	Tuaswran	5-Feb-16
K27	Pshdar	Sarnel	5-Feb-16
K28	Pshdar	Darwaza	5-Feb-16
K29	Ranya	Darband	5-Feb-16
K30	Pshdar	Esewa	21-Aug-15
K31	Pshdar	Esewa	21-Aug-15
K32	Pshdar	Dawzhan	21-Aug-15

Table 3.2. Selective media used to isolate members of *Streptomyces* groups from Pshdar and Ranya soil

Medium	Antibiotics	Reference
Starch-casein agar	Cycloheximide (50 µg ml^{-1}); Nystatin (50 µg ml^{-1}) & Novobiocin (25 µg ml^{-1})	(Vickers et al. 1984)
Raffinose-histidine agar	Cycloheximide (50 µg ml^{-1}) & Nystatin (50 µg ml^{-1})	Küster & Williams (1964), Sveshnikova et al. (1976)

Table 3.3. Diagnostic test used to obtain data for computer assisted identification and test strains

No.	Tests	No.	Tests
1.	Dextran	21.	Lecithinase activity
2.	D Fructose	22.	Lipolysis activity
3.	Lactose	23.	Crystal violet (0.0001%)
4.	D Mannitol	24.	Phenol (0.1%)
5.	D Raffinose	25.	Sodium azide (0.01%)
6.	Sucrose	26.	Sodium chloride (7%)
7.	Maltose	27.	50°C
8.	D Mannose	28.	30°C
9.	Sodium acetate	29.	25°C
10.	Sodium citrate	30.	*Escherichia coli*
11.	Sodium propionate	31.	*Staphylococcus aureus*
12.	L-Histidine	32.	*Candida* sp.
13.	Potassium nitrate	33.	*Pseudomonas fluorescens*
14.	L-Tyrosine	34.	*Bacillus subtitous*
15.	Xanthine (1%)	35.	*Klebsiella pneumoniae*
16.	Casein (1%)	36.	Amoxicillin AMC (30mg)
17.	Starch (1%)	37.	Rifampicin RA (5mg)
18.	Gelatin (1%)	38.	Ampicillin SAM (20mg)
19.	Hydrogen sulphide production	39.	Gentamycin CN (10mg)
20.	Urea Hydrolysis	40.	Erythromycin E (15mg)

Table 3.4: Test strains used in molecular and chemotaxonomic study

Test strains	Sources	Test strains	Sources
K10088 (**1K**)	Pshdar-Sndolan	K10090 (**11K**)	Pshdar-Sndolan
K14102 (**2K**)	Pshdar-Shex mahmud	K09072 (**12K**)	Ranya-Darband
K19128 (**3K**)	Pshdar-Darwina	K02008 (**13K**)	Pshdar-Delo
K20134 (**4K**)	Pshdar-Badawa	K08052 (**14K**)	Pshdar-Bnawshan
K08049 (**5K**)	Pshdar-Bnawshan	K03026 (**15K**)	Pshdar-Kashwna
K18126 (**6K**)	Pshdar-Kuratawl	K21147 (**16K**)	Ranya-Qamtaran
K17123 (**7K**)	Pshdar-Nuraddin	K21139 (**17K**)	Ranya-Qamtaran
K09081 (**8K**)	Ranya-Darband	K02005 (**18K**)	Pshdar-Delo
K14108 (**9K**)	Pshdar-Shex mahmud	K10087 (**19K**)	Pshdar-Sndolan
K16111 (**10K**)	Pshdar-Mamanda	K10093 (**20K**)	Pshdar-Sndolan

3.9. Nutritional test

3.9.1. Carbone source

The strains capability to use eleven different carbon sources (Table 3.3.) for growth and energy were examined Wiliams et al. (1983a, b). The carbon compounds were added to carbon utilization agar (ISP 9) to give a final concentration either (0.1% or 1%, w/v). Tyndallisation technique was used for sterilization of each carbon sources. The sterilized basal medium complemented with (1%, w/v) of D-glucose used as a positive control and the free basal medium as a negative control. The strains were inoculated on free basal medium with D-glucose and medium supplemented with tests carbon source for 4-7 days at 28°C. The growths of the strains were read for comparing of medium with carbon source alone against control plate. The result registered as a positive when growth on positive control was less than the test plate and the result recorded as a negative when growth on positive control equal or better than negative control. Experiment was repeated in case of non-growing of strains of positive control.

3.9.2. Nitrogen source

The strains capability to utilize three nitrogen compounds (Table 3.3.) are examined. Test compounds were added to nitrogen utilization medium to give final concentration (1% w/v). After that Tyndallisation technique was used for sterilization of the nitrogen compounds. Although the sterilized basal medium supplemented with L-Proline (1%, w/v) was used as a positive control and the free nitrogen basal medium as a negative control. The strains were inoculated on the positive control medium and the basal medium supplemented with test nitrogen sources at 28°C for 4-7 days. The growth of the strains was read for comparing the positive control with negative plates. The result registered as a positive when growth on control less than test plate and result registered as a negative when growth on control equal or better than negative plates. Tests were repeated in case of the non-growing of strains of positive control.

3.10. Biochemical test

3.10.1. Urea hydrolysis

Urea reduction in liquid medium was used for hydrolysis of urea by test strains. Alkaline response was produced during production of urease enzymes by adding an indicator. The resultant was change the pH from neural to alkaline by altering color of the indicator recorded as a positive result.

3.10.2. Degradation tests

Degradation of xanthine, casein, starch and gelatin, Tyndallisation were performed to detect enzymes production by test strains on Modified Bennett's agar. Positive results were recorded when under or around the inoculated strain clear zone produced. All compounds were added as 1% concentration into Modified Bennett's agar.

Lecithinase and lipolysis test: Egg yolk agar medium was used for inoculation of strains and 10 strains were inoculated per plate. The inoculated plates were incubated for 4-7 days at 28°C for enzyme activity and the result examined by eyes. Lecithinase action was examined when around the inoculated strains 6-10 mm clear zone were investigated during degradation of lecithin exist in the medium and dissolution of fat in the medium (lipolysis) were performed by production of clear zone around the inoculated strain 10-16 mm and below reflected light zones were investigated.

3.10.3. Hydrogen sulphide production test

Nitrate agar were used in additional test tube to test the production Filter paper saturated with lead acetate hanged over edge of test tube inoculated with strains. The results were examined after incubation of inoculated test tube with lead acetate paper for 5 days at 28°C. Produced of H_2S was recorded as a positive result by formation black color on the filter paper.

3.11. Tolerance tests

3.11.1. Resistance to chemical inhibitors

Growing of test strains was controlled against 4 different inhibitor compounds Determined the capability of isolates to grow on Modified Bennett's agar supplemented with some chemical inhibitor including sodium azide (0.01%, w/v), sodium chloride (7%, w/v), phenol (0.1%, w/v) and crystal violate (0.0001%, w/v) were inoculated with test strains. Inoculated test strains were examined after incubation at 28°C for 2 weeks. Modified Bennett's agar alone was used as a positive control. The growth of test strain on the test plates was recorded as a positive result.

3.11.2. Growth at 50°C

The test strains were examined by their ability to grow on Modified Bennett's agar at 50°C. Tests strains were examined for growth after incubation for 2 weeks. A positive result was recorded by visible growth by the naked eye.

3.11.3. Antibiotics susceptibility

Resistance and susceptibility of isolated strains were examined by disk diffusion method. Amoxicillin AMC 30 mg, Rifampicin RA 5 mg, Ampicillin SAM 20 mg, Gentamycin CN 10 mg and Erythromycin E 15 mg. Tests were performed on Muller Hinton agar and the results were detected by the formation of clear zone around the disc, if clear zone formed around the antibiotic disk it was recorded sensitive to this antibiotic, if the isolates strain could grow around the disk it was recorded resistance to that antibiotic.

3.12. Antimicrobial activity tests

Twenty test isolates were evaluated for their antimicrobial action against six different pathogenic bacteria including Gram negative bacteria (*Escherichia coli*, *Pseudomonas fluorescens* and *Klebsiella pneumoniae*), Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtitous*) and fungi (*Candida* sp.) by perpendiculars technique. Test strains

inoculated on Muller Hinton agar and incubated at 28°C for 7 days after total grow of isolates. The pathogenic strains were streaked around the test isolate 6 per plate and incubated at 37°C for 24 to 48 hours. The positive result was recorded when isolated strain inhibited growth of pathogenic strains by formed clear zone around the isolated strains and negative result was recorded when pathogenic strains able to grow around the isolated strains.

3.13. Numerical analysis of phenotypic characters

Most of tests were scored as two state characters and coded "–" for negative "+" for positive result. 20 test strains were examined for 40 diagnostic characters. Resultant data was typed in TAXON program. It is a program for data input and analysis of binary data and is run on a computer. After numerical analysis, a dendogram has been generated by the program and test strains were grouped. S_{SM} (Simple matching coefficients: Sokal and Michener 1958) used to analyse similarity of organisms. Test strains showing 80 S_{SM} level may be belong to same species..

3.14. Molecular characterization of test microorganisms

3.14.1. Isolation of genomic DNA

A DNA isolation test must be examined very accurately, and to carry it out 20 test strains representatives of color grouping were subject to DNA isolation. The method for DNA isolation of the test strains is described by Pitcher et al. (1989). Guanidine thiocyanate DNA isolation method and the DNA isolation kit (Invitrogen, Pure Link (R) Genomic DNA Kit) as described by (MZİOĞLU et al. 2014: Matsumoto et al. 1989) were used.

Cell harvesting for DNA isolation

The test organisms developed in pure cultures on glucose yeast malt extract agar were incubated at 28ºC for 10 days. Then test strains were inoculated into liquid medium under aseptic conditions and incubated 190 rpm for 10 days at 28°C in a shaking incubator (Excella, New Brunswick Scientific Co., Inc, NJ). 1 ml of each liquid culture was

transferred to sterile 1.5 ml Eppendorf tube by sterile automatic pipettes. The cell pellet was precipitated by centrifugation at 13000 rpm for 5 min. The remaining liquid phase was removed from the top of the cell pellet. The same procedure was repeated until sufficient cell pellet. Subsequently, the cells were washed at least 2 times with 300 µl sterile TE buffer (10 mM Tris, 1 mM EDTA, pH 8) and the cell pellets were stored at -20°C until DNA isolation.

Protocol for DNA extraction:

1. Cell pellets were taken out from the storage at -20°C and kept at room temperature until the ice was dissolved.
2. 180 µl of lysozyme added to the cell pellets and homogenizing with an automatic pipettor.
3. Incubated at 37°C overnight in dry incubator.
4. Added 2µl of Triton-X100 to the lysozyme supplemented pellet and incubate at 37°C for 30 minutes.
5. Added 20 µl Proteinase K and mix well by pipetting slowly.
6. Added 20 µl RNase and mix by pipetting slowly.
7. Added 200 µl of PureLink Genomic Lysis / Binding Buffer and mixed well.
8. Wait for 30 minutes at 55°C in dry incubator.
9. 200 µl 96% ethanol is added to Lysate and shaken thoroughly to obtain a homogenous solution
10. Remove the Spin Column from a package in a PureLink Collection Tube for each test strain.
11. Spin column tube placed on 2 ml collection tube
12. Mixed cell transferred from Eppendorf tube to PureLink Spin Column and centrifuged at room temperature for 1 minute at 13000 rpm.
13. After centrifugation, the collection tube is discarded and the spin column is placed in a clean PureLink Collection Tube.
14. Added 500 µl of genomic washing buffer 1 (previously ethanol was added).
15. Wash buffer 1 is centrifuged for 1 minute at 13000 rpm in the attached column at room temperature.
16. After centrifugation, the collection tube is discarded and the spin column is placed

in a clean collection tube.

17. 500 µl of genomic Wash buffer 2 added to spin column (previously ethanol was added).
18. Centrifuged the column at room temperature for 3 minutes at 13000 rpm and discard the collection tube
19. The spin column tube placed in new collection tube and centrifuged at 13000 rpm for 3 minute without adding anything until dry the matrix.
20. Place the spin column in a clean 1.5 ml micro-centrifuge tube.
21. 50 µl of elution buffer were added to the center of Spin column matrix and let it for 3 minute for absorption elution buffer by matrix.
22. Centrifuged at 13000 rpm for 60 second to elute the purified DNA.
23. Finally, the 1.5 ml micro-centrifuge tube contains pure DNA.
24. The purified DNA should be stored at -20°C.

3.14.2. Agarose gel preparation method

Agarose gel electrophoresis were used to run total genomic DNA, 1% agarose gel (60 ml 1X TBE buffer, 0.6 g agarose) supplemented with ethidium bromide 4 µl was prepared to run genomic DNA

The band of total genomic DNA was visualized under UV illuminator. Procedure for agarose gel is below:

- A glass form surrounded by tape across the end was prepared for gel electrophoresis tank and to make a pore for loading reactions a special comb was placed in the glass form
- 1 g of agarose were dissolved in 100 ml of 1X TBE, boil and heat continuously in micro-oven until the agarose is dissolved, left dissolved agarose cool until you can touch the flask comfortably
- Cooled formula was poured in to the form gently, and left it to solidified, remove tape and immersed the gel to 1X TBE in the gel electrophoresis tank and carefully removed the comb to form a pore.
- Loaded 3 µl of DNA sample were mixed with 2 µl of dye buffer to the wells in the gel.

- Loaded DNA run about 45 minutes
- The band of total genomic DNA gene was checked on a UV transilluminator.

DNA isolation control

1% agarose gel (60 ml 1X TBE buffer, 0.6 g agarose) supplemented with ethidium bromide 4 µl was prepared to control the presence of extracted DNA. The prepared gel was loaded with 3 µl total genomic DNA and 2 µl of dye was added. After DNA loaded, agarose gel placed in the electrophoresis tank and run at 100 volts for 45 minutes. DNA presence was checked under UV transilluminator (Vilber Lourmat, UV) and captured a photograph.

3.14.3. PCR amplification of 16S rDNA gene

After DNA samples were purified from the organisms, the region encoding 16S rRNA gene amplified by two universal primers 27f, (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1525r, (5'- AAG GAG GTG ATC CAG CCG CA-3') (Lane 1991). Stock solutions prepared for the PCR reaction were prepared with sterile ddH_2O. Stock solutions were separated in sterile Eppendorf tubes in small quantities 25-100 µl to remove the risk of contamination and stored at -20°C until use. Polymerase chain reaction procedures of 16S rDNA were carried out at a Thermal Cycler (MyGenie-96 Gradient Thermal Cycler, Korea) in a 0.2 ml PCR tube.

3.14.4. PCR mixture for 16S rDNA gene

50 µl of reaction mixture was prepared for 16S rDNA gene amplification. Preparation of mixed reaction is given on Table 3.5.

Protocol of PCR amplification is below.

1. Separately 1 µl of each DNA samples were transferred into sterile 0.2 ml PCR tubes.
2. The reaction mixture was prepared to give a total volume of 49 µl for each sample in 1.5 ml of Eppendorf tube.

3. 49 µl of reaction mixture were transferred to each PCR tube that contain 1 µl of pure DNA
4. Immediately after transfer, the PCR reaction (MyGenie-96 Gradient Thermal Cycler, Korea) was started working**.**

Table 3.5. PCR mixture for amplification of 16S rDNA.

Compounds	**Concentration**	**Volume (µl)**
DNA	50-100 ng	1 µl
27F	10 µM	1 µl
1525R	10 µM	1 µl
GoTaq® Hot Start Colorless Master Mix		25 µl
Free water		22 µl
Total volume		50 µl

3.14.5. PCR Program

After preparation of 50 µl PCR mix, time and temperature for 3 different PCR stages including denaturation, annealing and extension, respectively are given on Table 3.6. MyGenie-96 Gradient Thermal Cycler (Korea) was used to amplify the genes of test strains.

Table 3.6. PCR reaction conditions

Denaturation	**Amplification**			**Final**	**Hold**
	Denaturation	Annealing	Extinction	Extinction	
95°C	95°C	55°C	72°C	72°C	25°C
15 min	1 min	1 min	3 min	10 min	1 min
1 Cycle	35 Cycle				1 Cycle

3.14.6. Purification of PCR product of 16S rDNA

1.5% agarose gel (60 ml 1X TBE buffer, 0.9 g agarose) supplemented with ethidium bromide use for detection of the quality of PCR product. 4 µl PCR product was run to control the amplified 16S rDNA. The prepared gel was loaded with 16S rDNA and placed in the electrophoresis tank and run at 100 volts for 45 minutes. Finally, amplified 16S rDNA was checked under UV transilluminator (Vilber Lourmat, UV) and photograph was captured.

3.14.7. Gene sequence of 16S rDNA

Purification of PCR product of 16S rDNA gene was controlled and running on agarose gel. Purified DNA was sent to sequence using the ABI PRISM 3730XL Genetic Analyzer (PE Applied Biosystems) automated sequencing instrument with the three primers (Table 3.7.). These three primers were used for sequencing 16S rDNA gene. Sequence analysis of the 16S rDNA gene region was carried out by Macrogen Europe (Netherlands).

Table 3.7. 16S rRNA gene region amplification and sequence primers

Primer Name	Sequence row (5'-3 ')	Size	Reference
518F	CCAGCAGCCGCGGTAAT	17	Buchholz-Cleven et al. 1998
800R	TACCAGGGTATCTAATCC	18	Chun 1995
Mg5f	AAACTCAAAGGAATTGACGG	20	Chun 1995

3.15. Phylogenetic analysis of 16S rDNA sequence

Genomic DNA extracted from isolates strain and the 16S rDNA gene sequence was amplified method of Chun and Goodfellow (1995). The complete 16S rDNA sequences of all isolates strain were analyzed with Chromas version 1.7.5 (McCarthy, School of Health Sciences, Griffith University, Queensland, Australia) program. Sequence were compared to each other using EzTaxon-e server (http://www.ezbiocloud.net/eztaxon: Kim et al. 2012). The sequence of isolates type strains was retrieved from GenBank and aligned using CLUSTAL W in MEGA6 (Tamura et al. 2013). Construction of the phylogenetic trees was achieved by using the neighbor-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Fitch 1971) algorithms in the MEGA6 program (Tamura et al. 2013). The topology of the phylogenetic trees was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

3.16. Chemotaxonomic Chracterization

Chemotaxonomy is the study of the chemical difference in microbial cell and the use of chemical features in the classification and identification of bacteria including

Streptomyces and it is a crucial technique. It is concerned with the properties of specific chemicals of the cell envelope that given on Table 3.8. Biomass for chemotaxonomic analysis was prepared by growing 4 strains K14108 (9K), K08052 (14K), K21147 (16K) and K21139 (17K) in glucose yeast malt extract broth (150 ml) in Erlenmeyer flasks after 7 days incubation at 28°C in shaker incubator (200 rpm). Cells were harvested by centrifugation and washed twice in sterile distilled water and re-centrifuged and feezed at -80°C after that lyophilized at lyophilizator for 16 hours to getting dried bacterial cell mass.

Table 3.8. Chemotaxonomic markers applied in polyphasic approach of *Streptomyces*

Categories	**Site in cell**	**Composition**
Chemotaxonomic	Cell Cell wall	Sugars (LL, meso and hydroxy) DAP

3.16.1. Protocol for Diaminopimelic acid (DAP) analysis

Thin layer chromatography was used to characterize DAP and determine isomers (LL, meso and hydroxy) of 4 test strains K14108 (9K), K08052 (14K), K21147 (16K) and K21139 (17K), (Becker et al. 1965: Staneck and Roberts 1974) method were used and protocol for the study is as following:

1. Lyophilized isolates were put in room temperatures for 24 hours
2. Approximately 10 mg of lyophilized cell sample were transferred to 2 ml vials resistance temperature.
3. Added 200 µl of 6N HCl to cell sample and shaken very well
4. Kept it in oven at 100°C for 16 hours for hydrolysis.
5. The hydrolysates were cooled at room temperature
6. Transferred the dissolved part of hydrolysate cell to sterilized Eppendorf tube
7. Centrifuged at 13000 rpm for 10 minutes
8. Removed the participated part and transferred supernatant to new Eppendorf tube
9. Supernatant in a new Eppendorf tube were kept at 120°C for 2-3 hours, until the liquid was dried.

10. 100 µl of sterilized distilled water were added to dissolve hydrolysates and shaken very well by hand
11. Loaded 3 µl of isolates and standard of DAP to TLC sheet plate
12. After the sheet was thoroughly dried, it was run for 3 hours in the running phase
13. Thin-layer chromatography running phase were prepared: ddH_2O: Methanol: 6N HCl: Pyridine (26 ml: 80 ml: 4 ml: 10 ml)
14. The aluminum sheet from the solvent was dried in a fume hood for 15 minutes.
15. After thoroughly dried, it was sprayed with ninhydrin (0.2% w/v) and waited for 2 minutes
16. Dried by heat in oven at 100°C for 5 minutes, and photographed.

3.16.2. Protocol for whole sugar analysis

Whole sugar analysis of total 4 test strains K14108 (9K), K08052 (14K), K21147 (16K) and K21139 (17K) by thin layer chromatography method (TLC). were carried out and protocol for the study is as following:

1. Approximately 50 mg lyophilized dry cells were transferred to 2 ml vials.
2. Added 1.5 ml of 1N H_2SO_4 to cell sample, shaken very well and hydrolyzed at 100°C for 3 hours in hot air oven.
3. After hydrolyzed the pH was adjusted pH 5-5.5 with saturated $Ba(OH)_2$ in a small beaker.
4. The pH adjusted cell sample was transferred to large falcon tube.
5. The cell sample was centrifuged at 6000 rpm for 25 minutes in macro-centrifuge and the temperature of centrifuge adjusted at 4°C.
6. The supernatant was transferred to new falcon tube and precipitated part was removed.
7. The supernatant was carefully evaporated until the liquid was completely removed.
8. The residue part was dissolved by added 600 µl of ddH_2O to each cell sample.
9. Loaded each sample and standard mixture to the TLC sheet plate (7 µl and 5 µl), respectively.
10. After the aluminum sheet plate thoroughly dried it was carried out for 3 hours in the running phase.

11. TLC running phase was prepared: ddH_2O: Propanol: ethyl acetate (5 ml: 25 ml: 70 ml).
12. The aluminum sheet plate from the solvent was dried in a fume hood for 10 minutes.
13. TLC sheet plate then it was run for 1.5-2 hours in the running phase.
14. TLC plate was then dried for 15 minutes in the fume hood.
15. After totally dried, it was sprayed with aniline-phthalate and waited for 3 minutes.
16. Dried sheet plate at 100ºC for 4 minutes in hot air oven and captured a photograph

4. RESULTS

4.1. Physiochemical parameters of soil samples

In the following lines of this study, the physiochemical results of the soil samples were illustrated; including organic matter content, moisture contents, and pH of the soil samples (Table 4.1). The amount of organic matter content from all soil samples were ranged from 5.20 to 12.10% and the highest amount of organic matter content (12.10%) was recorded in soil sample number K05 collected from Esewa location and the lowest amount (5.20%) found in soil sample number K32 collected from Dawzhan location. Moisture content was ranged from 2.60 to 10.37% and the highest amount (10.37%) was recorded in soil sample number K21 collected from Qamtaran location and the lowest amount (2.60%) was recorded in soil sample number K01 collected from Delo location. The range of pH of all soil samples were recorded between 7.26 and 8.63 and the lowest pH 7.26 was recorded in soil sample number K20 in Badawa location and the highest pH 8.63 was recorded in soil sample number K01 in Delo location.

4.2. Distribution and numbers of *Streptomyces* microorganisms

According to the scientific discover (Atalan 1993) the Starch casein agars and Raffinose histidine agar were used to isolate *Streptomyces* bacteria. Even though, Soil sample was inoculated by serial dilution and petri plates prepared for the isolation which was incubated at 28°C for 14 days and 164 pure strains were isolated by streak plate method. The number of total *Streptomyces* were counted as a colony forming unit (CFU, Table 4.2.). Photographs of some isolation petri plates are shown on Figure 4.1. The highest number of colony were recorded from soil sample K09 and K14 which was 7.4×10^4 cfu. Also, *Streptomyces* colony appeared on both S.C.A & R.H.A plates such as K01, K02, K03, K05, K07, K08, K09, K14, K16, K17, K18, K19, K20 & K29, colony

appeared on only R.H agar plates such as K04, K10 & K26 and colony appeared on only S.C agar such as K11, K21, K23 & K25 Unfortunately, no *Streptomyces* colony were appeared on isolation plates inoculated from soil samples numbered K06, K12, K13, K15, K22, K24, K27, K28, K30, K31 & K32.

4.3. Colour grouping

In much early studies, most of the researchers arrived at the conclusion that morphology is the only suitable characteristic that can be used to describe taxa. The 164 isolates presumptively classified as *Streptomyces* and they were assigned to 10 color groups (Table 4.3). The color of aerial hyphae and substrate mycelium was used to group isolates. Isolates were inoculated on Oatmeal agar to examine aerial and mycelium color. In addition, isolates were inoculated on peptone yeast extract iron agar to observe melanin pigmentation. Inoculated plates were incubated for 2 weeks at 28ºC. Melanin production was positive in case of distinctive black formation in the reverse side of plate. The appearances of representative strains of same isolates growing on oatmeal, peptone yeast extract iron, modified Bennett's and glucose yeast malt extract agar are shown in Figure 4.2 and 4.3.

4.4. Grouping by numerical analysis

Scientifically, grouping the strains by color has already been explained. However, it is time to group and divide them by numerical analysis due to the most recent discoveries. Table 4.4 shows numerical and identification test scores for each 20 test strains. Data analyzed using TAXON program and a dendogram (Figure 4.5). It can be seen on dendogram that 20 test strains were assigned 10 cluster based on 80% simple matching coefficient. 5 out of 10 cluster were single member groups, while the other 5 cluster were contained 2 or more test strains. Traditional identification tests such as biochemical, carbon source, nitrogen source, chemical inhibitor, temperature, degradation, antibiotics, Antimicrobial activity, morphology, pigmentation growth tests are used for both identification and numerical analysis. Photographs of some tests are shown on Figure 4.4. In the following short paragraphs, some important tests are explained, the ability of 20 test microorganisms to utilize eleven different carbon compounds for energy and growth

were carried out after comparing growth of test microorganisms in test compounds between positive control and negative control groups (Figure 4.4a). The results show that the test strains have ability to assimilate at last one carbon source as energy.

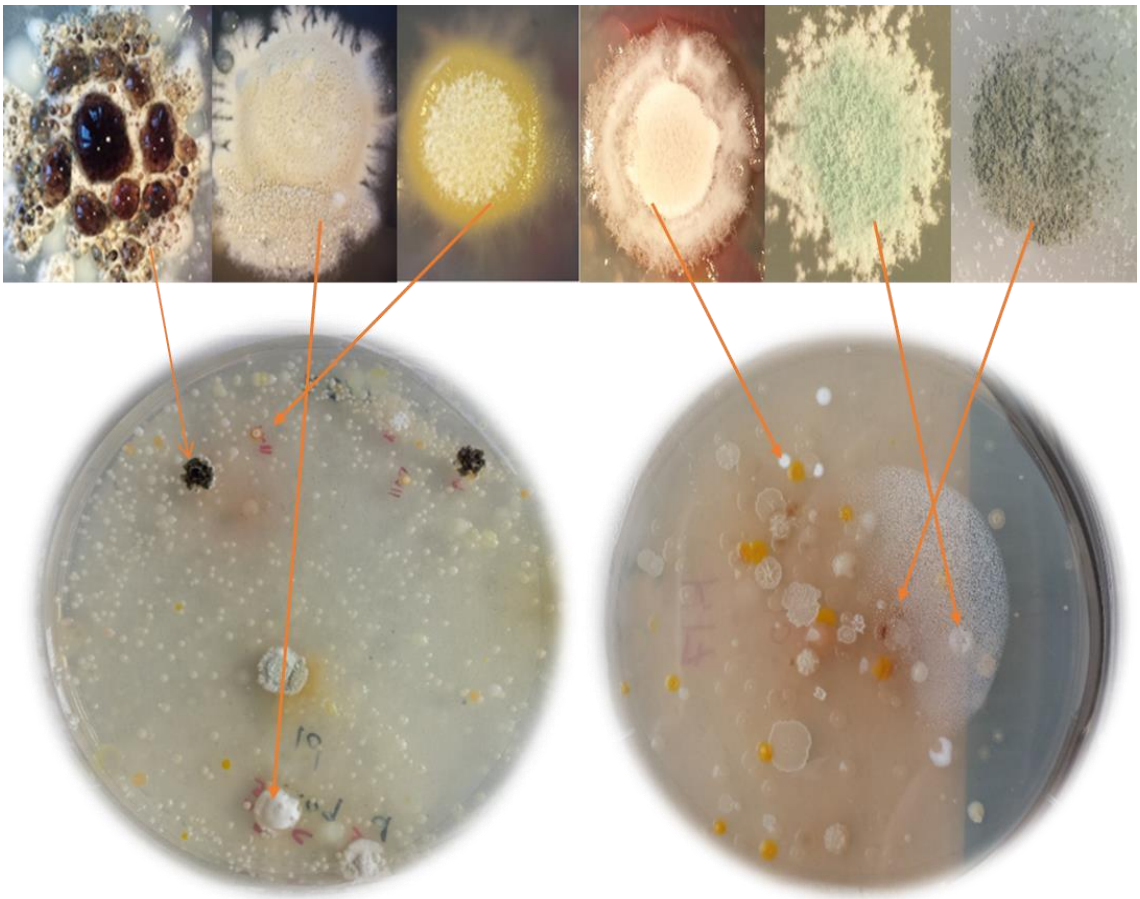
Although here is another test that is the capacity of 20 test microorganisms to utilize three different nitrogen compounds as a source of energy and growth was done. After compared growth of test strains in test compounds and compared to negative control and positive control (Figure 4.4b). The result was showed most test strains have the ability to assimilate all nitrogen source as energy. According to all test, only 3 test strains K02008 (13K), K03026 (15K) and K10087 (19K) have the inability to use histidine, 2 strains K08049 (5K) and K21147 (16K) have the inability to use potassium nitrate and 4 strains K10088 (1K), K08049 (5K), K09081 (8K) and K03026 (15K) have the inability to use tyrosine as a nitrogen source. All test strains were tested for their ability to grow in the presence of five different antibiotics in a different concentration on Muller Hinton agar (Figure 4.4c). The result showed that two test microorganisms K19128 (3K) and K03026 (15K) are resistance for all antibiotics, and 18 test microorganisms have resistance at least for two antibiotics for each strain.

The capacity of 20 test strain to grow on modified Bennett's agar supplemented with chemical inhibitor were tested (Figure4.4d). The result showed that all 20 test microorganisms were grown on medium supplemented withsodium azide, 19 test microorganisms were grown on medium supplemented with sodium chloride and any test strain couldn't grow on medium supplemented with phenol and crystal violet.

The ability of 20 test microorganisms were examined for their ability to inhibit the development of six pathogenic microorganisms gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtitous*), gram negative bacteria (*Escherichia coli*, *Pseudomonas floorescus*, and *Klebsiella pnaumoora*) and fungi (*Candida* sp.). The result was recorded as positive during formation of zone around the test strains and negative when pathogenic microorganisms were grown around the test strains. It observed that K19128 (3K) & K10093 (20K) were inhibited growth of all pathogenic microorganisms, K18126 (6K), K03026 (15K), K21147 (16K), K21139 (17K), K02008 (13K) & K02005 (18K) were unable to inhibit growth of any pathogenic microorganisms and. K10088 (1K), K14102

(2K), K20134 (4K), K08049 (5K), K17123 (7K), K09081 (8K), K14108 (9K), K16111 (10K), K10090 (11K), K09072 (12K), K08052 (14K) & K10087 (19K) were able to inhibit growth of at least one pathogenic microorganisms and some results are given on Figure 4.4.e.

20 test microorganisms were tested for their ability to production of enzyme by degradation of some different compounds (Figure 4.4f). The 20 test microorganisms were tested for their ability to grow at 50ºC any test strain couldn't grow at 50ºC (Figure 4.4g). The ability of 20 test microorganisms to produce H_2S Some results are given on Figure 4.4.h and their capacity to hydrolysis of urea by production of urease enzyme. (Figure 4.4i).



K09 (Darband) on S.C.A K17 (Nuraddin) on R.H.A

Figure 4.1. Colony appearance of *Streptomyces* on plates of starch-casein agar & raffinose histidine agar (SCA, Starch casein agar; RHA, Raffinose histidine agar)

Table 4.1. pH, moisture content and organic matter content of soil samples collected from Pshdar and Ranya, north of Iraq

Number of Soil samples	pH	Moisture content (%)	Organic matter content (%)
K01	**8.63**	**2.60**	9.10
K02	8.44	3.57	5.53
K03	8.30	4.03	8.37
K04	8.26	4.23	10.47
K05	8.46	3.00	**12.10**
K06	8.54	4.77	7.53
K07	8.40	5.87	8.73
K08	8.45	2.77	9.13
K09	8.55	5.10	10.20
K10	7.97	5.67	5.83
K11	8.25	7.43	10.17
K12	8.35	10.27	10.53
K13	7.60	7.33	6.27
K14	7.84	5.23	7.27
K15	8.04	7.07	8.83
K16	7.27	5.27	6.43
K17	7.78	6.80	10.5
K18	8.03	7.60	5.90
K19	8.20	9.63	7.37
K20	**7.26**	9.13	9.17
K21	8.14	**10.37**	8.63
K22	7.84	5.65	6.35
K23	7.93	5.97	7.23
K24	7.77	7.87	9.23
K25	8.24	8.10	8.20
K26	7.72	7.87	8.53
K27	8.32	7.67	9.28
K28	8.25	8.77	9.93
K29	8.35	8.03	9.57
K30	7.77	3.26	6.18
K31	7.81	3.34	6.28
K32	8.06	3.08	**5.20**

Table 4.2. Total number of *Streptomyces* (cfu/g dry weight soil) growing on starch casein agar supplemented with nystatin (50 µg/ml), cycloheximide (50 µg/ml) and novobiocin (25 µg/ml) and raffinose histidine agar supplemented with cycloheximide (50 µg/ml) and nystatin (50 µg/ml) seeded with soil suspension and incubated for 14 days at 28°C

Number of Soil samples	**Total *Streptomyces* on SCA 1 g soil sample X10^4 CFU**	**Total *Streptomyces* on RHA 1 g soil sample X10^4 CFU**
K01	1.0	2.1
K02	3.1	6.2
K03	5.2	7.3
K04	0.0	3.1
K05	1.0	1.0
K06	0.0	0.0
K07	4.2	5.3
K08	2.1	4.1
K09	**7.4**	**7.4**
K10	0.0	6.4
K11	3.2	0.0
K12	0.0	0.0
K13	0.0	0.0
K14	6.3	**7.4**
K15	0.0	0.0
K16	2.1	2.1
K17	6.4	4.3
K18	3.2	1.1
K19	2.2	3.3
K20	3.3	5.5
K21	6.7	0.0
K22	0.0	0.0
K23	3.2	0.0
K24	0.0	0.0
K25	4.4	0.0
K26	0.0	4.3
K27	0.0	0.0
K28	0.0	0.0
K29	2.2	2.2
K30	0.0	0.0
K31	0.0	0.0
K32	0.0	0.0

Table 4.3. Color grouping of isolates microorganisms on oatmeal agar and peptone yeast extract iron agar

Color group	Strain number	Color on Oatmeal agar		Melanin pigmentation on PYIA
		Aerial spore mass	Colony reverse	
1.	K02011, K03016, K09058, K09061, K09062, K09064, K09069, K09074, K09075, K09082, K10089, K14097, K14098, K14101, K14103, K14104, K14105, K14109, K19130, K21145, K25151, K25152, K25153, K25154, K26157, K26158, K26159, K29160, K14102 **(2K),** K14108 **(9K),** K19128 **(3K).**	Moderate Olive Green	Dark Olive Green	None
2.	K01001, K01003, K02010, K07036, K07044, K08047, K08048, K09066, K09068, K09084, K10-092, K17113, K17114, K17115, K17-116, K17118, K18124, K18125, K02008 **(13K),** K03026 **(15K),** K18126 **(6K)**.	White	Light Yellow	None
3.	K03021, K03024, K03025, K09054, K09055, K09056, K09057, K09059, K09060, K09063, K09065, K09067, K09070, K09071, K09073, K09076, K09077, K09078, K09079, K09080, K09085, K14099, K14100, K14106, K14107, K14110, K19129, K19132, K26155, K26156, K29161, K29162, K02005 **(18K)**, K09081 **(8K)**, K16111 **(10K)**.	Yellowish White	Strong Yellowish Brown	None
4.	K03015, K03017, K03018, K03019, K03022, K03023, K03028, K04031, K05032, K05033, K07040, K07041, K07043, K08050, K08051, K08053, K10086, K10091, K11094, K11095, K11096, K20133, K20136, K20137, K29164, K08049 **(5K),** K10087 **(19K)**, K10090 **(11K)**, K17123 **(7K)**.	Light gray	Pale Orange Yellow	None
5.	K01002, K02006, K02007, K02012, K02013, K09083, K09072 **(12K)**.	gray	Light Gray	None
6.	K02009, K03014, K03020, K03027, K07038, K08046, K16112, K17117, K17119, K17120, K17121, K17122, K18127, K19131, K20135, K20138, K23148, K23149, K23150, K29163, K08052 **(14K)**, K20134 **(4K)**.	Snowy	Light yellow	None
7.	K02004, K04029, K04030, K07034, K07035, K07037, K07039, K07042, K07045, K21147 **(16K)**.	Brilliant Orange	Dark Brown	None
8.	K21140, K21141, K21142, K21143, K21144, K21146, K21139 **(17K)**.	Pink	Light Orange Yellow	None
9.	K10088 **(1K).**	Moderate Olive Green	Dark Grayish Olive	Yes
10.	K10093 **(20K)**.	Grayish Olive	Dark Olive Green	Yes

Note: Bold strains were selected for numerical, chemotaxonomic and molecular analysis

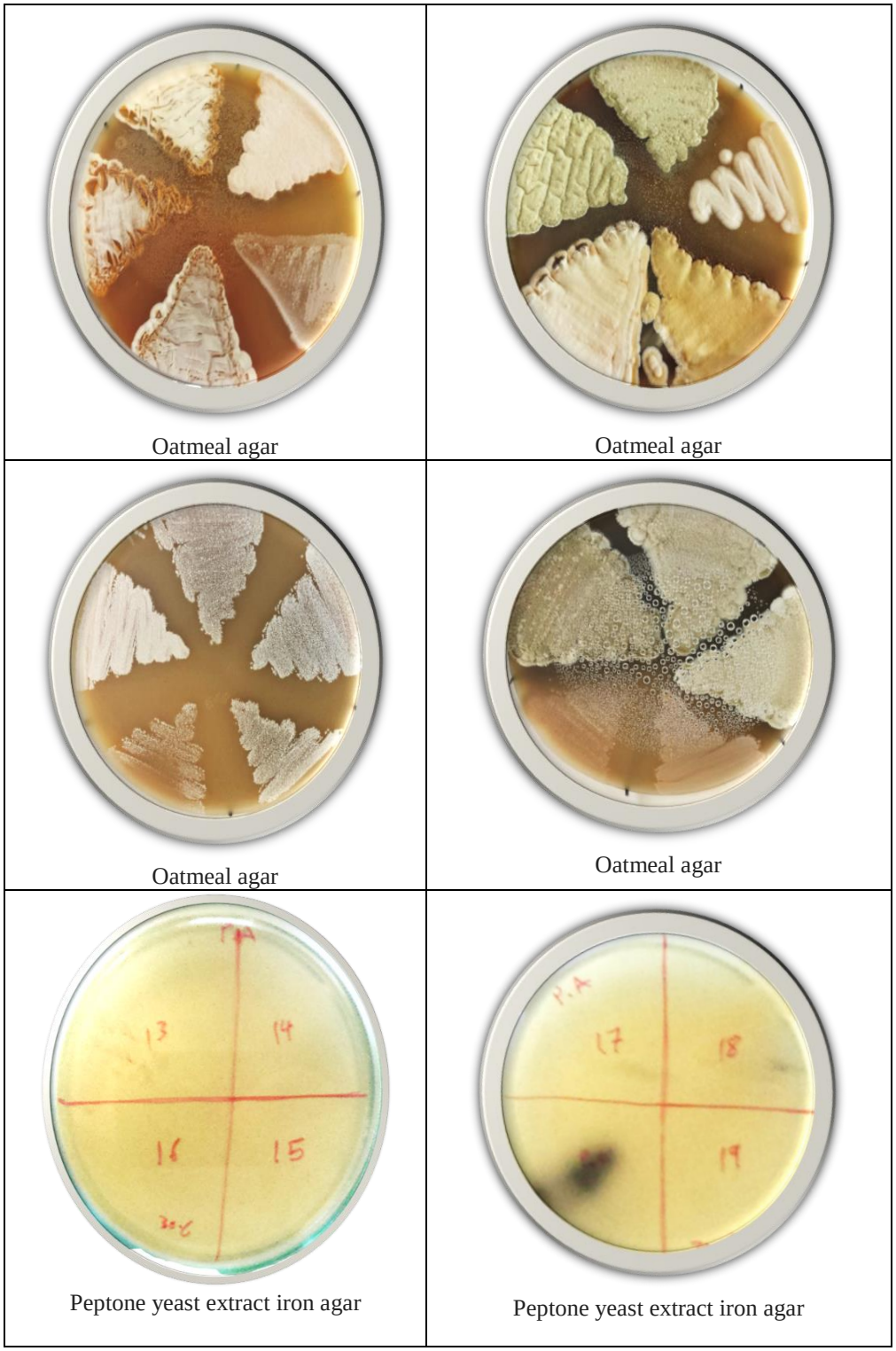


Figure 4.2. Representatives strains of *Streptomyces* strains growing on oatmeal agar and peptone yeast extract iron agar plates at 28ºC after 2 weeks growth

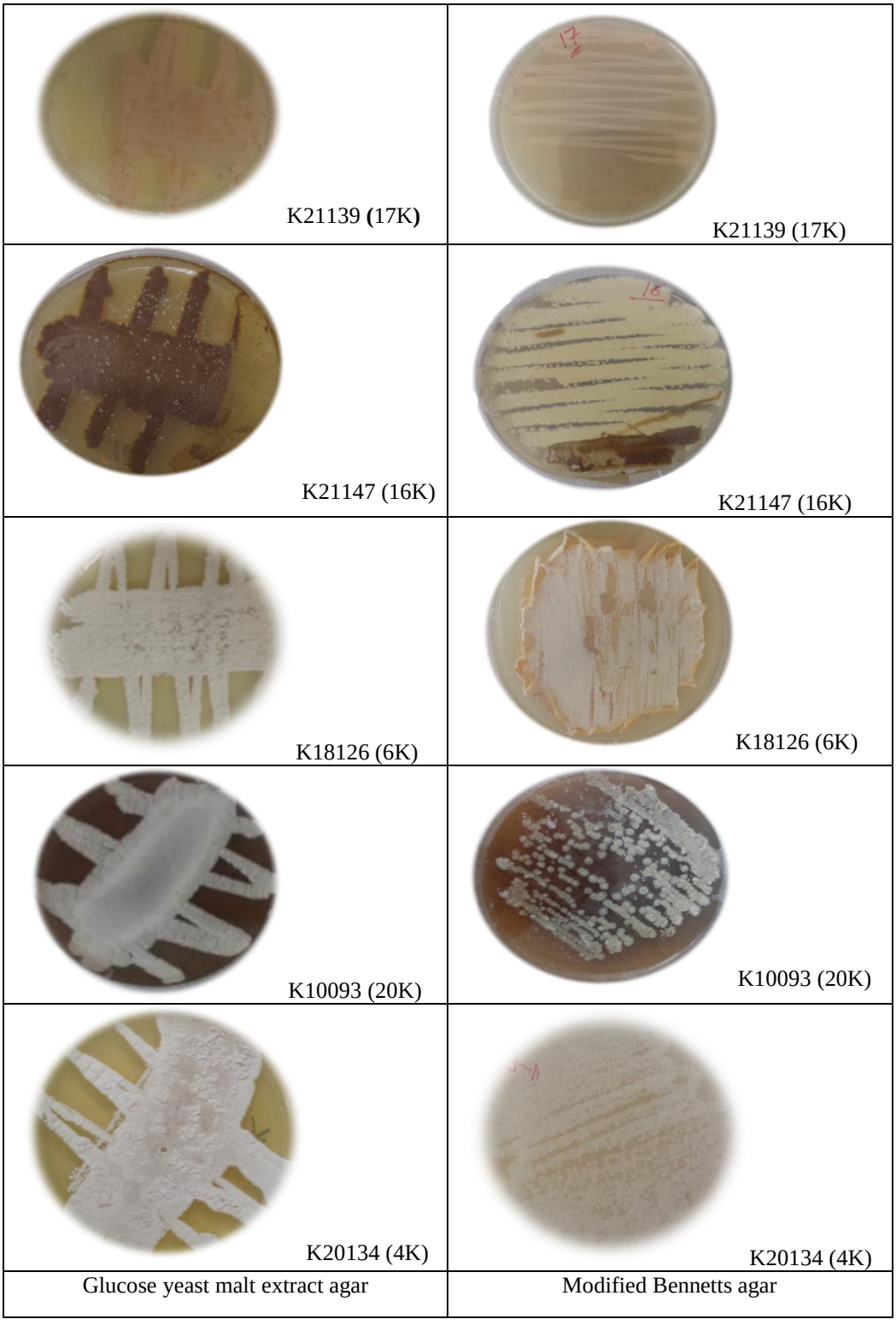
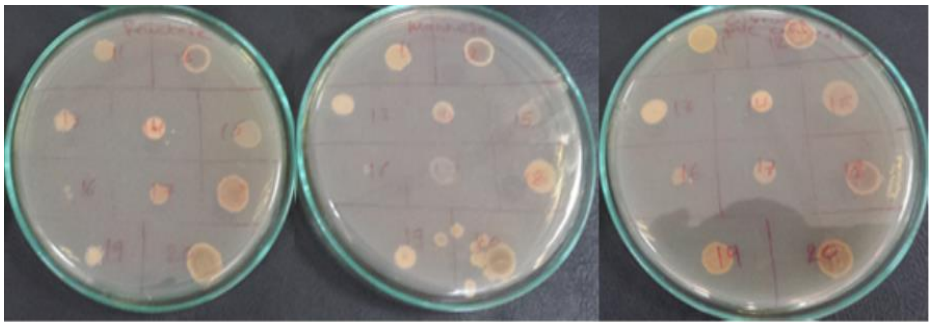


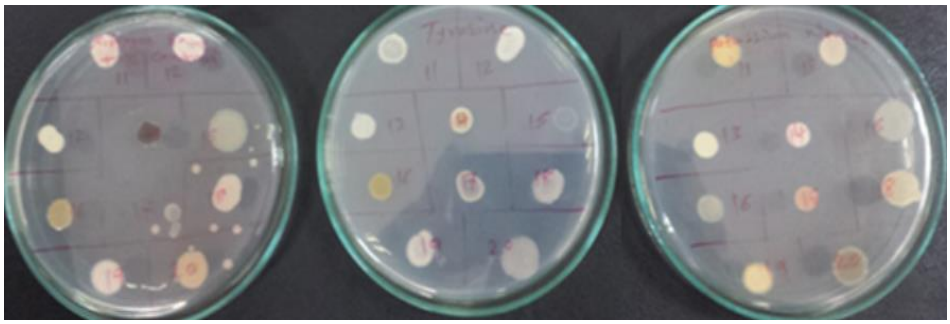
Figure 4.3. Representatives strains of *Streptomyces* strains growing on glucose yeast malt extract agar and Bennett's agar plates at 28ºC after 2 weeks growth

a. Carbon source tests



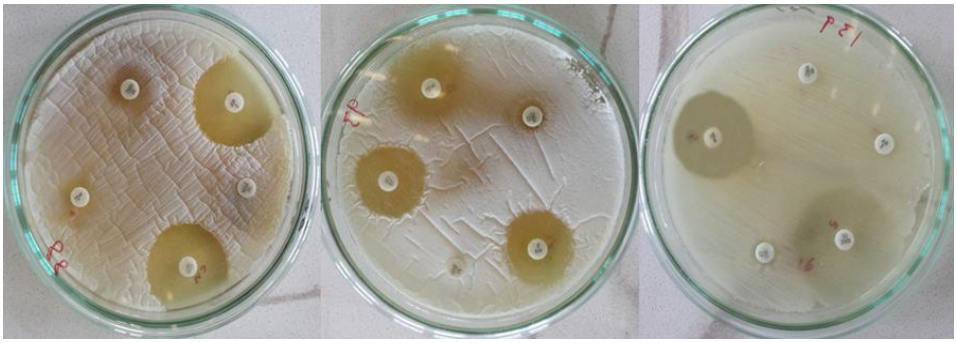
Fructose Mannose Glucose (+ve contrl)

b. Nitrogen source tests



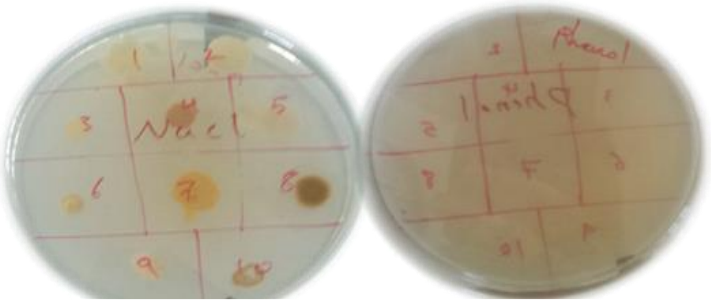
L-Proline (+ve contrl) Tyrosine Potassium nitrate

c. Antibiotic resistance tests



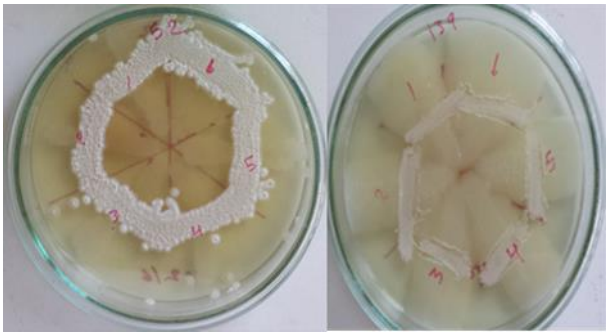
K10088 (1K) K10093 (20K) K21139 (17K)

d. Chemical inhibitor tests



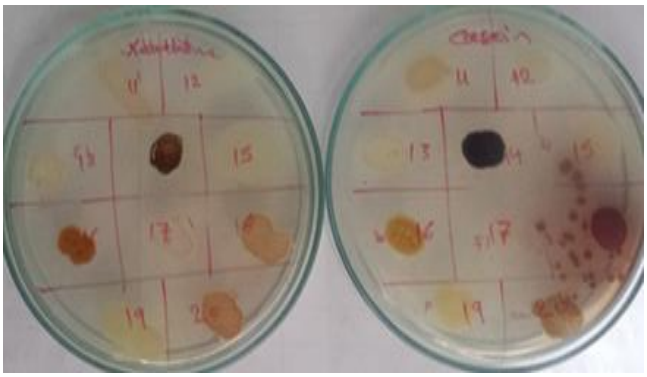
Sodium chloride (7%) Phenol (0.1%)

e. Antimicrobial activity test strains against pathogenic bacteria and fungi



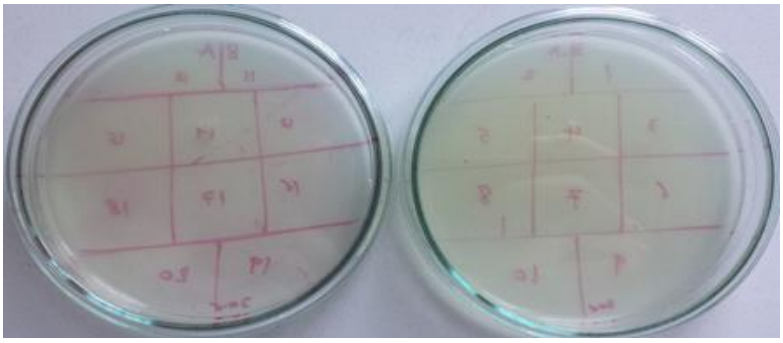
K08052 (14K) K21139 (17K)

f. Degradation tests

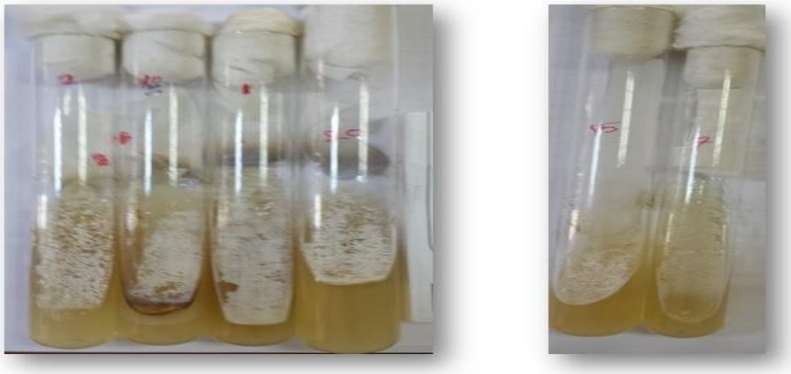


Xanthine Casein

g. Resistance to 50°C of temperature



h. H_2S production by test strains



i. Urease production by test strains

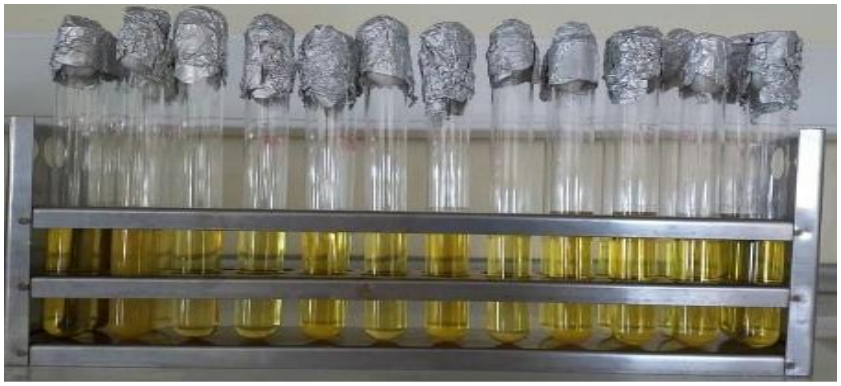


Figure 4.4. Photographs show growth of test isolates (a, b, c, d, e, f, g, h and i)

Table 4.4. Data obtained for numerical analysis of representative of *Streptomyces* strains from color grouping

Number of strain Tests	K10088	K14102	K19128	K20134	K08049	K18126	K17123	K09081	K14108	K16111	K10090	K09072	K02008	K08052	K03026	K21147	K21139	K02005	K10087	K10093
Nutritional tests																				
Growth on sole carbon source (1%, w/v)																				
1. Dextran	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-
2. D Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3. Lactose	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-
4. D Mannitol	+	+	-	+	+	-	+	+	+	+	+	+	-	+	-	-	-	+	+	-
5. D Raffinose	-	-	+	-	-	+	-	-	-	-	-	-	-	+	+	-	+	-	-	+
6. Sucrose	-	-	-	-	+	-	-	+	-	+	+	-	-	+	-	-	-	+	-	-
7. Maltose	+	+	-	-	+	-	-	+	-	+	+	+	-	+	-	-	-	+	+	-
8. D Mannose	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	-	+	+	+	+
Growth on sole carbon source (0.1%, w/v)																				
9. Sodium acetat	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
10. Sodium citrate	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
11. Sodium propionate	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+
Growth on sole nitrogen source (1% w/v)																				
12. L-Histidine	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	+
13. Potassium nitrate	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
14. L-Tyrosine	-	+	+	+	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+
Degradation tests																				
15. Xanthine (1%)	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16. Casein (1%)	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
17. Starch (1%)	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-
18. Gelatin (1%)	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
19. Lecithinase activity	-	-	-	+	-	+	+	-	-	+	+	+	-	+	+	+	-	-	+	-
20. Lipolysis activity	-	-	-	+	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+

Table 4.4. (continued)

Biochemical tests																				
21. Hydrogen sulphide production	-	-	+	+	-	+	-	+	+	+	+	-	-	-	+	-	-	+	-	+
22. Urea Hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Resistance to chemical inhibitors																				
23. Crystal violet (0.0001%)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24. Phenol (0.1%)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25. Sodium azide (0.01%)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
26. Sodium chloride (7%)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Resistance to temperature																				
27. 50ºC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28. 30ºC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
29. 25ºC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Resistance to antibiotics																				
30. Amoxicillin AMC (30mg)	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+
31. Rifampicin RA (5mg)	-	+	+	+	-	+	-	-	+	+	+	+	+	-	+	+	+	+	-	-
32. Ampicillin SAM (20mg)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+
33. Gentamycin CN (10mg)	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-
34. Erythromycin E (15mg)	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	+	+	-
Antimicrobial activity tests																				
35. *Escherichia coli*	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
36. Staphylococcus aureus	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
37. *Candida* sp.	+	+	-	-	-	+	-	+	-	-	+	+	+	-	+	+	+	+	-	-
38. *Pseudomonas fluorescens*	-	+	-	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
39. *Bacillus subtitous*	-	-	-	-	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-
40. *Klebsiella pneumoniae*	+	-	-	-	+	+	-	-	-	-	-	-	+	-	+	+	+	+	-	-

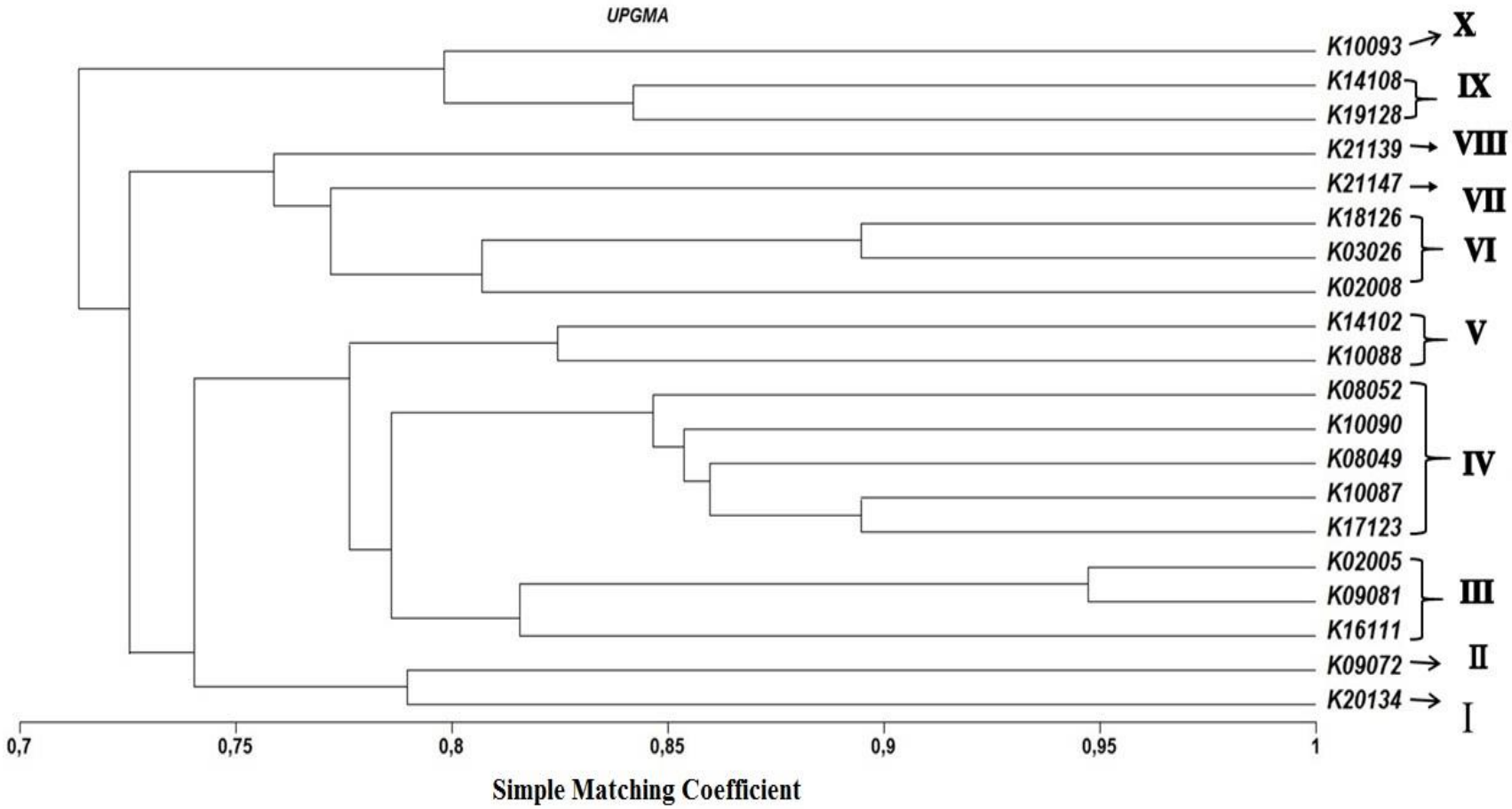
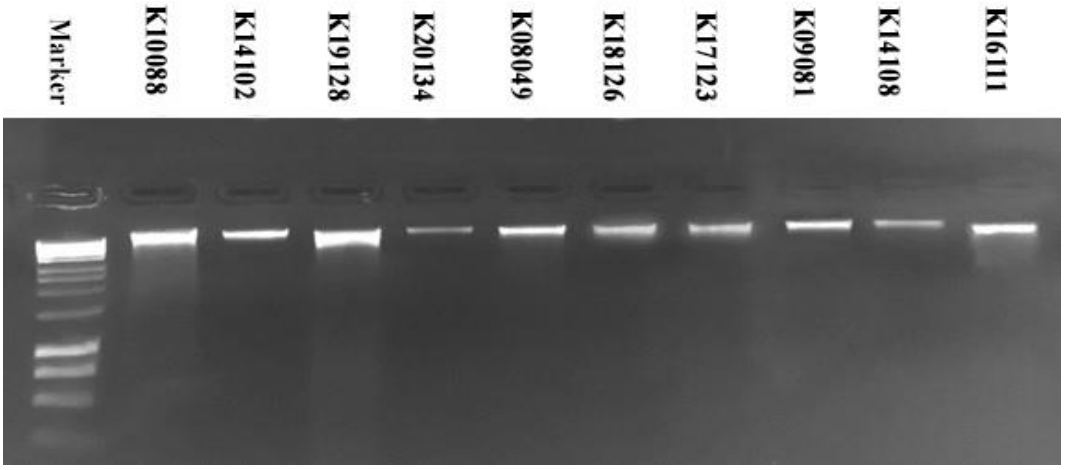


Figure 4.5. Dendogram showing relationships between representatives of *Streptomyces* groups analyzed data using S_{SM} (Simple matching coefficient) UPGMA algorithm

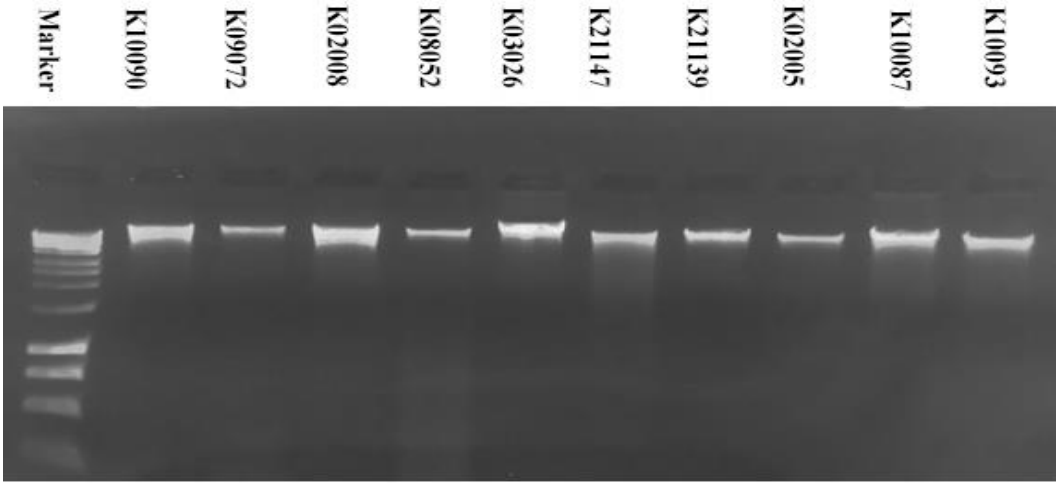
4.5. Molecular characterization

4.5.1. Genomic DNA extraction

The genomic DNA of 20 test strains were extracted by using a specific method that was described by Pitcher et al. (1989) and DNA isolation kit (Invitrogen, Pure Link (R) Genomic DNA Kit). The total genomic DNA on agarose gel electrophoresis are shown on Figure 4.6. As it is seen on Figures 4.6. pure genomic DNA for all test strains were yielded.



a.

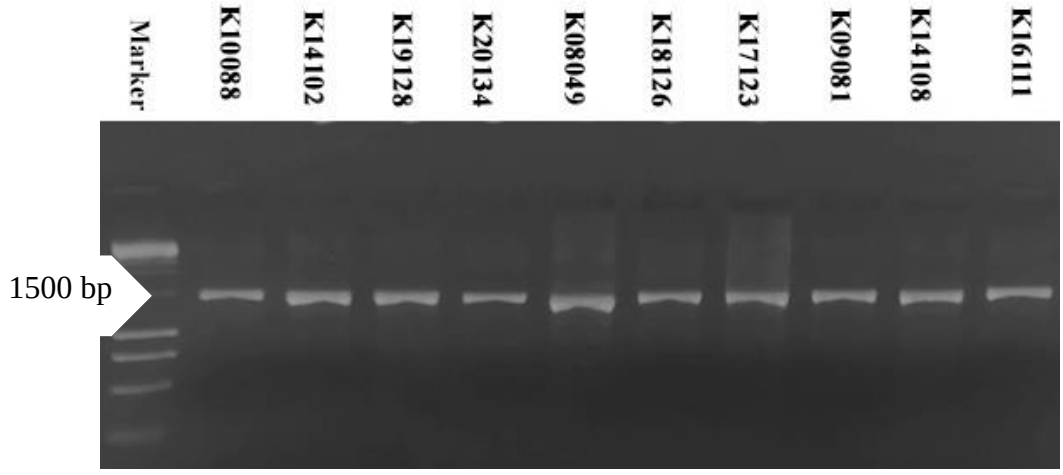


b.

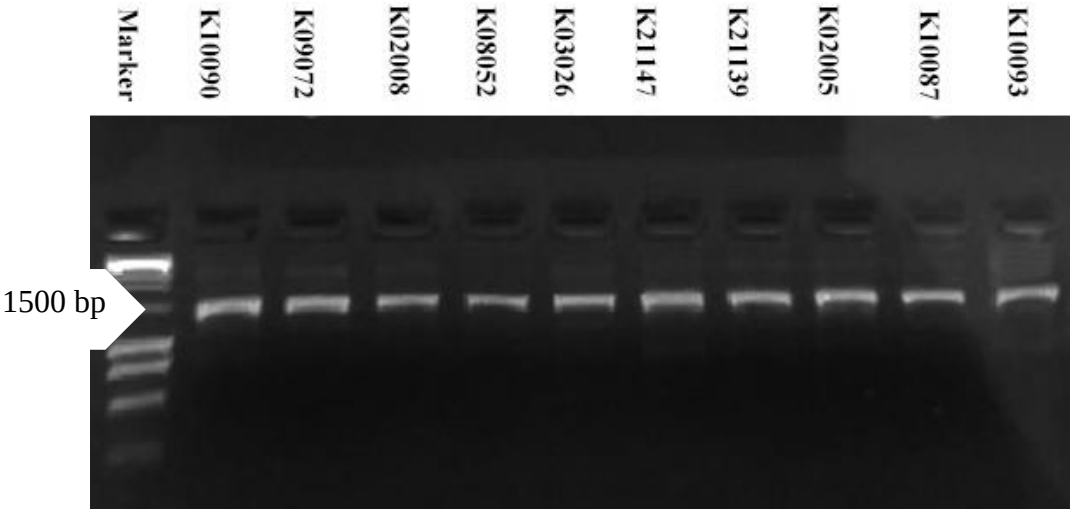
Figure 4.6. Whole genomic DNA bands of 20 test strains on 1% agarose gel electrophoresis image (Left side is marker 1kb DNA Ladder)

4.5.2. PCR product of 16S rDNA genes

16S rDNA gene region for 20 test strains was amplified using the Gradient PCR with the universal primers 27f and 1525r from genomic DNA. The size of 16S rDNA region were average 1500 base pairs. 16S rDNA bands amplified by PCR are seen on agarose gel electrophoresis (Figure 4.7).



a.



b

Figure 4.7. PCR amplified of the 16S rDNA gene region was performed using 1.5% agarose gel electrophoresis (Marker; Sigma, 1500 bp DNA Ladder)

4.6. Analysis of 16S rDNA sequence and phylogenetic tree

PCR amplifications of 16S rDNA gene region were performed by sequencing with primers 27f, 800r and MG5f after purification with the QIA quick PCR Purification Kit. The obtained sequence data was compared with the sequence data of the closest related species in the international databases using EzTaxon-e Server and % similarities were determined. Base sequence of 16S rDNA of 18 test strains are given at Appendix 2. A graph showing the distribution of isolates identified with their type strains are given in Figure 4.8. regarding to sequence analysis of base sequences of 16S rDNA. Dendogram were generated to determine phylogenetic positions of test isolates relative to 16S rDNA sequence data (Figures 4.9, 10, 11, 12, 13 and 14). The dendograms were drawn using the neighborhood-joining algorithm and the evolutionary distance matrix, Jukes and Cantor (1969). MEGA6 package program was used for phylogenetic analyzes (Tamura et al. 2013). The bootstrap analysis of the phylogenetic trees created (Felsenstein 1985) was done in 1000 replicates. *Kitasatospora nipponensis* HKI 0315^{T} (AY442263) used as an out group for all strains of *Streptomyces* dendogram, *Prauserella muralis* 05-Be-005^{T} (FM956091) used as an out group for *Amycolatopsis* dendogram, *Actinokineospora fastidiosa* IMSNU 20054T (AJ400710) used as an out group for *Lentzea* dendogram and *Rhodococcus kunmingensis* YIM 45607^{T} (DQ997045) was used as an out group for *Nocardia* dendogram.

As a result of phylogenetic analysis of sequence data, 11 isolates were identified as *Streptomyces* while 2 isolates were identified as *Lentzea* genus. Also 3 isolates were identified as *Amycolatopsis* genus and 2 isolate were identified as *Nocardia* genus. Phylogenetic similarities to the closest species of isolates according to 16S rDNA sequence results are given in Tables 4.5. Our findings are interesting showing to presence of different *Streptomyces*. Test strains were isolated on selective media for *Streptomyces*. So our expectation was that all test strains should be identify as *Streptomyces*. but the results showed that 2 *Lentzea*, 3 *Amycolatopsis* and 2 *Nocardia* species were present. Actually *Amycolatopsis, Lentzea* and *Nocardia* genera are belong to actinobacteria.

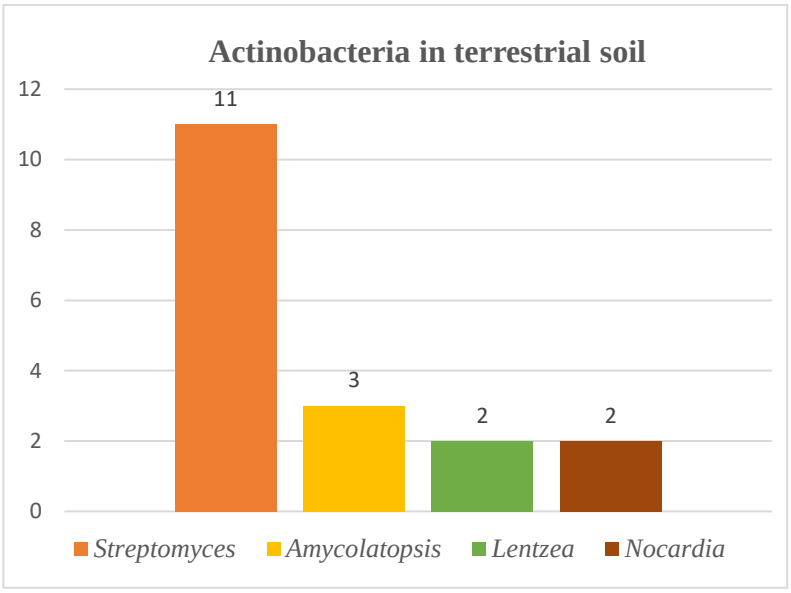


Figure 4.8. Distribution genus of test isolates according to the results of 16S rDNA sequence analysis

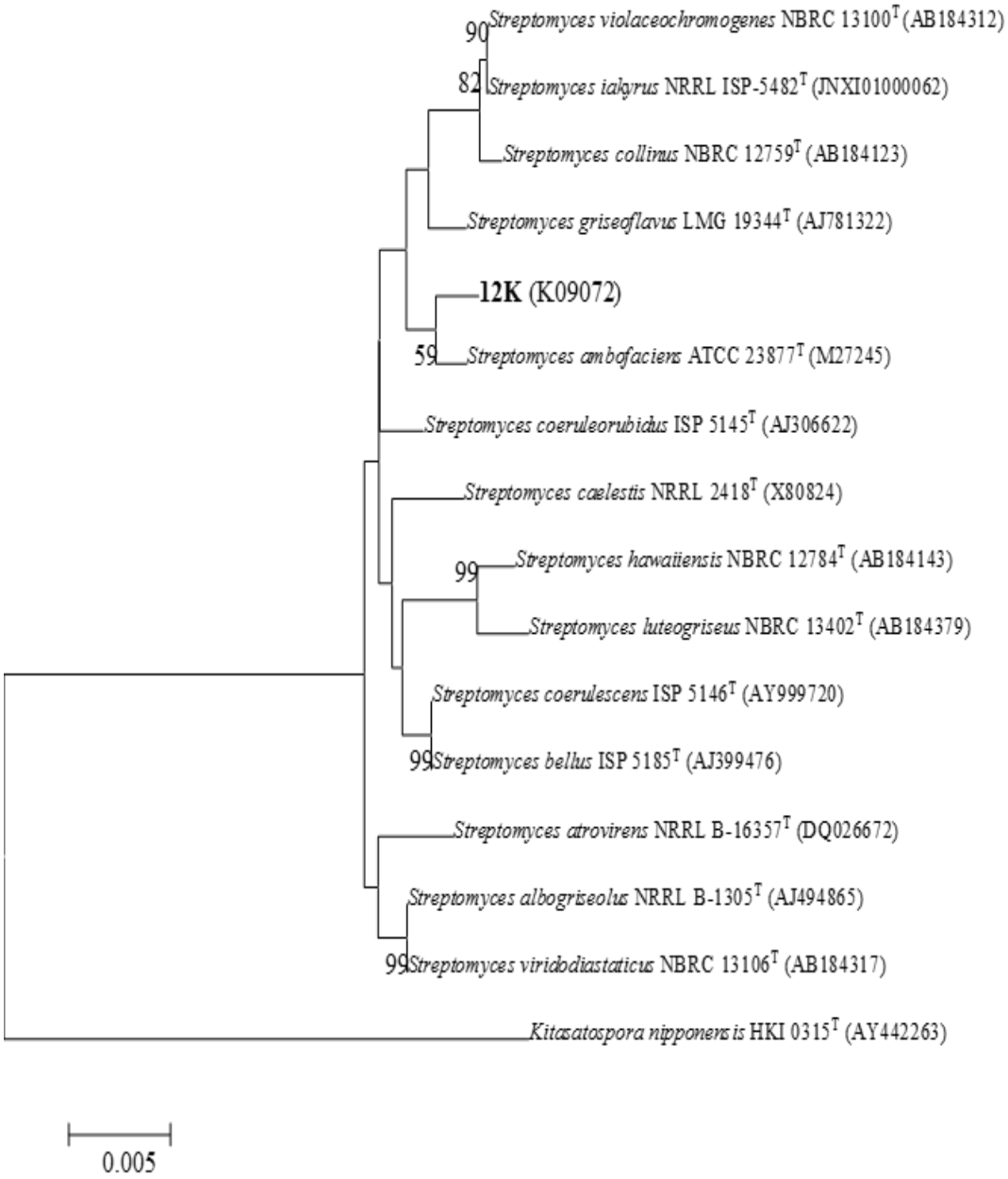


Figure 4.9. Phylogenetic dendogram showing the relation of one test strains of *Streptomyces* regarding base sequence of 16S rDNA gene. Dendogram produced neighboor-joining algorithms and numbers at nodes indicate the level of bootstrap support (%), only value ≥59% are shown. Genbank accession number are given in parentheses. Bar 0.005 substitutions per nucleotide position

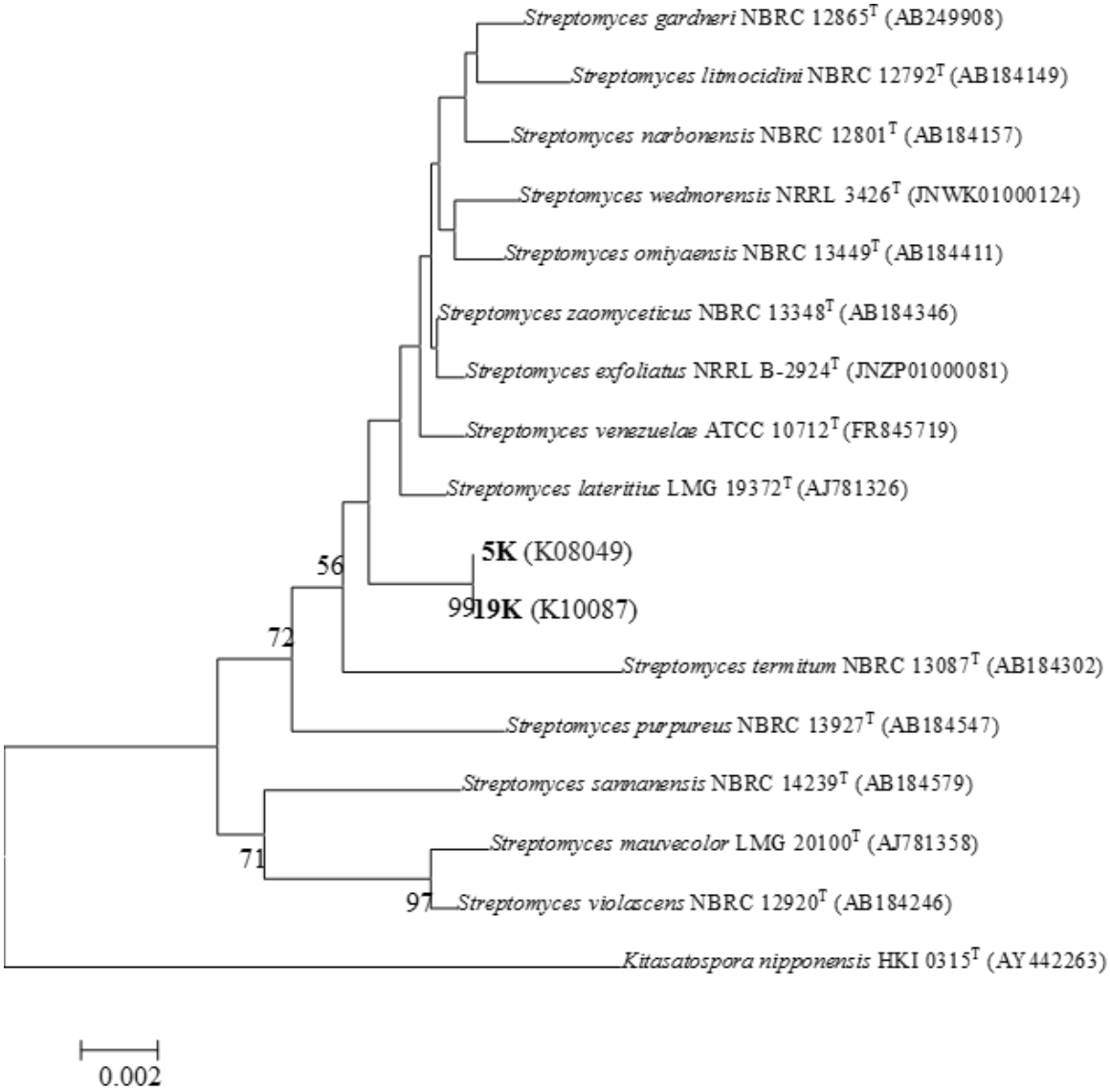


Figure 4.10. Phylogenetic dendogram showing the relation of 2 test strains of *Streptomyces* regarding base sequence of 16S rDNA gene. Dendogram produced neighboor joining algorithms and numbers at nodes indicate the level of bootstrap support (%), only value ≥56% are shown. Genbank accession number are given in parentheses. Bar 0.005 substitutions per nucleotide position

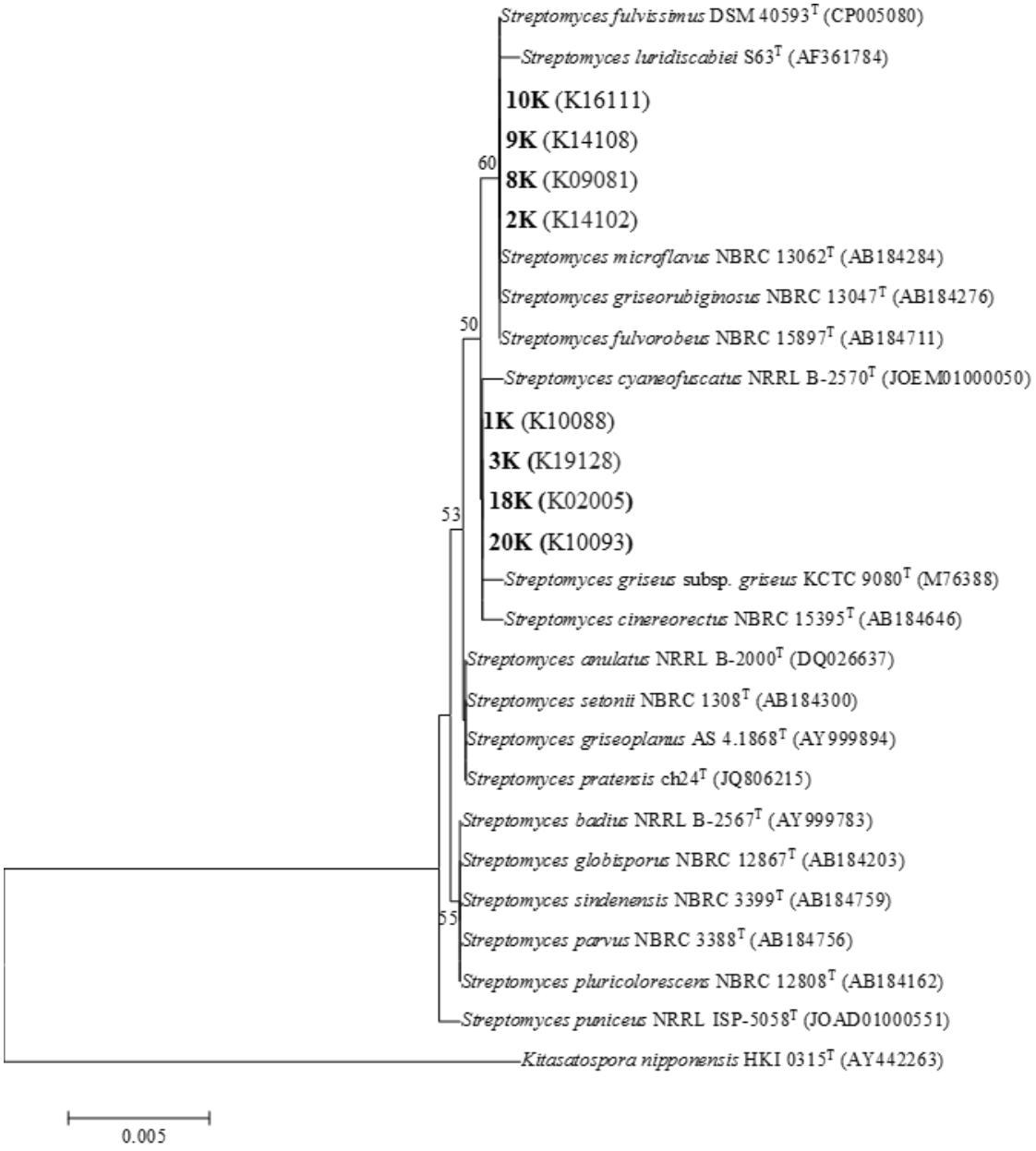


Figure 4.11. Phylogenetic dendogram showing the relation of 8 test strains of *Streptomyces* regarding base sequence of 16S rDNA gene. Dendogram produced neighboor-joining algorithms and numbers at nodes indicate the level of bootstrap support (%), only value ≥50% are shown. Genbank accession number are given in parentheses. Bar 0.005 substitutions per nucleotide position

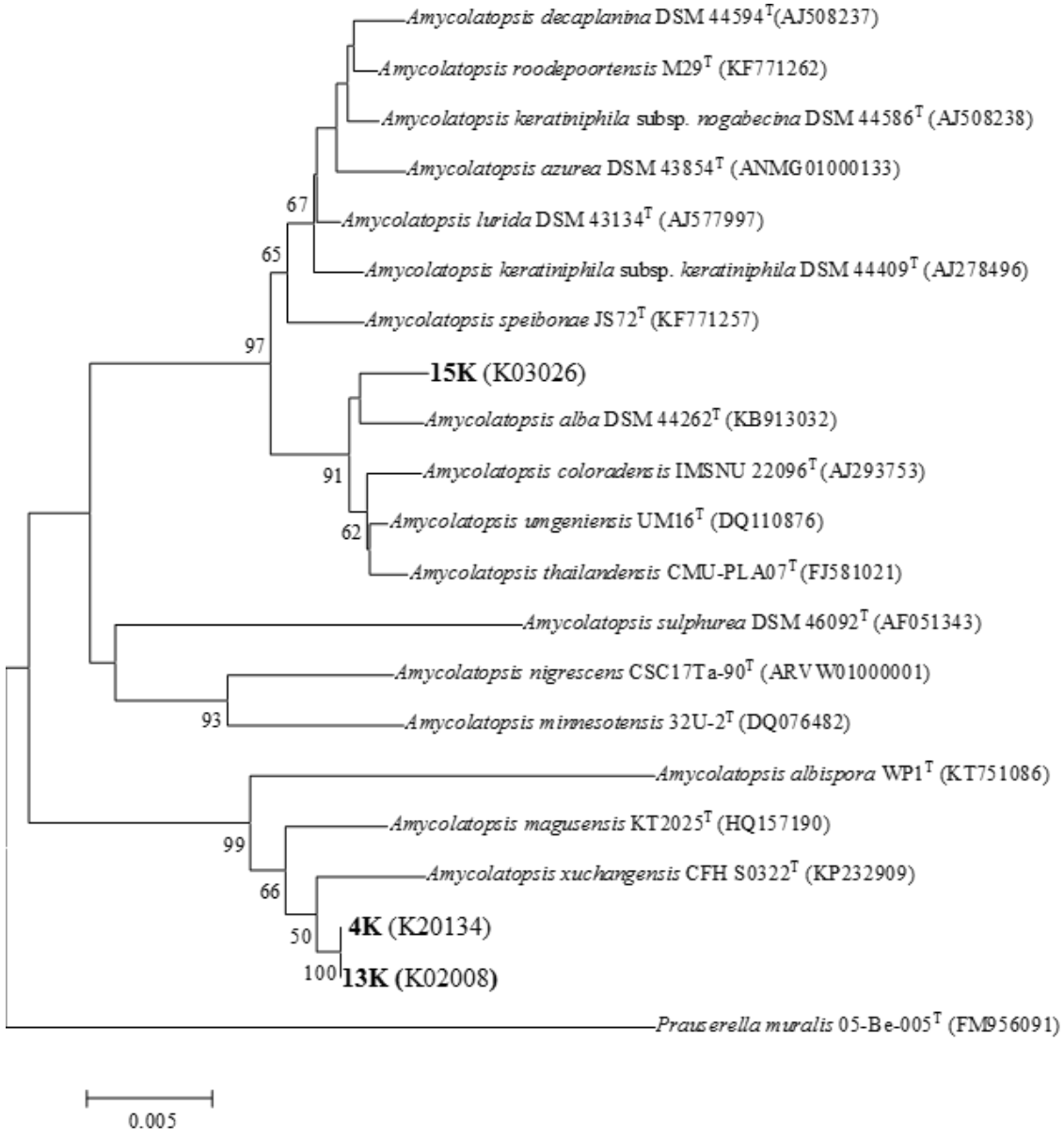


Figure 4.12. Phylogenetic dendogram showing the relation of 3 test strains of *Amycolatopsis* regarding base sequence of 16S rDNA gene. Dendogram produced neighboor-joining algorithms and numbers at nodes indicate the level of bootstrap support (%), only value ≥50% are shown. Genbank accession number are given in parentheses. Bar 0.005 substitutions per nucleotide position

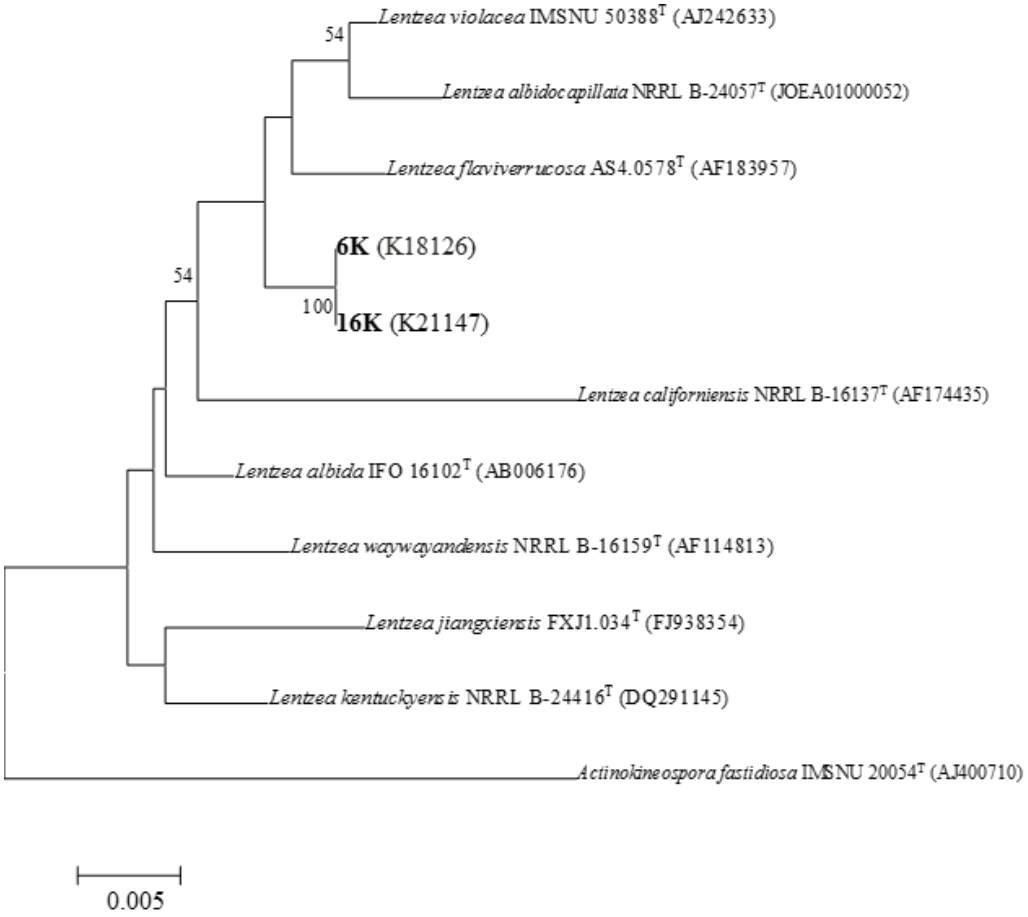


Figure 4.13. Phylogenetic dendogram showing the relation of 2 test strains of *Lentzea* regarding base sequence of 16S rDNA gene. Dendogram produced neighboor-joining algorithms and numbers at nodes indicate the level of bootstrap support (%), only value ≥54% are shown. Genbank accession number are given in parentheses. Bar 0.005 substitutions per nucleotide position

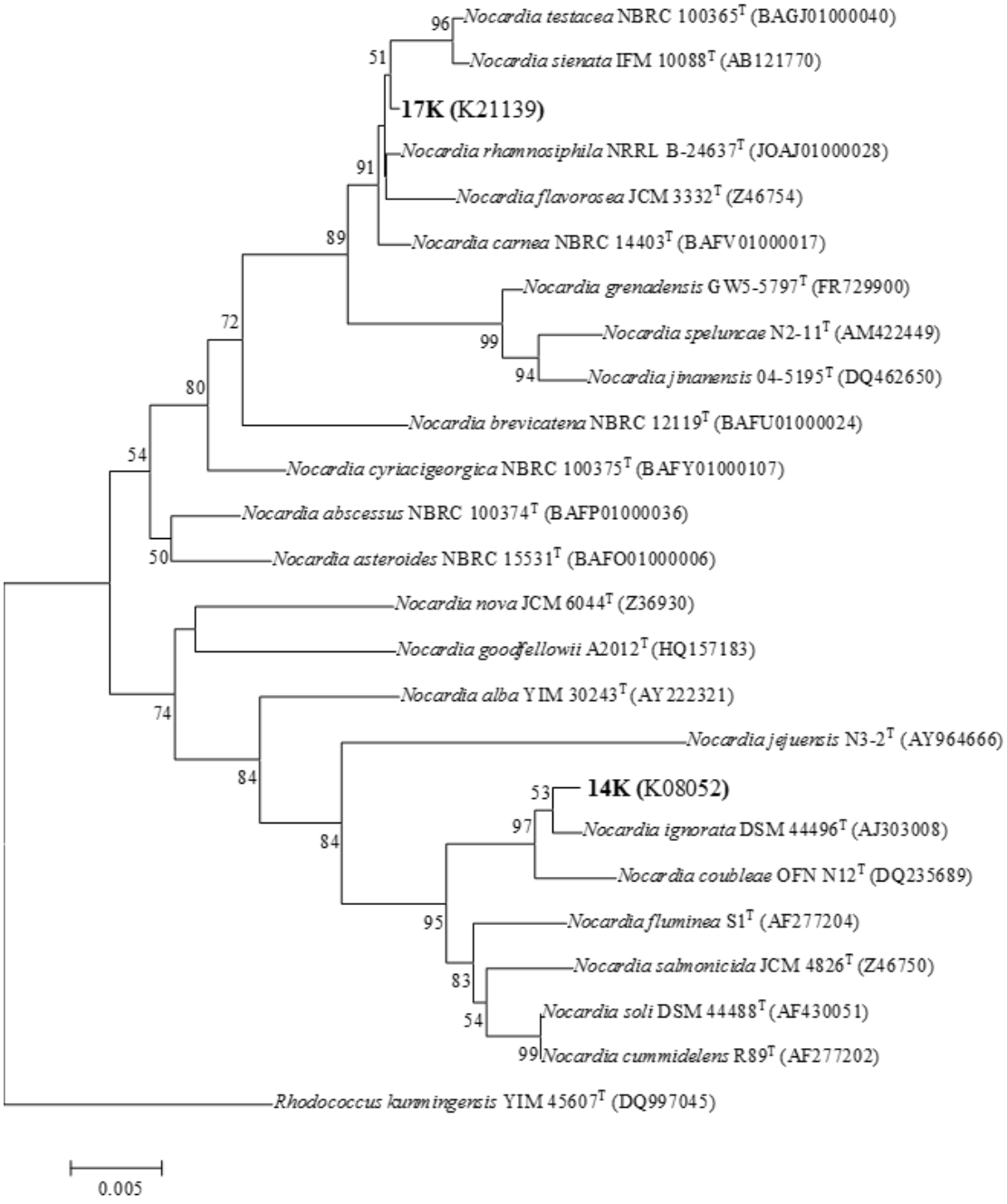


Figure 4.14. Phylogenetic dendogram showing the relation of 2 test strains of *Nocardia* regarding base sequence of 16S rDNA gene. Dendogram produced neighboor-joining algorithms and numbers at nodes indicate the level of bootstrap support (%), only value ≥50% are shown. Genbank accession number are given in parantheses. Bar 0.005 substitutions per nucleotide position

Table 4.5. Phylogenetic similarity with closely related species

Type strains	**The nearest type strain**	**% Similarity**
K10088 (1K)	*Streptomyces anulatus* NRRL B-2000(T)	99.93
K14102 (2K)	*Streptomyces fulvissimus* DSM 40593(T)	100.00
K19128 (3K)	*Streptomyces anulatus* NRRL B-2000(T)	99.93
K20134 (4K)	*Amycolatopsis xuchangensis* CFH S0322(T)	99.15
K08049 (5K)	*Streptomyces lateritius* LMG 19372(T)	99.32
K18126 (6K)	*Lentzea flaviverrucosa* AS4.0578(T)	99.15
K09081 (8K)	*Streptomyces fulvissimus* DSM 40593(T)	100.00
K14108 (9K)	*Streptomyces fulvissimus* DSM 40593(T)	100.00
K16111 (10K)	*Streptomyces fulvissimus* DSM 40593(T)	100.00
K09072 (12K)	*Streptomyces atrovirens* NRRL B-16357(T)	99.32
K02008 (13K)	*Amycolatopsis xuchangensis* CFH S0322(T)	99.15
K08052 (14K)	*Nocardia ignorata* DSM 44496(T)	99.73
K03026 (15K)	*Amycolatopsis umgeniensis* UM16(T)	99.38
K21147 (16K)	*Lentzea flaviverrucosa* AS4.0578(T)	99.15
K21139 (17K)	*Nocardia rhamnosiphila* NRRL B-24637(T)	99.73
K02005 (18K)	*Streptomyces anulatus* NRRL B-2000(T)	99.93
K10087 (19K)	*Streptomyces lateritius* LMG 19372(T)	99.32
K10093 (20K)	*Streptomyces anulatus* NRRL B-2000(T)	99.93

4.7. Chemotaxonomic analysis

Chemotaxonomic analyzes were performed to determine the characteristic chemical properties of the isolates. These analyzes include diaminopimelic acid and sugar analyzes (Table 4.6).

Table 4.6. Analysis of some chemotaxonomic feature of 4 test microorganisms

Type strains	**İdentified species**	**DAP**	**Sugar**
K14108 (**9K**)	*Streptomyces fulvissimus*	LL-A2pm	glucose
K08052 (**14K**)	*Nocardia ignorata*	meso-A2pm	glucose, galactose, arabinose and ribose
K21147 (**16K**)	*Lentzea flaviverrucosa*	meso-A2pm	glucose, galactose, mannose and ribose
K21139 (**17K**)	*Nocardia rhamnosiphila*	meso-A2pm	glucose, galactose, arabinose and ribose

4.7.1. Diaminopimelic acid test

The aim of this study is to determine DAP type in the cell wall of test strains. The spots were determined when the position of the bands formed in the one-dimensional thin layer chromatography by comparing with the standard A2pm solution. A one-dimensional TLC chromatogram show that one test strains contain the LL-A2pm content of isolates of the genus *Streptomyces* K14108 (9K) while 3 strains contained meso-A2pm which that two of them identified as *Nocardia* K08052 (14K) & K21139 (17K) genus and one as *Lentzea* K21147 (16K) genus from 16S rDNA analysis (Figure 4.15). Our results are disagreement with molecular identification based on 16S rDNA gene sequencing.

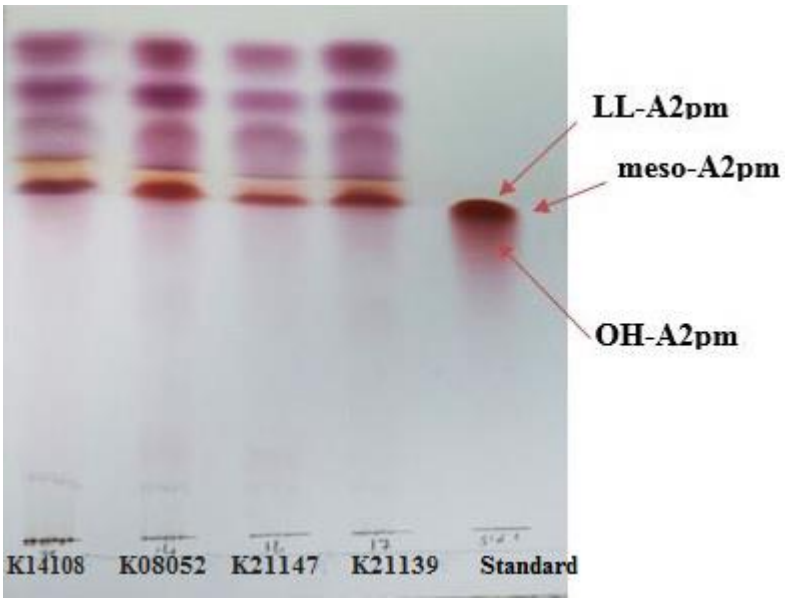


Figure 4.15. One-dimensional thin layer chromatogram of A2pm isomers of 4 isolates with standard A2pm (Diaminopimelic acid) that contains (LL-A2pm, meso-A2pm and OH-A2pm)– Sigma

4.7.2. Sugar analysis

The whole cell sugar profile in the cell wall chemo-type was determined by comparing two standards that contains seven sugars for test microorganisms in one dimensional thin layer chromatography. It is seen on Figure 4.16. that isolates of K14108 (9K) belonging to the genus *Streptomyces* was found to contain glucose. Unlike these isolates, strain K08052 (14K) & K21139 (17K) identified by molecular technique as *Nocardia* contains

glucose, galactose, arabinose and ribose. K21147 isolate identified as *Lentzea* were found to contain glucose, galactose, mannose and ribose. This findings support the result of molecular identification.

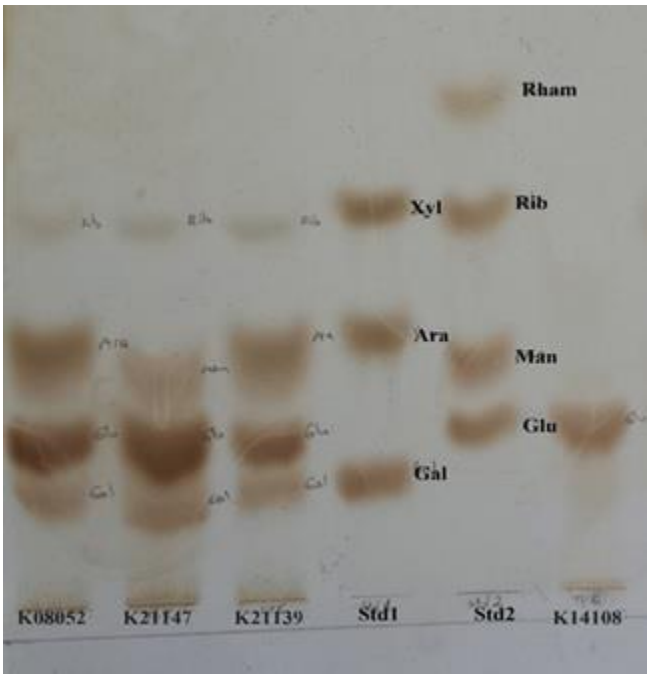


Figure 4.16. One-dimensional thin layer chromatogram of the sugar profile of 4 test isolates. Std1, standard 1: Gal, galactose; Ara, arabinose and Xyl; xylose. Std 2, standard 2: Glu; glucose; Man, mannose; Rib, ribose and Rham, rhamnnose

5. DISCUSSION

Streptomyces is one of genus within the class Actinobacteria is capable of extensive secondary metabolite production and encompass species possessing large genomes. *Streptomyces* bacteria are abundant and hugely exist in soils depending to pH, moisture content and organic matter content. (Schlatter et al. 2008). Also, it is well known that *Streptomyces* bacteria produce the vast secondary metabolism and physicochemical character of the soil that is affecting the distribution of *Streptomyces*. Chater et al. (2010) reported that over 70% of antibiotics are derived from *Streptomyces* sp.

Pshdar and Ranya are two of the places that has not been investigated for isolation of *Streptomyces* and other actinobacteria genera. So, it is really necessary to isolate *Streptomyces* from these soil samples because of recovering new *Streptomyces* species producing a new antimicrobial compound. pH of most of soils were alkali over 8 and only pH of 2 soil samples (K16 and K20) were neutral, about 7,3. It is thought that moisture content of soil samples was lowing down because of the climate change that Pshdar and Ranya faced a hot drought since the last recent years, Percentage of Moisture content of soil samples were less than 9% except (K12, K19, K20 & K21) were about 10%. Also, organic matter content of soil samples was low comparing to soil samples studied (Atalan et al. 2000; Tatar and Sahin 2015). Percentage of organic matter content of soil samples were less than 10% except K04, K05, K09, K11, K12 & K17 (Table 4.1.).

The physicochemical characters of soil samples affected the recovery of *Streptomyces* and other bacteria on selective media. However, cultivation based methods may recover less than 1% of total soil bacteria but a large number of bacteria can be recovered *Streptomyces* and other bacteria using different technique such as reciprocal shaking method (Atalan 1993). Also, the application of using selective media that primarily promote recovery of *Streptomyces*, adding cycloheximide, nystatin and novobiocin

antibiotics to reduce the proliferation of rapidly growing Gram-negative bacteria and fungi present in the sample, were used to optimize the isolation of streptomycetes. In our study, we used selective media, starch-casein agar supplemented with cycloheximide, nystatin and novobiocin and raffinose-histidine agar supplemented with cycloheximide and nystatin antibiotics to increase the count of *Streptomyces* and other actinobacteria genera. Total number of actinomycetes/*Streptomyces* were low comparing to other studies (Sahin, 1995) while no colony was appeared on isolation plates of neither SCA or RH agar inoculated from soil samples K06, K12, K13, K15, K22, K24, K27, K28, K30, K31 & K32 that is 11 soil samples out of 32 soil samples (Table 4.2). It can be said that media with high pH may promote to increase the number of colony because of high pH of soil samples. The number of soil *Streptomyces* were detected and different based on soil use and also parallel with soil pH. Watkins (2013) reported that organic matter content correlate with land use and pH but was not correlated with recovery of *Streptomyces*. Some studies reported that differences in the actinobacterial genera of the total bacterial community under various land uses (Hill et al. 2010), but a few studies have carried out to analyze bacterial distribution. It has been reported that number of Actinobacteria to be higher common in pasture and cultivated land compared to undeveloped or dried soils (Lauber et al. 2008), and Burke et al. (2003) found that higher number of actinomycete component of the microbial community in cultivated soils, rather than forested soils. Also, high number of Actinobacteria was found in agricultural, dust and street soils comparing to arid, dried and undeveloped soils (Hill et al. 2010). Finding our study is in good agreement with studies mentioned above. Watkins (2013) observed a larger number of *Streptomyces* in soils with a neutral to slightly alkaline pH.

The 164 streptomycetes isolates were assigned to 10 colour groups which were 8 major (7–35 isolates) and 2 single-membered colour-groups are shown in Table 4.3. The criteria used to colour group the 164 isolates are following; aerial spore mass colour, substrate mycelium colour, and melanin production. All of these groups contained strains isolated from more than one of the sampling sites except (group 9-10). In contrast, the overall grouping separated the groups into site-specific clusters (Table 4.3.). Antony-Babu et al. (2010) reported that colour grouping of 321 streptomycetes isolates grouped heterogeneous, isolates from different sampling sites and our results give similar colour groups. 2 single membered group (9th and 10th) were isolated from same soil sample of

K10 site. The example photographs of the colour-groups at the different sites is shown in Figure. 4.2 and 4.3).

Total 20 test strains selected from colour group as representatives of groups and 40 diagnostic tests (Table 4.4.) were carried out for numerical analysis by computer-assisted TAXON program. Final database contained information on 20 test strains and 40 unit characters. The numerical analysis based on S_{SM}, UPGMA analysis give good separation of cluster group and clusters was supported by high cophenetic correlation coefficient of 0,80. The 20 test strains were assigned to 5 multimembered and 5 single membered cluster-groups defined 80% similarity level (Figure 4.5). It is encouraging that good congruence was found between numerical analysis groups and colour groups. There was linear correlation between colourgroups and clusters of numerical analysis. 2 strains K14108 (9K) & K19128 (3K) of first colour group were grouped in same cluster IX while 3 strains K18126 (6K), K02008 (13K) & K03026 (15K) were grouped in same cluster VI. Also 3 strains K16111 (10K), K09081 (8K) & K02005 (18K) in third colour group were grouped in same cluster III while 2 strains K08049 (5K) & K10087 (19K) of fourth colour group were grouped in cluster IV. Antony-Babu et al (2010) suggested that the numerical analysis of colour group data support the visual display of data as dendrograms and highlights the recognition of taxa based on similar colour characteristics. Some researchers suggested that the ability to separate unknown streptomycetes to colour groups that can be equated with species and species-groups (Atalan et al. 2000; Sembiring et al. 2000; Tan et al. 2006; Goodfellow et al. 2007) can be used to choose isolates for screening programs that designed to detect novel secondary metabolites.

Identification of bacterial strains is still a difficult subject for microbiologist despite development of molecular biology techniques and the development of kits that are commercially available phenotype-based identification tests. Since the 16S rRNA gene is ubiquitous, stable, conserved and poorly subject to horizontal gene transfer, it is an effective molecular marker for identification of bacteria. It well known that analyses of 16S rRNA gene sequence of strains is fundamental technique for archaea and bacteria and is being used for the identification and classification of prokaryotes (Olsen & Woese 1993; Stackebrandt et al. 2002). So, sequencing and phylogenetic analysis of the 16S

rRNA genes easy and reliable method to classify and identify of bacterial isolates at various taxonomic levels. Cut off values is 98.7% for classification (Stackebrandt & Ebers 2006).

The 16S rRNA gene serve as the primary key for phylogeny-based identification when compared against well-conserved 16S rRNA gene sequence databases among the several thousand genes within a bacterial genome (Tindall et al. 2010: Kim et al. 2012). The correlation between the colour-group, numerical analysis, 16S rDNA gene analyses and chemotaxonomic analysis for test strains are presented on Table 5.1. It is apparent from Table 5.1. that there is a reasonable linear correlation between these datasets used to characterize and identify the tests strains.

The almost complete 16S rRNA gene sequence (about 1500 nts) of strain K09072 (12K) was compared with the corresponding sequences of representative members of colour groups using MEGA (Altschul et al. 1997), and the results revealed the highest similarity 99,32% with *Streptomyces atrovirens* (Figure 4.9) while K08049 (5K) and K10087 (19K) strains were identified with *Streptomyces lateritius* at 99,32% (Figure 4.10). K16111 (10K), K14108 (9K), K09081 (8K) and K14102 (2K) strains were identified with *Streptomyces fulvissimus* at 100% similarity level (Figure 4.11) while K10088 (1K), K19128 (3K), K02005 (18K) and K10093 (20K) were identified *Streptomyces anulatus* 99,93% similarity level (Figure 4.11). On the other hand, K03026 (15K) was identified as *Amycolatopsis umgeniensis* at 99,38% but K20134 (4K) and K02008 (13K) were identified to *Amycolatopsis xuchangensis* at 99,15% level (Figure 4.12). Interestingly K18126 (6K) and K21147 (16K) were identified as *Lentzea flaviverruosa* at 99,15% level (Figure 4.13) and K08052 (14K) was identified as *Nocardia ignorata* 99,73% level (Figure 4.14) while K21139 (17K) was identified to *Nocardia rhamnosiphilia* at 99,73% (Figure 4.14). Phylogenetic analyses based on 16S rRNA gene sequences showed that strain K18126 (6K) & K21147 (16K) fell into *Lentzea flaviverruosa* and K20134 (4K) & K02008 (13K) fell into *Amycolatopsis xuchangensis* but it may be new species.

Table 5.1. Comparison of different method applied to identify test strains

Type strains	**Color grouping**	**Numerical analysis**	**16S rDNA study**	**DAP type**	**Sugar type**
K14102 (**2K**)	1	V	*Streptomyces fulvissimus*		
K14108 (**9K**)	1	IX	*Streptomyces fulvissimus*	LL-A2pm	glucose
K19128 (**3K**)	1	IX	*Streptomyces anulatus*		
K18126 (**6K**)	2	VI	*Lentzea flaviverrucosa*		
K02008 (**13K**)	2	VI	*Amycolatopsis xuchangensis*		
K03026 (**15K**)	2	VI	*Amycolatopsis umgeniensis*		
K16111 (**10K**)	3	III	*Streptomyces fulvissimus*		
K09081 (**8K**)	3	III	*Streptomyces fulvissimus*		
K02005 (**18K**)	3	III	*Streptomyces anulatus*		
K08049 (**5K**)	4	IV	*Streptomyces lateritius*		
K10087 (**19K**)	4	IV	*Streptomyces lateritius*		
K09072 (**12K**)	5	II	*Streptomyces atrovirens*		
K08052 (**14K**)	6	IV	*Nocardia ignorata*	meso-A2pm	glucose, galactose, arabinose and ribose
K20134 (**4K**)	6	I	*Amycolatopsis xuchangensis*		
K21147 (**16K**)	7	VII	*Lentzea flaviverrucosa*	meso-A2pm	glucose, galactose, mannose and ribose
K21139 (**17K**)	8	VIII	*Nocardia rhamnosiphila*	meso-A2pm	glucose, galactose, arabinose and ribose
K10088 (**1K**)	9	V	*Streptomyces anulatus*		
K10093 (**20K**)	10	X	*Streptomyces anulatus*		

Addition, phenotypic and physiological characteristics showed differences between strain K18126 (6K), K21147 (16K), K20134 (4K) and K02008 (13K) strains with its closest neighbors (Table 4.4). Phylogenetic analyses based on 16S rRNA gene sequences from previous studies (Chomchoei et al. 2011; Guan et al. 2011) support that strains of K18126 (6K), K21147 (16K), K20134 (4K) and K02008 (13K) represents a novel species. Thus, it is suggested that strains of K18126 (6K), K21147 (16K), K20134 (4K) and K02008 (13K) represents a novel species on the basis of polyphasic taxonomic evidence.

Kim et al. (2012) reported that all species and phylotypes of prokaryotic microorganisms have been assigned to a complete hierarchical system (from species to phylum) by using comprehensive phylogenetic analyses based on 16S rRNA gene sequences. Therefore, our findings are reliable owing to identification the test strains using 16S rDNA database. The aim of this study was to identify bacteria isolated from Pshdar and Ranya soils. As result, test strains were identified as 11 *Streptomyces*, 3 *Amycolatopsis*, 2 *Lentzea* and 2 *Nocardia* species. Particularly K18126 (6K) & K21147 (16K) strains identified as *Lentzea flaviverrucosa* and K20134 (4K) & K02008 (13K) strains identified as *Amycolatopsis xuchangensis* should be correlated with DNA-DNA hybridization (DDH). DDH should be applied to these test strains assigned new species as suggestion of Stackebrandt & Ebers (2006) that propose 16S rRNA gene sequence identity might supported by DDH.

Whole-cell sugars were prepared according to Lechevalier and Lechevalier (1970b) and analyzed by thin-layer chromatography. The cell wall of K14108 (9K) was found to contain LL-diaminopimelic acid, but K08052 (14K), K21147 (16K) and K21139 (17K) strains contained meso- type. Test strains of K14108 (9K), K08052 (14K), K21147 (16K) and K21139 (17K) selected after phylogenetic analysis were analyzed for sugar type and DAP analysis (Table 4.6). The last three strains were distinguished from previously described species of the genus *Streptomyces*. Also, cell Wall of K14108 (9K) test strain contain glucose while K08052 (14K) and K21139 (17K) strains contained ribose, glucose, galactose and arabinose but K21147 (16K) strain contained ribose, glucose, galactose and mannose sugars (Figure 4.16). Cells of 1 strains were observed to contain LL-diaminopimelic acid as the diamino acid, indicating these strains is of cell wall chemotype I belong to *Streptomyces* (Lechevalier and Lechevalier 1970a, b).

There is no specific sugar type for *Streptomyces* cell Wall Whole-cell hydrolysates were found to contain glucose as these sugars are indication of *Streptomyces* but other strains were identified as *Amycolatopsis*, *Lentzea* and *Nocardia* species that cell walls of them contained 4 different sugar type. Sugar type and DAP type cell Wall and the phylogenetic analysis based on 16S rRNA gene sequences also support the differentiation of strains K14108 (9K), K08052 (14K), K21147 (16K) and K21139 (17K) from each other.

CONCLUSIONS

This investigation shows that the moisture content, organic matter content, and pH of each soil sample appear to influence the distribution of actinobacteria, particularly *Streptomyces* sp. It is possible that there are other edaphic factors including soil texture that influence recovery or distribution of bacteria. To overcome this problem, media with high pH (over 8) should be used for isolation of bacteria. It is clear that land use influences *Streptomyces* community composition. Additional studies are needed to isolate and study actinobacteria genera.

The findings of characterization studies using numerical analysis showed that isolated actinobacteria strains generate 10 colour groups and 10 clusters. It can be seen on Table 5.1 the colour groups and numerical analysis of the clusters were in agreement with each other. 2 test strains of 1st colour groups were IX clusters of numerical analysis. Similar result found for test strains of 2nd colour group which placed in VI cluster, and strains of 3rd colour group were assigned in same cluster of III. Also 2 strain of 4th colour group were assigned in cluster IV. Colour groups of 20 test strains were concurrence with the cluster generated after numerical analysis of diagnostic characters.

The phylogenetic analysis based on 16S rRNA gene sequences revealed that 11 test strains belong to genus *Streptomyces* and has high sequence similarity to *Streptomyces* (Table 4.5 and 5.1). The other 2 test strains belong to *Nocardia* while 2 test strains were identified as *Lentzea* genus and 3 were identified as *Amycolatopsis* genera.

The phylogenetic analysis showed that all 18 test strains form a distinct clade that is different from other closely related species of type strains (Figure 4.9-14). In conclusion, biodiversity of the soil samples was low, and a total of 164 strains of actinobacteria were isolated and purified. A total 20 isolates were selected based on colour grouping, and

numerical analysis dendogram to carried molecular study while phylogenetic analysis of 16S rDNA of 18 test strains were identified to 4 different genera those are *Nocardia*, *Lentzea*, *Amycolatopsis* and *Streptomyces* genera. It is clear that a further study of DNA DNA hybridization method can be applied to the strains of K18126 (6K), K02008 (13K), K20134 (4K) and K21147 (16K) owing to decide if they are new species.

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APPENDICES

Appendix 1: Media

Starch Casein Agar (Kuster and Williams 1964)

Starch casein agar was used as a basal medium for isolation of *Streptomyces* Sp.

Soluble Starch	5 g
Casein	0.15 g
KNO_3	1.0 g
NaCl	1.0 g
K_2HPO_4	1.0 g
$MgSO_4.\ 7H_2O$	0.001 g
$FeSO_4.\ 7H_2O$	0.005 g
$CaCO_3$	0.01 g
Agar	9 g
Distilled water	500 ml

Autoclaved at 121°C for 15 minutes. pH adjusted to 7.2-7.5

Raffinose Histidine Agar (Vickers et al. 1984)

Raffinose histidine agar was used as a basal medium for isolation of *Streptomyces* Sp.

Raffinose	5 g
L- Histidine	0.5 g
$MgSO_4.\ 7H_2O$	0.01 g
$FeSO_4.\ 7H_2O$	0.005 g
K_2HPO_4	0.5 g
Agar	7.5 g
Distilled water	500 ml

Autoclaved at 121°C for 15 minutes. pH adjusted to 7.2-7.5

Modified Bennett's Agar (Jones 1949)

Modified Bennett's agar was used as a basal medium for growth, Nitrogen source, degradation and chemical inhibitors.

Yeast extract	0.5 g
Lab-lemco	0.4 g
Bacto-casitone	1 g
Glycerol	5 ml
Agar	8 g
Distilled water	500 ml

Autoclaved at 121°C for 20 minutes. pH adjusted to 7.2-7.5

Oatmeal Agar (Shirling and Gottlieb 1966)

Oatmeal agar (ISP 3) medium was used to determine the color groups of *Streptomyces* Sp.

Oatmeal Agar	35 g
Distilled water	500 ml

Autoclaved at 121°C for 15 minutes. pH 7.2-7.5

Peptone Yeast Extract Iron Agar (Shirling and Gottlieb 1966)

Peptone yeast extract iron agar (ISP 6) medium was used to determine the ability of isolates to produce melanin pigment.

Peptone Iron Agar	18 g
Yeast Extract	1.0g
Distilled water	500 ml

Autoclaved at 121°C for 20 minutes. pH adjusted to 7.2-7.5

Glucose Yeast Malt Extract Agar (Rapp 1974)

Glucose yeast malt extract agar (ISP 2) was used in soil isolation and morphological examinations of microorganisms.

Glucose	2 g
Yeast-extract	2 g
Malt-extract	5 g
Agar	9 g

Distilled water 500 ml

Autoclaved at 121°C for 20 minutes. pH adjusted to 7.2-7.5

Muller Hinton agar (Mueller and Hinton 1941)

Muller Hinton agar was used to determine the Antimicrobial Spectrum of Isolates against the test organisms by using perpendicular method and Susceptibility test by (Antimicrobial-Sensitivity Discs)

Beef Extract powder	1.0 g
Acid Digest of Casein	8.75 g
Starch	0.75 g
Agar	8.5 g
Distilled water	500 mL

Autoclaved at 121°C for 20 minutes. pH adjusted to 7.2-7.5

Nitrogen Source Utilization Medium (Williams et al. 1983a)

Nitrogen source utilization medium was used as a basal medium for Nitrogen source.

Glucose	5 g
$MgSO_4.7H_2O$	0.25 g
$FeSO_4.7H_2O$	0.005
K_2HPO_4	0.5 g
NaCl	0.25 g
Agar	6 g
Distilled water	500 ml

Autoclaved at 121°C for 20 minutes. pH adjusted to 7.2-7.5

Nitrate Agar (Skerman 1967)

Nitrate agar was used determine hydrogen sulphide production by inserting sterile lead acetate filter paper strips into the necks of the tubes

KNO_3	0.5 g
Nutrient broth	500 ml
Agar	6.0 g

Autoclaved at 121°C for 20 minutes. pH adjusted to 7.2-7.5

Carbone source utilization medium (Shirling and Gottlieb 1966)

Carbone source utilization medium was used as a basal medium for Carbone source.

Ammonium Sulphate	1.32 g
KH_2PO_4	1.19 g
K_2HPO_4	2.83 g
$MgSO_4$	0.5 g
Pridham and Gottlieb's Trace salts solution	0.5 ml
Agar	9 g
Distilled water	500 ml

Autoclaved at 121°C for 20 minutes. pH adjusted to 7.2-7.5

Pridham and Gottlieb's Trace salts solution (Shirling and Gottlieb 1966)

$CuSO_4.\ 5H_2O$	0.64 g
$FeSO_4.7H_2O$	0.11 g
$MnCl_2.\ 4H_2O$	0.79 g
$ZnSO_4.7H_2O$	0.15 g
Distilled water	100 ml

20% Glycerol Stock Solution (Wellington and Williams 1978)

The glycerol suspension was prepared for stocking of spores or mycelium of test strains over a long period of time at -20 °C.

Glycerol	20 ml
Distilled water	80 ml

Sterilized at 121 °C for 15 min in autoclave.

Appendix 2: 16S rRNA gene sequences of test isolates

16S rRNA gene sequence of *Streptomyces* sp. 1K (K10088)

TCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAGC
CTTTCGGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCC
CTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAACACTCT
GTCCCGCATGGGACGGGGTTAAAAGCTCCGGCGGTGAAGGATGAGCCCGCG
GCCTATCAGCTTGTTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTAGC
CGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTC
CTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGC
AGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCA
GGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACG
TGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGG
GCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGATGTGAAAGCCCGGGGCT
TAACCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGGTAGGGGAGAT
CGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGT
GGCGAAGGCGGATCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGG
GGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGGGAAC
TAGGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACGCATTAAGTTC
CCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGG
GCCCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACC
TTACCAAGGCTTGACATATACCGGAAAGCATCAGAGATGGTGCCCCCCTTGT
GGTCGGTATACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTG
GGTTAAGTCCCGCAACGAGCGCAACCCTTGTTCTGTGTTGCCAGCATGCCCTT
CGGGGTGATGGGGACTCACAGGAGACTGCCGGGGTCAACTCGGAGGAAGGT
GGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTA
CAATGGCCGGTACAATGAGCTGCGATGCCGCGAGGCGGAGCGAATCTCAAA
AAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGA
GTTGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTT
GTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGC
CCAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGGCGATTGGGACGA
AGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGCT

16S rRNA gene base sequence of *Streptomyces* sp. 2K (K14102)

CGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAGCCTTTCG
GGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTTCAC
TCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAACACTCTGTCCTG
CATGGGACGGGGTTAAAAGCTCCGGCGGTGAAGGATGAGCCCGCGGCCTATC
AGCTTGTTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTG
AGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGG
AGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACG
CCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGA
AGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGC
AGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAA
GAGCTCGTAGGCGGCTTGTCACGTCGGATGTGAAAGCCCGGGGCTTAACCCC
GGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGGTAGGGGAGATCGGAATT
CCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAA
GGCGGATCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCG
AACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGGGAACTAGGTG
TTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACGCATTAAGTTCCCCGCC
TGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGC
ACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCA
AGGCTTGACATATACCGGAAAGCATCAGAGATGGTGCCCCCCTTGTGGTCGG
TATACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAA
GTCCCGCAACGAGCGCAACCCTTGTTCTGTGTTGCCAGCATGCCCTTCGGGGT
GATGGGGACTCACAGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGAC
GACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGG
CCGGTACAATGAGCTGCGATGCCGCGAGGCGGAGCGAATCTCAAAAAGCCG
GTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTTGCTA
GTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACAC
ACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACC
CCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGGCGATTGGGACGAAGTCGT
AACAAGGTAGCCGTACCGGAAGGTGCGG

16S rRNA gene base sequence of *Streptomyces* sp. 3K (K19128)

TCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAGC
CTTTCGGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCC
CTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAACACTCT
GTCCCGCATGGGACGGGGTTAAAAGCTCCGGCGGTGAAGGATGAGCCCGCG
GCCTATCAGCTTGTTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTAGC
CGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTC
CTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGC
AGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCA
GGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACG
TGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGG
GCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGATGTGAAAGCCCGGGGCT
TAACCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGGTAGGGGAGAT
CGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGT
GGCGAAGGCGGATCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGG
GGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGGGAAC
TAGGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACGCATTAAGTTC
CCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGG
GCCCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACC
TTACCAAGGCTTGACATATACCGGAAAGCATCAGAGATGGTGCCCCCCTTGT
GGTCGGTATACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTG
GGTTAAGTCCCGCAACGAGCGCAACCCTTGTTCTGTGTTGCCAGCATGCCCTT
CGGGGTGATGGGGACTCACAGGAGACTGCCGGGGTCAACTCGGAGGAAGGT
GGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTA
CAATGGCCGGTACAATGAGCTGCGATGCCGCGAGGCGGAGCGAATCTCAAA
AAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGA
GTTGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTT
GTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGC
CCAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGGCGATTGGGACGA
AGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGC

16S rRNA gene base sequence of *Amycolatopsis* sp. 4K (K20134)

CAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAGCC
CTTCGGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCC
TGTACTTTGGGATAAGCCCTGGAAACGGGGTCTAATACCGGATAGGACTGCG
CATCGCATGGTGTGTGGTGGAAAGCTCCGGCGGTACAGGATGAGCCCGCGGC
CTATCAGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCG
GCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTA
CGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGC
GACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCGACAGGG
ACGAAGGGTGAGTGACGGTACCTGTAGAAGAAGCACCGGCTAACTACGTGCC
AGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTGTCCGGAATTATTGGGCGT
AAAGAGCTCGTAGGCGGTTTGTCGCGTCGGCCGTGAAAACTGGAGGCTTAAC
CTTCAGCTTGCGGTCGATACGGGCAGACTTGAGTTCGGTAGGGGAGACTGGA
ATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCG
AAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAG
CGAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGTTGGGCGCTAGG
TGTGGGCGACATCCACGTTGTCCGTGCCGTAGCTAACGCATTAAGCGCCCCG
CCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCC
GCACAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGAAGAACCTTAC
CTGGGCTTGACATGCGCCAGACATCCCTAGAGATAGGGCTTCCCTTGTGGTTG
GTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTA
AGTCCCGCAACGAGCGCAACCCTTATCCTATGTTGCCAGCGGTTCGGCCGGG
GACTCGTGGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTC
AAGTCATCATGCCCCTTATGTCCAGGGCTTCACACATGCTACAATGGCTGGTA
CAGAGGGCTGCGATACCGTGAGGTGGAGCGAATCCCTTAAAGCCGGTCTCAG
TTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAGTCGCTAGTAATC
GCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
CGTCACGTCATGAAAGTCGGTAACACCCGAAGCCCATGGCCCAACCCGTAAG
GGGGGGAGTGGTCGAAGGTGGGACTGGCGATTGGGACGAAGTCGTAACAAG
GTAGCCGTACCGGAAGGTGCGGCTG

16S rRNA gene base sequence of *Streptomyces* sp. 5K (K08049)

CTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAA
CCCTTCGGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGC
CCTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAACACCG
GCTTCCGCATGGAAGCTGGTTGAAAGCTCCGGCGGTGAAGGATGAGCCCGCG
GCCTATCAGCTTGTTGGTGGGGTAATGGCCCACCAAGGCGACGACGGGTAGC
CGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTC
CTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGC
AGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCA
GGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACG
TGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGG
GCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGGTGTGAAAGCCCGGGGCT
TAACCCCGGGTCTGCATCCGATACGGGCAGGCTAGAGTGTGGTAGGGGAGAT
CGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGT
GGCGAAGGCGGATCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGG
GGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGGGAAC
TAGGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACGCATTAAGTTC
CCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGG
GCCCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACC
TTACCAAGGCTTGACATATACCGGAAACGGCCAGAGATGGTCGCCCCCTTGT
GGTCGGTATACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTG
GGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTGTGTTGCCAGCATGCCCTT
CGGGGTGATGGGGACTCACAGGAGACCGCCGGGGTCAACTCGGAGGAAGGT
GGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTA
CAATGGCCGGTACAAAGAGCTGCGATGCCGCGAGGCGGAGCGAATCTCAAA
AAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGA
GTTGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTT
GTACACACCGCCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGG
CCCAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGGCGATTGGGACG
AAGTCGTAACAAGGT

16S rRNA gene base sequence of *Lentzea* sp. 6K (K18126)

AGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGTAAGGCCC
TTCGGGGTACACGAGCGGCGAACGGGTGAGTAACACGTGGGTAACCTGCCCT
GTACTCTGGGATAAGCCTTGGAAACGAGGTCTAATACCGGATACGACCACTG
ATCGCATGATCGGTGGTGGAAAGTTCCGGCGGTATGGGATGGACCCGCGGCC
TATCAGCTTGTTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTAGCCGG
CCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTAC
GGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCG
ACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGA
CGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCACCGGCTAACTACGTGCC
AGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTGTCCGGAATTATTGGGCGT
AAAGAGCTCGTAGGCGGTTTGTCGCGTCGGCCGTGAAAACTTGGGGCTTAAC
TCCAAGCTTGCGGTCGATACGGGCAGACTTGAGTTCGGCAGGGGAGACTGGA
ATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCG
AAGGCGGGTCTCTGGGCCGACACTGACGCTGAGGAGCGAAAGCGTGGGGAG
CGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGTGCTAGG
TGTGGGGGGCTTCCACGCCCTCTGTGCCGCAGCTAACGCATTAAGCACCCCG
CCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCC
GCACAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGAAGAACCTTAC
CTGGGCTTGACATGGACTAGAAAGCTCTAGAGATAGAGCCTCCCTTGTGGCT
GGTTCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTT
AAGTCCCGCAACGAGCGCAACCCTCGTTCCATGTTGCCAGCACGTAATGGTG
GGGACTCATGGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACG
TCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACACATGCTACAATGGCCGG
TACAAAGGGCTGCTAAGCCGTGAGGTGGAGCGAATCCCATAAAGCCGGTCTC
AGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGCTAGTAA
TCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCG
CCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCCGTGGCCCAACTCGCA
AGAGGGGGAGCGGTCGAAGGTGGGACTGGCGATTGGGACGAAGTCGTAACA
AGGTAGCCGTACCGGAAGGTGCGGCT

16S rRNA gene base sequence of *Streptomyces* sp. 8K (K09081)

CAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAGCC
TTTCGGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCC
TTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAACACTCTG
TCCTGCATGGGACGGGGTTAAAAGCTCCGGCGGTGAAGGATGAGCCCGCGGC
CTATCAGCTTGTTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTAGCCG
GCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTA
CGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGC
GACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGG
AAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGC
CAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCG
TAAAGAGCTCGTAGGCGGCTTGTCACGTCGGATGTGAAAGCCCGGGGCTTAA
CCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGGTAGGGGAGATCGG
AATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGC
GAAGGCGGATCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGA
GCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGGGAACTAG
GTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACGCATTAAGTTCCCC
GCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCC
CGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTA
CCAAGGCTTGACATATACCGGAAAGCATCAGAGATGGTGCCCCCCTTGTGGT
CGGTATACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGT
TAAGTCCCGCAACGAGCGCAACCCTTGTTCTGTGTTGCCAGCATGCCCTTCGG
GGTGATGGGGACTCACAGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGG
GACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAA
TGGCCGGTACAATGAGCTGCGATGCCGCGAGGCGGAGCGAATCTCAAAAAG
CCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTT
GCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTA
CACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCA
ACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGGCGATTGGGACGAAGT
CGTAACAAGGTAGCCGTACCGGAAGGTGCGGC

16S rRNA gene base sequence of *Streptomyces* sp. 9K (K18108)

TCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAGC
CTTTCGGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCC
CTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAACACTCT
GTCCTGCATGGGACGGGGTTAAAAGCTCCGGCGGTGAAGGATGAGCCCGCGG
CCTATCAGCTTGTTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTAGCC
GGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCT
ACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAG
CGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGG
GAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTG
CCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGC
GTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGATGTGAAAGCCCGGGGCTTA
ACCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGGTAGGGGAGATCG
GAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGG
CGAAGGCGGATCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGG
AGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGGGAACTA
GGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACGCATTAAGTTCCC
CGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGC
CCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTT
ACCAAGGCTTGACATATACCGGAAAGCATCAGAGATGGTGCCCCCCTTGTGG
TCGGTATACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGG
TTAAGTCCCGCAACGAGCGCAACCCTTGTTCTGTGTTGCCAGCATGCCCTTCG
GGGTGATGGGGACTCACAGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGG
GGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACA
ATGGCCGGTACAATGAGCTGCGATGCCGCGAGGCGGAGCGAATCTCAAAAA
GCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGT
TGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGT
ACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCC
AACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGGCGATTGGGACGAA
GTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGG

16S rRNA gene base sequence of *Streptomyces* sp. 10K (K16111)

TCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAGC
CTTTCGGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCC
CTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAACACTCT
GTCCTGCATGGGACGGGGTTAAAAGCTCCGGCGGTGAAGGATGAGCCCGCGG
CCTATCAGCTTGTTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTAGCC
GGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCT
ACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAG
CGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGG
GAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTG
CCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGC
GTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGATGTGAAAGCCCGGGGCTTA
ACCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGGTAGGGGAGATCG
GAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGG
CGAAGGCGGATCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGG
AGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGGGAACTA
GGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACGCATTAAGTTCCC
CGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGC
CCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTT
ACCAAGGCTTGACATATACCGGAAAGCATCAGAGATGGTGCCCCCCTTGTGG
TCGGTATACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGG
TTAAGTCCCGCAACGAGCGCAACCCTTGTTCTGTGTTGCCAGCATGCCCTTCG
GGGTGATGGGGACTCACAGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGG
GGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACA
ATGGCCGGTACAATGAGCTGCGATGCCGCGAGGCGGAGCGAATCTCAAAAA
GCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGT
TGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGT
ACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCC
AACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGGCGATTGGGACGAA
GTCGTAACAAGGTAGCCGTACCGGAAGGTGCG

16S rRNA gene base sequence of *Streptomyces* sp. 12K (K09072)

AGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCAC
TTCGGTGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCT
GCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGATCCGC
CTGGGCATCCAGGCGGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGC
CTATCAGCTTGTTGGTGAGGTAGTGGCTCACCAAGGCGACGACGGGTAGCCG
GCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTA
CGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGC
GACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGG
AAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGC
CAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCG
TAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTAA
CCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGG
AATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGC
GAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGA
GCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAG
GTGTGGGCAACATTCCACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCC
GCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCC
CGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTA
CCAAGGCTTGACATACACCGGAAACGTCTGGAGACAGGCGCCCCCTTGTGGT
CGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGT
TAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAAGCCCTTCG
GGGTGTTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGG
GGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACA
ATGGCCGGTACAATGAGCTGCGATACCGCAAGGTGGAGCGAATCTCAAAAA
GCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGT
CGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGT
ACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCC
AACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGGCGATTGGGACGAA
GTCGTAACAAGGTAGCCGTACCGGAAGGTGCG

16S rRNA gene sequence of *Amycolatopsis* sp. 13K (K02008)

TCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAGC
CCTTCGGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCC
CTGTACTTTGGGATAAGCCCTGGAAACGGGGTCTAATACCGGATAGGACTGC
GCATCGCATGGTGTGTGGTGGAAAGCTCCGGCGGTACAGGATGAGCCCGCGG
CCTATCAGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCC
GGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCT
ACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAG
CGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCGACAGG
GACGAAGGGTGAGTGACGGTACCTGTAGAAGAAGCACCGGCTAACTACGTG
CCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTGTCCGGAATTATTGGGC
GTAAAGAGCTCGTAGGCGGTTTGTCGCGTCGGCCGTGAAAACTGGAGGCTTA
ACCTTCAGCTTGCGGTCGATACGGGCAGACTTGAGTTCGGTAGGGGAGACTG
GAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGG
CGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGG
AGCGAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGTTGGGCGCTA
GGTGTGGGCGACATCCACGTTGTCCGTGCCGTAGCTAACGCATTAAGCGCCC
CGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGC
CCGCACAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGAAGAACCTT
ACCTGGGCTTGACATGCGCCAGACATCCCTAGAGATAGGGCTTCCCTTGTGGT
TGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGT
TAAGTCCCGCAACGAGCGCAACCCTTATCCTATGTTGCCAGCGGTTCGGCCG
GGGACTCGTGGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACG
TCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACACATGCTACAATGGCTGG
TACAGAGGGCTGCGATACCGTGAGGTGGAGCGAATCCCTTAAAGCCGGTCTC
AGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAGTCGCTAGTAA
TCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCG
CCCGTCACGTCATGAAAGTCGGTAACACCCGAAGCCCATGGCCCAACCCGTA
AGGGGGGGAGTGGTCGAAGGTGGGACTGGCGATTGGGACGAAGTCGTAACA
AGGTAGCCGTACCGGAAGGTGC

16S rRNA gene sequence of *Nocardia* sp. 14K (K08052)

TCAGGACGAACGCTAGCGGCGTGCTTAACACATGCAAGTCGAGCGGTAAGGC
CCTTCGGGGTACACGAGCGGCGAACGGGTGAGTAACACGTGGGTGATCTGCC
TTGCACTTCGGGATAAGCCTGGGAAACTGGGTCTAATACCGGATATGACCTC
GGGATGCATGTTCTGGGGTGGAAAGATTTATCGGTGCGAGATGGGCCCGCGG
CCTATCAGCTTGTTGGTGAGGTAACGGCTCACCAAGGCGACGACGGGTAGCC
GACCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCT
ACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAG
CGACGCCGCGTGCGGGATGACGGCCTTCGGGTTGTAAACCGCTTTTGACAGG
GACGAAGCGCAAGTGACTGTACCTGTAGAATAAGGACCGGCCAACTACGTGC
CAGCAGCCGCGGTAATACGTAGGGTCCGAGCGTTGTCCGGAATTACTGGGCG
TAAAGAGCTTGTAGGCGGTTCGTCGCGTCGTTTGTGAAAACTTGGGGCTCAA
CCTTAAGCTTGCAGGCGATACGGGCGGACTAGAGTACTTCAGGGGAGACTGG
AATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGC
GAAGGCGGGTCTCTGGGAAGTAACTGACGCTGAGAAGCGAAAGCATGGGTA
GCAAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGGTGGGTACTAG
GTGTGGGGCTCCTTCCACGGACTCCGTGCCGTAGCTAACGCATTAAGTACCCC
GCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGAAATTGACGGGGGCC
CGCACAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGAAGAACCTTA
CCTGGGTTTGACATACACCAGAAAGCCGTAGAGATACGGCCCCCCTTGTGGT
TGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGT
TAAGTCCCGCAACGAGCGCAACCCTTATCTTATGTTGCCAGCGCGTAATGGC
GGGGACTCGTGAGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGACGAC
GTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACACATGCTACAATGGCCG
GTACAGAGGGCTGCGATACCGTGAGGTGGAGCGAATCCCTTAAAGCCGGTCT
CAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTTGGAGTCGCTAGTA
ATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACC
GCCCGTCACGTCATGAAAGTCGGTAACACCCGAAGCCGGTGGCCTAACCCCT
TGTGGGAGGGAGCCGTCGAAGGTGGGATCGGCGATTGGGACGAAGTCGTAA
CAAGGTAGCCGTACCGGAAGGTGC

16S rRNA gene sequence of *Amycolatopsis* sp. 15K (K03026)

TCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAGC
CTTCGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCT
GTACTTTGGGATAAGCCTGGGAAACTGGGTCTAATACCGGATATCACTGACT
CTCGCATGGGGGTTGGTTGAAAGTTCTGGCGGTACAGGATGAGCCCGCGGCC
TATCAGCTTGTTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTAGCCGG
CCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTAC
GGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCG
ACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCGCCAGGGA
CGAAGCGCAAGTGACGGTACCTGGATAAGAAGCACCGGCTAACTACGTGCCA
GCAGCCGCGGTAATACGTAGGGTGCAAGCGTTGTCCGGAATTATTGGGCGTA
AAGAGCTCGTAGGCGGTTTGTCGCGTCGTTCGTGAAAACTCCACGCTTAACGT
GGAGCGTGCGGGCGATACGGGCAGACTTGAGTTCGGTAGGGGAGACTGGAA
TTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGA
AGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGC
GAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGT
GTGGGCGACATCCACGTTGTCCGTGCCGTAGCTAACGCATTAAGCGCCCCGC
CTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCG
CACAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGAAGAACCTTACC
TGGGCTTGACATGCGCCAGACATCCCTAGAGATAGGGCTTCCCTTGTGGTTGG
TGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAA
GTCCCGCAACGAGCGCAACCCTTATCCTACGTTGCCAGCGCGTTATGGCGGG
GACTCGTGGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTC
AAGTCATCATGCCCCTTATGTCCAGGGCTTCACACATGCTACAATGGCTGGTA
CAGAGGGCTGCGATACCGCGAGGTGGAGCGAATCCCTTAAAGCCGGTCTCAG
TTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAGTCGCTAGTAATC
GCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
CGTCACGTCATGAAAGTCGGTAACACCCGAAGCCCACGGCCCAACCCGCAAG
GGAGGGAGTGGTCGAAGGTGGGACTGGCGATTGGGACGAAGTCGTAACAAG
GTAGCCGTACCGGAAGGTGCGG

16S rRNA gene sequence of *Lentzea* sp. 16K (K21147)

CTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGTAAGG
CCCTTCGGGGTACACGAGCGGCGAACGGGTGAGTAACACGTGGGTAACCTGC
CCTGTACTCTGGGATAAGCCTTGGAAACGAGGTCTAATACCGGATACGACCA
CTGATCGCATGATCGGTGGTGGAAAGTTCCGGCGGTATGGGATGGACCCGCG
GCCTATCAGCTTGTTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTAGC
CGGCCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCC
TACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCA
GCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAG
GGACGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCACCGGCTAACTACGT
GCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTGTCCGGAATTATTGGG
CGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCGGCCGTGAAAACTTGGGGCTT
AACTCCAAGCTTGCGGTCGATACGGGCAGACTTGAGTTCGGCAGGGGAGACT
GGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTG
GCGAAGGCGGGTCTCTGGGCCGACACTGACGCTGAGGAGCGAAAGCGTGGG
GAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGTGCT
AGGTGTGGGGGGCTTCCACGCCCTCTGTGCCGCAGCTAACGCATTAAGCACC
CCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGG
CCCGCACAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGAAGAACCT
TACCTGGGCTTGACATGGACTAGAAAGCTCTAGAGATAGAGCCTCCCTTGTG
GCTGGTTCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGG
GTTAAGTCCCGCAACGAGCGCAACCCTCGTTCCATGTTGCCAGCACGTAATG
GTGGGGACTCATGGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATG
ACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACACATGCTACAATGGC
CGGTACAAAGGGCTGCTAAGCCGTGAGGTGGAGCGAATCCCATAAAGCCGGT
CTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGCTAG
TAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACA
CCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCCGTGGCCCAACTC
GCAAGAGGGGGAGCGGTCGAAGGTGGGACTGGCGATTGGGACGAAGTCGTA
ACAAGGTAGCCGTACCGGAAGGTGC

16S rRNA gene base sequence of *Nocardia* sp. 17K (K21139)

TCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGTAAGGC
CCTTCGGGGTACACGAGCGGCGAACGGGTGAGTAACACGTGGGTGATCTGCC
TTGCACTCTGGGATAAGCCTGGGAAACTGGGTCTAATACCGGATATGACCTT
CGGTTGCATGACCGGGGGTGGAAAGATTTATCGGTGCAAGATGAGCCCGCGG
CCTATCAGCTTGTTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTAGCC
GGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCT
ACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAG
CGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCGACTCC
GACGAAGCGAGAGTGACGGTAGGAGTATAAGAAGCACCGGCCAACTACGTG
CCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTGTCCGGAATTACTGGGC
GTAAAGAGCTTGTAGGCGGCTTGTCGCGTCGATCGTGAAAACTCGGGGCTCA
ACCCCGAGCTTGCGGTCGATACGGGCAGGCTTGAGTACTTCAGGGGAGACTG
GAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGG
CGAAGGCGGGTCTCTGGGAAGTAACTGACGCTGAGAAGCGAAAGCGTGGGT
AGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGTACTA
GGTGTGGGTTTCCTTCCACGGGATCCGTGCCGTAGCTAACGCATTAAGTACCC
CGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGC
CCGCACAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGAAGAACCTT
ACCTGGGTTTGACATACACCGGAAACCTGCAGAGATGTAGGCCCCCTTGTGG
TCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGG
TTAAGTCCCGCAACGAGCGCAACCCTTATCTTATGTTGCCAGCGCGTAATGGC
GGGGACTCGTGAGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGACGAC
GTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACACATGCTACAATGGCCG
GTACAGAGGGCTGCGATACCGTGAGGTGGAGCGAATCCCTTAAAGCCGGTCT
CAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTTGGAGTCGCTAGTA
ATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACC
GCCCGTCACGTCATGAAAGTCGGTAACACCCGAAGCCGGTGGCCTAACCCTT
GTGGAGGGAGCCGTCGAAGGTGGGATCGGCGATTGGGACGAAGTCGTAACA
AGGTAGCCGTACCGGAAGGTGCGGC

16S rRNA gene sequence of *Streptomyces* sp. 18K (K02005)

TCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAGC
CTTTCGGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCC
CTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAACACTCT
GTCCCGCATGGGACGGGGTTAAAAGCTCCGGCGGTGAAGGATGAGCCCGCG
GCCTATCAGCTTGTTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTAGC
CGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTC
CTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGC
AGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCA
GGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACG
TGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGG
GCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGATGTGAAAGCCCGGGGCT
TAACCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGGTAGGGGAGAT
CGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGT
GGCGAAGGCGGATCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGG
GGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGGGAAC
TAGGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACGCATTAAGTTC
CCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGG
GCCCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACC
TTACCAAGGCTTGACATATACCGGAAAGCATCAGAGATGGTGCCCCCCTTGT
GGTCGGTATACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTG
GGTTAAGTCCCGCAACGAGCGCAACCCTTGTTCTGTGTTGCCAGCATGCCCTT
CGGGGTGATGGGGACTCACAGGAGACTGCCGGGGTCAACTCGGAGGAAGGT
GGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTA
CAATGGCCGGTACAATGAGCTGCGATGCCGCGAGGCGGAGCGAATCTCAAA
AAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGA
GTTGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTT
GTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGC
CCAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGGCGATTGGGACGA
AGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGG

16S rRNA gene sequence of *Streptomyces* sp. 19K (K10087)

TCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAAC
CCTTCGGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCC
CTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAACACCGG
CTTCCGCATGGAAGCTGGTTGAAAGCTCCGGCGGTGAAGGATGAGCCCGCGG
CCTATCAGCTTGTTGGTGGGGTAATGGCCCACCAAGGCGACGACGGGTAGCC
GGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCT
ACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAG
CGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGG
GAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTG
CCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGC
GTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGGTGTGAAAGCCCGGGGCTTA
ACCCCGGGTCTGCATCCGATACGGGCAGGCTAGAGTGTGGTAGGGGAGATCG
GAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGG
CGAAGGCGGATCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGG
AGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGGGAACTA
GGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACGCATTAAGTTCCC
CGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGC
CCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTT
ACCAAGGCTTGACATATACCGGAAACGGCCAGAGATGGTCGCCCCCTTGTGG
TCGGTATACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGG
TTAAGTCCCGCAACGAGCGCAACCCTTGTCCTGTGTTGCCAGCATGCCCTTCG
GGGTGATGGGGACTCACAGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGG
GGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACA
ATGGCCGGTACAAAGAGCTGCGATGCCGCGAGGCGGAGCGAATCTCAAAAA
GCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGT
TGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGT
ACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCC
AACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGGCGATTGGGACGAA
GTCGTAACAAGGTAGCCGTACCGGAAGGTGCGG

16S rRNA gene sequence of *Streptomyces* sp. 20K (K10093)

TCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAGC
CTTTCGGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCC
CTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAACACTCT
GTCCCGCATGGGACGGGGTTAAAAGCTCCGGCGGTGAAGGATGAGCCCGCG
GCCTATCAGCTTGTTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTAGC
CGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTC
CTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGC
AGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCA
GGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACG
TGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGG
GCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGATGTGAAAGCCCGGGGCT
TAACCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGGTAGGGGAGAT
CGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGT
GGCGAAGGCGGATCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGG
GGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGGGAAC
TAGGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACGCATTAAGTTC
CCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGG
GCCCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACC
TTACCAAGGCTTGACATATACCGGAAAGCATCAGAGATGGTGCCCCCCTTGT
GGTCGGTATACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTG
GGTTAAGTCCCGCAACGAGCGCAACCCTTGTTCTGTGTTGCCAGCATGCCCTT
CGGGGTGATGGGGACTCACAGGAGACTGCCGGGGTCAACTCGGAGGAAGGT
GGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTA
CAATGGCCGGTACAATGAGCTGCGATGCCGCGAGGCGGAGCGAATCTCAAA
AAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGA
GTTGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTT
GTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGC
CCAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGGCGATTGGGACGA
AGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGG

Table A. Similarity values and nucleotide number differences of 16S rRNA gene region sequence between test isolates and type species.

No. Isolate	Name	Accession	Pairwise Similarity (%)
16K (K21147) & 6K (K18126)	*Lentzea flaviverrucosa*	AF183957	99.15 (12/1406)
	Lentzea violacea	AJ242633	98.96 (15/1437)
	Lentzea albida	AB006176	98.61 (20/1437)
	Lentzea waywayandensis	AF114813	98.54 (21/1438)
	Lentzea albidocapillata	JOEA01000052	98.47 (22/1439)
13K (K02008) & 4K (K20134)	*Amycolatopsis xuchangensis*	KP232909	99.15 (12)
	Amycolatopsis magusensis	HQ157190	98.82 (17/1438)
	Amycolatopsis lurida	AJ577997	97.83 (30/1385)
	Amycolatopsis nigrescens	ARVW01000001	97.71 (33/1438)
	Amycolatopsis keratiniphila	LQMT01000206	97.5 (36/1438)

CURRICULUM VITAE

I was born in Pshdar on the 26th of August 1988. I have finished primary, secondary and high school in a small town called Qaladze. I have completed my undergraduate education (2008-2011) at Sulaimani University- College of Science, Biology Department.

In 2012 & 2013, I have taught biology at Azadi evening school, and in 2014 I have taught biology at Nawroz high school in Qaladze. In 2015 I applied to study a Masters Degree in microbiology at Bingol University/ Turkey and I am currently at the final stages of my studies there.

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