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Scanning Electron Microscopy of Selected Members of the *Streptomyces hygroscopicus* Group

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Distinctive variations in whole spore morphology and spore surface morphology of *Streptomyces hygroscopicus* strains, which had been observed previously by the authors by the preshadowed carbon replica technique, were confirmed by observations with the scanning electron microscope.

Previous publications by the authors (1, 2) described the similarities in spore morphology among certain members of the *Streptomyces hygroscopicus* group and other *Streptomyces* species which are not classified as *S. hygroscopicus*. We also showed that five cultures designated as varieties of *Streptomyces hygroscopicus* had spores which differed from the holotype.

The present study, describing the in situ scanning electron microscopy of six members of the *S. hygroscopicus* group, was done to evaluate this new technique and to determine the validity of previous observations of spore morphology.

Six cultures selected for this study were *S. hygroscopicus* CBS; *S. halstedii* CBS; *S. hygroscopicus* var. *decoyicus* NRRL 2666; *S. hygroscopicus* var. *glebosus* ATCC 14607; *S. hygroscopicus* var. *odoratus* IFO 1545; *S. hygroscopicus* var. *ossamyceticus* ATCC 15420. These organisms were seeded on Czapek's sucrose agar in petri plates containing inclined coverslips by the method of Williams and Davies (3). After 10 days of incubation, the coverslips were carefully withdrawn, leaving a narrow band of agar and attendant growth attached to the coverslip. These coverslips were cemented to aluminum stubs and coated with a thin film of gold in a vacuum evaporator provided with a variable tilt, rotary specimen support. The metal-plated coverslips were examined in a Stereoscan scanning electron microscope (Cambridge Scientific Instruments, Ltd.); instrument time was generously made available by Engis Equipment Co., Morton Grove, Ill.

The holotype of *S. hygroscopicus* has the same spiral spore chain as shown by carbon repligraphy with the transmission electron microscope (Fig. 1 and 2). The rugose surface, consisting of numerous pits bordered by ridges of spore wall material, is identical to the surface found by carbon repligraphy.

S. halstedii is again shown to be identical in spore morphology to the holotype, *S. hygroscopicus* (Fig. 3 and 4). This is further evidence that this organism should be redescribed and reclassified as a member of the *S. hygroscopicus* group.

S. hygroscopicus var. *decoyicus* (Fig. 5 and 6), *S. hygroscopicus* var. *glebosus* (Fig. 7 and 8), *S. hygroscopicus* var. *odoratus* (Fig. 9 and 10), and *S. hygroscopicus* var. *ossamyceticus* (Fig. 11 and 12) are all shown to have spores that differ from the spores of the holotype (Fig. 1 and 2). This confirms our previous observation (1, 2) by carbon repligraphy that these cultures do not conform in spore morphology to *S. hygroscopicus*, and thus warrant redescription and reclassification. *S. hygroscopicus* var. *decoyicus* (Fig. 5 and 6) presents a chain of smooth, unsymmetrical, individual spores arranged in a spiral fashion. *S. hygroscopicus* var. *glebosus* (Fig. 7 and 8) has a similar chain of smooth, individual spores in spiral configuration. However, these spores are more symmetrical in appearance. *S. hygroscopicus* var. *odoratus* (Fig. 9 and 10) has smooth, individual spores in short spiral groupings. *S. hygroscopicus* var. *ossamyceticus* (Fig. 11 and 12) has rough surfaced spores arranged in straight or slightly curved chains. These chains appear to have been twisted.

We present these pictures as evidence that scanning electron microscopy is a valuable, new technique in studying spore morphology of the streptomycetes and in determining the in situ relationship of spores to the aerial mycelium. Such depth of field is not available with any other microscope. We believe that the scanning electron microsope will prove to be invaluable for definitive streptomycete characterization.

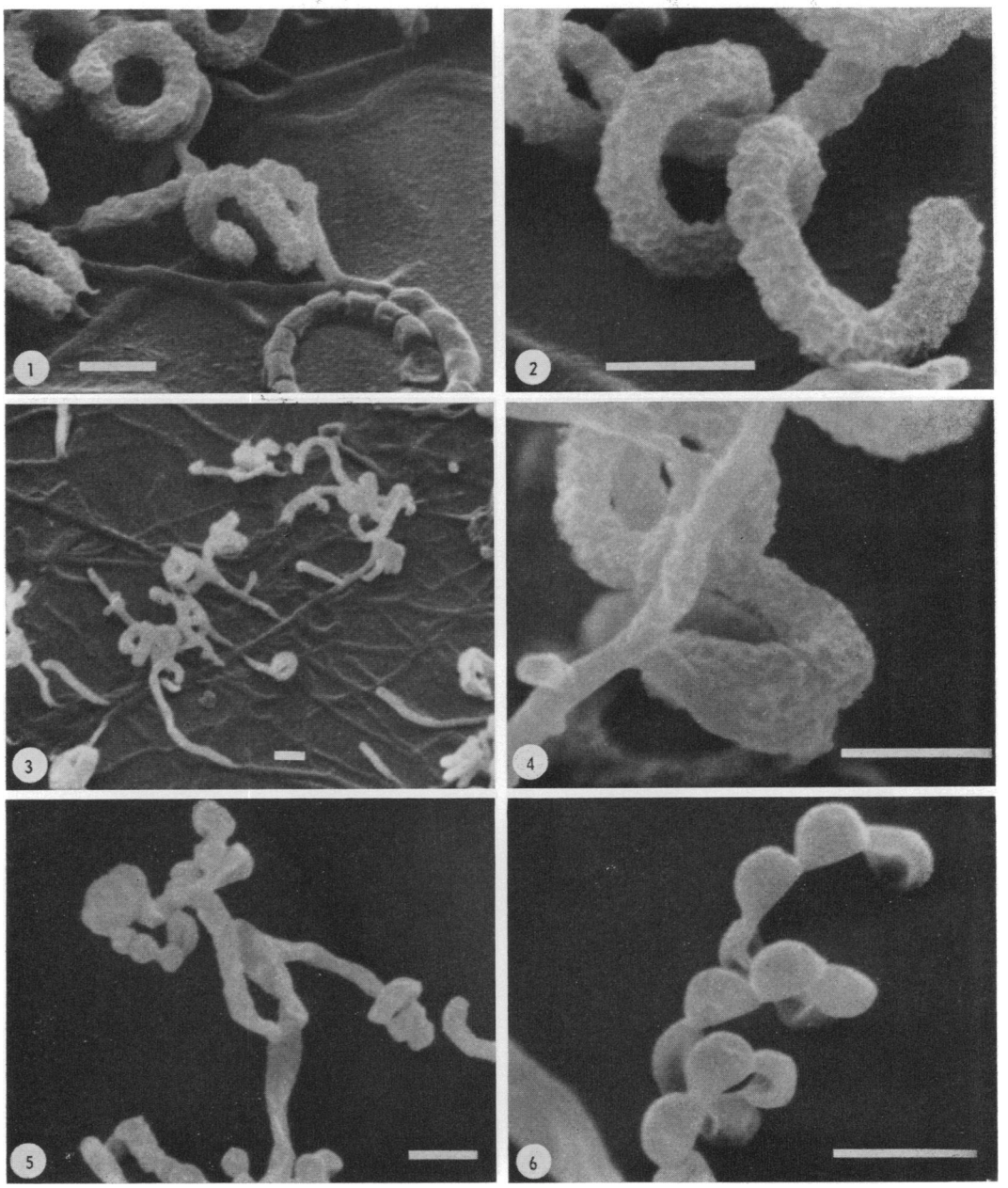

FIG. 1–6. *Fig. 1, Streptomyces hygroscopicus CBS; Fig. 2, Streptomyces hygroscopicus CBS; Fig. 3, Streptomyces halstedii CBS; Fig. 4, Streptomyces halstedii CBS; Fig. 5, Streptomyces hygroscopicus var. decoyicus NRRL 2666; Fig. 6, Streptomyces hygroscopicus var. decoyicus NRRL 2666. Each index mark represents 2 μm.*

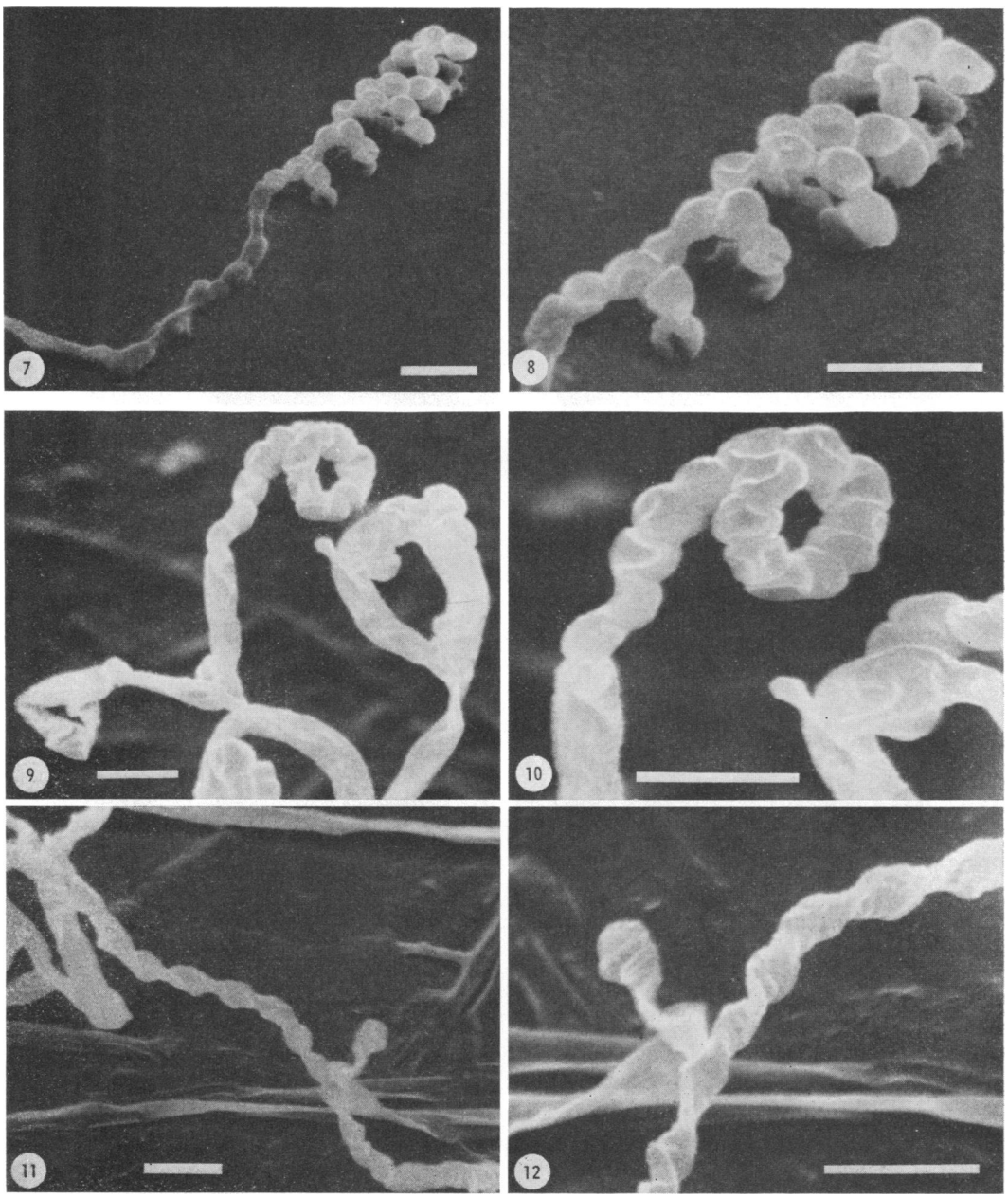

FIG. 7–12. *Fig. 7, Streptomyces hygroscopicus var. glebosus ATCC 14607; Fig. 8, Streptomyces hygroscopicus var. glebosus ATCC 14607; Fig. 9, Streptomyces hygroscopicus var. odoratus IFO 1545; Fig. 10, Streptomyces hygroscopicus var. odoratus IFO 1545; Fig. 11, Streptomyces hygroscopicus var. ossamyceticus ATCC 15420; Fig. 12, Streptomyces hygroscopicus var. ossamyceticus ATCC 15420. Each index mark represents 2 μm.*

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