INTRODUCTION

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What are actinomycetes

Actinomyctes are considered true bacteria that exhibit wide morphological differences that range 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 (sporoactinomycetes), 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 monocentric (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 for 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 streptomycetes have been reviewed by Nüesch (1965) and Williams and Cross (1971). Isolation and also enumeration of actinomycetes are almost invariably achieved by a dilution plate psocedure.

Direct observation experemints have indicated that most colonies on soil dilution plates orignate from spores or other resting propagules (Lloyd, 1969: Mayfield et al., 1972).

Streptomycetes 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 Streptomycetes is their general lack of specific nutrient requirements, which excludes the possibility of formulating media on which other bacteria and fungi grow or spread more rapidly than Streptomycetes 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 Streptomycetes is extensive (Kutzner, 1981), Whatever, the composition of the isolation medium it is usually necessary to increase its effeciency by selective inhibitors.

Competition from fungi is dealt with most effectively by incorporation of antifungal antibiotics in the medium. Cycloheximide (aclidione) has been widely used. In contrast, use of antibiotics selectively to isolate Streptomycetes in the presence of other bacteria is less effective (Williams

and Davies, 1965) because their sensitivity spectra often overlap.

For obtaining as many streptomycetes 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 streptomycetes 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 decade great revolutionary changes. 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 of differentiation of genera and species 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 Streptomycetes (ISCP, 1964), describes a method for the direct microscopy of streak cultures of Streptomycetes. Kutzner (1981) reviewed the different methods of microscopic examinations of cultures.

In the last edition of Bergey's manual, Cross (1989) emphasizing the significance of morphological criteria stated that the morphology of an actinomycete provides useful and 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 Streptomycetes 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, colours of aerial mycelium, substrate mycelium and pigments diffusing into the medium.

Colour of aerial mycelium

Species of the actinomycete genera capable of producing aerial mycelium show a definite colour of their aerial mycelium. Experience with thousands of cultures shows that

the innumerable shades or tinges of colours do not form a continuous spectrum but rather clusters around few basic colours, besides that the colour of the aerial mycelium is an easy feature, which was conveniently used for the first grouping of isolates (Flaig and Kutzner, 1954, 1960; Hesseltine et al., 1954; Ettlinger et al., 1958; Pridham et al., 1958; Gauze et al., 1957).

In the international Streptomycetes project (ISP), the following seven colour series were used: yellow, violet, red, blue, grey and white. It is impossible to find a universal medium, which would suffice the requirements of all Streptomycetes species as they vary greatly in their nutritional requirements. Different sets of media were suggested for culture description (Hesseltine et al., 1954; ISP, 1964).

Colour of substrate mycelium and soluble pigments

Numerous actinomycetes of different genera possess a strikingly coloured substrate mycelium. Kutzner (1981) differentiates pigments of actinomycetes into :
(a) endopigments, which are bound to certain cell structure and confer a striking colour to the colonies of the organism, (b) exopigments and these are excreted into the surrounding medium and colour it in the corresponding colour. Both types of pigments may be pH indicators and change their colour according to pH.

Since pigments are secondary metabolites, their production depends on medium composition. The ISP method manual suggests a standard set of media for colour determination of pigments. The colours are observed after 1, 2 and 3 weeks.

Actinomycete taxonomists differ in their opinion concerning the value of pigmentation in actinomycete taxonomy. Conn and Conn (1941); Baldacci et al. (1954); Preobrajenskaya et al. (1960); Krassilnikov, (1960); Hussein (1965,1971) considered pigmentation a reliable taxonomic criterion, however, another group of taxonomists (Pridham et al., 1956; Hutter, 1962; Sanchez Maroquin, 1962; Trejo and Bennet, 1963) opposed this idea. The reasons for this criticism were discussed thoroughly by Kutzner (1981).

Progress has been made in the last decade in the elucidation of the chemical structure of pigments as well as simple chromatographic procedures for pigment characterization. (Lechevalier et al., 1971; Krassilnikov, 1970; Hussein, 1971; Blinov and Khokhlov, 1970).

Production of Melanin Pigments

Melanin pigments are often produced on proteinacous media, however some actinomycetes can produce such pigments on synthetic media. The ISP method manual describes two media for testing the ability of cultures to produce melanin pigments. The significance of melanin pigment production in

actinomycete taxonomy was emphasized by many authors (Waksman, 1959; Krassilnikov, 1960; Gauze et al., 1957; Hutter, 1962 and Bergey's manual, 1974).

Physiological tests

Physiological tests were early used as differentiating criteria in bacteriology and were applied by the first pioneers of actinomycetes, (Waksman, 1919; Lieske, 1921 and Krassilnikov, 1938).

Of these tests one can mention protein hydrolyses, starch hydrolyses, coagulation and peptonization of milk, liquefiaction of gelatin, reduction of nitrates to nitrites, decomposition of cellulose and production of H_2S (Tresner and Danga, 1958). The stability and diagnostic value of such physiological reactions were discussed by many authors. Baldacci et al. (1954); Hesseltine et al. (1954). Krassilnikov (1961) considered that most of these tests are of nonsignificant taxonomic value.

Physiological tests other than melanin production were considered by Sveshnikova et al. (1960); Gottlieb (1961); Preobrazhenskaya (1968); and Shirling and Gottlieb (1968) as of low significant taxonomic value. The results of five years of colloberative research carried out by the participants of the I.S.P. coincided with these conclusions.