Characterization of Hairy-Spored Streptomycetes

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Eight *Streptomyces* species having hairy-surfaced spores were examined by transmission electron microscopy and scanning electron microscopy. On the basis of the flexibility of the spore chains and the appearance of the spores, three subgroups are proposed.

Streptomycete spore surfaces have been classified into five groups by the present authors (4). This paper is concerned with hairy-spored streptomycetes. We classify as hairy those spores whose ornamentation consists of long, flexible filaments. Length may vary from less than one spore diameter to five or more spore diameters. Flexibility is evidenced by the ability to bend without breaking and by movement while being observed in the electron microscope. Most hairs are nontapered, although a few cultures have spores with slightly tapered hairs. Occasionally hairs are seen which are flattened in cross section as evidenced by their twisted ribbon appearance. Pictures of spores produced by eight *Streptomyces* species are presented, and three subgroups of hairy spores are proposed.

Cultures chosen for study were: *Streptomyces glaucus* ATCC (American Type Culture Collection) 12730 (UC [Upjohn Culture] 5153); *S. pactum* NRRL (Northern Regional Research Laboratory) 2939 (UC 2432); *Streptomyces* sp. UC 2677; *Streptomyces* sp. F-57 (UC 2768); *Streptomyces prasinus* ATCC 15825 (UC 5344); *S. prasinopilosus* ATCC 19092 (UC 5343); *Streptomyces* sp. no. 284 (UC 5187); and *S. werraensis* ATCC 14424 (UC 5345). The conditions of culture growth and the preparation for microscopy have been described previously (1-3, 5). For direct examination of whole spores, carbon-filmed grids were touched to the surface of mature aerial growth. Large numbers of spores adhered to the carbon film and could be observed with the transmission electron microscope (TEM). For scanning electron microscopy, cover slips with adherent culture were cemented to stubs, coated with evaporated gold, and examined in a Stereoscan scanning electron microscope (SEM; Cambridge Scientific Instruments, Ltd.) in the laboratory of Kent Cambridge Scientific, Inc., Morton Grove, Ill.

The degree of coiling of the spore chain is best observed by the SEM, although light microscopy may suffice, both methods allowing observation of the undisturbed and intact culture. Observation by TEM involves touching the sporulated culture with a carbon-filmed grid, which may lead to disruption of the original spatial arrangement. Therefore, one must make conclusions regarding degree of coiling based on SEM or light microscope observations.

The accompanying figures show the appearance of the spores of cultures of eight *Streptomyces* species. The spores of each culture have ornamentation which fits our criteria for inclusion in the hairy group; namely, long, flexible filaments. Within the hairy groups, three subgroups (A, B, and C) are delineated on the basis of sporophore, spore, and hair morphology.

Subgroup A. Subgroup A is characterized by tightly coiled spiral spore chains. Generally, these spores are joined closely and have large numbers of long hairs. Representative cultures are shown in Fig. 1 to 8; these include cultures of *S. glaucus* and *S. pactum*.

Subgroup B. Subgroup B has fairly straight spore chains. The spores are tightly joined and frequently difficult to distinguish. The hairs are fewer and shorter than those of cultures in subgroup A. Representatives of this subgroup (Fig. 9 to 12) include cultures of *S. prasinus* and *S. prasinopilosus*.

Subgroup C. Subgroup C has flexuous spore chains. The spores are more or less spherical and have a "bald-headed" appearance. Representatives of this subgroup (Fig. 13 to 16) include a culture of *S. werraensis*.

Of special interest are features shown in Fig. 11 and 17. Figure 11 is in focus, but two hairs (arrows) appear fuzzy or out of focus. This is due to movement of these hairs at the moment the photograph was taken. Such movement is frequently seen during observation of hairy

FIG. 1-2. *Streptomyces glaucus* UC 5153. 1, Whole spores by TEM; 2, intact culture by SEM. FIG. 3-4. *Streptomyces pactum* UC 2432. 3, Whole spores by TEM; 4, intact culture by SEM. FIG. 5-6. *Streptomyces* sp. UC 2677. 5, Whole spores by TEM; 6, intact culture by SEM. Each bar represents 1 μ m.

FIG. 7-8. *Streptomyces* sp. UC 2768. 7, Whole spores by TEM; 8, intact culture by SEM. FIG. 9-10. *Streptomyces prasinus* UC 5344. 9, Whole spores by TEM; 10, intact culture by SEM. FIG. 11-12. *Streptomyces prasinopilosus* UC 5343. 11, Whole spores by TEM; 12, intact culture by SEM. Each bar represents 1 μ m.

FIG. 13-14. *Streptomyces* sp. no. 284 UC 5187. 13, Whole spores by TEM; 14, intact culture by SEM.
 FIG. 15-16. *Streptomyces werraensis* UC 5345. 15, Whole spores by TEM; 16, intact culture by SEM.
 FIG. 17. *Streptomyces* sp. F-57 UC 2768. Intact culture showing two types of spores by SEM. Each bar represents 1 μ m.

spores. Figure 17 is an SEM photograph of spores of *Streptomyces* sp. F-57. Two types of spores are evident: those with hairs and those without hairs. The spores without hairs are in close contact with the agar surface. This moist

condition may prevent the formation of hairs, or it may cause the hairs to adhere to the surface of the spore and thus cause the spore to appear hairless. (A similar phenomenon was noted by the authors for the rugose type [4].)

These observations cover eight cultures of the group having hairy spores. Other cultures of this group will be included in a future study.

LITERATURE CITED

1. Dietz, A., and J. Mathews. 1962. Taxonomy by carbon replication. I. An examination of *Streptomyces hygroscopicus*. Appl. Microbiol. **10**:258-263.
2. Dietz, A., and J. Mathews. 1968. Taxonomy by carbon replication. II. Examination of eight additional cultures of *Streptomyces hygroscopicus*. Appl. Microbiol. **16**:935-941.
3. Dietz, A., and J. Mathews. 1969. Scanning electron microscopy of selected members of the *Streptomyces hygroscopicus* group. Appl. Microbiol. **18**:694-696.
4. Dietz, A., and J. Mathews. 1971. Classification of *Streptomyces* spore surfaces into five groups. Appl. Microbiol. **21**:527-533.
5. Williams, S. T., and F. L. Davies. 1967. Use of a scanning electron microscope for the examination of actinomycetes. J. Gen. Microbiol. **48**: 171-177.