Taxonomy by Carbon Replication

II. Examination of Eight Additional Cultures of *Streptomyces hygroscopicus*

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Received for publication 14 March 1968

Carbon repligraphic comparison of eight cultures described as *Streptomyces hygroscopicus* showed four distinct types. Five of the cultures could be considered *S. hygroscopicus* by spore type (a relatively nonsegmented spore structure with an extremely wrinkled surface); three of the cultures had spore types that differed from one another and from the *hygroscopicus* holotype. Differentiation was confirmed by Ektacolor pattern.

The preshadowed carbon replica technique was applied to five designated cultures by Dietz and Mathews (1). The present paper presents the evaluation by this technique of eight additional cultures described as *Streptomyces hygroscopicus*.

**MATERIALS AND METHODS**

Eight cultures were examined with an electron microscope by observation of whole spores and preshadowed carbon replicas of spore surfaces. Whole-spore preparations were observed and photographed at 5,000 X. Carbon replicas of spore surfaces were photographed at 10,000 X. Cultures were grown on six selected media and were photographed in color after 7 days of growth. These conditions and procedures were described fully in the first paper of this series (1).

The eight cultures studied were (I) *S. hygroscopicus* (Jensen) Waksman CBS, (II) *S. hygroscopicus* NRRL 2751, (III) *S. hygroscopicus* NRRL 2387, (IV) *S. hygroscopicus* var. ascymeticus ATCC 14891, (V) *S. hygroscopicus* var. glebosus ATCC 13810, (VI) *S. hygroscopicus* var. ascomyceticus ATCC 14607, (VII) *S. hygroscopicus* var. ossamyceticus ATCC 15420, (VIII) *S. hygroscopicus* var. odoratus IFO 1545. These will be referred to in this paper by the Roman numeral preceding each organism.

**RESULTS AND DISCUSSION**

Electron micrographs of I, the holotype, are shown in Fig. 1 and 2. The picture of whole spores shows a compact, spiral growth with an irregular, possibly warty, surface. The carbon repligraph of the spores shows a rugose surface and relatively nonsegmented appearance. This does not imply that these spore chains cannot dissociate into individual spores. Electron micrographs of II (Fig. 3 and 4), III (Fig. 5 and 6), IV (Fig. 7 and 8), and V (Fig. 9 and 10) show great similarity to the holotype. The compact spiral growth and rugose surfaces indicate that these are five cultures producing nearly identical spore chains.

Electron micrographs of VI (Fig. 11 and 12), VII (Fig. 13 and 14), and VIII (Fig. 15 and 16) show spore chains that are distinctly different from the holotype. The pictures of VI show easily recognizable spores with ridged, detailed surfaces and smooth, oval outline. The spores of VII are electron-lucent and have less surface detail. The spores of VIII are also distinctly different, with ridged surfaces and squared ends. Ektacolor prints (Fig. 17–24) show that the eight cultures designated *S. hygroscopicus* produce different color patterns. I, considered the holotype, serves as the reference for comparison of the other cultures. IV and V closely resemble the holotype in color pattern, with only minor differences noted. II and III show more deviation from I, but are similar in enough respects to be included in the group. The lack of conformity of the color patterns of VI, VII, and VIII is the basis for excluding them from this group.

Using data obtained by electron microscopy and color photography of growth on six agar media, one can conclude that I, II, III, IV, and V are closely related members of the *S. hygroscopicus* group. Using these two criteria, it would be nearly impossible to differentiate these five organisms. VI, VII, and VIII show sufficient dissimilarity from the holotype and each other that redescriptions are merited.

We believe that the preshadowed carbon replica technique should be more widely used in charac-
Fig. 1. Streptomyces hygroscopicus CBS, whole-spore micrograph. (All markers represent 1 μ.)
Fig. 2. S. hygroscopicus CBS, carbon repligraph.
Fig. 3. S. hygroscopicus NRRL 2751, whole-spore micrograph.
Fig. 4. S. hygroscopicus NRRL 2751, carbon repligraph.
FIG. 5. *Streptomyces hygroscopicus* NRRL 2387, whole-spore micrograph.
Fig. 6. *S. hygroscopicus* NRRL 2387, carbon repligraph.
Fig. 7. *S. hygroscopicus* var. *ascomyceticus* ATCC 14891, whole-spore micrograph.
Fig. 8. *S. hygroscopicus* var. *ascomyceticus* ATCC 14891, carbon repligraph.
Fig. 9. *Streptomyces* hygroscopicus ATCC 13810, whole-spore micrograph.
Fig. 10. *S. hygroscopicus* ATCC 13810, carbon repligraph.
Fig. 11. *S. hygroscopicus* var. glebosus ATCC 14607, whole-spore micrograph.
Fig. 12. *S. hygroscopicus* var. glebosus ATCC 14607, carbon repligraph.
FIG. 13. Streptomyces hygroscopicus var. ossamyceticus ATCC 15420, whole-spore micrograph.
FIG. 14. S. hygroscopicus var. ossamyceticus ATCC 15420, carbon repligraph.
FIG. 15. S. hygroscopicus var. odoratus IFO 1545, whole-spore micrograph.
FIG. 16. S. hygroscopicus var. odoratus IFO 1545, carbon repligraph.
terizing new isolates and in the redescription of previously described species.

**ACKNOWLEDGMENTS**

We are indebted to N. Drake for the color prints.

**LITERATURE CITED**