

Classification of *Streptomyces* Spore Surfaces into Five Groups

ALMA DIETZ AND JOHN MATHEWS

Research Laboratories, The Upjohn Company, Kalamazoo, Michigan 49001

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Streptomyces spore surfaces have been classified into five groups, smooth, warty, spiny, hairy, and rugose, by examination of carbon replicas of spores with the transmission electron microscope and by direct examination of spores with the scanning electron microscope.

Kriss et al. (12) were probably the first to do electron microscopy of streptomycete spores. Their finding of smooth spores was followed by those of Carvajal (3) and Bringman (2), who also found smooth spores on the cultures they studied. Flaig et al. (10) described thorny or spiny spores from certain streptomycete species. Küster (13) confirmed the presence of spiny spores on certain species and also found hairy spores and warty spores on other species. Baldacci and Grein (1) observed smooth, spiny, and hairy spores but failed to mention warty spores. Enghusen (8) suggested that the warty surface of spores was an artefact of preparation. Flaig and Kutzner (11), Ettlinger et al. (9), Preobrazhenskaya et al. (15), and Tresner et al. (17) found and discussed four spore surface types: smooth, warty, spiny, and hairy. Lechevalier and Tikhonenko (14) studied the formation of spines during spore maturation. The concept of smooth, warty, spiny, and hairy spore surfaces was sufficiently established that Cross and MacIver (4) and Shirling and Gottlieb (16) listed this as one of the criteria to be used in characterizing species.

These studies were based on the direct observation by electron microscopy of whole streptomycete spores. Instead of spore surfaces these workers were looking at silhouettes of spores. The size and density of whole spores prevent penetration of the electron beam with sufficient strength to reveal surface structure. Information about the spore surface obtained by studying the silhouette is at best conjecture and may be misleading.

In 1961, the authors began to use preshadowed carbon replicas in studying streptomycete spore surfaces. This technique provides more information about gross characteristics of the spore surface and permits study of the fine detail of the spore coat. We have examined more than 500 cultures by this method. Our findings (5, 6) show that, in addition to the four recognized spore

surface types, a fifth type should be designated, namely "rugose."

This paper presents pictures of the five spore surface types as revealed by transmission electron microscopy (TEM) of whole spores and preshadowed carbon replicas, scanning electron microscopy (SEM) of intact cultures, and phase-contrast microscopy of intact cultures. Characteristics of the fifth spore surface type will be discussed.

MATERIALS AND METHODS

Based on previous information, cultures examined in this study were chosen to represent the four established types and the proposed new type. These cultures and the types they represent are: smooth, *Streptomyces albus*, ATCC 3004; warty, *S. steffisburgensis*, NRRL 3193; spiny, *S. echinatus*, NRRL 2587; hairy, *S. pactum*, NRRL 2939; rugose, *S. hygroscopicus*, (Jensen) Waksman, CBS.

Conditions of culture and growth have been described previously (5, 7). Whole spore mounts and preshadowed carbon replicas were prepared for TEM examination by the procedures previously described (5). Cultures for examination by SEM were prepared by the method of Williams and Davies (18) as described previously (7). Cover slips with adherent culture were cemented to stubs, coated with a thin film of evaporated aluminum, and examined with a Stereoscan SEM (Cambridge Scientific Instruments, Ltd.) in the laboratories of Alpha Research and Development, Inc., Blue Island, Ill. Extra cover slips with adherent growth were placed, growth side up, on glass slides and examined by phase-contrast microscopy. Appropriate, representative photographs were made by each of the three methods and are used to substantiate our designation of a fifth spore surface type.

RESULTS AND DISCUSSION

Figures 1 to 5 show the five species studied as seen by (A) phase-contrast, (B) low-magnification SEM, (C) medium-magnification SEM, (D) whole spores by TEM, and (E) preshadowed

carbon replicas of spores by TEM. The phase-contrast pictures, designated part A, in the figures show the maximum information that can be obtained by this method with its relatively poor resolution and shallow depth of field. In compari-

son, the low-magnification SEM pictures, designated section B, show much more concerning spatial arrangement, attachment of spores to mycelium, and development of spores. At higher magnification (the sections designated C), SEM

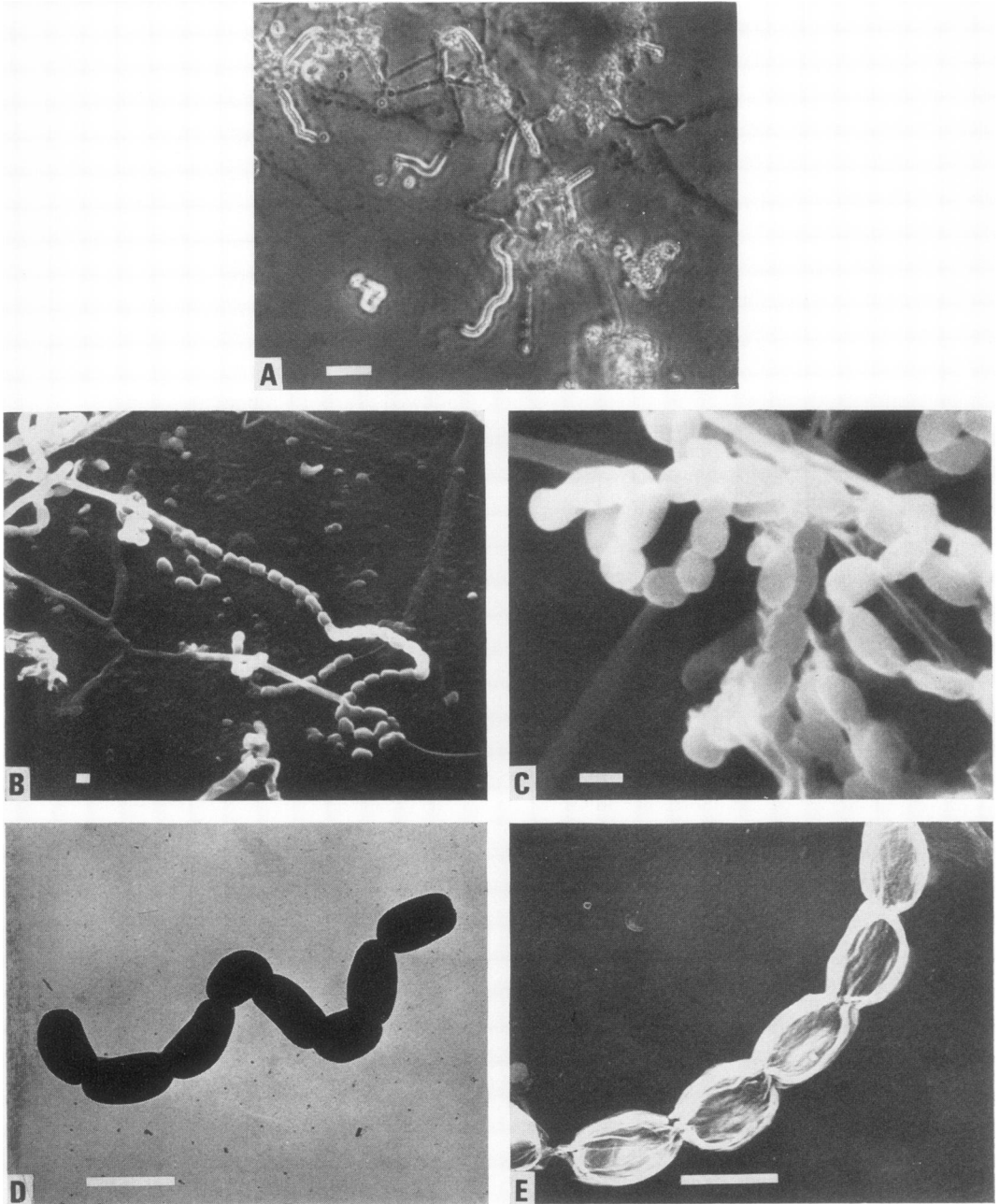


FIG. 1. *Streptomyces albus*. (A) Phase-contrast microscopy; (B) low-magnification SEM; (C) medium-magnification SEM; (D) whole spores by TEM; (E) preshadowed carbon replica of spores by TEM. In part A of all figures, the bar represents 10 μm . In all sections marked B, C, D, and E, the bar represents 1 μm .

reveals information concerning surface structure and detail. Some alteration of surface structures, which is evident, may be caused by the aluminum coating process, charging in the electron beam, or a combination of these factors. The sections design-

ated D show the well-known pictures of whole spores observed by TEM. These pictures provide information concerning size and shape. The pre-shadowed carbon replicas observed by TEM (section E) show, in addition to size and shape,

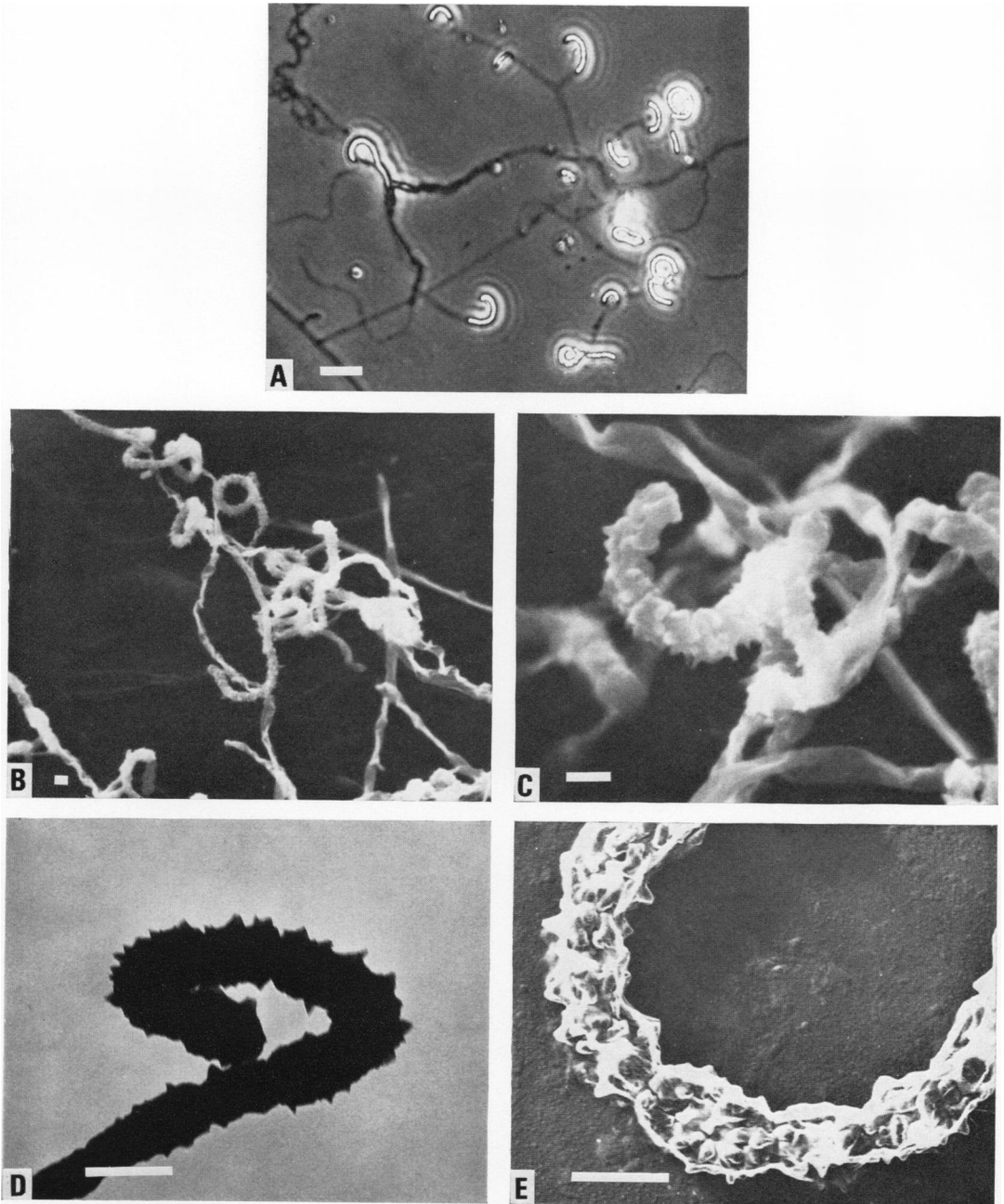


FIG. 2. *Streptomyces steffisburgensis*. (A) Phase-contrast microscopy; (B) low-magnification SEM; (C) medium-magnification SEM; (D) whole spores by TEM; (E) preshadowed carbon replica of spores by TEM.

details of the structures present on the surface of the spores. Magnifications of these pictures were kept low enough to show several spores per field. At higher magnification, details of surface subunits become apparent.

Looking at Fig. 2D and 5D, one sees spore chains with poorly defined segmentation and many short protrusions. One could easily classify both of these cultures as the warty spore type based on these two pictures alone. Figures 2C,

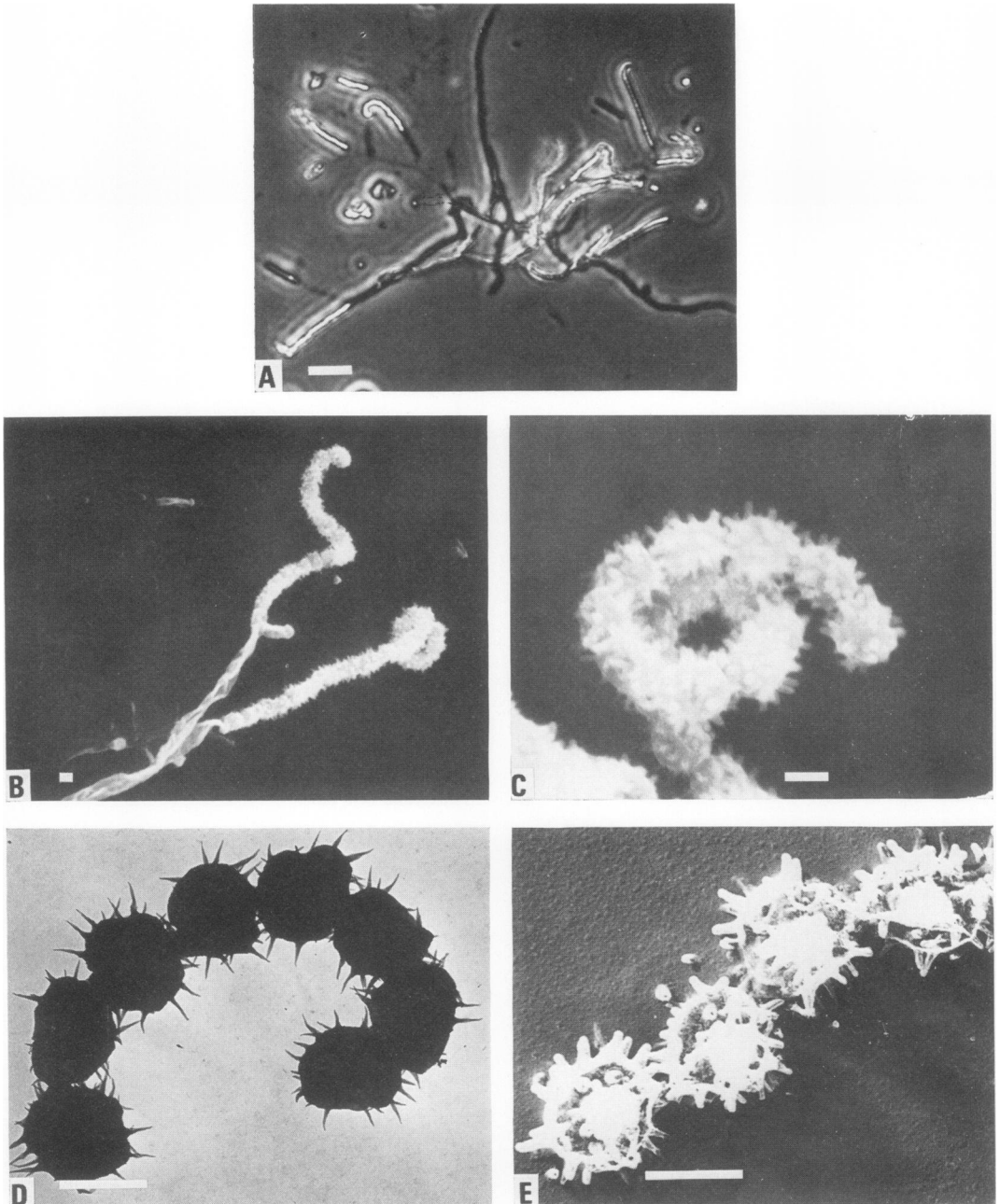


FIG. 3. *Streptomyces echinatus*. (A) Phase-contrast microscopy; (B) low-magnification SEM; (C) medium-magnification SEM; (D) whole spores by TEM; (E) preshadowed carbon replica of spores by TEM.

2E, 5C, and 5E show surface detail of the spores of these two cultures and enable one to differentiate between the two. Figures 2C and 2E show spores with short, individual protrusions, i.e., warts, on the surface. Figures 5C and 5E show

spores with a wrinkled surface, i.e., rugose, and no individual protrusions.

Figure 5B shows what are apparently two types of spores. These are only found by SEM. We believe that the light-colored spirals of unseg-

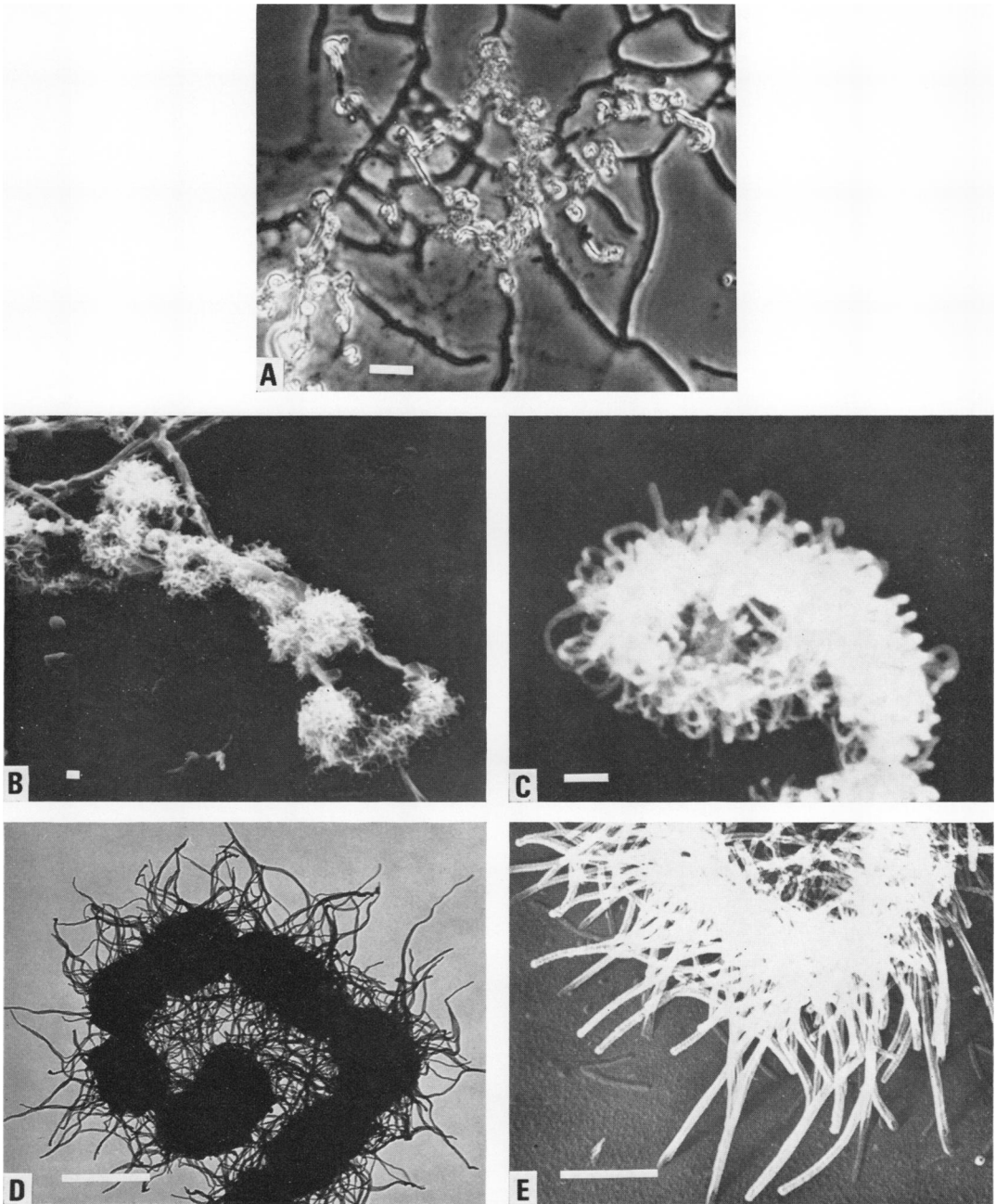


FIG. 4. *Streptomyces pactum*. (A) Phase-contrast microscopy; (B) low-magnification SEM; (C) medium-magnification SEM; (D) whole spores by TEM; (E) preshadowed carbon replica of spores by TEM.

mented, rugose-surfaced spore chains are those which develop as aerial spores with no direct contact with the agar surface. The darker, segmented, smooth spores are those which have developed while in direct contact with the moist

agar surface. This latter spore configuration is never found on replicas or whole spore mounts by TEM, since such spores would be difficult to pick up on a filmed grid or a glass disc.

On the basis of our previously reported obser-

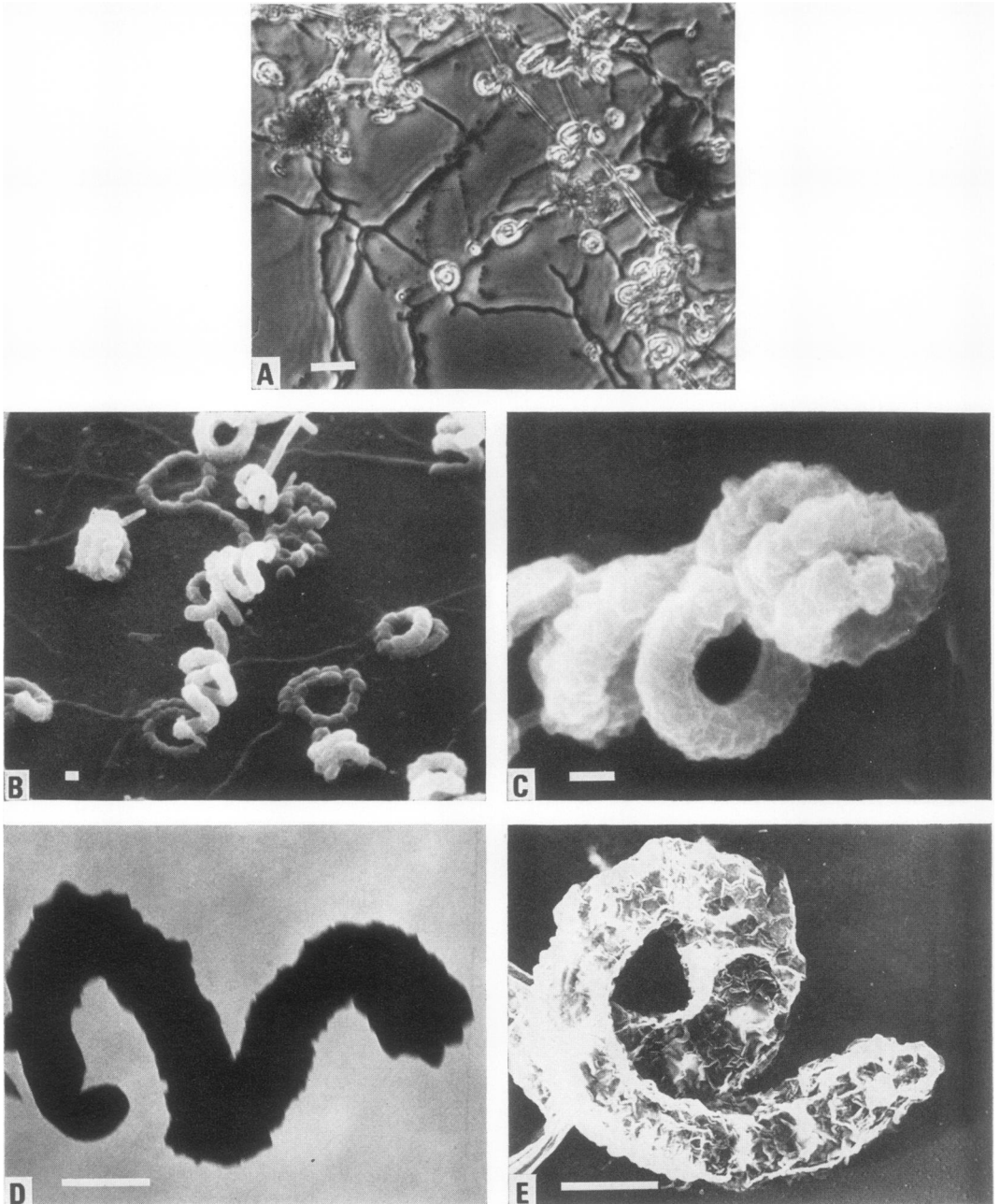


FIG. 5. *Streptomyces hygrosopicus*. (A) Phase-contrast microscopy; (B) low-magnification SEM; (C) medium-magnification SEM; (D) whole spores by TEM; (E) preshadowed carbon replica of spores by TEM.

vations (5-7) plus the pictures presented here, we believe that in addition to the smooth, warty, spiny, and hairy spore surface types a fifth type should be recognized. Because of the nature of the surface we have designated this the rugose type.

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