Actinomycetes

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Actinomycetes are best known for their ability to produce antibiotics and are gram positive bacteria which comprise a group of branching unicellular microorganisms. They produce branching mycelium which may be of two kinds viz., substrate mycelium and aerial mycelium. Among actinomycetes, the streptomycetes are the dominant. The non-streptomycetes are called rare actinomycetes, comprising approximately 100 genera.

Members of the actinomycetes, which live in marine environment, are poorly understood and only few reports are available pertaining to actinomycetes from mangroves (Siva Kumar, 2001; Vikineswari et al. 1997; Rathana Kala & Chandrika, 1993; Lakshmanaperumalsamy, 1978).

Isolation of Actinomycetes from Sediments

For actinomycetes, the samples can be collected by inserting a polyvinyl corer (10cm dia.) (previously sterilized with alcohol) into the sediments. The corer is sterilized with alcohol before sampling at each station. The central portion of the top 2 cm sediment sample can be taken out with the help of a sterile spatula. This sample can be transferred to a sterile polythene bag and transported immediately to the laboratory. The sediment samples thus collected are air-dried aseptically. After a week, the sediment samples are to be incubated at 55°C for 5 min (Balagurunathan, 1992). Then, 10-fold serial dilutions of the sediment samples should be prepared, using filtered and sterilized 50% seawater. One ml of the serially diluted samples should be plated in the Kuster’s Agar (Siva Kumar, 2001) in triplicate petriplates. To minimize fungal contamination, all agar plates should be supplemented with 50 µg/ml of nystatin. The actinomycete colonies that appear on the petriplates can be counted from 5th day onwards, upto 28th day. All the colonies that are growing on the petriplates can be separately streaked in petriplates, sub-cultured, ensured for their axenicity and maintained in slants.
Identification

Various approaches for the identification of actinomycetes are given briefly below:

a) Molecular Approach

The most powerful approaches to taxonomy are through the study of nucleic acids. Because these are either direct gene products or the genes themselves and comparisons of nucleic acids yield considerable information about true relatedness.

Molecular systematics, which includes both classification and identification, has its origin in the early nucleic acid hybridization studies, but has achieved a new status following the introduction of nucleic acid sequencing techniques (O’Donnell et al., 1993). Significance of phylogenetic studies based on 16S rDNA sequences is increasing in the systematics of bacteria and actinomycetes (Yokota, 1997). Sequences of 16S ribosomal DNA have provided actinomycetologists with a phylogenetic tree that allows the investigation of evolution of actinomycetes and also provides the basis for identification.

Analysis of the 16S rDNA begins by isolating DNA (Hapwood, 1985) and amplifying the gene coding for 16S rRNA using the polymerase chain reaction (e.g. Siva Kumar, 2001). The purified DNA fragments are directly sequenced. The sequencing reactions are performed using DNA sequencer in order to determine the order in which the bases are arranged within the length of sample (Xu Li-Hua, et al., 1999) and a computer is then used for studying the sequence for identification using phylogenetic analysis procedures. However, analysis of 16S rDNA generally allows us to identify the organisms up to the genus level only.

b) Chemotaxonomical Approach

Chemotaxonomy is the study of chemical variation in organisms and the use of chemical characters in the classification and identification. It is one of the valuable methods to identify the genera of actinomycetes.

Studies of Cummins and Harris (1956) established that actinomycetes have a cell wall composition akin to that of gram-positive bacteria, and also indicated that the chemical composition of the cell wall might furnish practical methods of differentiating various types of actinomycetes. This is because of the fact that chemical components of the organisms that satisfy the following conditions, have significant meaning in systematics.
i. They should be distributed universally among the microorganisms studied; and,

ii. The components should be homologous among the strains within a taxon, while significant differences exist between the taxa to be differentiated.

Presence of Diaminopimelic Acid (DAP) isomers is one of the most important cell-wall properties of gram-positive bacteria and actinomycetes. Most bacteria have a characteristic wall envelope, composed of peptidoglycan. The 2,6-Diaminopimelic Acid (DAP) is widely distributed as a key aminoacid and it has optical isomers. The systematic significance lies mostly in the key aminoacid with two amino bases, and determination of the key aminoacid is usually sufficient for characterisation. If DAP is present, bacteria generally contain one of the isomers, the LL-form or the meso-form, mostly located in the peptidoglycan.

Major constituents of cell wall of actinomycetes (Lechevalier and Lechevalier, 1970) are as follows:

<table>
<thead>
<tr>
<th>Cell wall</th>
<th>glycine</th>
<th>meso-DAP</th>
<th>LL-DAP</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>II</td>
<td>+</td>
<td>+**</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

**hydroxy DAP (may also be present)**

The sugar composition often provides valuable information on the classification and identification of actinomycetes. Actinomycete cells contain some kinds of sugars, in addition to the glucosamine and muramic acid of peptidoglycan. The sugar pattern plays a key role in the identification of sporulating actinomycetes which have meso-DAP in their cell walls. However, the actinomycetes which have LL-DAP along with glycine (wall chemo type-I) have no characteristic pattern of sugars (Lechevalier and Lechevalier, 1970) and hence the whole cell sugar test has not received much attention here.

c) Classical Approach

Classical approaches for classification make use of morphological, physiological, and biochemical characters. The classical method
described in the identification key by Nonomura (1974) and Bergey’s Manual of Determinative Bacteriology (Buchanan & Gibbons, 1974) is very much useful in the identification of streptomycetes. These characteristics have been commonly employed in taxonomy of streptomycetes for many years. They are quite useful in routine identification. They are as follows.

1. **Aerial Mass Colour**
   The colour of the mature sporulating aerial mycelium is recorded in a simple way (White, grey, red, green, blue and violet). When the aerial mass colour falls between two colour series, both the colours are recorded. If the aerial mass colour of a strain to be studied shows intermediate tints, then also, both the colour series are noted.

2. **Melanoid Pigments**
   The grouping is made on the production of melanoid pigments (i.e. greenish brown, brownish black or distinct brown, pigment modified by other colours) on the medium. The strains are grouped as melanoid pigment produced (+) and not produced (‐).

3. **Reverse Side Pigments**
   The strains were divided into two groups, according to 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 such as pale yellow, olive or yellowish brown occurs, it is included in the latter group (‐).

4. **Soluble Pigments**
   The strains are divided into two groups by their ability to produce soluble pigments other than melanin: namely, produced (+) and not produced (‐). The colour is recorded (red, orange, green, yellow, blue and violet).

5. **Spore Chain Morphology**
   With regard to spore chains, the strains can be grouped into ‘sections’. The species belonging to the genus *Streptomyces* are divided into three sections (Shirling & Gottlieb, 1966), namely rectiflexibles (RF), retinaculiaperti (RA) and Spirales (S). When a strain forms two types of spore chains, both are noted (e.g. SRA).
Characteristics of the spore-bearing hyphae and spore chains should be determined by using direct microscopic examination of the culture surface. Adequate magnification (400x) could be used to establish the presence or absence of spore chains and to observe the nature of sporophores.

Spore morphological characters of the strains can be studied by inoculating a loopful of one week old cultures into 1.5% agar medium contained in test tubes at 37°C. The actinomycete should be suspended and thoroughly mixed in the semisolid agar medium and 1 or 2 drops of the medium could be aseptically pipetted on to a sterile glass slide. A drop of agar should be spread well on the slide and allowed to solidify into a thin film so as to facilitate direct observation under microscope. The cultures should be incubated at 28 ± 2°C and examined periodically for the formation of aerial mycelium, sporophore structure and spore morphology.

6. **Spore Surface**

Spore morphology and its surface features should be observed under the scanning electron microscope. The cross hatched cultures prepared for observation under the light microscope can be used for this purpose.
The electron grid should be cleaned and adhesive tape should be placed on the surface of the grid. The mature spores of the strain should be carefully placed on the surface of the adhesive tape and gold coating should be applied for half an hour and the specimen can be examined under the electron microscope at different magnifications. The spore silhouettes can be characterized as smooth, spiny, hairy and warty.

7. Assimilation of Carbon Source

The ability of different actinomycete strains in utilizing various carbon compounds as source of energy should be 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 or contaminating materials, should be used for this purpose. Carbon sources for this test could be arabinose, xylose, inositol, mannitol, fructose, rhamnose, sucrose and raffinose. These carbon sources should be sterilized by ether sterilization without heating.

Comparing the properties of the isolated strain with the representative species found in the key of Nonomura (1974) and Bergey’s Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974) can help in the species level identification. If the isolated strain could not be assigned to any of the valid representatives listed in the key of Nonomura (1974) and Bergey’s Manual of Determinative Bacteriology (Buchanan & Gibbons, 1974), then it can be identified based on the numerical taxonomic studies.

d) Numerical Taxonomic Approach

Numerical taxonomy involves examining many strains for a large number of characters prior to assigning the test organism to a cluster based on shared features. The numerically defined taxa are polythetic; so, no single property is either indispensable or sufficient to entitle an organism for membership of a group. Once classification has been achieved, cluster-specific or predictive characters can be selected for identification (Williams et al, 1983).

Numerical taxonomy was first applied to Streptomyces by Silvestri et al. (1962). The numerical taxonomic study of the genus Streptomyces by Williams et al. (1983) involves determination of 139 unit characters for 394 type cultures of Streptomyces; clusters were defined at 77.5% or 81% Ssn and 63% Sj similarity levels, and the former co-efficient
is being used to define the clusters. His study includes 23 major, 20 minor and 25 single member clusters.

The numerical classification of the genus *Streptomyces* by Kampfer *et al.* (1991) involves determination of 329 physiological tests. His study includes 15 major clusters, 34 minor clusters and 40 single member clusters which are defined at 81.5% similarity level $S_{sm}$ using the simple matching coefficient (Sokal and Michener, 1958) and 59.6 to 64.6% similarity level $S_{j}$ using Jaccard coefficient (Sneath, 1957). Thus, numerical taxonomy provides us with an invaluable framework for *Streptomyces* taxonomy, including identification of species.

**Preservation**

The preservation methods are similar to that of bacteria such as subculturing, freezing especially in liquid nitrogen, freeze-drying and maintenance of strains in mineral oil.

**Conclusion**

Studies on actinomycetes are very limited and the actinomycetes have been mentioned incidentally, on the microbial community of marine habitats. Further, only little information is available on the actinomycetes of the mangrove environment (which is one among the most productive coastal ecosystems) with regard to their occurrence and distribution (Vikineswari *et al.*, 1997; Rathna Kala and Chandrika, 1993; Lakshmanaperumalsamy, 1978).

**References**


204 Actinomycetes


