Sporulation by Some Strains of Nocardiae and Streptomyces

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One of the fundamental taxonomic criteria of the genus *Streptomyces* is the formation of specialized aerial hyphae which produce chains of spores. By definition, members of the genus *Nocardia* usually do not produce aerial hyphae, and when aerial hyphae are formed, they are unspecialized and are asporogenous (Waksman and Henrici, 1943). In the laboratory, streptomycetes have been observed to lose the ability to produce aerial hyphae. These “degenerate” streptomycetes cannot be distinguished from nocardiae (Erikson, 1953). Therefore the axiom has been widely adopted that streptomycetes may be asporogenous but nocardiae are always asporogenous. Recently, however, electron micrographs of aerial hyphae from *Nocardia asteroides* revealed chains of spores (Gordon and Mihm, 1958).

This report confirms that *N. asteroides* is sporogenous and presents evidence that other species of *Nocardia* produce spores. Sporogenesis by streptomycetes and nocardiae is compared.

**Materials and Methods**

The strains of *Nocardia* and *Streptomyces* used in this investigation have been previously described (Bradley, 1957; Bradley and Anderson, 1958a). *Streptomyces griseus* strain S104 was obtained through the courtesy of Dr. Elizabeth McCoy, University of Wisconsin. The aerial mycelium of strain S104 is abundant, powdery, and olive-buff in color; the sporophores are straight and produced in tufts. There is no soluble pigment produced on nutrient agar; the vegetative mycelium is cream-colored. Strain S104 liquefies gelatin, peptonizes milk, hydrolyzes starch, and produces the antibiotic streptomycin. The optimal temperature for growth is 30°C.

*Streptomyces coelicolor* strain S16 was obtained from the Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Illinois, as strain NRRL-B-1257. The aerial mycelium of strain S16 is short-napped, powdery, and mouse-gray in color; the sporophores are spiraled. The vegetative mycelium is red to blue; the pigment is blue in alkaline substrates and red in acidic substrates. Strain S16 liquefies gelatin, hydrolyzes starch, and produces the antibiotic coeliacolorin. The optimal temperature for growth is 35°C. *S. coelicolor* strain S114 is an asporogenous variant of strain S16, which was obtained subsequent to ultraviolet irradiation.

The strains of *Nocardia* were kindly supplied by Dr. Norman F. Conant, Duke University. *N. asteroides* strain N300 forms waxy, folded, orange-colored colonies; scant tan-colored aerial mycelia develop on old colonies. Strain N300 does not liquefy gelatin, does not hydrolyze starch and does not coagulate milk; hyphae grown in milk are acid-fast. Strain N300 is pathogenic for the mouse when injected intraperitoneally, with 5 per cent gastric mucin as an adjuvant. The optimal temperature for growth is 35°C.

*Nocardia brasiliensis* strain N301 forms waxy, folded, orange-colored colonies which quickly develop extensive short-napped aerial mycelia. Strain N301 liquefies gelatin, hydrolyzes starch slowly, and coagulates milk; hyphae grown in milk are acid-fast. The optimal temperature for growth is 35°C.

The colonies of *Nocardia madurae* strain N302 are waxy, smooth, cream-colored and without any indication of aerial mycelia. Strain N302 does not liquefy gelatin or coagulate milk; hyphae grown in milk are not acid-fast. The optimal temperature for growth is 35°C.

The colonies of *Nocardia paraguayensis* strain N303 are glabrous, dark cream to gray in color, with scant white aerial mycelia which are formed only at the periphery of old colonies. Strain N303 liquefies gelatin, coagulates milk, and is not acid-fast. The optimal temperature for growth is 35°C.

The media used in this investigation included a modified Sabouraud’s agar, a modified Czapek’s agar, and a complex medium. The modified Sabouraud’s agar was composed of glucose, 20 g; peptone (Difco), 2 g; agar (Difco), 1 g; deionized water, 1 L. The modified Czapek’s agar contained glucose, 20 g; yeast extract (Difco), 2 g; NaNO₃, 2 g; K₂HPO₄, 2 g; MgSO₄·7H₂O, 0.5 g; agar (Difco), 15 g; deionized water, 1 L. The complex medium was composed of glucose, 1 g; peptone (Difco), 5 g; yeast extract (Difco), 3 g; beef extract (Armour), 2 g; Casamino acids (Difco), 1 g; agar (Difco), 15 g; deionized water, 1 L. The results reported herein were essentially the same irrespective of the medium employed. Moreover, stocks which were maintained in continuous culture on these media behaved identically to those which had been lyophilized.

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2 Difco Laboratories, Inc., Detroit, Michigan.

3 Armour and Co., Chemical Division, Chicago, Illinois.
The photomicrographs presented here show 3-week-old cultures which were grown on modified Czapek’s agar. Small colonies, grown on agar, were photographed in situ at a magnification of 200X. Preparations of aerial hyphae were obtained by gently pressing a slide against the surface of a colony. These slides were observed and photographed at a magnification of 2000X as unstained preparations using dark phase contrast or as stained preparations. The staining techniques employed were (1) Gram’s stain, (2) Congo red negative stain, and (3) nigrosin stain (Conn et al., 1957).

**RESULTS**

*S. coelicolor* strain S16 and *S. griseus* strain S104 grew rapidly, producing abundant sporulating aerial hyphae within 2 days (figures 1 to 4). *S. coelicolor* strain S114, an asporogenous variant of strain S16, grew slowly and did not produce aerial hyphae, even after 6 weeks (figures 5 and 6). Similarly, *N. madurae* did not produce aerial hyphae or spores (figures 15 and 16). Contrariwise, *N. brasiliensis* produced extensive aerial growth within 3 days and sporulated after 7 days (figures 7 to 9). *N. paraguayensis* did not form aerial hyphae until the second week of incubation, and then aerial hyphae developed only at the periphery of the colony (figures 13 and 14). Spore formation by *N. paraguayensis* is quite irregular. *N. asteroides* produced aerial hyphae after 1 week of incubation and sporulation occurred somewhat later (figures 10 to 12). *N. asteroides* regularly produced infrequent chains of spores, contrasted to *N. brasiliensis* which consistently sporulated extensively.

The mechanism of sporogenesis seemed to be identical for nocardiae and streptomycetes. The aerial hyphae were twice the diameter of the vegetative hyphae, and initially there were few or no cross walls. Next, cross walls formed at regular intervals in the aerial hyphae (Gregory, 1956), followed by contraction of the protoplasm into refractile units, first at the tips of the aerial hyphae and then progressively toward the base. The collapsed residual lateral walls of the aerial hyphae could be seen between the maturing spores. Later when this “sheath” disappeared, the chains of spores would fragment readily thereby releasing their individual elements.

**DISCUSSION**

In view of the vast discrepancies, which are frequently encountered, between the characteristics of cultures labeled with species designations and the accepted descriptions of these species (Gordon and Mihm, 1957), it is necessary to consider the validity of the strains used in this study. *S. griseus* strain S104 not only closely matches the accepted description with respect to morphology and biochemical tests, but produces strepto- mycin as well (Waksman and Lechevalier, 1953). *S. coelicolor* strain S16 is correctly identified, according to similar criteria. *N. asteroides* strain N300 and *N. madurae* strain N302 closely resemble the descriptions given by Waksman (1957). *N. paraguayensis* strain N303 and *N. brasiliensis* strain N301 are not recognized by Waksman (1957), however these strains are nearly identical with the descriptions given by Conant et al. (1954). Because *N. brasiliensis* strain N301 and *N. asteroides* strain N300 are acid-fast, there is little doubt as to the nocardial nature of these organisms. The fact that strain N300 is pathogenic for the mouse corroborates the diagnosis of this strain as *N. asteroides*.

It is now apparent that sporulation is not unique to the genus *Streptomyces*. Furthermore the mechanism of sporogenesis within aerial hyphae of streptomycetes and nocardiae seems to be identical. The close biological affinities of the nocardiae and streptomycetes are also manifested by cross-susceptibility to bacteriophages (Bradley and Anderson, 1958b).

However, the delimitation of the genera *Nocardia* and *Streptomyces* is also based upon mycelial fragmentation. Because the term mycelium has not been adequately defined, the usefulness of this characteristic is limited (Umbreit, 1939). Moreover, McClung (1954) points out that members of the genus *Nocardia* constitute a continuous series ranging from those which fragment immediately to those which have never been observed to undergo fragmentation. Although more study is required on this aspect of the life histories of these organisms, it should be noted that there is remarkable similarity between coccoid formation in *Nocardia corallina* (Webb and Clark, 1957) and sporulation in submerged culture by *S. griseus* (Wilkin and Rhodes, 1955).

Even though the International Bacteriological Code of Nomenclature (Buchanan et al., 1948) permits each taxonomist to evolve and apply his own definition of a genus, the definition of a genus should show natural affinities and aid in the correct identification of the organisms (Buchanan, 1957). The present status of the taxa *Nocardia* and *Streptomyces* may not reflect biological affinities and certainly does not aid in the identification of the group (Jensen, 1953), therefore the taxonomic validity of these two genera must be re-evaluated. Lacaz (1956) has already placed *N. madurae*, *N. paraguayensis*, *N. brasiliensis*, *S. griseus* and *S. coelicolor* in the same genus (*Actinomyces* of Lacaz = *Streptomyces* of Waksman and Henrici) but has retained *N. asteroides* in a separate genus (*Proactinomyces* of Lacaz = *Nocardia* of Waksman and Henrici). The synonymy which was used above, and which illustrates the complex semantic problems confronting actinomycete classification, has been amply discussed by Baldaeci et al. (1953).
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Figure 1. Colony of Streptomyces griseus showing abundant aerial hyphae. 178 X.
Figure 2. Aerial hyphae of Streptomyces griseus, as seen with dark phase contrast, showing mature spores. 1780 X.
Figure 3. Colony of Streptomyces coelicolor strain S16 showing aerial hyphae. 178 X.
Figure 4. Aerial hyphae of Streptomyces coelicolor strain S16, stained by Gram’s method, showing spores. 1780 X.
Figure 5. Colony of Streptomyces coelicolor strain S114 showing only vegetative mycelium. 178 X.
Figure 6. Vegetative hyphae of Streptomyces coelicolor strain S114, as seen with dark phase contrast. Note that the diameter of vegetative hyphae is only half the diameter of aerial hyphae. 1780 X.
Figure 7. Colony of Nocardia brasiliensis showing abundant aerial hyphae. 178 X.
Figure 8. Aerial hyphae of Nocardia brasiliensis, as seen with dark phase contrast, showing spores. 1780 X.
Figure 9. Aerial hyphae of Nocardia brasiliensis, prepared with Congo red negative stain, showing a distinct chain of spores. 1780 X.
Figure 10. Colony of *Nocardia asteroides* showing aerial hyphae. 184 X.

*Figure 11.* Aerial hyphae of *Nocardia asteroides*, as seen with dark phase contrast, showing spores. 1840 X.

*Figure 12.* Aerial hyphae of *Nocardia asteroides*, prepared with nigrosin negative stain, showing distinct chains of spores. 1840 X.

*Figure 13.* Colony of *Nocardia paraguayensis* showing aerial hyphae. 184 X.

*Figure 14.* Aerial hyphae of *Nocardia paraguayensis*, stained by the Gram's method, showing spores. 1840 X.

*Figure 15.* Colony of *Nocardia madurae* showing only vegetative mycelium. 184 X.

*Figure 16.* Vegetative hyphae of *Nocardia madurae*, stained by the Gram's method. 1840 X.
Summary

Nocardia brasiliensis, Nocardia asteroides, and Nocardia paraguayensis have been shown to produce aerial hyphae bearing chains of spores. Sporogenesis in streptomycetes and nocardiae was found to be similar. The validity of the taxons Nocardia and Streptomyces is questioned.

REFERENCES


BUCHANAN, R. E. 1957 How bacteria are named and identified. In Bergey's manual of determinative bacteriology, 7th ed. The Williams & Wilkins Co., Baltimore, Maryland.


