

# INTRODUCTION

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## **What are Actinomycetes**

*Actinomycetes* are considered true gram positive bacteria that exhibit wide morphological differences ranging from relatively simple rods and cocci to complex mycelial organizations, similar to that of some Eukaryotes (Locci and Sharples, 1984). The majority of spore-forming *Actinomycetes* (Sporo'actinomycetes), including the genus *Streptomyces* produce a nonfragmenting branched substrate mycelium. Such a mycelium, which represents the most advanced conditions for *actinomycetes* (Prauser, 1978) is typically fungal in organization, i.e. it is moncentric (Locci, 1976). It develops from the growth extension of a single propagule and all portions of the growth remain in filamentous continuity with the original element (Erikson, 1953).

## **Isolation from natural habitats**

The procedure of the isolation of a microorganism from its habitat varies with: (i) the nature of the microorganism and (ii) the number of germs relative to the other microbes within the habitat. Isolation of *Actinomycetes* requires either enrichment and/or use of more or less selective media. The methods for the isolation of *Streptomyces* have been reviewed by Nuesh (1965) and Williams and Cross (1971). Isolation and also enumeration of *Actinomycetes* are almost invariably achieved by a dilution plate procedure. Direct observation experiments have indicated that most colonies on soil dilution plates originate from spores or other resting propagules (Lloyd, 1969; Mayfield et al., 1972).

*Streptomyces* as a group do not have specific growth factor requirements and are able to use a wide range of carbon and nitrogen sources. However, the major problem in devising media for the selective isolation of *Streptomyces* is their general lack of specific nutrient requirements, which excludes the possibility of formulating media on which other bacteria and fungi grow or spread less rapidly than *Streptomyces* on isolation plates. Selectivity of isolation procedure may be influenced by: (a) pretreatment of samples, (b) selection of medium nutrient sources, (c) addition of selective inhibitors to the medium and (d) incubation conditions.

The range of media that have been used to isolate *Streptomyces* is extensive (Kutzner, 1981), whatever, the composition of the isolation medium, it is usually necessary to increase its efficiency by selective inhibitors.

Competition from fungi is dealt with, most effectively, by incorporation of antifungal antibiotics in the medium. Cycloheximide (actidione) has been widely used. In contrast, use of antibiotics selectively to isolate *Streptomyces* in the presence of other bacteria is less effective (Williams and Davies, 1965), because their sensitivity spectra often overlap.

For obtaining as many *Streptomyces* as possible from their habitat the following four principles of enrichment and isolation have been successfully employed, single or in combination:

1. Enrichment within the substrate before isolation.
2. Treatment of the sample to remove other microbes that may render isolation difficult.
3. Encouragement of the development of *Streptomyces* on isolation plates by choosing carbon and nitrogen sources preferred by these organisms.

4. Inhibition of the accompanying flora by the incorporation of selective substances into the nutrient agar, used for isolation (Kutzner, 1981).

## **Taxonomy of Actinomycetes**

The taxonomy of *Actinomycetes* suffered in the last two decades great revolutionary change. This is mainly due to the reevaluation of the value and significance of criteria used for the characterization of genera and species of *Actinomycetes*.

### **Classical criteria applied in the taxonomy of Actinomycetes**

The classical criteria used for the differentiation of genera and species of *Actinomycetes* were thoroughly reviewed and discussed by Burkholder et al., (1954) and Hesseltine et al., 1954). These are morphological, cultural, physiological and ecological criteria.

### **Morphological criteria**

*Burkholder et al., (1954)* described the direct microscopy of agar cultures media suggested for such techniques. The methods manual of the International Cooperative Project for Description and Deposition of Type Cultures of *Streptomyces* (ISCP, 1964), describes a method for the direct microscopy of streak cultures of *Streptomyces*. Kutzner (1981) reviewed the different methods of microscopy of cultures.

In the last edition of *Bergey's manual*, Cross (1989) emphasizing the significance of morphological criteria stated that rapid clues to its

identity and he advised the examination of organisms streaked in a cross-hatched pattern on the surface of the agar using a transmitted light microscope with a long working distance objective.

### **Morphological features to be recorded**

These are:

- 1) Length, mode of branching and fragmentation of substrate mycelium, type of spores and spore chains or sporangia, possibly carried on substrate hyphae.
- 2) Length, mode of branching and fragmentation of aerial mycelium and type of spores, spore chains or sporangia carried on the aerial hyphae.

### **Morphology of spores**

On examining spores under the transmission electron microscope, four types of silouettes can be distinguished: smooth, warty, spiny and hairy. Kriss et al (1945) were the first to use electron microscope for the study of the spores of *Streptomyces* species. Flaig et al., (1952-1955) followed by several workers (Kutzner, 1981) realized the taxonomic value of morphology of spores.

### **Cultural properties**

More than almost other bacteria, *Actinomycetes* show a striking appearance of macroscopic features: texture of growth, colors of aerial mycelium, substrate mycelium and pigments diffusing into the medium.