7. **Culturable diversity: Identification of actinobacteria**

### 7.1. Introduction

Microorganisms especially actinomycetes should be characterized with multiple, readily decipherable and practically stable properties (Burkholder *et al.* 1954), including their morphological features. Because, single character alone will not be sufficient for the identification of the organism up to species level (Krasilnikov, 1960) and a well-defined laboratory procedure and combined technological advances would help define a species (Waksman, 1961).

In the early 1950s, it was felt that improved taxonomic criteria were necessary for classifying the actinomycetes. In this respect, advances made in microbial biochemistry have provided the basis for chemotaxonomy (cell wall analysis), and successfully used in microbial systematics. Analysis of cell wall components like amino acids and whole cell sugars helps the actinomycetes taxonomists to classify these organisms. In fact the cell wall and amino acid determination (Lechevalier and Lechevalier, 1970) has led to the reassessment of actinobacterial systematics and the assignment of taxa to several wall chemotypes (Suzuki *et al.*, 1993).

Molecular techniques are the major tools for the analysis of microorganisms. Hence, microbial taxonomists have taken up these advanced methods to classify, differentiate and define a species (Anderson and Wellington, 2001). Additionally, in the current classification scheme, all the taxa higher than the rank of a genus are distinguished primarily on the basis of taxon-specific 16S rRNA signature nucleotides (Zhi *et al.*, 2009).

In the present study, all the important characteristics (colony morphology, chemotaxonomy, cultural characteristics, micromorphology and molecular
characteristics) have been used to identify the strains (AUNIA-1, AUNIA-2, AUNIA-3, AUNIA-4, AUNIA-5, AUNIA-6, AUNIA-7, AUNIA-8, AUNIA-9, AUNIA-10, AUNIA-11, AUNIA-12, AUNIA-13, AUNIA-14, AUNIA-15, AUNIA-16, AUNIA-17, AUNIA-18, AUNIA-19, AUNIA-20 and AUNIA-21), isolated from the different coastal habitats of the Neil island.

7.2. Materials and methods

7.2.1. Taxonomic identification

Characterization and subsequent identification of the strains to genus level were made based on the criteria of Shirling and Gottlieb (1966), Lechevalier and Lechevalier (1970), Nonomura (1974) and Cummins and Harris (1958).

7.2.1.1. Genus level identification

7.2.1.1.1. Whole Cell Hydrolysis

Whole cell hydrolysis was made to release the amino acids. Harvested cells of each strain weighing 2 mg were placed in a culture tube and 2 ml of 6 N HCl was added. The samples were kept for 4 h in a sand bath. The culture tubes were cooled by keeping them at a room temperature of 28±2°C.

Hydrolysis was also done for releasing sugars. Harvested cells of each strain weighing 50 mg were placed in a culture tube and 2 ml of 1N H2SO4 was added. The samples were kept for 4 h in a sand bath. The culture tubes were cooled by keeping them at a room temperature of 28±2°C.

7.2.1.1.2. Thin Layer Chromatography (TLC)

Whole cell hydrolysate was spotted carefully on the TLC plates using a micropipette. Spots were of 2-5 mm in diameter. This was done by multiple applications on the same spot of very small portions of the sample, which were dried by hand drier.
7.2.1.3. Amino acids

Each sample of 2 µl was applied on the base lines of cellulose pre-coated TLC plate (20 cm x 20 cm); 1µl mixture of DAP isomers (Sigma) and 1 µl of amino acetic acid (glycine) were used as standards. Samples on TLC plate were allowed to run on the solvent system containing methanol: distilled water: 6 N HCl: pyridine (80:26:4:10). It took more than 4 hrs for development of spots. The spots were visualized by spraying with 0.2% Ninhydrin solution in acetone, followed by drying in hot air oven; spot of amino acid ran faster than DAP. The sample spots were immediately compared with the spots of the standards since spots gradually disappeared in few hours.

7.2.1.4. Whole-cell sugars

On a TLC plate (procedure as done for DAP), 2 µl of sample was spotted, along with 1 µl of sugar solutions as standards on the silica precoated TLC plates. Galactose, arabinose, xylose and mannose were the sugars used as standards. TLC plate was developed with the solvent mixture containing n-butanol: distilled water: pyridine: toluene (10:6:6:1 v/v). The developing time was more than four hours. Spots were visualized by spraying with aniline pthalate reagent (2.5 g of pthalic acid dissolved in 2 ml of aniline and made up to 100 ml with water saturated n-butanol). The sprayed plate was dried in a hot air oven. Hexoses appeared as yellowish brown spots and pentoses, as maroon coloured spots.

7.2.1.2. Molecular identification

The genus status was also confirmed based on the 16S rRNA gene sequencing, using the following methodology.
7.2.1.2.1. DNA extraction

Total genomic DNA was extracted from the actinobacterial broth by phenol-chloroform isoamyl alcohol method, which removes the protein and other cellular components from the nucleic acid to obtain the pure DNA.

Log phase culture (2 to 4 ml) was taken and centrifuged at 10,000 rpm for 10 minutes at 4°C. Centrifugation was repeated to wash the cells twice with 500 µl of TE buffer. The pellet was resuspended in 500 µl of TE buffer and incubated for 10 minutes in boiling water bath and centrifuged. After centrifugation, equal volume of Phenol: chloroform: isoamyl alcohol was added to the supernatant and centrifuged. To the aqueous phase, 0.1 volume of 3 M ammonium acetate (pH 5.2) and 2.5 volume of ice-cold ethanol were added and incubated at -20°C overnight. After incubation, sample was centrifuged at 10,000 rpm for 10 min at 4°C and 70% ethanol was added to wash the pellet and air dried. After drying, the DNA was resuspended in TE buffer (pH 8.0) and stored at 4°C. DNA sample (10 µl) was mixed with 2 µl of 6x loading dye and loaded in 1% agarose gel. The separated DNA was visualized by UV transilluminator.

7.2.1.2.2. Amplification of 16S rDNA

Each 50 µl amplification reaction contained 1 µl template DNA (50–200 ng), 5 µl 10 x PCR buffer, 1 µl each PCR primer (20 mM) (27F, 1492R), 1 µl dNTP mix (10 mM), 6 µl MgCl₂ (25 mM), 2.5 U Taq DNA polymerase, 2.5 µl DMSO and 31.5 µl sterile MilliQ water. The reaction conditions were initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 90 s. A final extension was performed at 72°C for 10 min
Culturable diversity: Identification of actinobacteria (Karuppiah et al., 2011). Reaction products were electrophoresed on a 1% agarose gel and checked with ethidium bromide under UV light and then purified.

7.2.1.2.3. 16S rDNA sequencing

The purified fragment was directly sequenced using a Ampli Tag FS DNA sequencing Kit (Applied Biosystem). The data were analyzed using applied biosystem DNA editing and assembly software and sequence comparisons were obtained using the Micro Seq Software.

7.2.1.2.4. Phylogenetic analysis

Sequence similarity search was made for the 16S rDNA sequence of all isolates by applying their sequence to BLAST search of the NCBI (National Centre for Biotechnological Information, USA). Phylogenetic analysis was performed using the software package MEGA (Molecular Evolutionary Genetics Analysis) version 5 (Kumar et al., 2001) after multiple alignment of data by CLUSTAL_X (Thompson et al., 1997). A phylogenetic tree was constructed using the neighbor-joining method of Saitou and Nei (1987) from $K_{\text{nucl}}$ values (Kimura, 1980). Topology of the phylogenetic tree was evaluated, using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

7.2.1.3. Species level identification

An identification key was developed by Nonomura (1974) to identify 458 species of actinomycetes belonging to the genus Streptomyces and Streptoverticillium has been referred. The key deals with the cultural characteristics such as aerial mass colour, melanoid pigments, reverse side pigments and soluble pigments. The key also gives the details of the micro morphological characteristics such as spore chain morphology. The biochemical character such as carbon source utilization has also been included in this identification key. The Nonomura (1974) key has been used to
identify the members of the genus *Streptomyces* at species level. Further, the characteristic feature of *Streptomyces* mentioned by Sezaki *et al.*, 1967, Hatano *et al.*, 1980, Park *et al.*, 2003, Laidi *et al.*, 2006, Goodfellow *et al.*, 2007 and Ostash *et al.*, 2011 has also been referred.

To identify the species belonging to the *Nocardiopsis*, the description mentioned in the Bergy’s Manual of Determinative Bacteriology (Lechevalier, 1989); Chun *et al.* (2000) and Chen *et al.* (2010) were referred. To identify the species belonging to *Nocardia* the description mentioned in the Cui *et al.* (2005); Xu *et al.* (2006) and Everest *et al.* (2011) were referred.

7.2.1.3.1. Aerial mass colour

Colour of the mature sporulating aerial mycelium was recorded in a simple way (white, grey, red, blue and violet).

7.2.1.3.2. Melanoid pigment

Grouping was made on the production of melanoid pigments (*i.e.* greenish brown, brownish black or distinct brown, pigment modified by other colours) on the medium.

7.2.1.3.3. Reverse side pigment

Actinobacterial strains were examined for their ability to produce characteristic pigments on the reverse side of the colony, namely, distinctive (+) and not distinctive or none (-). In case, a colour with low chroma as pale yellow, olive or yellowish brown occurred, it was included in the latter group (-).

7.2.1.3.4. Soluble pigment

Strains were examined for their ability to produce soluble pigments other than melanin: namely, produced (+) and not produced (-). The colour was recorded (red, orange, yellow, blue, green and violet).
7.2.1.3.5. Spore chain morphology

Characteristics of the spore bearing hyphae and spore chains were determined using direct microscopic examination of the culture surface. Adequate magnification (400X) was used to establish the presence or absence of spore chains and to observe the nature of sporophores.

Spore morphological characters of the strains were studied by inoculating a loopful of one week old cultures into 15% agar medium contained in test tubes, at 37°C. The actinobacteria were suspended and thoroughly mixed in the semisolid medium and one or two drops of the medium were aseptically pipetted on to a sterile glass slide. A drop of agar was spread well on the slide and allowed to solidify into a thin film so as to facilitate direct observation under the microscope. The cultures were incubated at 28 ± 2°C and examined periodically for the formation of aerial mycelium, sporophore structure and spore morphology.

7.2.1.3.6. Assimilation of carbon source

Ability of the actinobacterial strains in utilizing various carbon compounds as source of energy was studied, following the method recommended by International Streptomyces Project (Shirling and Gottlieb, 1966). Chemically pure carbon sources certified to be free of admixture with other carbohydrates and contaminating materials were used for this purpose. Carbon sources used for this test were arabinose, xylose, inositol, mannitol, rhamnose, sucrose, sorbitol and raffinose. These carbon sources were sterilized by ether sterilization without heating.

Media and plates were prepared and inoculated according to the convention of ISP project (Shirling and Gottlieb, 1966). For each of the carbon sources, utilization is expressed as positive (+), negative (-), or doubtful (±). In the doubtful strains, only a trace of growth slightly greater than that of the control was noticed.
7.3. Results

A total of 21 actinobacterial strains were isolated from the Neil island. Majority of the actinobacterial isolates belonged to the genus *Streptomyces* (53%), followed by *Nocardiopsis* (33%) and *Nocardia* (14%) (Fig. 7.1).

![Percentage composition of actinobacterial genera isolated from the Neil island.](image)

7.3.1. Taxonomical identification of actinobacteria

Out of the 21 strains isolated in the present study, 11 strains (AUNIA-2, AUNIA-4, AUNIA-5, AUNIA-8, AUNIA-11, AUNIA-12, AUNIA-13, AUNIA-14, AUNIA-15, AUNIA-17 and AUNIA-18) showed the presence of LL-DAP along with glycine of peptidoglycan layer with no characteristic sugar pattern, indicating that these strains belong to the cell wall chemotype I (Table 7.1). The genera belonging to the cell wall type-I are *Streptomyces, Streptoverticillium, Chainia, Actinopycnidium, Actinosporangium, Elyptosporangium, Microellobosporia, Sporichthya* and *Intrasporangium* (Lechevalier and Lechevalier, 1970). It is important to note that the
presence of spores in a long chain occurring on the aerial mycelium and branched nature of the substrate mycelium in the 11 strains eliminates all the genera having the cell wall type-I except *Streptomyces* (Lechevalier and Lechevalier, 1970). This clearly indicated that these strains belong to the genus *Streptomyces*.

Out of the 21 strains isolated, the whole cell hydrolysates of 7 strains (AUNIA-1, AUNIA-3, AUNIA-6, AUNIA-7, AUNIA-9, AUNIA-19 and AUNIA-20) possessed meso-diaminopimelic acid as the only amino acid of peptidoglycan, but no diagnostic sugar was present indicating that these strains belong to the cell wall chemotype III (Lechevalier and Lechevalier, 1970), (Table 7.2). Based on the micromorphological and molecular characteristics studies, these strains were assigned to the genus, *Nocardiopsis*.

The whole-cell hydrolysates of the other 3 strains (AUNIA-10, AUNIA-16 and AUNIA-21) contained meso-DAP with arabinose and galactose as the characteristic sugar pattern and no xylose, indicating that these strains belong to the cell wall chemotype IV (Lechevalier and Lechevalier, 1970), (Table 7.3). Based on the micromorphological and molecular characteristics studies, these strains were assigned to the genus, *Nocardia*. 
Table 7.1. Chemo taxonomical characteristics of *Streptomyces* strains isolated from six stations of the Neil Island.

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Cell wall amino acids</th>
<th>Whole cell sugar</th>
<th>Cell wall chemo type</th>
<th>Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LL-DAP</td>
<td>Meso DAP</td>
<td>Glycine</td>
<td></td>
</tr>
<tr>
<td>AUNIA-2</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>* I</td>
</tr>
<tr>
<td>AUNIA-4</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>* I</td>
</tr>
<tr>
<td>AUNIA-5</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>* I</td>
</tr>
<tr>
<td>AUNIA-8</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>* I</td>
</tr>
<tr>
<td>AUNIA-11</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>* I</td>
</tr>
<tr>
<td>AUNIA-12</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>* I</td>
</tr>
<tr>
<td>AUNIA-13</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>* I</td>
</tr>
<tr>
<td>AUNIA-14</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>* I</td>
</tr>
<tr>
<td>AUNIA-15</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>* I</td>
</tr>
<tr>
<td>AUNIA-17</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>* I</td>
</tr>
<tr>
<td>AUNIA-18</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>* I</td>
</tr>
</tbody>
</table>

* indicates Presence, - indicates Absence, * No characteristic sugar pattern

Table 7.2. Chemo taxonomical characteristics of *Nocardiopsis* strains isolated from six stations of the Neil Island.

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Cell wall amino acids</th>
<th>Whole cell sugar</th>
<th>Cell wall chemo type</th>
<th>Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LL-DAP</td>
<td>Meso DAP</td>
<td>Glycine</td>
<td></td>
</tr>
<tr>
<td>AUNIA-1</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>* III</td>
</tr>
<tr>
<td>AUNIA-3</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>* III</td>
</tr>
<tr>
<td>AUNIA-6</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>* III</td>
</tr>
<tr>
<td>AUNIA-7</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>* III</td>
</tr>
<tr>
<td>AUNIA-9</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>* III</td>
</tr>
<tr>
<td>AUNIA-19</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>* III</td>
</tr>
<tr>
<td>AUNIA-20</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>* III</td>
</tr>
</tbody>
</table>

* indicates Presence, - indicates Absence, * No diagnostic sugar
Table 7.3. Chemo taxonomical characteristics of *Nocardia* strains isolated from six stations of the Neil Island.

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Cell wall amino acids</th>
<th>Whole cell sugars</th>
<th>Cell wall chemo type</th>
<th>Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LL-DAP</td>
<td>Meso DAP</td>
<td>Glycine</td>
<td>Arabinose</td>
</tr>
<tr>
<td>AUNIA-10</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>AUNIA-16</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>AUNIA-21</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+ indicates Presence, -indicates Absence

AUNIA-1

AUNIA-1 is a mesophilic actinobacterium which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into long and branched spore chains. A white colour aerial spore mass was formed on ISP2 agar. Reverse side pigment and soluble pigment were not produced on peptone yeast extract iron agar. Melanin pigment was also absent on ISP7 agar. Culture grew well when it was supplemented with the carbon sources viz. xylose and rhamnose. No growth was observed in arabinose, inositol, mannitol, sucrose and raffinose.

A 1171 bp of 16S rDNA sequence was determined for the strain AUNIA-1, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and an accession number (KF548134) was obtained. The sequence similarity studies of the strain AUNIA-1 exhibited close phylogenetic relationship with the members of the genus *Nocardiopsis*. Comparison of the 16S rRNA gene sequence (1171 bp) of the strain AUNIA-1 with the previously obtained sequences of *Nocardiopsis* and its related species deposited in GenBank (NCBI) was made and the rooted phylogenetic tree (Fig. 7.2) indicated that the strain AUNIA-1 forms a branch with *N. dassonvillei* subsp. *dassonvillei* with 99.8% similarity (Table 7.4). The *Actinoplanes* sp. (L41047.1) served as an outgroup.
Strain AUNIA-1

White coloured aerial mycelium

Long and branched spore chains under 400X

Fig. 7.2. Phylogenetic tree based on 16S rRNA gene sequences showing the positions of AUNIA-1 and related strains. Only bootstrap values expressed as percentages of 1000 replications are shown at the branch points.

Table 7.4. Levels of 16S rDNA sequence similarity between the strain AUNIA-1 and the representative species.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Representative species</th>
<th>Accession Number (Gen Bank)</th>
<th>Similarity level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUNIA-1</td>
<td><em>N. dassonvillei</em> subsp. <em>dassonvillei</em></td>
<td>KF306367.1</td>
<td>99.8</td>
</tr>
<tr>
<td>AUNIA-1</td>
<td><em>N. lucentensis</em></td>
<td>KC759324.1</td>
<td>99.8</td>
</tr>
<tr>
<td>AUNIA-1</td>
<td><em>N. synnemataformans</em></td>
<td>KF384488.1</td>
<td>99.5</td>
</tr>
<tr>
<td>AUNIA-1</td>
<td><em>N. dassonvillei</em></td>
<td>KC493994.1</td>
<td>99.5</td>
</tr>
<tr>
<td>AUNIA-1</td>
<td><em>Actinoplanes</em> sp.</td>
<td>L410447.1</td>
<td>87</td>
</tr>
</tbody>
</table>
So, a comparison was made between the strain AUNIA-1 and its closest phylogenetic member *N. dassonvillei subsp. dassonvillei* for their cultural, morphological and biochemical properties. Results showed that *N. dassonvillei subsp. dassonvillei* differed from the strain AUNIA-1 in the production of reverse side and soluble pigment. Utilization of carbon sources (arabinose, mannitol, rhamnose and sucrose) also varied (Table 7.5). All the other characters were similar to those of *N. dassonvillei*. Hence, the strain AUNIA-1 is considered to be closely related to *N. dassonvillei*.

### Table 7.5. Cultural, morphological and biochemical characteristics of the strain AUNIA-1 and the closely related *Nocardiopsis* species.

<table>
<thead>
<tr>
<th>Characters studied (as per Bergy’s Manual of Determinative Bacteriology)</th>
<th>Strain AUNIA-1</th>
<th><em>N. dassonvillei subsp. dassonvillei</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>White</td>
<td>White</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reverse side pigment</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Soluble pigment</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Spore chain</td>
<td>Long and branched</td>
<td>Long and branched</td>
</tr>
</tbody>
</table>

**Carbon source assimilation**

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Strain AUNIA-1</th>
<th><em>N. dassonvillei subsp. dassonvillei</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabinose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Xylose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*+ indicates Positive, - indicates Negative*
AUNIA-2

AUNIA-2 is a mesophilic actinobacterium which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into short spiral spore chains. A white colour aerial spore mass was formed on ISP2 agar. Reverse side pigment and soluble pigment were not produced on peptone yeast extract iron agar. Melanin pigment was also absent on ISP7 agar. Culture grew well when it was supplemented with the carbon sources viz. inositol, mannitol and sorbitol. No growth was observed in xylose.

A 1288 bp of 16S rDNA sequence was determined for the strain AUNIA-2, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and an accession number (KF548139) was obtained. The sequence similarity studies of the strain AUNIA-2 exhibited close phylogenetic relationship with the members of the genus *Streptomyces*. Comparison of the 16S rRNA gene sequence (1288 bp) of the strain AUNIA-2 with the previously obtained sequences of *Streptomyces* and its related species deposited in GenBank (NCBI) was made and the rooted phylogenetic tree (Fig. 7.3) indicated that the strain AUNIA-2 forms a branch with *S. misawanensis* with 99.6% similarity (Table 7.6). The *Actinoplanes* sp. (L41047.1) served as an outgroup.
**Strain AUNIA-2**

**White coloured aerial mycelium**

**Spiral spore chains under 400X**

**Fig. 7.3. Phylogenetic tree based on 16S rRNA gene sequences showing the positions of AUNIA-2 and related strains. Only bootstrap values expressed as percentages of 1000 replications are shown at the branch points.**

**Table 7.6. Levels of 16S rDNA sequence similarity between the strain AUNIA-2 and the representative species.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Representative species</th>
<th>Accession Number (Gen Bank)</th>
<th>Similarity level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUNIA-2</td>
<td><em>S. misawanensis</em></td>
<td>AB184533.1</td>
<td>99.6</td>
</tr>
<tr>
<td>AUNIA-2</td>
<td><em>S. triostinicus</em></td>
<td>EU635725.1</td>
<td>98.1</td>
</tr>
<tr>
<td>AUNIA-2</td>
<td><em>S. recifensis</em></td>
<td>EU216596.1</td>
<td>98.1</td>
</tr>
<tr>
<td>AUNIA-2</td>
<td><em>S. niveoruber</em></td>
<td>FN178390.1</td>
<td>97.7</td>
</tr>
<tr>
<td>AUNIA-2</td>
<td><em>Actinoplanes</em> sp.</td>
<td>L41047.1</td>
<td>88.5</td>
</tr>
</tbody>
</table>
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So, a comparison was made between the strain AUNIA-2 and its closest phylogenetic member *S. misawanensis* for their cultural, morphological and biochemical properties. Results showed that *S. misawanensis* differed from the strain AUNIA-2 in the utilization of carbon source *viz.* xylose (Table 7.7) and all the other characters were similar to the characters of the strain, *S. misawanensis*. Hence, the strain AUNIA-2 has been tentatively identified as *S. misawanensis*.

**Table 7.7. Cultural, morphological and biochemical characteristics of the strain AUNIA-2 and the closely related *Streptomyces* species.**

<table>
<thead>
<tr>
<th>Characters studied (as per Sezaki <em>et al.</em>, 1967)</th>
<th>Strain AUNIA-2</th>
<th><em>S. misawanensis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>White</td>
<td>White to light grayish (Calcium malate agar)</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reverse side pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soluble pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spore chain</td>
<td>Spiral</td>
<td>Spiral</td>
</tr>
<tr>
<td><strong>Carbon source assimilation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ *indicates* Positive, - *indicates* Negative

**AUNIA-3**

AUNIA-3 is a mesophilic actinobacterium which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into long and branched spore chains. A white colour aerial spore mass was formed on ISP2 agar. Reverse side pigment and soluble pigments were not produced on peptone yeast extract iron agar. Melanin pigment was also absent on ISP7 agar. Culture grew well when it was supplemented with the carbon sources *viz.* xylose, inositol, sucrose, mannitol and rhamnose. No growth was observed in arabinose and raffinose.
A 1433 bp of 16S rDNA sequence was determined for the strain AUNIA-3, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and an accession number (KF548135) was obtained. The sequence similarity studies of the strain AUNIA-3 exhibited close phylogenetic relationship with the members of the genus *Nocardiopsis*. Comparison of the 16S rRNA gene sequence (1433 bp) of the strain AUNIA-3 with the previously obtained sequences of *Nocardiopsis* and its related species deposited in GenBank (NCBI) was made and the rooted phylogenetic tree (Fig. 7.4) indicated that the strain AUNIA-3 forms a branch with *N. dassonvillei* with 98.5% similarity (Table 7.8). The *Actinoplanes* sp. (L41047.1) served as an outgroup.

**Strain AUNIA-3**

*White coloured aerial mycelium*  
*Long and branched spore chains under 400X*
Culturable diversity: Identification of actinobacteria

Fig. 7.4. Phylogenetic tree based on 16S rRNA gene sequences showing the positions of AUNIA-3 and related strains. Only bootstrap values expressed as percentages of 1000 replications are shown at the branch points.

Table 7.8. Levels of 16S rDNA sequence similarity between the strain AUNIA-3 and the representative species.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Representative species</th>
<th>Accession Number (Gen Bank)</th>
<th>Similarity level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUNIA-3</td>
<td><em>N. dassonvillei</em></td>
<td>FJ486356.1</td>
<td>98.5</td>
</tr>
<tr>
<td>AUNIA-3</td>
<td><em>N. lucentensis</em></td>
<td>KC759324.1</td>
<td>98.5</td>
</tr>
<tr>
<td>AUNIA-3</td>
<td><em>N. synnemataformans</em></td>
<td>KF384488.1</td>
<td>98.3</td>
</tr>
<tr>
<td>AUNIA-3</td>
<td><em>N. antartica</em></td>
<td>X97885.1</td>
<td>98.3</td>
</tr>
<tr>
<td>AUNIA-3</td>
<td>Actinoplanes sp.</td>
<td>L41047.1</td>
<td>87.8</td>
</tr>
</tbody>
</table>

So, a comparison was made between the strain AUNIA-3 and its closest phylogenetic member *N. dassonvillei* for their cultural, morphological and biochemical properties. Results showed that *N. dassonvillei* differed from the strain AUNIA-3 in the production of reverse side pigment and utilization of carbon sources also varied (arabinose, inositol and rhamnose) (Table 7.9). All the other characters were similar to those of *N. dassonvillei*. Hence, the strain AUNIA-3 is considered to be closely related to *N. dassonvillei*. 
Table 7.9. Cultural, morphological and biochemical characteristics of the strain AUNIA-3 and the closely related Nocardiopsis species.

<table>
<thead>
<tr>
<th>Characters studied (as per Bergy’s Manual of Determinative Bacteriology)</th>
<th>Strain AUNIA-3</th>
<th>N. dassonvilllei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>White</td>
<td>White</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reverse side pigment</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Soluble pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spore chain</td>
<td>Long and branched</td>
<td>Long and branched</td>
</tr>
</tbody>
</table>

**Carbon source assimilation**

<table>
<thead>
<tr>
<th>Carbon sources</th>
<th>Strain AUNIA-3</th>
<th>N. dassonvilllei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabinose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Xylose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*+ indicates Positive, - indicates Negative*

**AUNIA-4**

AUNIA-4 is a mesophilic actinobacterium which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into rectiflexible spore chains. A white colour aerial spore mass was formed on ISP2 agar. Reverse side pigment and soluble pigments were not produced on peptone yeast extract iron agar. Melanin pigment production was absent on ISP7 agar. Culture grew well when it was supplemented with the carbon sources viz. arabinose, xylose, inositol, sucrose and raffinose. No growth was observed in mannitol and rhamnose.

A 1266 bp of 16S rDNA sequence was determined for the strain AUNIA-4, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and an accession number (KF548129) was obtained. The sequence similarity studies of the strain AUNIA-4 exhibited close phylogenetic relationship with the members of the genus *Streptomyces*. Comparison of the 16S rRNA gene sequence (1266 bp) of the strain AUNIA-4 with the previously obtained sequences of
Streptomyces and its related species deposited in GenBank (NCBI) was made and the rooted phylogenetic tree (Fig. 7.5) indicated that the strain AUNIA-4 forms a branch with S. puniceus with 98.4% similarity (Table 7.10). The Actinoplanes sp. (L41047.1) served as an outgroup.

**Strain AUNIA-4**

![White coloured aerial mycelium](image1)

![Rectiflexible spore chains under 400X](image2)

Fig. 7.5. Phylogenetic tree based on 16S rRNA gene sequences showing the positions of AUNIA-4 and related strains. Only bootstrap values expressed as percentages of 1000 replications are shown at the branch points.
Table 7.10. Levels of 16S rDNA sequence similarity between the strain AUNIA-4 and the representative species.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Representative species</th>
<th>Accession Number (Gen Bank)</th>
<th>Similarity level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUNIA-4</td>
<td><em>S. puniceus</em></td>
<td>KC790270.1</td>
<td>98.4</td>
</tr>
<tr>
<td>AUNIA-4</td>
<td><em>S. alboloncus</em></td>
<td>JN609385.1</td>
<td>98.4</td>
</tr>
<tr>
<td>AUNIA-4</td>
<td><em>S. cavourensis</em></td>
<td>HQ610450.1</td>
<td>98.4</td>
</tr>
<tr>
<td>AUNIA-4</td>
<td><em>S. fulvorobeus</em></td>
<td>NR041196.1</td>
<td>98.3</td>
</tr>
<tr>
<td>AUNIA-4</td>
<td>Actinoplanes sp.</td>
<td>L41047.1</td>
<td>87.1</td>
</tr>
</tbody>
</table>

So, a comparison was made between the strain AUNIA-4 and its closest phylogenetic member *S. puniceus* for their cultural, morphological and biochemical properties. Results showed that *S. puniceus* differed from the strain AUNIA-4 in the aerial mass color and production of melanoid pigment and utilization of carbon sources (mannitol and rhamnose) (Table 7.11). All the other characters were similar to those of *S. puniceus*. Hence, the strain AUNIA-4 is considered to be closely related to *S. puniceus*.

Table 7.11. Cultural, morphological and biochemical characteristics of the strain AUNIA-4 and the closely related *Streptomyces* species.

<table>
<thead>
<tr>
<th>Character studied (as per Park et al., 2003)</th>
<th>Strain AUNIA-4</th>
<th><em>S. puniceus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>White</td>
<td>Purple (or) Red</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Reverse side pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soluble pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spore chain</td>
<td>Rectiflexible</td>
<td>Rectiflexible</td>
</tr>
<tr>
<td><strong>Carbon source assimilation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Xylose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*+ indicates Positive, - indicates Negative*
AUNIA-5

AUNIA-5 is a mesophilic actinobacterium which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into rectiflexible chains. A white colour aerial spore mass was formed on ISP2 agar. Reverse side pigment and soluble pigments were not produced on peptone yeast extract iron agar. Melanin pigment was also absent on ISP7 agar. Culture grew well when it was supplemented with the carbon sources viz. inositol, sucrose and raffinose. No growth was observed in arabinose, xylose, mannitol and rhamnose.

A 1428 bp of 16S rDNA sequence was determined for the strain AUNIA-5, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and an accession number (KF548128) was obtained. The sequence similarity studies of the strain AUNIA-5 exhibited close phylogenetic relationship with the members of the genus Streptomyces. Comparison of the 16S rRNA gene sequence (1428 bp) of the strain AUNIA-5 with the previously obtained sequences of Streptomyces and its related species deposited in GenBank (NCBI) was made and the rooted phylogenetic tree (Fig. 7.6) indicated that the strain AUNIA-5 forms a branch with S. griseobrunneus with 98.7% similarity (Table 7.12). The Actinoplanes sp. (L41047.1) served as an outgroup.
Strain AUNIA-5

White coloured aerial mycelium

Rectiflexible spore chains under 400X

Fig. 7.6. Phylogenetic tree based on 16S rRNA gene sequences showing the positions of AUNIA-5 and related strains. Only bootstrap values expressed as percentages of 1000 replications are shown at the branch points.

Table 7.12. Levels of 16S rDNA sequence similarity between the strain AUNIA-5 and the representative species.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Representative species</th>
<th>Accession Number (Gen Bank)</th>
<th>Similarity level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUNIA-5</td>
<td><em>S. griseobrunneus</em></td>
<td>AB249912.1</td>
<td>98.7</td>
</tr>
<tr>
<td>AUNIA-5</td>
<td><em>S. tsusimaensis</em></td>
<td>AB184478.1</td>
<td>98.7</td>
</tr>
<tr>
<td>AUNIA-5</td>
<td><em>S. bacillaris</em></td>
<td>NR041146.1</td>
<td>98.1</td>
</tr>
<tr>
<td>AUNIA-5</td>
<td><em>S. badius</em></td>
<td>AB184114.1</td>
<td>98.1</td>
</tr>
<tr>
<td>AUNIA-5</td>
<td><em>Actinoplanes sp.</em></td>
<td>L41047.1</td>
<td>87.1</td>
</tr>
</tbody>
</table>
So, a comparison was made between the strain AUNIA-5 and its closest phylogenetic member *S. griseobrunneus* for their cultural, morphological and biochemical properties. Results showed that *S. griseobrunneus* differed from the strain AUNIA-5 in the aerial mass color and production of melanoid pigment. Utilization of carbon also varied (xylose, inositol, mannitol, sucrose and raffinose) (Table 7.13). All the other characters were similar to those of *S. griseobrunneus*. Hence, the strain AUNIA-5 is considered to be closely related to *S. griseobrunneus*.

**Table 7.13. Cultural, morphological and biochemical characteristics of the strain AUNIA-5 and the closely related *Streptomyces* species.**

<table>
<thead>
<tr>
<th>Character studied (as per Nonomura key)</th>
<th>Strain AUNIA-5</th>
<th><em>S. griseobrunneus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>White</td>
<td>Yellow</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Reverse side pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soluble pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spore chain</td>
<td>Rectiflexible</td>
<td>Rectiflexible</td>
</tr>
<tr>
<td><strong>Carbon source assimilation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xylose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

*indicates Positive, - indicates Negative

**AUNIA-6**

AUNIA-6 is a mesophilic actinobacterium which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into long and branched spore chains. A greyishwhite colour aerial spore mass was formed on ISP2 agar. Reverse side pigment and soluble pigments were not produced on peptone yeast extract iron agar. Melanin pigment was also absent on ISP7 agar. Culture grew well
Culturable diversity: Identification of actinobacteria

when it was supplemented with the carbon sources *viz.* mannitol, sucrose and raffinose. No growth was observed in arabinose, xylose, inositol and rhamnose.

A 1206 bp of 16S rDNA sequence was determined for the strain AUNIA-6, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and an accession number (KF548126) was obtained. The sequence similarity studies of the strain AUNIA-6 exhibited close phylogenetic relationship with the members of the genus *Nocardiopsis*. Comparison of the 16S rRNA gene sequence (1206 bp) of the strain AUNIA-6 with the previously obtained sequences of *Nocardiopsis* and its related species deposited in GenBank (NCBI) was made and the rooted phylogenetic tree (Fig. 7.7) indicated that the strain AUNIA-6 forms a branch with *Nocardiopsis dassonvillei* subsp. *dassonvillei* with 98.8% similarity (Table 7.14). The *Actinoplanes* sp. (L41047.1) served as an outgroup.

**Strain AUNIA-6**

Greyishwhite coloured aerial mycelium

Long and branched spore chains under 400X
Table 7.14. Levels of 16S rDNA sequence similarity between the strain AUNIA-6 and the representative species.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Representative species</th>
<th>Accession Number (Gen Bank)</th>
<th>Similarity level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUNIA-6</td>
<td>N. dassonvillei subsp. dassonvillei</td>
<td>KF306364.1</td>
<td>98.8</td>
</tr>
<tr>
<td>AUNIA-6</td>
<td>N. lucentensis</td>
<td>KC759324.1</td>
<td>98.8</td>
</tr>
<tr>
<td>AUNIA-6</td>
<td>N. dassonvillei</td>
<td>EU8496121.1</td>
<td>98.5</td>
</tr>
<tr>
<td>AUNIA-6</td>
<td>Nocardiopsis sp.</td>
<td>JF793519.1</td>
<td>98.5</td>
</tr>
<tr>
<td>AUNIA-6</td>
<td>Actinoplanes sp.</td>
<td>L41047.1</td>
<td>88.1</td>
</tr>
</tbody>
</table>

So, a comparison was made between the strain AUNIA-6 and its closest phylogenetic member *N. dassonvillei* subsp. *dassonvillei* for their cultural and morphological properties. Results showed that *N. dassonvillei* subsp. *dassonvillei* differed from the strain AUNIA-6 in the aerial mass colour, reverse side and soluble pigment production. Utilization of carbon sources (arabinose, xylose and raffinose) also varied (Table 7.15). All the other characters were similar to those of *N. dassonvillei* subsp. *dassonvillei*. Hence, the strain AUNIA-6 is considered to be closely related to *N. dassonvillei* subsp. *dassonvillei*.
Table 7.15. Cultural, morphological and biochemical characteristics of the strain AUNIA-6 and the closely related *Nocardiopsis* species.

<table>
<thead>
<tr>
<th>Character studied (as per Bergey’s manual)</th>
<th>Strain AUNIA-6</th>
<th><em>N. dassonvillei subsp. dassonvillei</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>Greyish white</td>
<td>White</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reverse side pigment</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Soluble pigment</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Spore chain</td>
<td>Long and branched</td>
<td>Long and branched</td>
</tr>
</tbody>
</table>

**Carbon source assimilation**

<table>
<thead>
<tr>
<th></th>
<th>Strain AUNIA-6</th>
<th><em>N. dassonvillei subsp. dassonvillei</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabinose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Xylose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

*+ indicates Positive, - indicates Negative*

**AUNIA-7**

AUNIA-7 is a mesophilic actinobacterium which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into long and branched spore chains. A yellow colour aerial spore mass was formed on ISP2 agar. Reverse side pigment and soluble pigments were not produced on peptone yeast extract iron agar. Melanin pigment was also absent on ISP7 agar. Culture grew well when it was supplemented with the carbon sources *viz.* xylose and sucrose. No growth was observed in arabinose, mannitol and raffinose.

A 1146 bp of 16S rDNA sequence was determined for the strain AUNIA-7, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and an accession number (KF548132) was obtained. The sequence similarity studies of the strain AUNIA-7 exhibited close phylogenetic relationship with the members of the genus *Nocardiopsis*. Comparison of the 16S rRNA gene sequence (1146 bp) of the strain AUNIA-7 with the previously obtained sequences of
*Nocardiopsis* and its related species deposited in GenBank (NCBI) was made and the rooted phylogenetic tree (Fig. 7.8) indicated that the strain AUNIA-7 forms a branch with *N. terrae* with 98.6% similarity (Table 7.16). The *Actinoplanes* sp. (L41047.1) served as an outgroup.

**Strain AUNIA-7**

![Yellow coloured aerial mycelium](image1)

![Long and branched spore chains under 400X](image2)

Fig. 7.8. Phylogenetic tree based on 16S rRNA gene sequences showing the positions of AUNIA-7 and related strains. Only bootstrap values expressed as percentages of 1000 replications are shown at the branch points.
Table 7.16. Levels of 16S rDNA sequence similarity between the strain AUNIA-7 and the representative species.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Representative species</th>
<th>Accession Number (Gen Bank)</th>
<th>Similarity level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUNIA-7</td>
<td><em>N. terrae</em></td>
<td>DQ387958.1</td>
<td>98.6</td>
</tr>
<tr>
<td>AUNIA-7</td>
<td><em>N. salina</em></td>
<td>JF445136.1</td>
<td>98.5</td>
</tr>
<tr>
<td>AUNIA-7</td>
<td><em>N. alba</em></td>
<td>KF445136.1</td>
<td>98.4</td>
</tr>
<tr>
<td>AUNIA-7</td>
<td><em>N. exhalans</em></td>
<td>EU430537.1</td>
<td>98.3</td>
</tr>
<tr>
<td>AUNIA-7</td>
<td><em>Actinoplanes sp.</em></td>
<td>L41047.1</td>
<td>91.7</td>
</tr>
</tbody>
</table>

So, a comparison was made between the strain AUNIA-7 and its closest phylogenetic member *N. terrae* for their cultural, morphological and biochemical properties. Results showed that *N. terrae* differed from the strain AUNIA-7 in the aerial mass color and production of soluble pigment. Utilization of carbon source (raffinose) also varied (Table 7.17). All the other characters were similar to those of *N. terrae*. Hence, the strain AUNIA-7 is considered to be closely related to *N. terrae*.

Table 7.17. Cultural, morphological and biochemical characteristics of the strain AUNIA-7 and the closely related Nocardiopsis species.

<table>
<thead>
<tr>
<th>Character studied (as per Chen et al., 2010)</th>
<th>Strain AUNIA-7</th>
<th><em>N. terrae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>Yellow</td>
<td>White</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reverse side pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soluble pigment</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Spore chain</td>
<td>Long and branched</td>
<td>Long and branched</td>
</tr>
<tr>
<td><strong>Carbon source assimilation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xylose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

* + indicates Positive, - indicates Negative
AUNIA-8

AUNIA-8 is a mesophilic actinobacterium which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into retinaculiaperti spore chains. A white colour aerial spore mass was formed on ISP2 agar. Reverse side pigment and soluble pigments were not produced on peptone yeast extract iron agar. Melanin pigment was also absent on ISP7 agar. Culture grew well when it was supplemented with the carbon sources *viz.* inositol, mannitol and rhamnose. No growth was observed in arabinose, xylose, sucrose and raffinose.

A 1126 bp of 16S rDNA sequence was determined for the strain AUNIA-8, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and an accession number (KF548130) was obtained. The sequence similarity studies of the strain AUNIA-8 exhibited close phylogenetic relationship with the members of the genus *Streptomyces*. Comparison of the 16S rRNA gene sequence (1126 bp) of the strain AUNIA-8 with the previously obtained sequences of *Streptomyces* and its related species deposited in GenBank (NCBI) was made and the rooted phylogenetic tree (Fig. 7.9) indicated that the strain AUNIA-8 forms a branch with *S. tendae* with 99.2% similarity (Table 7.18). The *Actinoplanes* sp. (L41047.1) served as an outgroup.
Culturable diversity: Identification of actinobacteria

Strain AUNIA-8

![White coloured aerial mycelium](image1)

![Retinaculiaperti spore chains under 400X](image2)

Fig. 7.9. Phylogenetic tree based on 16S rRNA gene sequences showing the positions of AUNIA-8 and related strains. Only bootstrap values expressed as percentages of 1000 replications are shown at the branch points.
Table 7.18. Levels of 16S rDNA sequence similarity between the strain AUNIA-8 and the representative species.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Representative species</th>
<th>Accession Number (Gen Bank)</th>
<th>Similarity level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUNIA-8</td>
<td><em>S. tendae</em></td>
<td>KF454841.1</td>
<td>99.2</td>
</tr>
<tr>
<td>AUNIA-8</td>
<td><em>S. griseorubens</em></td>
<td>EU570365.1</td>
<td>99.2</td>
</tr>
<tr>
<td>AUNIA-8</td>
<td><em>S. fragilis</em></td>
<td>NR043381.1</td>
<td>99.2</td>
</tr>
<tr>
<td>AUNIA-8</td>
<td><em>S. violaceorubidus</em></td>
<td>NR042309.1</td>
<td>99.2</td>
</tr>
<tr>
<td>AUNIA-8</td>
<td>Actinoplanes sp.</td>
<td>L41047.1</td>
<td>91.2</td>
</tr>
</tbody>
</table>

So, a comparison was made between the strain AUNIA-8 and its closest phylogenetic member *S. tendae* for their cultural and morphological properties. Results showed that *S. tendae* differed from the strain AUNIA-8 in the aerial mass color and utilization of carbon sources (arabinose, xylose, sucrose and raffinose) also varied (Table 7.19). All the other characters were similar to those of *S. tendae*. Hence, the strain AUNIA-8 is considered to be closely related to *S. tendae*.

Table 7.19. Cultural, morphological and biochemical characteristics of the strain AUNIA-8 and the closely related *Streptomyces* species.

<table>
<thead>
<tr>
<th>Character studied (as per Laidi et al., 2006)</th>
<th>Strain AUNIA-8</th>
<th><em>S. tendae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>White</td>
<td>Grey</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reverse side pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soluble pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spore chain</td>
<td>Retinaculiaperti</td>
<td>Retinaculiaperti</td>
</tr>
<tr>
<td>Carbon source assimilation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Xylose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

*+ indicates Positive, - indicates Negative*
AUNIA-9

AUNIA-9 is a mesophilic actinobacterium which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into long and branched spore chains. A white colour aerial spore mass was formed on ISP2 agar. Reverse side pigment and soluble pigments were not produced on peptone yeast extract iron agar. Melanin pigment was also absent on ISP7 agar. Culture grew well when it was supplemented with the carbon sources \textit{viz.} inositol, mannitol and sucrose. No growth was observed in arabinose, xylose, rhamnose and raffinose.

A 1432 bp of 16S rDNA sequence was determined for the strain AUNIA-9, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and an accession number (KF548133) was obtained. The sequence similarity studies of the strain AUNIA-9 exhibited close phylogenetic relationship with the members of the genus \textit{Nocardiopsis}. Comparison of the 16S rRNA gene sequence (1432 bp) of the strain AUNIA-9 with the previously obtained sequences of \textit{Nocardiopsis} and its related species deposited in GenBank (NCBI) was made the rooted phylogenetic tree (Fig. 7.10) indicated that the strain AUNIA-9 forms a branch with \textit{N. alba} with 99.7\% similarity (Table 7.20). The \textit{Actinoplanes} sp. (L41047.1) served as an outgroup.
Strain AUNIA-9

Fig. 7.10. Phylogenetic tree based on 16S rRNA gene sequences showing the positions of AUNIA-9 and related strains. Only bootstrap values expressed as percentages of 1000 replications are shown at the branch points.
Table 7.20. Levels of 16S rDNA sequence similarity between the strain AUNIA-9 and the representative species.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Representative species</th>
<th>Accession Number (Gen Bank)</th>
<th>Similarity level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUNIA-9</td>
<td>N. alba</td>
<td>FJ481638.1</td>
<td>99.7</td>
</tr>
<tr>
<td>AUNIA-9</td>
<td>N. metallicus</td>
<td>JX254903.1</td>
<td>98.7</td>
</tr>
<tr>
<td>AUNIA-9</td>
<td>N. lucentensis</td>
<td>NR026342.1</td>
<td>98.6</td>
</tr>
<tr>
<td>AUNIA-9</td>
<td>N. terrae</td>
<td>KC493982.1</td>
<td>98.2</td>
</tr>
<tr>
<td>AUNIA-9</td>
<td>Actinoplanes sp</td>
<td>L41047.1</td>
<td>87.9</td>
</tr>
</tbody>
</table>

So, a comparison was made between the strain AUNIA-9 and its closest phylogenetic member *N. alba* for their cultural, morphological and biochemical properties. Results showed that *N. alba* differed from the strain AUNIA-9 in the production of soluble pigment and utilization of carbon sources (inositol, mannitol and sucrose) (Table 7.21). All the other characters were similar to those of *N. alba*. Hence, the strain AUNIA-9 is considered to be closely related to *N. alba*.

Table 7.21. Cultural, morphological and biochemical characteristics of the strain AUNIA-9 and the closely related *Nocardiopsis* species.

<table>
<thead>
<tr>
<th>Character studied (as per Chun <em>et al.</em>, 2000)</th>
<th>Strain AUNIA-9</th>
<th><em>N. alba</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>White</td>
<td>White</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reverse side pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soluble pigment</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Spore chain</td>
<td>Long and branched</td>
<td>Long and branched</td>
</tr>
<tr>
<td>Carbon source assimilation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aarbinose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xylose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Inositol</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Raffinose</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ indicates Positive, - indicates Negative
AUNIA-10

AUNIA-10 is a mesophilic actinobacterium which forms an extensively branched substrate mycelium and aerial hyphae. A whitishyellow colour aerial spore mass was formed on ISP2 agar. Reverse side pigment and soluble pigments were not produced on peptone yeast extract iron agar. Melanin pigment was also absent on ISP7 agar. Culture grew well when it was supplemented with the carbon sources viz. mannitol, rhamnose and raffinose. No growth was observed in arabinose, xylose, inositol and sucrose.

A 1031 bp of 16S rDNA sequence was determined for the strain AUNIA-10, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and an accession number (KF668281) was obtained. The sequence similarity studies of the strain AUNIA-10 exhibited close phylogenetic relationship with the members of the genus *Nocardia*. Comparison of the 16S rRNA gene sequence (1031 bp) of the strain AUNIA-10 with the previously obtained sequences of *Nocardia* and its related species deposited in GenBank (NCBI) was made and the rooted phylogenetic tree (Fig. 7.11) indicated that the strain AUNIA-10 forms a branch with *Nocardia rhamnosiphila* with 99.9% similarity (Table 7.22). The *Actinoplanes* sp. (L41047.1) served as an outgroup.
Strain AUNIA-10

Spore chains under 400X

Fig. 7.11. Phylogenetic tree based on 16S rRNA gene sequences showing the positions of AUNIA-10 and related strains. Only bootstrap values expressed as percentages of 1000 replications are shown at the branch points.

Table 7.22. Levels of 16S rDNA sequence similarity between the strain AUNIA-10 and the representative species.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Representative species</th>
<th>Accession Number (Gen Bank)</th>
<th>Similarity level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUNIA-10</td>
<td>N. rhamnosiphila</td>
<td>JX860357.1</td>
<td>99.9</td>
</tr>
<tr>
<td>AUNIA-10</td>
<td>N. flavorosea</td>
<td>GQ376168.1</td>
<td>99.9</td>
</tr>
<tr>
<td>AUNIA-10</td>
<td>N. sienata</td>
<td>JF797316.1</td>
<td>99.8</td>
</tr>
<tr>
<td>AUNIA-10</td>
<td>N. cyriacigeorgica</td>
<td>KC577151.1</td>
<td>98.8</td>
</tr>
<tr>
<td>AUNIA-10</td>
<td>Actinoplanes sp.</td>
<td>L41047.1</td>
<td>89.7</td>
</tr>
</tbody>
</table>
So, a comparison was made between the strain AUNIA-10 and its closest phylogenetic member *Nocardia rhamnosiphila* for their cultural, morphological and biochemical properties. Results showed that *Nocardia rhamnosiphila* differed from the strain AUNIA-10 in the aerial mass color and in the utilization of carbon sources (mannitol and raffinose) (Table 7.23). All the other characters were similar of the strain *Nocardia rhamnosiphila*. Hence, the strain AUNIA-10 has been tentatively identified as *Nocardia rhamnosiphila*.

Table 7.23. Cultural, morphological and biochemical characteristics of the strain AUNIA-10 and the closely related *Nocardia* species.

<table>
<thead>
<tr>
<th>Character studied (as per Everest <em>et al.</em>, 2011)</th>
<th>Strain AUNIA-10</th>
<th><em>N. rhamnosiphila</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>Whitish yellow</td>
<td>White to Pink</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reverse side pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soluble pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Carbon source assimilation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xylose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Inositol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

*+ indicates Positive, - indicates Negative*

AUNIA-11

AUNIA-11 is a mesophilic actinobacterium which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into spiral spore chains. A grey colour aerial spore mass was formed on ISP2 agar. Reverse side pigment was absent and a soluble pigment was produced on peptone yeast extract iron agar. Melanin pigment was also absent on ISP7 agar. Culture grew well when it was
supplemented with the carbon sources *viz.* arabinose, xylose, mannitol, sucrose and raffinose. No growth was observed in inositol and rhamnose.

A 1403 bp of 16S rDNA sequence was determined for the strain AUNIA-11, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and an accession number (KF548131) was obtained. The sequence similarity studies of the strain AUNIA-11 exhibited close phylogenetic relationship with the members of the genus *Streptomyces*. Comparison of the 16S rRNA gene sequence (1403 bp) of the strain AUNIA-11 with the previously obtained sequences of *Streptomyces* and its related species deposited in GenBank (NCBI) was made and the rooted phylogenetic tree (Fig. 7.12) indicated that the strain AUNIA-11 forms a branch with *S. collinus* subsp. *albescens* with 99.6% similarity (Table 7.24). The *Actinoplanes* sp. (L41047.1) served as an outgroup.

**Strain AUNIA-11**

/  

Grey coloured aerial mycelium  

Spiral spore chains under 400X
So, a comparison was made between the strain AUNIA-11 and its closest phylogenetic member *S. collinus* subsp. *albescens* for their cultural, morphological and biochemical properties. Results showed that *S. collinus* subsp. *albescens* differed from the strain AUNIA-11 in the utilization of carbon sources *viz.* inositol and raffinose (Table 7.25) all the other characters were similar to those of the *S. collinus* subsp. *albescens*. Hence, the strain AUNIA-11 has been tentatively identified as *S. collinus* subsp. *albescens*.
Table 7.25. Cultural, morphological and biochemical characteristics of the strain AUNIA-11 and the closely related *Streptomyces* species.

<table>
<thead>
<tr>
<th>Character studied (as per Hatano <em>et al.</em>, 1980)</th>
<th>Strain AUNIA-11</th>
<th><em>S. collinus</em> subsp. <em>albescens</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>Grey</td>
<td>Grey</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reverse side pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soluble pigment</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Spore chain</td>
<td>Spiral</td>
<td>Spiral</td>
</tr>
<tr>
<td>Carbon source assimilation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Xylose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*+ indicates Positive, - indicates Negative*

**AUNIA-12**

AUNIA-12 is a mesophilic actinobacterium which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into spiral spore chains. A grey colour aerial spore mass was formed on ISP2 agar. Reverse side pigment was present and soluble pigments were not produced on peptone yeast extract iron agar. Melanin pigment was also absent on ISP7 agar. Culture grew well when it was supplemented with the carbon sources *viz.* xylose and mannitol. No growth was observed in inositol.

A 1156 bp of 16S rDNA sequence was determined for the strain AUNIA-12, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and an accession number (KF548140) was obtained. The sequence similarity studies of the strain AUNIA-12 exhibited close phylogenetic relationship with the members of the genus *Streptomyces*. Comparison of the 16S rRNA gene
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sequence (1156 bp) of the strain AUNIA-12 with the previously obtained sequences of *Streptomyces* and its related species deposited in GenBank (NCBI) was made and the rooted phylogenetic tree (Fig. 7.13) indicated that the strain AUNIA-12 forms a branch with *S. misawanensis* with 99.9% similarity (Table 7.26). The *Actinoplanes* sp. (L41047.1) served as an outgroup.

**Strain AUNIA-12**

![Grey coloured aerial mycelium](image1)

![Spiral spore chains under 400X](image2)

*Fig. 7.13. Phylogenetic tree based on 16S rRNA gene sequences showing the positions of AUNIA-12 and related strains. Only bootstrap values expressed as percentages of 1000 replications are shown at the branch points.*
Table 7.26. Levels of 16S rDNA sequence similarity between the strain AUNIA-12 and the representative species.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Representative species</th>
<th>Accession Number (Gen Bank)</th>
<th>Similarity level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUNIA-12</td>
<td>S. misawanensis</td>
<td>AB184533.1</td>
<td>99.9</td>
</tr>
<tr>
<td>AUNIA-12</td>
<td>S. fulvoviolaceus</td>
<td>AB184573.1</td>
<td>99.8</td>
</tr>
<tr>
<td>AUNIA-12</td>
<td>S. niveoruber</td>
<td>AB741463.1</td>
<td>99.5</td>
</tr>
<tr>
<td>AUNIA-12</td>
<td>S. ginsengisoli</td>
<td>AB245393.1</td>
<td>98.8</td>
</tr>
<tr>
<td>AUNIA-12</td>
<td>Actinoplanes sp.</td>
<td>L41047.1</td>
<td>88.6</td>
</tr>
</tbody>
</table>

So, a comparison was made between the strain AUNIA-12 and its closest phylogenetic member S. misawanensis for their cultural, morphological and biochemical properties. Results showed that S. misawanensis differed from the strain AUNIA-12 in the utilization of carbon source viz. inositol (Table 7.27) and the remaining characters were similar to the characters of the strain S. misawanensis. Hence, the strain AUNIA-12 has been tentatively identified as S. misawanensis.

Table 7.27. Cultural, morphological and biochemical characteristics of the strain AUNIA-12 and the closely related Streptomyces species.

<table>
<thead>
<tr>
<th>Character studied (as per Sezaki et al., 1967)</th>
<th>Strain AUNIA-12</th>
<th>S. misawanensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>Grey</td>
<td>White to light grayish (Calcium malate agar)</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reverse side pigment</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Soluble pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spore chain</td>
<td>Spiral</td>
<td>Spiral</td>
</tr>
</tbody>
</table>

**Carbon source assimilation**

<table>
<thead>
<tr>
<th></th>
<th>Strain AUNIA-12</th>
<th>S. misawanensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*+ indicates Positive, - indicates Negative*
AUNIA-13

AUNIA-13 is a mesophilic actinobacterium which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into short spiral spore chains. A white colour aerial spore mass was formed on ISP2 agar. Reverse side pigment and soluble pigments were produced on peptone yeast extract iron agar. Melanin pigment was also absent on ISP7 agar. Culture grew well when it was supplemented with the carbon sources *viz.* inositol, rhamnose and sucrose. No growth was observed in mannitol.

A1028 bp of 16S rDNA sequence was determined for the strain **AUNIA-13**, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and an accession number (KF548141) was obtained. The sequence similarity studies of the strain AUNIA-13 exhibited close phylogenetic relationship with the members of the genus *Streptomyces*. Comparison of the 16S rRNA gene sequence (1028 bp) of the strain AUNIA-13 with the previously obtained sequences of *Streptomyces* and its related species deposited in GenBank (NCBI) was made and the rooted phylogenetic tree (Fig. 7.14) indicated that the strain AUNIA-13 forms a branch with *S. albiflaviniger* with 99.9% similarity (Table 7.28). The *Actinoplanes* sp. (L41047.1) served as an outgroup.
Strain AUNIA-13

White coloured aerial mycelium

Spiral spore chains under 400X

Fig. 7.14. Phylogenetic tree based on 16S rRNA gene sequences showing the positions of AUNIA-13 and related strains. Only bootstrap values expressed as percentages of 1000 replications are shown at the branch points.
Culturable diversity: Identification of actinobacteria

Table 7.28. Levels of 16S rDNA sequence similarity between the strain AUNIA-13 and the representative species.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Representative species</th>
<th>Accession Number (Gen Bank)</th>
<th>Similarity level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUNIA-13</td>
<td><em>S. albiflaviniger</em></td>
<td>JX020704.1</td>
<td>99.9</td>
</tr>
<tr>
<td>AUNIA-13</td>
<td><em>S. yogyakartensis</em></td>
<td>HQ244470.1</td>
<td>99.7</td>
</tr>
<tr>
<td>AUNIA-13</td>
<td><em>S. javensis</em></td>
<td>HQ244469.1</td>
<td>99.7</td>
</tr>
<tr>
<td>AUNIA-13</td>
<td><em>S. roseogriseolus</em></td>
<td>EF371441.1</td>
<td>99.7</td>
</tr>
<tr>
<td>AUNIA-13</td>
<td><em>S. hygroscopicus</em></td>
<td>AJ391821.1</td>
<td>99.6</td>
</tr>
<tr>
<td>AUNIA-13</td>
<td><em>Actinoplanes sp.</em></td>
<td>L41047.1</td>
<td>94.1</td>
</tr>
</tbody>
</table>

So, a comparison was made between the strain AUNIA-13 and its closest phylogenetic member *S. albiflaviniger* for their cultural, morphological and biochemical properties. Results showed that *S. albiflaviniger* differed from the strain AUNIA-13 in the aerial mass color and utilization of carbon sources (inositol and sucrose) (Table 7.29) and the remaining characters were similar the characters of the strain *S. albiflaviniger*. Hence, the strain AUNIA-13 has been tentatively identified as *S. albiflaviniger*.

Table 7.29. Cultural, morphological and biochemical characteristics of the strain AUNIA-13 and the closely related *Streptomyces* species.

<table>
<thead>
<tr>
<th>Character studied (as per Goodfellow et al., 2007)</th>
<th>Strain AUNIA-13</th>
<th><em>S. albiflaviniger</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>White</td>
<td>Yellow</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reverse side pigment</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Soluble pigment</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Spore chain</td>
<td>Spiral</td>
<td>Spiral</td>
</tr>
<tr>
<td><strong>Carbon source assimilation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inositol</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

*+ indicates Positive, - indicates Negative*
AUNIA-14

AUNIA-14 is a mesophilic actinobacterium which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into short spiral spore chains. A grey colour aerial spore mass was formed on ISP2 agar. Reverse side pigment was produced and soluble pigments were absent on peptone yeast extract iron agar. Melanin pigment was also absent on ISP7 agar. Culture grew well when it was supplemented with the carbon sources viz. arabinose and mannitol. No growth was observed in xylose, inositol, rhamnose, sucrose and raffinose.

A 1255 bp of 16S rDNA sequence was determined for the strain AUNIA-14, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and an accession number (KF548125) was obtained. The sequence similarity studies of the strain AUNIA-14 exhibited close phylogenetic relationship with the members of the genus Streptomyces. Comparison of the 16S rRNA gene sequence (1255 bp) of the strain AUNIA-14 with the previously obtained sequences of Streptomyces and its related species deposited in GenBank (NCBI) was made and the rooted phylogenetic tree (Fig. 7.15) indicated that the strain AUNIA-14 forms a branch with Streptomyces fradiae with 99.8% similarity (Table 7.30). The Actinoplanes sp. (L41047.1) served as an outgroup.
Strain AUNIA-14

Grey coloured aerial mycelium

Spiral spore chains under 400X

Fig. 7.15. Phylogenetic tree based on 16S rRNA gene sequences showing the positions of AUNIA-14 and related strains. Only bootstrap values expressed as percentages of 1000 replications are shown at the branch points.

Table 7.30. Levels of 16S rDNA sequence similarity between the strain AUNIA-14 and the representative species.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Representative species</th>
<th>Accession Number (Gen Bank)</th>
<th>Similarity level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUNIA-14</td>
<td><em>S. fradiae</em></td>
<td>AB184060.1</td>
<td>99.8</td>
</tr>
<tr>
<td>AUNIA-14</td>
<td><em>S. tritolerans</em></td>
<td>NR043751.1</td>
<td>99.8</td>
</tr>
<tr>
<td>AUNIA-14</td>
<td><em>S. rubrogriseus</em></td>
<td>AJ781373.1</td>
<td>99.3</td>
</tr>
<tr>
<td>AUNIA-14</td>
<td><em>Actinoplanes sp.</em></td>
<td>L41047.1</td>
<td>94.4</td>
</tr>
</tbody>
</table>
So, a comparison was made between the strain AUNIA-14 and its closest phylogenetic member *Streptomyces fradiae* for their cultural, morphological and biochemical properties. Results showed that *Streptomyces fradiae* differed from the strain AUNIA-14 in the aerial mass color, reverse side pigment and spore chain morphology. Utilization of carbon sources (xylose and mannitol) also varied (Table 7.31). All the other characters were similar to those of *S. fradiae*. Hence, the strain AUNIA-14 is considered to be closely related to *S. fradiae*.

**Table 7.31. Cultural, morphological and biochemical characteristics of the strain AUNIA-14 and the closely related *Streptomyces* species.**

<table>
<thead>
<tr>
<th>Character studied (as per Nonomura key)</th>
<th>Strain AUNIA-14</th>
<th>S. fradiae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>Grey</td>
<td>Red</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reverse side pigment</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Soluble pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spore chain</td>
<td>Spiral</td>
<td>Retinaculiaperti</td>
</tr>
<tr>
<td><strong>Carbon source assimilation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Xylose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Raffinose</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*+ indicates Positive, - indicates Negative*

**AUNIA-15**

AUNIA-15 is a mesophilic actinobacterium which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into rectiflexible spore chains. A white colour aerial spore mass was formed on ISP2 agar. Reverse side pigment and soluble pigments were absent on peptone yeast extract iron agar. Melanin pigment was also absent on ISP7 agar. Culture grew well when it was
supplemented with the carbon sources viz. arabinose, xylose, inositol, rhamnose, sucrose and raffinose. No growth was observed in mannitol.

A 1051 bp of 16S rDNA sequence was determined for the strain AUNIA-15, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and an accession number (KF573609) was obtained. The sequence similarity studies of the strain AUNIA-14 exhibited close phylogenetic relationship with the members of the genus *Streptomyces*. Comparison of the 16S rRNA gene sequence (1054 bp) of the strain AUNIA-15 with the previously obtained sequences of *Streptomyces* and its related species deposited in GenBank (NCBI) was made and the rooted phylogenetic tree (Fig. 7.16) indicated that the strain AUNIA-15 forms a branch with *S. puniceus* with 99.8% similarity (Table 7.32). The Actinoplanes sp. (L41047.1) served as an outgroup.

**Strain AUNIA-15**

- White coloured aerial mycelium
- Retiflexible spore chains under 400X
Fig. 7.16. Phylogenetic tree based on 16S rRNA gene sequences showing the positions of AUNIA-15 and related strains. Only bootstrap values expressed as percentages of 1000 replications are shown at the branch points.

Table 7.32. Levels of 16S rDNA sequence similarity between the strain AUNIA-15 and the representative species.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Representative species</th>
<th>Accession Number (Gen Bank)</th>
<th>Similarity level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUNIA-15</td>
<td>S. puniceus</td>
<td>KC790270.1</td>
<td>99.8</td>
</tr>
<tr>
<td>AUNIA-15</td>
<td>S. californicus</td>
<td>FJ486387.1</td>
<td>99.3</td>
</tr>
<tr>
<td>AUNIA-15</td>
<td>S. flavogriseus</td>
<td>KX857622.1</td>
<td>99.1</td>
</tr>
<tr>
<td>AUNIA-15</td>
<td>S. globisporus</td>
<td>JQ066793.1</td>
<td>99.1</td>
</tr>
<tr>
<td>AUNIA-15</td>
<td>Actinoplanes sp.</td>
<td>L41047.1</td>
<td>94.1</td>
</tr>
</tbody>
</table>

So, a comparison was made between the strain AUNIA-15 and its closest phylogenetic member *S. puniceus* for their cultural, morphological and biochemical properties. Results showed that *S. puniceus* differed from the strain AUNIA-15 in the aerial mass color and production of melanoid pigment. Utilization of carbon source (mannitol) also varied (Table 7.33). All the other characters were similar to those of *S. puniceus*. Hence, the strain AUNIA-15 is considered to be closely related to *S. puniceus*. 
Table 7.33. Cultural, morphological and biochemical characteristics of the strain AUNIA-15 and the closely related *Streptomyces* species.

<table>
<thead>
<tr>
<th>Character studied (as per Park <em>et al.</em>, 2003)</th>
<th>Strain AUNIA-15</th>
<th><em>S. puniceus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>White</td>
<td>Purple or Red</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Reverse side pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soluble pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spore chain</td>
<td>Rectiflexible</td>
<td>Rectiflexible</td>
</tr>
<tr>
<td><strong>Carbon source assimilation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Xylose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ indicates Positive, - indicates Negative

**AUNIA-16**

AUNIA-16 is a mesophilic actinobacterium which forms an extensively branched substrate mycelium and aerial hyphae. A yellowish white colour aerial spore mass was formed on ISP2 agar. Reverse side pigment was absent and soluble pigments were present on peptone yeast extract iron agar. Melanin pigment was also absent on ISP7 agar. Culture grew well when it was supplemented with the carbon sources *viz.* xylose, inositol, sucrose and raffinose. No growth was observed in arabinose, mannitol and rhamnose.

A 1256 bp of 16S rDNA sequence was determined for the strain AUNIA-16, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and an accession number (KF548136) was obtained. The sequence similarity studies of the strain AUNIA-16 exhibited close phylogenetic relationship with the members of the genus *Nocardia*. Comparison of the 16S rRNA gene
sequence (1256 bp) of the strain AUNIA-16 with the previously obtained sequences of *Nocardia* and its related species deposited in GenBank (NCBI) was made and the rooted phylogenetic tree (Fig. 7.17) indicated that the strain AUNIA-16 forms a branch with *Nocardia lijiangensis* with 99.8% similarity (Table 7.34). The *Actinoplanes* sp. (L41047.1) served as an outgroup.

**Strain AUNIA-16**

![Spore chains under 400X](image)

Fig. 7.17. Phylogenetic tree based on 16S rRNA gene sequences showing the positions of AUNIA-16 and related strains. Only bootstrap values expressed as percentages of 1000 replications are shown at the branch points.
Table 7.34. Levels of 16S rDNA sequence similarity between the strain AUNIA-16 and the representative species.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Representative species</th>
<th>Accession Number (Gen Bank)</th>
<th>Similarity level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUNIA-16</td>
<td><em>N. lijiangensis</em></td>
<td>NR043185.1</td>
<td>99.8</td>
</tr>
<tr>
<td>AUNIA-16</td>
<td><em>N. xishanensis</em></td>
<td>NP024832.1</td>
<td>99.0</td>
</tr>
<tr>
<td>AUNIA-16</td>
<td><em>N. polyresistens</em></td>
<td>AY626158.1</td>
<td>98.9</td>
</tr>
<tr>
<td>AUNIA-16</td>
<td><em>N. exalbida</em></td>
<td>JF797308.1</td>
<td>98.4</td>
</tr>
<tr>
<td>AUNIA-16</td>
<td><em>N. takedensis</em></td>
<td>NR024832.1</td>
<td>98.2</td>
</tr>
<tr>
<td>AUNIA-16</td>
<td><em>Actinoplaes sp.</em></td>
<td>L41047.1</td>
<td>89.0</td>
</tr>
</tbody>
</table>

So, a comparison was made between the strain AUNIA-16 and its closest phylogenetic member *Nocardia lijiangensis* for their cultural, morphological and biochemical properties. Results showed that *Nocardia lijiangensis* differed from the strain AUNIA-16 in the aerial mass color and production of melanoid and soluble pigment (Table 7.35). All the other characters were similar to those of *Nocardia lijiangensis*. Hence, the strain AUNIA-16 is considered to be closely related to *Nocardia lijiangensis*.

Table 7.35. Cultural, morphological and biochemical characteristics of the strain AUNIA-16 and the closely related *Nocardia* species.

<table>
<thead>
<tr>
<th>Character studied (as per Xu et al., 2006)</th>
<th>Strain AUNIA-16</th>
<th><em>N. lijiangensis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>Yellowish white</td>
<td>White</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Reverse side pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soluble pigment</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Carbon source assimilation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Xylose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*indicates Positive, - indicates Negative*
AUNIA-17

AUNIA-17 is a mesophilic actinobacterium which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into short spiral spore chains. A grey colour aerial spore mass was formed on ISP2 agar. Reverse side pigment was absent and soluble pigments were present on peptone yeast extract iron agar. Melanin pigment was also absent on ISP7 agar. Culture grew well when it was supplemented with the carbon sources \textit{viz.} arabinose and xylose. No growth was observed in rhamnose, sucrose and raffinose.

A 1299 bp of 16S rDNA sequence was determined for the strain AUNIA-17, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and an accession number (KF573608) was obtained. The sequence similarity studies of the strain AUNIA-17 exhibited close phylogenetic relationship with the members of the genus \textit{Streptomyces}. Comparison of the 16S rRNA gene sequence (1299 bp) of the strain AUNIA-17 with the previously obtained sequences of \textit{Streptomyces} and its related species deposited in GenBank (NCBI) was made and the rooted phylogenetic tree (Fig 7.18) indicated that the strain AUNIA-17 forms a branch with \textit{S. globisporus} with 99.8\% similarity (Table 7.36). The \textit{Actinoplanes} sp. (L41047.1) served as an outgroup.
Strain AUNIA-17

Grey coloured aerial mycelium

Spiral spore chains under 400X

Fig. 7.18. Phylogenetic tree based on 16S rRNA gene sequences showing the positions of AUNIA-17 and related strains. Only bootstrap values expressed as percentages of 1000 replications are shown at the branch points.

Table 7.36. Levels of 16S rDNA sequence similarity between the strain AUNIA-17 and the representative species.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Representative species</th>
<th>Accession Number (Gen Bank)</th>
<th>Similarity level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUNIA-17</td>
<td><em>S. globisporus</em></td>
<td>JX535034.1</td>
<td>99.8</td>
</tr>
<tr>
<td>AUNIA-17</td>
<td><em>S. celluloflavus</em></td>
<td>JQ066794.1</td>
<td>99.5</td>
</tr>
<tr>
<td>AUNIA-17</td>
<td><em>S. anulatus</em></td>
<td>EU647478.1</td>
<td>99.2</td>
</tr>
<tr>
<td>AUNIA-17</td>
<td><em>S. mediolani</em></td>
<td>FJ486429.1</td>
<td>99.2</td>
</tr>
<tr>
<td>AUNIA-17</td>
<td><em>Actinoplanes sp.</em></td>
<td>L41047.1</td>
<td>89.6</td>
</tr>
</tbody>
</table>
So, a comparison was made between the strain AUNIA-17 and its closest phylogenetic member *S. globisporus* for their cultural, morphological and biochemical properties. Results showed that *S. globisporus* differed from the strain AUNIA-17 in the aerial mass color and spore chain morphology (Table 7.37). All the other characters were similar to those of *S. globisporus*. Hence, the strain AUNIA-17 is considered to be closely related to *S. globisporus*.

**Table 7.37. Cultural, morphological and biochemical characteristics of the strain AUNIA-17 and the closely related *Streptomyces* species.**

<table>
<thead>
<tr>
<th>Character studied (as per Ostash <em>et al.</em>, 2011)</th>
<th>Strain AUNIA-17</th>
<th><em>S. globisporus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>Grey</td>
<td>Grey, White</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reverse side pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soluble pigment</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Spore chain</td>
<td>Spiral</td>
<td>Rectiflexible</td>
</tr>
<tr>
<td><strong>Carbon source assimilation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Xylose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Raffinose</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*+ indicates Positive, - indicates Negative*

**AUNIA-18**

AUNIA-18 is a mesophilic actinobacterium which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into short spiral spore chains. A grey colour aerial spore mass was formed on ISP2 agar. Reverse side pigment absent and soluble pigment present on peptone yeast extract iron agar. Melanin pigment was also absent on ISP7 agar. Culture grew well when it was
supplemented with the carbon sources *viz.* arabinose, xylose and rhamnose. No growth was observed in inositol, mannitol, raffinose and sucrose.

A 1069 bp of 16S rDNA sequence was determined for the strain AUNIA-18, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and an accession number (KF548142) was obtained. The sequence similarity studies of the strain AUNIA-18 exhibited close phylogenetic relationship with the members of the genus *Streptomyces*. Comparison of the 16S rRNA gene sequence (1069 bp) of the strain AUNIA-18 with the previously obtained sequences of *Streptomyces* and its related species deposited in GenBank (NCBI) was made and the rooted phylogenetic tree (Fig. 7.19) indicated that the strain AUNIA-18 forms a branch with *S. pactum* with 99.9% similarity (Table 7.38). The *Actinoplanes* sp. (L41047.1) served as an outgroup.

**Strain AUNIA-18**

Grey coloured aerial mycelium  
Spiral spore chains under 400X
Fig. 7.19. Phylogenetic tree based on 16S rRNA gene sequences showing the positions of AUNIA-18 and related strains. Only bootstrap values expressed as percentages of 1000 replications are shown at the branch points.

Table 7.38. Levels of 16S rDNA sequence similarity between the strain AUNIA-18 and the representative species.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Representative species</th>
<th>Accession Number (Gen Bank)</th>
<th>Similarity level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUNIA-18</td>
<td>S. pactum</td>
<td>NR041134.1</td>
<td>99.9</td>
</tr>
<tr>
<td>AUNIA-18</td>
<td>S. carnosus</td>
<td>KC522300.1</td>
<td>99.9</td>
</tr>
<tr>
<td>AUNIA-18</td>
<td>S. olivaceus</td>
<td>KF312278.1</td>
<td>99.9</td>
</tr>
<tr>
<td>AUNIA-18</td>
<td>S. litmocidini</td>
<td>EF654101.1</td>
<td>99.9</td>
</tr>
<tr>
<td>AUNIA-18</td>
<td>Actinoplanes sp.</td>
<td>L41047.1</td>
<td>89.1</td>
</tr>
</tbody>
</table>

So, a comparison was made between the strain AUNIA-18 and its closest phylogenetic member *S. pactum* for their cultural, morphological and biochemical properties. Results showed that *S. pactum* differed from the strain AUNIA-18 in the utilization of carbon sources (arabinose, xylose and rhamnose) (Table 7.39). Hence, the strain AUNIA-18 has been tentatively identified as *S. pactum*. 
Table 7.39. Cultural, morphological and biochemical characteristics of the strain AUNIA-18 and the closely related *Streptomyces* species.

<table>
<thead>
<tr>
<th>Character studied (as per Nonomura key)</th>
<th>Strain AUNIA-18</th>
<th><em>S. pactum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>Grey</td>
<td>Grey</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reverse side pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soluble pigment</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Spore chain</td>
<td>Spiral</td>
<td>Spiral</td>
</tr>
</tbody>
</table>

**Carbon source assimilation**

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Strain AUNIA-18</th>
<th><em>S. pactum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabinose</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Xylose</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Inositol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Raffinose</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* indicates Positive, - indicates Negative

AUNIA-19

AUNIA-19 is a mesophilic actinobacterium which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into long and branched spore chains. A white colour aerial spore mass was formed on ISP2 agar. Reverse side pigment and soluble pigments production was absent on peptone yeast extract iron agar. Melanin pigment was also absent on ISP7 agar. Culture grew well when it was supplemented with the carbon sources *viz*. rhamnose and sucrose. No growth was observed in arabinose, xylose, inositol, mannitol and raffinose.

A 1249 bp of 16S rDNA sequence was determined for the strain AUNIA-19, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and an accession number (KF548127) was obtained. The sequence similarity studies of the strain AUNIA-19 exhibited close phylogenetic relationship with the members of the genus *Nocardiopsis*. Comparison of the 16S rRNA gene sequence (1249 bp) of the strain AUNIA-19 with the previously obtained sequences
of *Nocardiopsis* and its related species deposited in GenBank (NCBI) was made and the rooted phylogenetic tree (Fig. 7.20) indicated that the strain AUNIA-19 forms a branch with *N. alba* with 99.3% similarity (Table 7.40). The *Actinoplanes* sp. (L41047.1) served as an outgroup.

**Strain AUNIA-19**

![White coloured aerial mycelium](image1.png) ![Long and branched spore chains under 400X](image2.png)

**Fig. 7.20.** Phylogenetic tree based on 16S rRNA gene sequences showing the positions of AUNIA-19 and related strains. Only bootstrap values expressed as percentages of 1000 replications are shown at the branch points.
So, a comparison was made between the strain AUNIA-19 and its closest phylogenetic member *N. alba* for their cultural, morphological and biochemical properties. Results showed that *N. alba* differed from the strain AUNIA-19 in the utilization of carbon sources (rhamnose and sucrose) (Table 7.41) and the remaining characters were similar to the characters of the strain *N. alba*. Hence, the strain AUNIA-19 has been tentatively identified as *N. alba*.

Table 7.41. Cultural, morphological and biochemical characteristics of the strain AUNIA-19 and the closely related *Nocardiopsis* species.

<table>
<thead>
<tr>
<th>Character studied (as per Chun et al., 2000)</th>
<th>Strain AUNIA-19</th>
<th><em>N. alba</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>White</td>
<td>White</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reverse side pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soluble pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spore chain</td>
<td>Long and branched</td>
<td>Not mentioned</td>
</tr>
<tr>
<td><strong>Carbon source assimilation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xylose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Inositol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Raffinose</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*indicates Positive, - indicates Negative*
AUNIA-20

AUNIA-20 is a mesophilic actinobacterium which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into long and branched spore chains. A white colour aerial spore mass was formed on ISP2 agar. Reverse side pigment and soluble pigments production was absent on peptone yeast extract iron agar. Melanin pigment was also absent on ISP7 agar. Culture grew well when it was supplemented with the carbon source mannitol. No growth was observed in xylose, inositol and raffinose.

A 1313 bp of 16S rDNA sequence was determined for the strain AUNIA-20, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and an accession number (KF548143) was obtained. The sequence similarity studies of the strain AUNIA-20 exhibited close phylogenetic relationship with the members of the genus Nocardiopsis. Comparison of the 16S rRNA gene sequence (1313 bp) of the strain AUNIA-20 with the previously obtained sequences of Nocardiopsis and its related species deposited in GenBank (NCBI) was made and the rooted phylogenetic tree (Fig. 7.21) indicated that the strain AUNIA-20 forms a branch with Nocardiopsis metallicus with 97.1% similarity (Table 7.42). The Actinoplanes sp. (L41047.1) served as an outgroup.
Strain AUNIA-20

![White coloured aerial mycelium](image1)

![Long and branched spore chains under 400X](image2)

Fig. 7.21. Phylogenetic tree based on 16S rRNA gene sequences showing the positions of AUNIA-20 and related strains. Only bootstrap values expressed as percentages of 1000 replications are shown at the branch points.

**Table 7.42. Levels of 16S rDNA sequence similarity between the strain AUNIA-20 and the representative species.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Representative species</th>
<th>Accession Number (Gen Bank)</th>
<th>Similarity level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUNIA-20</td>
<td>N. metallicus</td>
<td>JX254903.1</td>
<td>97.1</td>
</tr>
<tr>
<td>AUNIA-20</td>
<td>Nocardiopsis sp</td>
<td>FJ982381.1</td>
<td>96.9</td>
</tr>
<tr>
<td>AUNIA-20</td>
<td>N. lucentensis</td>
<td>EF392847.1</td>
<td>96.9</td>
</tr>
<tr>
<td>AUNIA-20</td>
<td>Actinoplanes sp.</td>
<td>L41047.1</td>
<td>88.3</td>
</tr>
</tbody>
</table>
So, a comparison was made between the strain AUNIA-20 and its closest phylogenetic member *Nocardiopsis metallicus* for their cultural and morphological properties. Results showed that *Nocardiopsis metallicus* differed from the strain AUNIA-20 in the utilization of carbon sources viz. xylose, inositol and raffinose (Table 7.43) and all the other characters were similar to those of the strain *Nocardiopsis metallicus*. Hence, the strain AUNIA-20 has been tentatively identified as *Nocardiopsis metallicus*.

### Table 7.43. Cultural, morphological and biochemical characteristics of the strain AUNIA-20 and the closely related *Nocardiopsis* species.

<table>
<thead>
<tr>
<th>Characters studied (as per Bergy’s Manual of Determinative Bacteriology)</th>
<th>Strain AUNIA-20</th>
<th>N. mellaticus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>White</td>
<td>White</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reverse side pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soluble pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spore chain</td>
<td>Long and branched</td>
<td>Long and branched</td>
</tr>
<tr>
<td><strong>Carbon source assimilation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylose</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

*+ indicates Positive, - indicates Negative*

### AUNIA-21

AUNIA-21 is a mesophilic actinobacterium which forms an extensively branched substrate mycelium and aerial hyphae. A whitish grey colour aerial spore mass was formed on ISP2 agar. Reverse side pigment and soluble pigments production was absent on peptone yeast extract iron agar. Melanin pigment was also present on ISP7 agar. Culture grew well when it was supplemented with the carbon sources *viz.* xylose, inositol and rhamnose. No growth was observed in arabinose, mannitol, sucrose and raffinose.
A 1042 bp of 16S rDNA sequence was determined for the strain **AUNIA-21**, which was submitted to the GenBank (National Center for Biotechnology Information, USA) and an accession number (KF668282) was obtained. The sequence similarity studies of the strain AUNIA-21 exhibited close phylogenetic relationship with the members of the genus *Nocardia*. Comparison of the 16S rRNA gene sequence (1042 bp) of the strain AUNIA-21 with the previously obtained sequences of *Nocardia* and its related species deposited in GenBank (NCBI) was made and the rooted phylogenetic tree (Fig. 7.22) indicated that the strain AUNIA-21 forms a branch with *Nocardia miyunensis* with 100% similarity (Table 7.44). The *Actinoplanes* sp. (L41047.1) served as an outgroup.

**Strain AUNIA-21**

![Spore chains under 400X](image_url)
Culturable diversity: Identification of actinobacteria

Fig. 7.22. Phylogenetic tree based on 16S rRNA gene sequences showing the positions of AUNIA-21 and related strains. Only bootstrap values expressed as percentages of 1000 replications are shown at the branch points.

Table 7.44. Levels of 16S rDNA sequence similarity between the strain AUNIA-21 and the representative species.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Representative species</th>
<th>Accession Number (Gen Bank)</th>
<th>Similarity level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUNIA-21</td>
<td>N. miyunensis</td>
<td>GQ376179.1</td>
<td>100</td>
</tr>
<tr>
<td>AUNIA-21</td>
<td>N. jiangxiensis</td>
<td>DQ840027.1</td>
<td>99.9</td>
</tr>
<tr>
<td>AUNIA-21</td>
<td>N. vermiculata</td>
<td>JF797321.1</td>
<td>98.1</td>
</tr>
<tr>
<td>AUNIA-21</td>
<td>N. niigatensis</td>
<td>AB092562.1</td>
<td>98.0</td>
</tr>
<tr>
<td>AUNIA-21</td>
<td>Actinoplanes sp.</td>
<td>L41047.1</td>
<td>90.3</td>
</tr>
</tbody>
</table>

So, a comparison was made between the strain AUNIA-21 and its closest phylogenetic member *Nocardia miyunensis* for their cultural, morphological and biochemical properties. Results showed that *Nocardia miyunensis* differed from the strain AUNIA-21 in the aerial mass and utilization of carbon sources (inositol and rhamnose) (Table 7.45) all the other characters were similar to the characters of the strain *Nocardia miyunensis*. Hence, the strain AUNIA-21 has been tentatively identified as *Nocardia miyunensis*. 
Table 7.45. Cultural, morphological and biochemical characteristics of the strain AUNIA-21 and the closely related Nocardia species.

<table>
<thead>
<tr>
<th>Character studied (as per Cui et al., 2005)</th>
<th>Strain AUNIA-21</th>
<th>N. miyunensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>Whitish grey</td>
<td>White</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reverse side pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soluble pigment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbon source assimilation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xylose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Raffinose</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ indicates Positive, - indicates Negative

7.4. Discussion

In the present study, 21 actinobacterial strains were selected based on their distinct colony morphology and among them, color of the aerial mycelium was white (11 isolates), grey (5 isolates), greyishwhite (1 isolate), yellow (1 isolate), whitishyellow (1 isolate), whitishgrey (1 isolate) and yellowishwhite (1 isolate). Further, out of the 21 isolates, none of them produced melanoid pigment on ISP7 medium; 5 isolates produced soluble pigments and 4 isolates produced reverse side pigment, on the peptone yeast exact agar and ISP2 medium, respectively. Isolated actinobacterial colonies were elevated, convex and powdery in nature and such morphological features are common in most actino (Sivakumar et al., 2005; Fguira et al., 2005; Baskaran et al., 2011).

Spore chain and spore morphology form an important characteristic in the identification of actinobacteria as they determine the culture surface and it could vary greatly in different species (Sivakumar, 2001). In the present study, majority of the
isolates produced spores in Spiral (7 isolates), Rectiflexible (3 isolates), Retinaculiaperti (1 isolate), long and branched (7 isolates) and long, curled and branched (3 isolates) spore chains.

Moreover, utilization of carbon sources also plays a major role in the identification of actinobacteria at the genus level (Meena et al., 2013). Based on the results of the chemotaxonomy, cultural characteristics, micromorphology and carbon source utilization, three genera (Streptomyces, Nocardiopsis and Nocardia) were identified.

Molecular characteristics are uniquely shared by different groups of organisms and provide us with important information for the identification of different clades and an understanding of their evolutionary relationship (Gao and Gupta, 2012). From the 16S rRNA gene sequence analysis, undertaken in the present study, it can be concluded that the isolates belonged to Streptomyces, Nocardiopsis and Nocardia, with the closest species, sharing high similarity levels (Table 7.46).
Table 7.46. Levels of 16S rRNA gene sequence similarity between 21 isolates and their closest representative species.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Closest representative species</th>
<th>Accession number (Gen Bank)</th>
<th>Similarity level %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUNIA-1</td>
<td>N. dassonvillei subsp. dassonvillei</td>
<td>KF306367.1</td>
<td>99.8</td>
</tr>
<tr>
<td>AUNIA-2</td>
<td>S. misawanensis</td>
<td>AB184533.1</td>
<td>99.6</td>
</tr>
<tr>
<td>AUNIA-3</td>
<td>N. dassonvillei</td>
<td>FJ486356.1</td>
<td>98.5</td>
</tr>
<tr>
<td>AUNIA-4</td>
<td>S. puniceus</td>
<td>KC790270.1</td>
<td>98.4</td>
</tr>
<tr>
<td>AUNIA-5</td>
<td>S. griseobrunneus</td>
<td>AB249912.1</td>
<td>98.7</td>
</tr>
<tr>
<td>AUNIA-6</td>
<td>N. dassonvillei subsp. dassonvillei</td>
<td>KF306364.1</td>
<td>98.8</td>
</tr>
<tr>
<td>AUNIA-7</td>
<td>N. terrae</td>
<td>DQ387958.1</td>
<td>98.6</td>
</tr>
<tr>
<td>AUNIA-8</td>
<td>S. tendae</td>
<td>KF454841.1</td>
<td>99.2</td>
</tr>
<tr>
<td>AUNIA-9</td>
<td>N. alba</td>
<td>FJ481638.1</td>
<td>99.7</td>
</tr>
<tr>
<td>AUNIA-10</td>
<td>N. rhamnosiphila</td>
<td>JX860357.1</td>
<td>99.9</td>
</tr>
<tr>
<td>AUNIA-11</td>
<td>S. collinus subsp. albescens</td>
<td>KF454841.1</td>
<td>99.6</td>
</tr>
<tr>
<td>AUNIA-12</td>
<td>S. misawanensis</td>
<td>AB184533.1</td>
<td>99.9</td>
</tr>
<tr>
<td>AUNIA-13</td>
<td>S. albiflavinger</td>
<td>JX020704.1</td>
<td>99.9</td>
</tr>
<tr>
<td>AUNIA-14</td>
<td>S. fradiae</td>
<td>AB184060.1</td>
<td>99.8</td>
</tr>
<tr>
<td>AUNIA-15</td>
<td>S. puniceus</td>
<td>KC790270.1</td>
<td>99.8</td>
</tr>
<tr>
<td>AUNIA-16</td>
<td>N. lijiangensis</td>
<td>NR043185.1</td>
<td>99.8</td>
</tr>
<tr>
<td>AUNIA-17</td>
<td>S. globisporus</td>
<td>JX535034.1</td>
<td>99.8</td>
</tr>
<tr>
<td>AUNIA-18</td>
<td>S. pactum</td>
<td>NR041134.1</td>
<td>99.9</td>
</tr>
<tr>
<td>AUNIA-19</td>
<td>N. alba</td>
<td>FJ481638.1</td>
<td>99.3</td>
</tr>
<tr>
<td>AUNIA-20</td>
<td>N. metallicus</td>
<td>JX254903.1</td>
<td>97.1</td>
</tr>
<tr>
<td>AUNIA-21</td>
<td>N. miyunensis</td>
<td>GQ376179.1</td>
<td>100</td>
</tr>
</tbody>
</table>

Streptomyces was the dominant genus of the culturable actinobacteria, isolated from the different coastal habitats of the Neil Island. Dominance of Streptomyces in marine samples has also been reported by many workers: Barcina et al. (1987) from seawater and sediments; Jensen et al. (1991) from near-shore tropical marine environment of Bahamas; Aarthi (2009) from the mud volcano of the Bartang island, the Andamans; Gupta et al. (2009) from the plant, sediments and water of Bhitarkanika mangroves; Raja et al. (2010) from the Vellar estuary; Schneemann et al. (2010) from Baltic Sea; Swarnakumar (2010) from the Great Nicobar Island. Fan et al. (2011) from the Baltic sea; Sivakumar (2011) from the Gulf of Mannar; Karruppiah (2011) from the Gulf of Mannar, Sethubathi (2011) from the Little
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Andaman; Sivalingam *et al.* (2012) from the mangrove sediment of Pitchavaram; Aarthi (2013) from the mud volcano of the Baratang island; Mohanraj and Sekar (2013) from the Bay of Bengal; Williams *et al.* (2013) from the tropical marine sediments and Sivasankar *et al.* (2013a) from the Uppanar estuary, east coast of India.

*Nocardiopsis* was first described by Meyer (1976) and, it comprises 28 recognized species (Zhang *et al.*, 2008). Recently, Vimal *et al.* (2009) from Puducherry coast, Chandrakumar (2010) from the Andamans; Khopade *et al.* (2012) from Mumbai coastal region of India and Aarthi (2013) from Barantang Island, have reported the occurrence of *Nocardiopsis* species; Manivasagan *et al.* (2013) have also recently reported the occurrence of *Nocardiopsis* in the marine sediments of Busan coast, South Korea.

The genus, *Nocardiopsis* is of interest for both its ecological versality and its ability to produce a rich array of bioactive metabolites. Numerous studies have shown that *Nocardiopsis* species are ubiquitously distributed across a diverse range of environments, such as saline or alkaline habitats, deserts, marine habitats, plant tissues, animal guts and indoor environment (Kroppenstedt and Evtushenko, 2002; Li *et al.*, 2012a). Members of this genus also produce bioactive metabolites such as methylpendolmycin (Sun *et al.*, 1991), apoptolidin (Kim *et al.*, 1997), griseusin D (Li *et al.*, 2007), lipopeptide biosurfactants (Gandhimathi *et al.*, 2009), antimicrobial (Vimal *et al.*, 2009), thiopeptides (Engelharat *et al.*, 2010) and naphthospironone A (Ding *et al.*, 2010; Li *et al.*, 2013). They have been also a source of antibacterial compounds such as the nocathiacins (Li *et al.*, 2003), nocardithiocin (Mukai *et al.*, 2009) and chemomicin A (Sun *et al.*, 2007).

As compared to *Streptomyces*, reports on the occurrence of *Nocardia* from the marine samples are very limited (Lakshmanaperumalsamy, 1978; Ellaiah and
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Reddy, 1987; Kokare et al., 2004; El-Sery and Elela, 2006; Zhang et al., 2006; Remya and Vijayakumar, 2008; Sun et al., 2010; Yamamura et al., 2010; Vyas and Dave (2011) and Sirisha et al., 2013). Five new lipopeptides, peptidolipins B-F (1-5) have been isolated from the marine Nocardia sp. (strain WMMB215) (Wyche et al., 2012). But, this genus and its species have not been extensively studied as other actinobacteria

In conclusion, it can be stated that the Neil island of the Andamans is a good source for the isolation of cultivable actinobacteria viz. species of Streptomyces, Nocardiosis and Nocardia, implying that they can be further studied for their diversity, novelty, bioactivity and bioremediation potentials.