OBSERVATIONS ON THE LIFE CYCLE OF THE *NOCARDIA*

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(With 4 Figures in the Text)

INTRODUCTION

The *Nocardia* form part of the ill-defined 'aerobic *Actinomyces*', and many workers, notably Naselund (1925), Lord & Trevett (1936), Cope (1938), Bibby & Knighton (1941), Crowley (1941), and Bartels (1943), claim that they differ from the *Actinomyces* proper only by their ability to tolerate oxygen.

The genus is defined in Bergey's *Manual* (1948) as aerobic and as producing, in young cultures, a non-septate mycelium which may later become septate, and ultimately break down into rod-shaped or spherical fragments. The generic name, *Proactinomyces*, was used by Umbreit (1939), and he divided the genus into two groups, *alpha* and *beta*. Umbreit believed that the former, which have a short unstable mycelium, were possibly related to the *Corynebacteria* or the *Mycobacteria*, while the latter exhibit some of the cultural and morphological characters of the *Actinomyces*. These two colony forms were recognized by Jensen (1934), but he claimed that intermediate forms can be distinguished, and that the gradation is compatible with this group of organisms being a true genus.

A cytological study of the cell walls and the mode of branching was made by Bisset & Moore (1949), and their observations are in general agreement with those described in the present paper.

MATERIAL

Ten strains were examined. Nine were isolated from apparently healthy mouths, and one was a saprophyte used by Moore (1949) in his experiments on the utilization of pyridine by micro-organisms. All the strains were aerobic.

The colonies of young cultures all had features resembling the matt type of *A. bovis* (Erikson, 1949; Morris, 1951*a*), but, as the cultures aged, the colonies became more wrinkled than those of the *Actinomyces*. The oral strains were all white or cream in colour, and often semi-transparent, but the saprophytic strain produced a pink pigment. All were non-motile and Gram positive. In young cultures staining with Gram's stain was uniform; but in older cultures the organisms showed the beading that is seen in the *Actinomyces*. Only one strain was slightly acid-fast, resisting decolorization by 1 % sulphuric acid in water.

The morphology of all the organisms investigated was in accordance with Bergey's description, and the time at which fragmentation was observed varied from 2 to 9 days. Apparent branching was observed in all strains.

Although in heat-fixed Gram-stained preparations of young cultures many features simulating the *Actinomyces* were seen, cytologically the *Nocardia* were, in fact, very different from *Actinomyces bovis* (Morris, 1951*a*).

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METHODS

The staining techniques employed were the acid Giemsa method for nuclear structures, and the tannic acid violet method for cell walls, as described by Robinow (1942).

Direct smears on cover-slips, impression colonies (Klieneberger-Nobel, 1934; Bisset, 1938), and colonies from Brewer's medium, were used for the preparation of material for examination.

As in the case of the cytological studies of *A. bovis* (Morris, 1951*a*), the depth of focus of the preparations made photomicrographs unsuitable for the study of the life cycle, and therefore all observations were recorded by drawings. The illustrations in this paper are composite figures taken from these records.

OBSERVATIONS

It was noticed that ageing cultures were composed of spherical bodies, lying either singly or in chains. These were shown to be microcysts, similar to those of the *Eubacteria* (Bisset, 1950), exhibiting the morphology of spherical or ovoid cells with a small eccentrically placed nucleus. Such cultures were used as a starting-point for the study of the life cycle of these organisms.

When subcultured on to a fresh medium the microcysts (Figs. 1 A and 2 A) germinate, and the first manifestation of this is the rapid enlargement of the cells. During this period, the nucleus expands and becomes vesicular (Figs. 1 C, D and 2 C). The next stage consists of the protrusion of buds from the spherical cell (Figs. 1 E and 2 D), which develop into club-shaped filaments. Two or three buds may arise from a single microcyst. As the bud grows, nuclear material from the microcyst streams into it, and the separate portions appear to divide repeatedly. The arrangement of these nuclear bodies is at first haphazard (Figs. 1 G and 2 E, *a*, and E, *c*). The microcyst becomes absorbed into the filament which becomes less club-shaped and more filamentous. The nuclear bodies then arrange themselves in pairs, or less commonly in threes, the regular pattern developing from the end distal to the microcyst (Figs. 1 H and 2 E, and E, *b*). Elongation of the filament continues and transverse cell walls are formed, giving rise to individual cells, each of which has two or three nuclei. Each nucleus then divides to give a pair of chromosomal bodies lying across the long axis of the cell (Figs. 1 I, J and 2 E, *b* and F, G). Further development is identical with that of the *Eubacteria* (Bisset, 1950), except that branching is common.

There are two ways by which a branch may be formed: (*a*) The branch starts as a bud from the mature vegetative cell, while the pair of chromosomes nearer the bud fuses, divides, and one part passes into the bud. This stage is followed by a rapid division of the nucleus within the bud, so that a multinucleated filament is produced, which then repeats the development of the filament derived from the microcyst (Figs. 1 S-U and 2 G). (*b*) The second type of branching arises from the young multinuclear filament, commencing as a bud into which some of the nuclear material from the parent filament passes. This is followed by repeated division of the nuclei, after which the development is identical with that described for the maturing filament from the microcyst, and the (*a*) type of branching.

The cells which have three pairs of nuclear rods (Figs. 1 J and 3 A) then undergo complex vegetative reproduction (Bisset, 1950). The rods move to the centre of the cell, and there form a fusion nucleus; two successive divisions take place, and eventually the products of the divisions arrange themselves in pairs, giving rise to a cell with twelve sets of paired chromatinic rods (Figs. 1 V-Y and 3 B-E). Finally transverse walls are formed, cutting off six mature vegetative cells (Figs. 1 Z and 3 F).

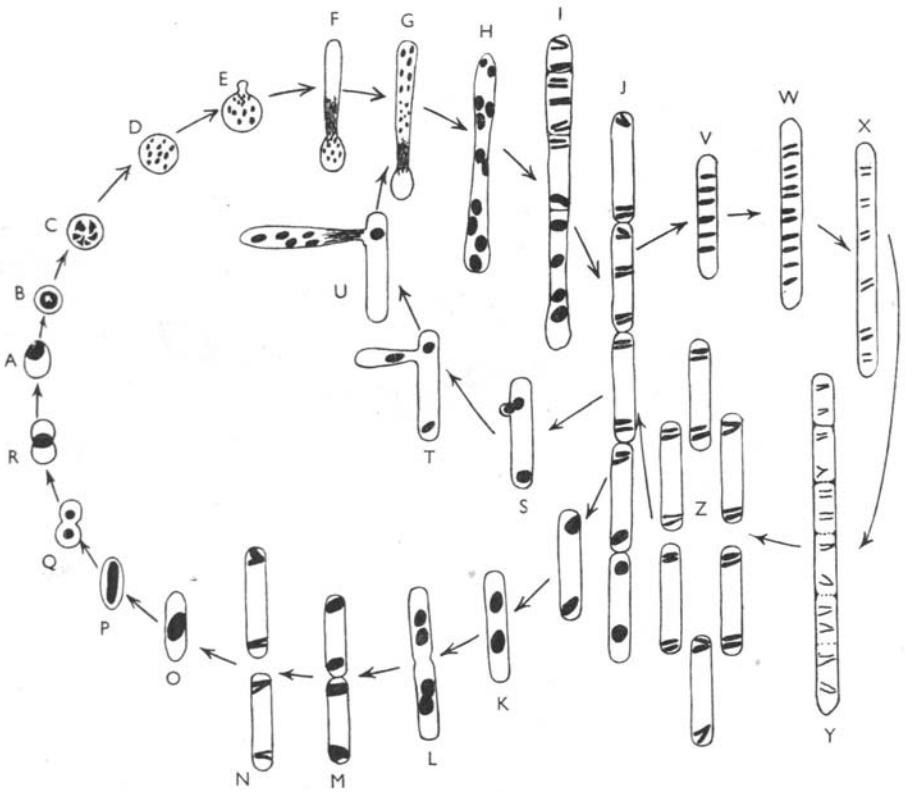

Fig. 1. Life cycle of the *Nocardia*. A–D, germinating microcyst, showing fragmentation of the nucleus; E–G, budding of the microcyst; H–J, arrangement of nuclear material, and development of transverse cell walls; K–N, binary fission; O–A, microcyst formation; S–U, branch formation from a mature vegetative cell; V–Z, complex reproductiv vegetation.

The return to the microcyst stage is heralded by a shortening of the cell with each successive division (Fig. 4 A). The cells become ovoid and later spherical in shape. During these changes, the pairs of chromatinic rods fuse and move to the centre of the cell, where a single nucleus is formed (Fig. 1 O). The single nucleus then elongates (Figs. 1 P and 4 C), divides in two, and at the same time a constriction appears in the cell wall (Figs. 1 Q and 4 D). Finally the two nuclear bodies again fuse to form a mature microcyst (Figs. 1 R, A and 4 E, F). The changes described in the production of the microcyst are identical with those seen in the *Eubacteria* (Bisset, 1950).

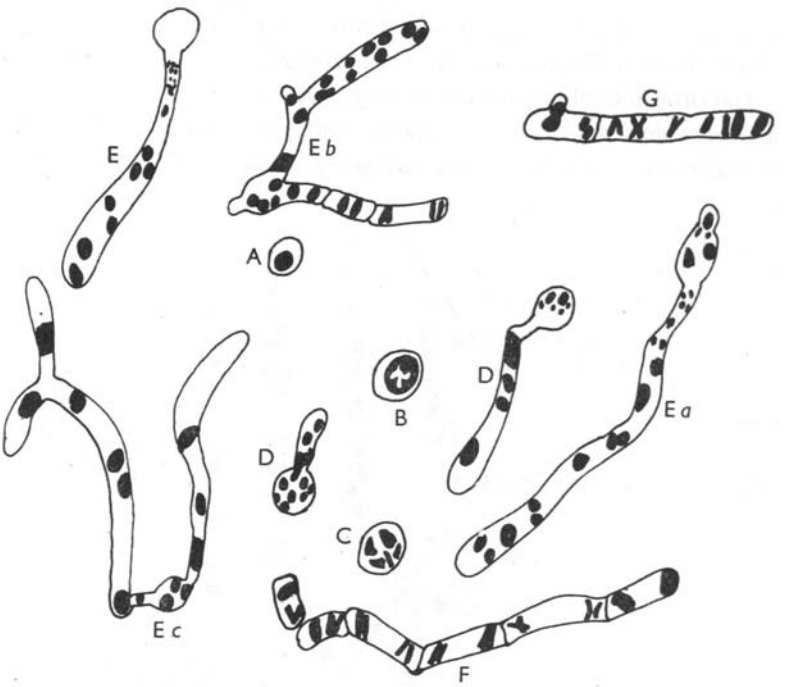

Fig. 2. A, a mature microcyst; B, microcyst showing a vesicular nucleus; C, fragmentation of the nucleus in the microcyst; D, budding of the microcyst; E, the beginning of regular arrangement in the multinucleated filament; E, *a*, as in E, but there are two buds arising from the microcyst; E, *b*, as in E and E, *a*, but showing the beginning of branch formation, and the development of cell walls; E, *c*, branching further advanced; F, a multicellular filament arising from the multinucleated filament; G, as in F, but branching is starting from a mature vegetative cell.

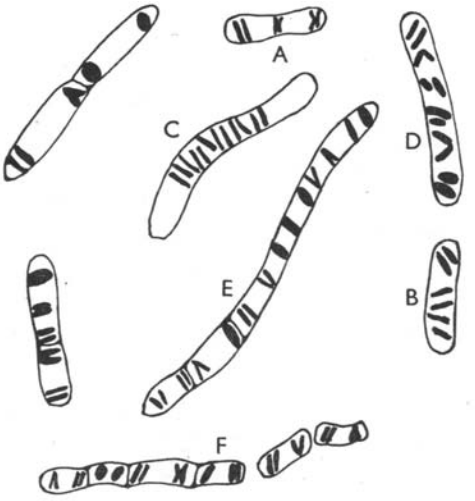

Fig. 3. Stages in the complex vegetative reproduction cycle.

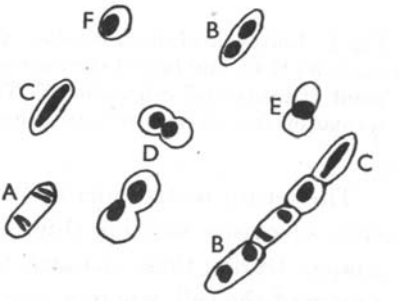

Fig. 4. Development of the microcyst from the simple vegetative cell.

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DISCUSSION

The above life cycle is in agreement with the morphological changes described by Bergey (1948). The non-septate filaments described in his *Manual* are undoubtedly the young multinucleate filaments arising from the germination of the microcyst, while the rod-shaped fragments are the individual vegetative cells, and the spherical forms are the microcysts.

From the above observations it is evident that, while heat-fixed Gram-stained preparations of *Nocardia* and *Actinomyces* may resemble each other to such an extent as to make differentiation almost impossible, cytologically they are markedly different. This difference is so distinct that there can be no doubt that the *Nocardia* constitute a separate genus. The differences between the aerobic *Nocardia* and the anaerobic *Actinomyces* are so marked that it would appear to be improbable that one is an adaptation of the other.

The germination of the microcyst by budding is comparable to the germination of the spore in *Streptomyces* (Klieneberger-Nobel, 1947), *Micromonospora* (Morris, 1951*b*), and *Actinomyces bovis* (Morris, 1951*a*); but it differs from the microcyst of *Jensenia* (Morris, 1951*b*), which germinates in a manner similar to that of the *Eubacteria* (Bisset, 1950).

The vegetative cells of *Nocardia* resemble those of *Jensenia* and *Eubacteria* more closely than they do the cells of *Streptomyces* or *Actinomyces*. Also, while the complex vegetative reproduction characteristic of *Eubacteria* is present in *Nocardia*, it has not been observed to occur in the *Streptomycetales*, *Actinomyces bovis*, or *Jensenia*.

A very important difference between *Actinomyces* and *Nocardia* is that the former produces true reproductive spores and has a prolonged diploid phase (Morris, 1951*a*), while the latter has a microcyst and a greatly reduced diploid phase, as happens in the *Eubacteria*.

When branching occurs it is of the impermanent type, resembling that seen in *Actinomyces* (Bisset & Moore, 1949; Morris, 1951*a*), thus being differentiated from the permanent branching seen in the *Streptomycetales* (Klieneberger-Nobel, 1947; Bisset & Moore, 1949; Morris, 1951*b*).

SUMMARY

The life cycle of *Nocardia* starts with the microcyst, which germinates by budding. The bud develops into a long multinucleate filament, which becomes divided into individual cells by the formation of transverse cell walls. Microcysts are formed in the same manner as in the *Eubacteria*. Multiplication of the cells takes place by simple fission, by complex vegetative reproduction, and by branching. They do not have a prolonged diploid phase like the *Actinomyces*.

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