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Surface Structure of Streptomycete Spores as Revealed by Negative Straining and Freeze-Etching

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The application of a simple negative staining technique and freeze-etching has revealed detailed information on the species-specific surface structure of spores and aerial hyphae of streptomycetes.

The walls of spores and aerial hyphae of streptomycetes are covered with a thin, easily detached surface layer which is responsible for the surface structure (4, 6, 9). This has been investigated in a few species with the aid of carbon replica methods (1, 3, 6). However, this technique allows only limited resolution and it is fairly laborious, which might be the reason why it has not become a standard method for the morphological characterization of streptomycetes in taxonomic investigations.

In the present study, a simple technique was applied to obtain detailed information on the surface layer. Vigorously sporulating colonies of four different species grown on chemically defined minimal medium (5) at 30 C were gently touched with a carbon-coated grid, and the specimen was negatively stained with a droplet of potassium-phosphotungstate or ammonium-molybdate (2, 7). During preparation, parts of the surface layer were often displaced and thus could be examined free from the spores and hyphae. Details of the surface coat were also visible on disintegrating hyphae, which had become sufficiently electron-transparent. The results obtained from negatively stained specimens were compared with those of freeze-etching, a technique which provides excellent information about the arrangement of morphological components in situ; this technique is, however, very exacting and requires complex apparatus. Electron microscopy was carried out on a Siemens Elmiskop Ia electron microscope operating at 80 kv at an instrumental magnification of 10,000 to 40,000.

Streptomyces violaceoruber [*S. coelicolor* (8), strain A3(2) (5)] has smooth spores (Fig. 1). Its surface pattern consists of a network of rodlike components (Fig. 2, 3) appearing in pairs, as demonstrated previously in carbon replicas (6). In negatively stained preparations, the original rod-like pattern (clearly seen in freeze-etchings; Fig. 3) was often disturbed.

S. spadicus (strain ISP 5476) is another species with smooth spores (Fig. 4); in silhouette pictures it cannot be distinguished from *S. violaceoruber* (Fig. 1). However, its surface structure is completely different in that it consists of long, very fine parallel ribs which are slightly curved (Fig. 5, 6).

The spores of *S. viridochromogenes* (number 93 of Krassilnikov's collection) have spines (Fig. 7) produced by the surface layer (9). Negative staining (Fig. 8) and freeze-etching (Fig. 9) revealed that they consist of striated bundles slightly twisted around each other. Similar bundles were also found lying between the spines. These might be produced by a star-shaped configuration of bundles arranged in a cone.

S. glaucescens (strain E.T.H. 24204) has "hairy" spores (Fig. 10). The electron micrographs thus far published are silhouettes, similar to Fig. 10, obtained from untreated whole mounts (11). When negatively stained (Fig. 11), the "hairs" appear as bundles of wide, straight hollow tubes with a regular substructure. Freeze-etchings (Fig. 12) confirmed that the protrusions consist of a cluster of such tubes, twisted around each other.

The results of this study demonstrate that the two techniques provide detailed information about the surface structure of the spores (and also of the hyphae, which often have a different structure) of streptomycetes. Freeze-etchings reveal valuable information about the topography of the surface layers but do not allow such a high resolution as negative staining. A further advantage of negative staining is its simplicity.

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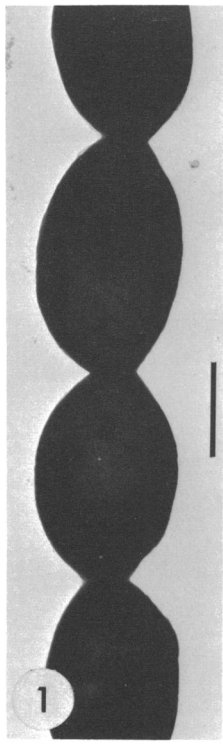


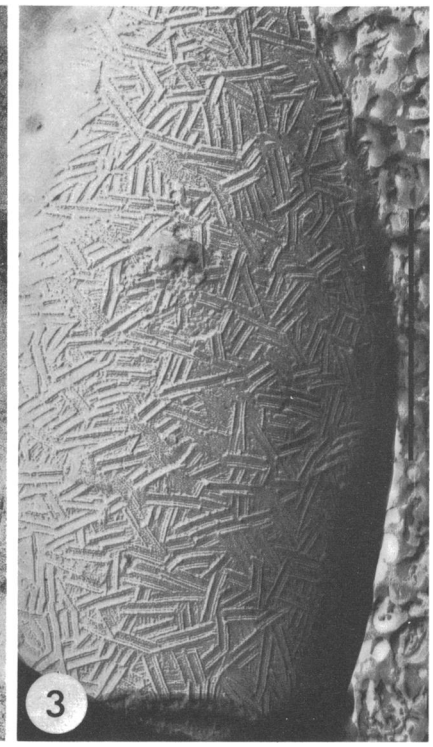
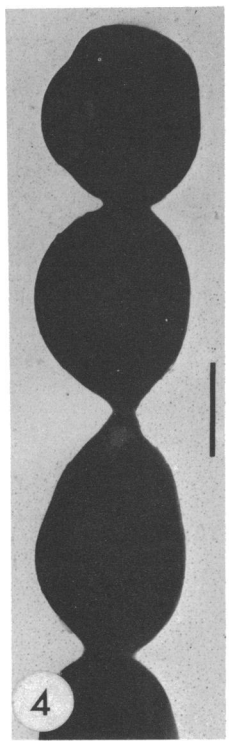


FIG. 1. *Silhouette of spore chain (untreated whole mount) of Streptomyces violaceoruber. In all micrographs the marker represents 0.5 μm.*

FIG. 2. *Negatively stained surface layer of Streptomyces violaceoruber showing the rod-like structure.*

FIG. 3. *Surface of freeze-etched spore of Streptomyces violaceoruber. Note that the rod-like structures appear in pairs.*



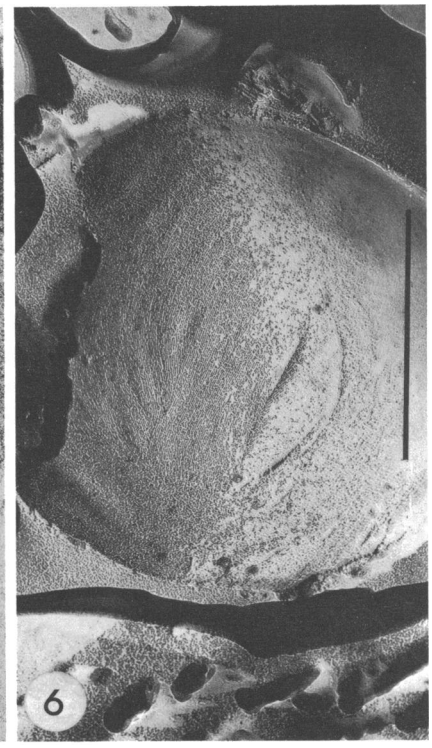


FIG. 4. *Silhouette of spore chain (untreated whole mount) of Streptomyces spadicus.*

FIG. 5. *Negatively stained surface layer of Streptomyces spadicus showing the long curved ribs.*

FIG. 6. *Surface of freeze-etched spores of Streptomyces spadicus.*

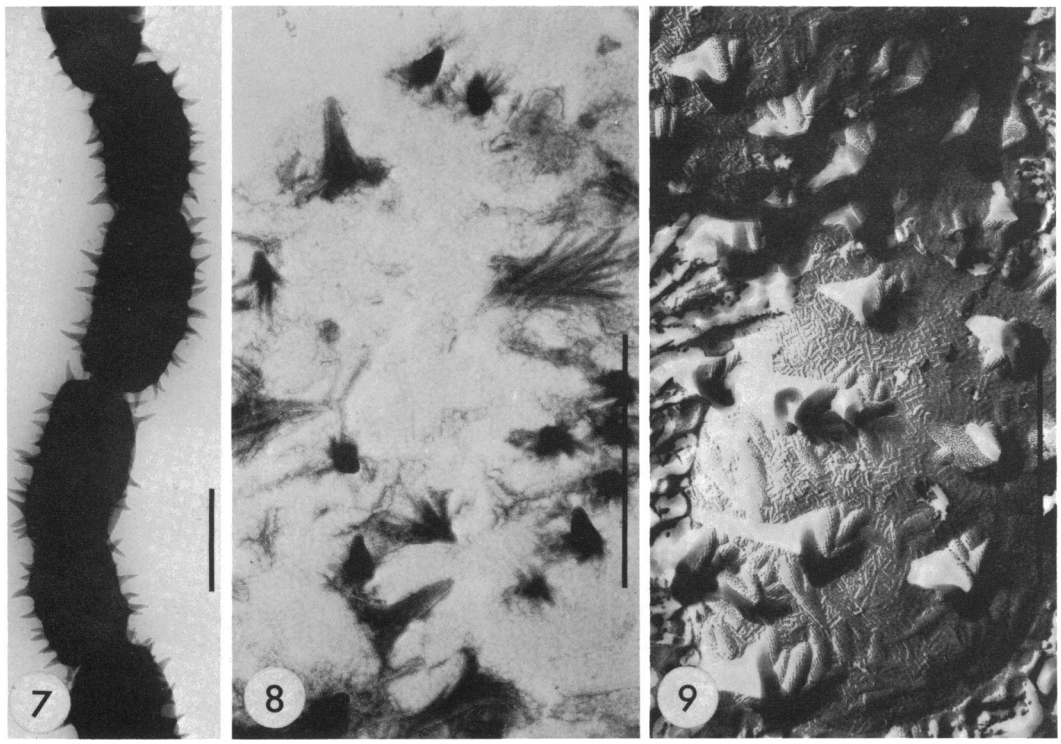


FIG. 7. *Silhouette of spore chain (untreated whole mount) of Streptomyces viridochromogenes.*

FIG. 8. *Negatively stained surface layer of Streptomyces viridochromogenes showing the fine structure of the spines.*

FIG. 9. *Surface of freeze-etched spore of Streptomyces viridochromogenes. Note the striated bundles constituting the spines.*

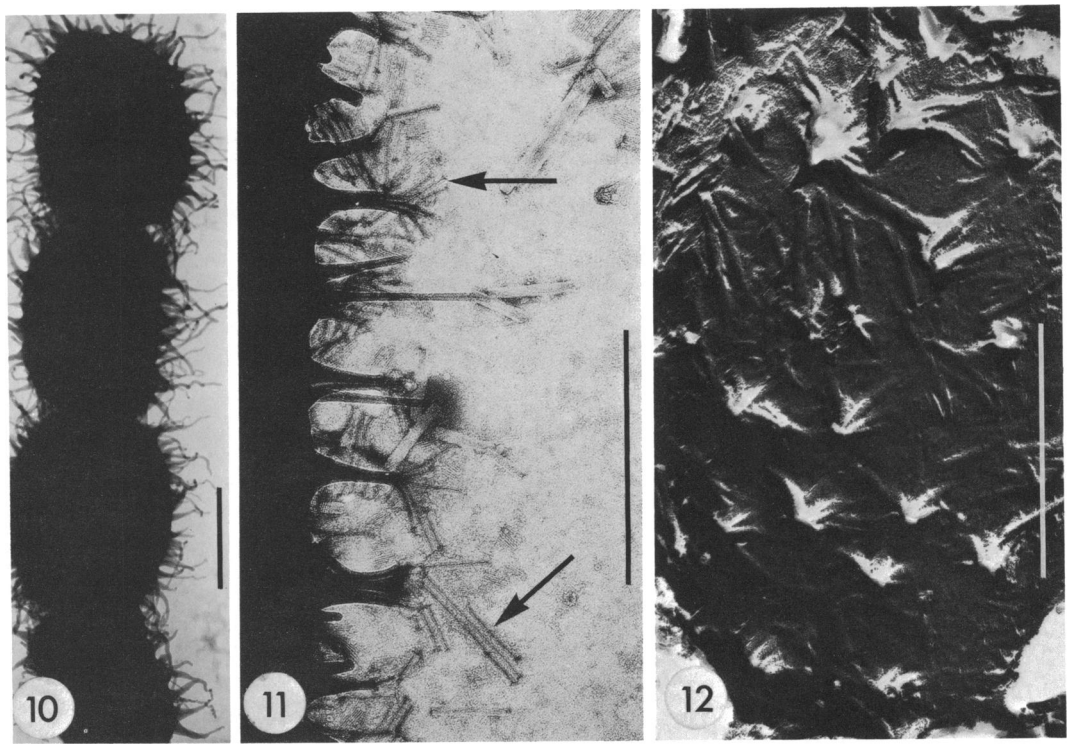


FIG. 10. *Silhouette of spore chain (untreated whole mount) of Streptomyces glaucescens.*

FIG. 11. *Edge of negatively stained spore of Streptomyces glaucescens showing that protrusions are made of several straight tubelike subunits (arrows).*

FIG. 12. *Surface of freeze-etched spore of Streptomyces glaucescens. The protrusions broke off during preparation leaving stumps which show the arrangement of the subunits.*

enabling a rapid morphological characterization (10) of species which have not been before distinguished microscopically.

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