ELECTRON MICROSCOPY OF STREPTOMYCES SPORE MORPHOLOGY
AND ITS ROLE IN SPECIES DIFFERENTIATION

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The widespread search for new antibiotic-producing Streptomyces unleashed during the
last two decades also brought with it the problems of their classification. In the early attempts at
species differentiation excessive use was made of highly variable cultural and physiological prop-
erties as taxonomic criteria and too little attention was paid the more stable morphological
characteristics. However, in some of the more recently derived Streptomyces classification sys-
tems there has been increasing evidence that certain morphological properties are finally and
properly being accorded major status as criteria for species differentiation.

Pridham, Hesselteine, and Benedict (1958) assigned prime importance to the morphology of
Streptomyces sporophores and grouped species into primary morphological sections on this basis. In the system of Ettlinger, Corbaz, and Hütter (1958) the type of sporophore (spore chain) was also one of the characteristics given
major emphasis but the morphology of the spores themselves, as observed by electron microscopy,
was given equal weight. In recent years several other investigators (Flaig et al., 1952; Küster,
1953; Flaig and Kutzer, 1954; Flaig, Küster, and Beutelspacher, 1955; Baldaeci and Grein,
1955; Baldaeci, Gilardi, and Amici, 1956; Kutzer, 1956; and Preobrajenskaya et al., 1959) using
the electron microscope have studied the details of Streptomyces spore structure and have shown
that an apparently distinctive spore surface configuration exists in many of the species. Provided with this encouraging prospect, it seemed highly desirable to expand these studies to include mass collections of strains of individual
Streptomyces species, for it is only by demonstration of constancy from strain to strain within a
species that the true value of any criterion for species differentiation can be firmly established.
Having available substantial numbers of strains of many species of this genus, which in the past
were used to demonstrate that H₂S production merits major status in Streptomyces systematics
(Tresner and Danga, 1958), it was logical to make use of this assemblage of organisms again in
the further evaluation of spore ornamentation as a criterion.

MATERIALS AND METHODS

The RCA EMU 3C electron microscope was employed in the present study for observing
Streptomyces spore micromorphology. Preparations for examination were made by a simple
spore print technique in which Formvar-covered copper grids (200 mesh) (Shawinigan Resin
Corporation, Springfield, Massachusetts) were gently pressed to the sporulating surface of the
organisms. Spore chains adhering to the surface of the grids could be viewed in the microscope
without the necessity of fixing or shadowing the material. Because of the simplicity of this
method, as many as 10 cultures per hour could be studied. Most observations were made at about
8,000 enlargements which permitted easy visibility of the nature of the spore surfaces, and which
was generally satisfactory for making electron micrographs.

Cultures for study were grown in petri dishes for 14 days at 28 C. A yeast-malt agar medium
[0.4 per cent yeast extract (Difco), 1.0 per cent malt extract (Difco), 0.4 per cent glucose, 2.0
per cent agar, and 1.0 liter distilled H₂O; adjust to pH 7.0] that usually induced good sporulation
was used routinely; however, in some instances when sporulation was scanty or absent on this
medium, a Waksman's starch agar (Waksman, 1957) or a tomato paste oatmeal agar (Pridham
et al., 1957) was employed. Mature cultures were preserved with formalin (Tresner and Backus,
1957) and stored at 4 C until needed.

RESULTS

During the course of our study approximately 600 Streptomyces strains were examined, among
which were present some 118 described species
or varieties. About 350 of the strains were assignable to a group of 25 species, in which each species was represented by a block of 5 to 40 (average 14) carefully selected strains that generally included one or more reference cultures obtained from various culture repositories. It was observed that the spores of all the cultures studied could be classified according to
one or another of four morphological types, smooth, warty, spiny or hairy; this substantiates the findings of Kutzner (1956). By definition, we considered spores as smooth whenever there was no surface ornamentation present (fig. 1). Also included here were spores that sometimes showed considerable lumpiness or irregularities within the confines of the cell wall, but in which the
surface contour was unmodified. Warty spores, on the other hand, had distinct, obtuse protuberances that were uniformly present on the wall surfaces (figure 2). Spiny spores, distinguished by acute, rigid appendages varying in length and thickness (figures 3, 4, and 5), were differentiated from hairy spores; the latter possessed thin, flexible, filiform outgrowths that sometimes
# Table 1

**Electron microscope studies of Streptomyces spore morphology**

## A. Smooth-spored Streptomyces

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<td><em>S. rubrocyangodiastaticus var. piger</em></td>
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<td><em>S. rutgersensis</em></td>
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<td>YCB</td>
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<tr>
<td><em>S. salmonicida</em></td>
<td>2</td>
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<td>YCB</td>
</tr>
<tr>
<td><em>S. sulphureus</em></td>
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<td>YCB</td>
</tr>
<tr>
<td><em>S. tanashiensis</em></td>
<td>11</td>
<td>Hata #144</td>
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<td><em>S. venezuelae</em></td>
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<td></td>
<td>Waksman 3627</td>
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<tr>
<td><em>S. verticillatus</em></td>
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<tr>
<td><em>S. violaceoniger</em></td>
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<td>NRRL B-205</td>
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<td></td>
<td>NRRL B-1476</td>
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<td>NRRL B-1477</td>
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TABLE 1—(Continued)

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<tr>
<th>Organism</th>
<th>Total No. Strains Studied</th>
<th>Culture Collection Source and No.</th>
<th>Spore Color Group*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. violaceoruber</strong></td>
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<td>Cochrane strain A25S</td>
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<td></td>
<td>Sermonti strain P (1)</td>
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<td>ATCC 10147</td>
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<tr>
<td><strong>S. virginiæ</strong></td>
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<td>ATCC 12630</td>
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<td>IFO 3392</td>
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<td><strong>S. viridis</strong></td>
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<td><strong>S. willmorei</strong></td>
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B. Spiny-spored Streptomyces

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total No. Strains Studied</th>
<th>Culture Collection Source and No.</th>
<th>Spore Color Group*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. albocallus</strong></td>
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<td>ATCC 12626</td>
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<tr>
<td><strong>S. albus</strong></td>
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<td>NRRL B-2490</td>
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<td><strong>S. arabicus</strong></td>
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<td>NRRL B-1733</td>
<td>G-B</td>
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<td><strong>S. chartreusis</strong></td>
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<td>BL-G</td>
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<tr>
<td><strong>S. diastatochromogenes</strong></td>
<td>1</td>
<td>NRRL B-1698</td>
<td>G-B</td>
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<td><strong>S. erythraeus</strong></td>
<td>5</td>
<td>NRRL B-2338</td>
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<td></td>
<td>NRRL B-2359</td>
<td>TAN</td>
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<tr>
<td><strong>S. filipinensis</strong></td>
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<td><strong>S. gilvosporeus</strong></td>
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<td><strong>S. griseoflavus</strong></td>
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<td>CBS (Ciferri)</td>
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<td><strong>S. noursei</strong></td>
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<td><strong>S. pseudogriseolus</strong></td>
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<td><strong>S. purpurascens</strong></td>
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<td><strong>S. viridochromogenes</strong></td>
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<td><strong>Streptomyces sp.</strong></td>
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<td>Gottlieb (Levomycin)</td>
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<tr>
<td><strong>Streptomyces sp.</strong></td>
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<td>Kuroya F-300 (Phagostatin)</td>
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<td><strong>Streptomyces sp.</strong></td>
<td>1</td>
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</table>

C. Hairy-spored Streptomyces

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total No. Strains Studied</th>
<th>Culture Collection Source and No.</th>
<th>Spore Color Group*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. albogriseolus</strong></td>
<td>12</td>
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<td>G-B</td>
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<tr>
<td><strong>S. calvus</strong></td>
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<td>ATCC 13382</td>
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<tr>
<td><strong>S. flaveolus</strong></td>
<td>1</td>
<td>ATCC 3319</td>
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<tr>
<td><strong>Streptomyces sp.</strong></td>
<td>1</td>
<td>Robbins A419 (Chrysomycin)</td>
<td>G-B</td>
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</tbody>
</table>

D. Warty-spored Streptomyces

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total No. Strains Studied</th>
<th>Culture Collection Source and No.</th>
<th>Spore Color Group*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. diastaticus</strong></td>
<td>1</td>
<td>NRRL B-2650</td>
<td>G-B</td>
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<tr>
<td><strong>S. griseoplanus</strong></td>
<td>4</td>
<td>Lederle AA-223</td>
<td>G-B</td>
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<tr>
<td><strong>S. olivaceus</strong></td>
<td>1</td>
<td>ATCC 12019</td>
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</tbody>
</table>

* G-B = spores in shades of gray, gray-brown or brown; YCB = spores in shades of yellow, cream or buff; TAN = spores in shades of pinkish-tan to pinkish-cinnamon; BL-G = spores in shades of blue to bluish-green; and W = spores white or cultures with pure white aerial mycelium and not sufficient sporulation to impart a color.
attained lengths several times the greatest dimension of the spores (figure 6).

From an examination of table 1 it will be noted that smooth spores were by far the most commonplace and were characteristic of 80 per cent of the species studied. In streptomycetes having spore color in yellow to cream to buff or white shades, only smooth spores were found. Although smooth spores were the rule in the organisms having pinkish-cinnamon to pinkish-tan sporulation, *Streptomyces erythraeus* (Waksman) Waksman and Henrici, *Streptomyces purpurascens* Lindenbein, and two undetermined species belonging to this spore color group were outstandingly different with their spiny spores. About one-third of the gray to brownish-spored forms possessed distinctive spines, hairs or warts, although the latter two types were relatively rare. All of the cultures having blue to blue-green spore masses consistently produced spiny spores.

In keeping with the findings of Ettlinger et al. (1958) as well as other investigators, we observed that organisms having ornamented spores, produced them in the form of spiralled chains (figure 7) or less commonly in loops or coils, i.e., belonging in the sections “Spira” or “Retinaculum-Apertum” of the Pridham et al. (1958) classification. However, the converse of this was not necessarily true, since several species having a spiralled-spore apparatus had smooth spores. In no instance did Streptomycetes with straight to flexous sporophores have other than smooth spores.

In table 1 it may also be observed that a remarkably constant spore surface morphology existed in most of the species studied. In a few instances, e.g., with *Streptomyces albus* (Rossi-Doria emend. Krainsky) Waksman and Henrici, *Streptomyces diastaticus* (Krainsky) Waksman and Henrici, *Streptomyces diastatochromogenes* (Krainsky) Waksman and Henrici, *Streptomyces olivaceus* (Waksman) Waksman and Henrici, and *Streptomyces erythraeus*, it would appear that this conformity did not hold, since reference strains of these species were found to have spores of different morphological types. However, upon closer examination the disparity between strains received from various collections under the same species name proved to be attributable to major taxonomic differences. In the case of the groups of *S. albus*, *S. diastaticus*, and *S. erythraeus* cultures, for instance, such profound differences as spore color and sporophore morphology were observed to exist between strains, and it was obvious that misidentifications were responsible for all the anomalies.

*Figure 7. Spiral chains of ornamented spores of Streptomyces viridochromogenes* (Lederle AS-361)
Minor variations in spore surface morphology between strains of some species have been observed, but these have been mostly a matter of degree. For instance, the amount of, or length of spines or hairs on the spores of certain organisms may differ from strain to strain, but never have the variations been sufficiently great as to alter the general spore morphological type.

The spores themselves have shown considerable diversity of form among the different species ranging from globose to elliptical to elongate or cylindrical. Although their size and shape has been found quite uniform in strains of some Streptomyces, these features are not generally constant enough to provide reliable taxonomic criteria. Both the size and shape of spores can be modified in the electron microscope, the amount depending upon the intensity of the electron beam. Furthermore, the position of spores on the sporophore can be an influencing factor in their form and dimensions. Frequently, the terminally located spores are smaller and more uniform in size and shape than those more basally located; the basal spores often appear enlarged and misshapen.

It has been suggested that the nutritional environment may influence the surface configuration of Streptomyces spores. Lechevalier and Tihonienko (1960), in a study of two spiny-spored species on several defined and organic media, found that variation in the appearance of the spores occurred from one medium to another affecting the clarity with which spines could be demonstrated. Hence, it was of interest to us to determine whether morphological differences were produced by any of the media used in the present study. Several species representing the various spore morphological types were compared on the three agar media—yeast-malt, Waksman's starch, and tomato paste oatmeal. Insofar as could be observed there were no significant modifications in spore morphology of cultures brought about by these cultivation media.

**DISCUSSION**

In our experience, spore surface morphology as a taxonomic criterion is most valuable in two of the groups of Streptomyces, namely those with spores in gray to gray-brown to brown shades and to a more limited extent, those with pinkish-cinnamon to pinkish-tan shades. Especially fortunate is the diversity of morphological types found in the larger gray to brownish-spored group. Since all of the four basic spore morphological types are present in this group, it can be conveniently divided into as many taxonomic sections. We have not considered it advisable, however, to make further subdivisions on the basis of the length of spore appendages, as was recommended by Ettlinger et al. (1958), because of the variability in this regard which we have encountered between strains of some species.

Ornamentation of spores among the pinkish-cinnamon to pinkish-tan species has been observed quite rarely and has always been of the spiny type. In *S. erythraeus* of this spore color group the spines not only cover the spores, but likewise appear prominently on the intercalary sheath between the often widely separated spores (figure 5). This has also been illustrated by Dolezilova, Vanek, and Kralik (1959). Spiny spores and sheaths have similarly been observed in strains of *S. purpurascens* of this same spore color group.

The taxonomic value of spore surface configuration in the other Streptomyces does not appear to be quite so significant. In the blue to blue-green-spored members, all have spiny spores, and in the remaining spore color groups, all have had smooth spores. Despite the fact this is a limitation on the use of spore surface morphology as a taxonomic criterion, we do not find it a serious objection, because the majority of the species fall within the gray to brown or the pinkish-cinnamon to pinkish-tan spore color groups.

In general, we have noted that our observations of Streptomyces spore surface morphology have corroborated those of other investigators, and it would appear that the consensus of these workers endorses spore morphology as a constant feature within the species. In our present evaluation of this criterion we too have concluded that herein lies a reliable and convenient tool, made possible through electron microscopy, for systematizing this group of organisms.

**ACKNOWLEDGMENT**

The authors wish to express their thanks to Miss Mary Englert for the assistance given during this study.

**SUMMARY**

Spore morphology of the Streptomyces was studied by means of electron microscopy to
determine the reliability of this feature as a criterion for species differentiation in this genus. Approximately 600 cultures assignable to one or another of some 120 described species or varieties were included in the study. Twenty-five of the species were represented by an average of 14 strains each.

Spores of all the Streptomyces studied were found to conform to one of four types—smooth, warty, spiny, or hairy. About one-third of the gray to brownish-spored species had either spiny, warty, or hairy spores; the remaining members of this spore color group were smooth-spored. All of the blue to blue-green-spored forms had spiny spores. Those having spore masses in white, or yellow to cream or buff shades had smooth-walled spores. All of the pinkish-cinnamon to pinkish-spored group had smooth spores with the exception of Streptomyces erythraeus, Streptomyces purpurascens, and two undetermined species which had spiny spores.

The size and shape of spores in most species tended to be variable and appeared to be of limited usefulness for taxonomic differentiation. On the other hand, surface configuration of the spores was observed to be a remarkably constant species characteristic and promises to provide a reliable and useful taxonomic aid.

REFERENCES


