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CHAPTER 21

Application of Electron Microscopy to Cultures of Industrial Significance

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Since characterization of cultures is a necessary part of a patent application disclosure of microbial products, it is imperative for an industrial taxonomist to obtain as many type cultures as possible, study them under standardized conditions, and develop a practical in-house identification scheme. Both light and electron microscopic observations are significant in the identification scheme. Electron microscope studies often show important details not detectable with the routinely used light microscope. Illustrations will be used to indicate the value of electron microscopy for identifying cultures of industrial significance.

INTRODUCTION

Industrial microbial taxonomists must determine or verify culture identity and characterize cultures for patent applications. Reliable, reproducible procedures must be applied to resolve culture identification problems. In industry, time and manpower are costly. Therefore, it is beneficial to obtain significant information with minimal effort. Minimal time is required for preparation and direct examination of specimens with the electron microscope.

METHODS

Many different, simple preparatory methods for electron microscopic examination of microbes are given in the literature. The following references provide useful methodology: Bulba (1968), Dietz and Mathews (1962, 1969, 1971, Baldacci et al. (1971 a,b), Holt and Leadbetter (1969), Locci (1971, 1972), Locci and Baldan (1971 a,b), and Murphy and Campbell (1969). Simple, reliable methods of preparation of unicellular and filamentous microbes for direct examination with the transmission electron microscope (TEM) and the scanning electron microscope (SEM) are presented in Table 1.

RESULTS AND DISCUSSION

Light microscopic examination is essential to the characterization, classification, and taxonomy of a culture. However, the limitations of the magnification available impose a severe restriction on evaluation of microscopic material. Surface structures, for all microbes, and spatial arrangement in filamentous forms are seen best by examination with the higher resolution electron microscopes. Direct examination with the TEM provides silhouette information only. Direct examination with the SEM provides surface structure as well as spatial arrangement information. In certain situations (e.g., confirmation of surface details seen by SEM) it may be desirable or preferable to study surface detail with the TEM, using the more tedious techniques of carbon replication or freeze etching.

The need for reliable and reproducible conditions for examination of microbes for identification and characterization must be continually stressed. One group of microbiologists attempted to bring order out of chaos in characterization of the actinomycete genus *Streptomyces*. Over 400 species of *Streptomyces* were studied by 40 collaborators. Standardized

TABLE 1. *Preparation of microbes for direct examination by electron microscopy*

	TEM	SEM
Unicellular Forms		
Bacteria Yeasts		
18 h growth on agar slant or plate or optimum time for development of culture for a particular study.	Triple wash and centrifuge in sterile saline. Resuspend in saline and put drop on carbon-coated grid. Examine	Put loopful of growth in 5 ml sterile distilled water. Mix gently. Put drop on cover glass, polished stub, or surface of choice and spread. Mount cover glass or other material on SEM stub with double-backed scotch tape. Make contact between specimen and stub with conductive coating to prevent electrical charging. Coat with gold-palladium (preferably with a sputter-coater). Examine.
Filamentous Forms		
Actinomycetes Fungi		
Streak inoculum (preferably blended 48 h shake flask growth) in cross hatch on agar in petri plate and across cover glass inserted at 45 degrees in agar. Incubate length of time for desired observation.	Touch carbon-coated grid to growth. Examine.	Mount cover glass on SEM stub with double-backed scotch tape. Make contact between specimen and stub with conductive coating to prevent electrical charging. Coat with gold-palladium (preferably with a sputter-coater). Examine. OR Remove small amount of material with fine pointed object and smash or spread on double-backed scotch tape on SEM stub. Treat as cover glass.

procedures (Shirling and Gottlieb 1966) included observations of spore chains by light microscopy and spore silhouettes by TEM. In these studies the spore silhouettes were photographed and reproduced in plates accompanying the published descriptions (Shirling and Gottlieb 1968 a,b, 1969, 1972). It is unfortunate that these plates are not reproduced in *Bergey's Manual,* 8th edition (Buchanan and Gibbons 1974). Photomicrographs of microbes are "thumb prints" for the individual concerned with microbial identification.

Many industrial microbiologists have developed in-house keys, based on their own standardized conditions, to supplement published keys. Defined media and growth conditions are essential for biochemical and physiological tests. However, any medium supporting good growth of a microbe may be used for growth for TEM or SEM examination. This should not obviate the responsibility for reporting the medium used.

Results obtained by light microscopy and electron microscopy generally are reproducible. Significant differences seen by direct examination of specimens with the light microscope, the TEM, and the SEM are summarized in Table 2. The SEM is a valuable aid to the industrial

TABLE 2. *Comparative benefits of direct observation of microbes by light microscopy (LM) and electron microscopy (TEM and SEM)*

	Light Microscopy	Electron Microscopy	
		TEM	SEM
Unicellular Forms			
Bacteria	Gram stain Motility Type by special stains Spores By special stain Drug effects if length or width affected	Silhouette Type of motility Spore silhouette Drug effects if length or width affected	Surface pattern Type of motility Spore surface pattern Drug effects on surface as well as growth (Klainer and Geis 1973)
Yeasts	Same as for bacteria where applicable	Same as for bacteria where applicable	Same as for bacteria where applicable
Filamentous Forms			
Actinomycetes Fungi	Disrupted growth unless special slide cultures are prepared. (Loose interpretation of growth). Motility as for bacteria Spore surface pattern: Impossible for actinomycetes At upper limits for some fungi Surface detail for mycelium and spore bearing structures: Impossible for actinomycetes At upper limits for some fungi (Raper and Fennell 1965)	Same as above	Same as above PLUS Spatial arrangement and Mycelial development Spore surface pattern: Good to excellent. Much more detail for fungal spores. (Locci 1972; Sands 1976) Surface detail for mycelium and spore-bearing structures: Good to excellent.

microbiologist. Characteristics of structures concerned with motility, surface pattern, and, in the case of filamentous forms, spatial arrangement can be noted without laborious procedures which may cause alteration of material. Examples of details to be found in simple SEM preparations are given in Figs. 1-10. The value of SEM to the industrial microbiologist is documented in the following explanations of the figures.

Two species of *Pseudomonas*, *Ps. aeruginosa* and *Ps. fluorescens*, which we had obtained from the American Type Culture Collection and had carried in our collection by the species designation applied when received, were selected for SEM studies to show difference in flagellation. *Ps. aeruginosa* has polar monotrichous flagellation and *Ps. fluorescens* has polar multitrichous flagellation. Figure 1 demonstrates both cultures showed polar monotrichous flagellation. On checking the 12th edition of the ATCC catalog, we noted the culture designated *Ps. fluorescens* was considered by R. R. Colwell to be *Ps. aeruginosa*. Thus we confirmed Colwell's notation by finding polar monotrichous flagellation on the so-called *Ps. fluorescens*. SEM examination of motile cultures can give information in minutes as opposed to the laborious work involved in demonstrating flagellation by staining techniques or TEM examination.

Escherichia coli ATCC 4157 is reported to be the original Escherich strain, producing rough and smooth forms. This was confirmed by direct SEM examination of the culture (Fig. 2).

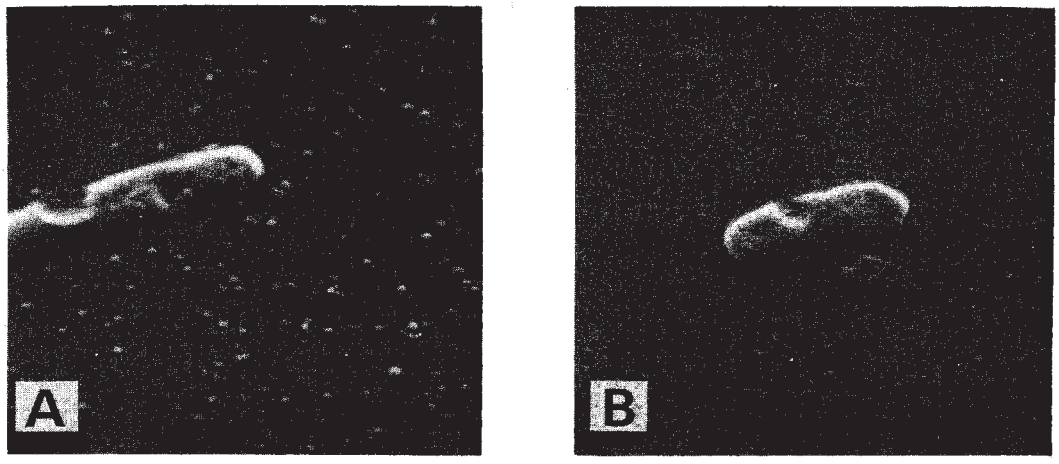

FIG. 1.
a. *Pseudomonas aeruginosa* ATCC® 10145 (UC® 104) - Neotype.
b. *Pseudomonas fluorescens* ATCC 12121 (UC 3058) - *Pseudomonas aeruginosa* per R. R. Colwell (ATCC Cat. 12th ed.). *Ps. aeruginosa* has polar monotrichous flagellation; true *Ps. fluorescens* has polar multitrichous flagellation.

In Fig. 3 differences are shown in the surface appearance of the parent and mutant of a strain of *Saccharomyces cerevisiae.* The parent (Fig. 3a) is well defined; the mutant (Fig. 3b) is collapsed.

In Fig. 4 the various significant morphological characteristics of *Aspergillus echinulatus* are depicted. Slide preparations and examination for the details of foot cell, development of conidial head, conidial surface, and production of ascospores can be very time-consuming. With direct SEM examination, the fine details one needs for characterization can be found and permanently recorded with ease. In addition, details not previously reported may be noted. In Fig. 4 we show the details of a developing head and warty surface protuberances on the conidiophore.

In Figs. 5-9 are shown very different spore chains and spore surfaces of five *Streptomyces* species. These cultures can be confused if their spore chain type and spore surface type are not known. All have blue aerial growth and are melanin positive. The SEM's pictured illustrate the value of the SEM in actinomycete taxonomy.

In Fig. 10 we have depicted a mass of material to show that microbial cells can be seen in detail and to indicate the value of the SEM in checking products, raw materials, etc., as well as in confirmation of identity or newness of a microbe.

In this presentation examples have been given of information that can be obtained from SEM examination of very simple preparations of microorganisms or of material that may be of concern to an industrial microbiologist. In conclusion, it must be stressed that microbial examination must be done by a competent and concerned microbiologist if the full value of any microscopic study is to be realized.

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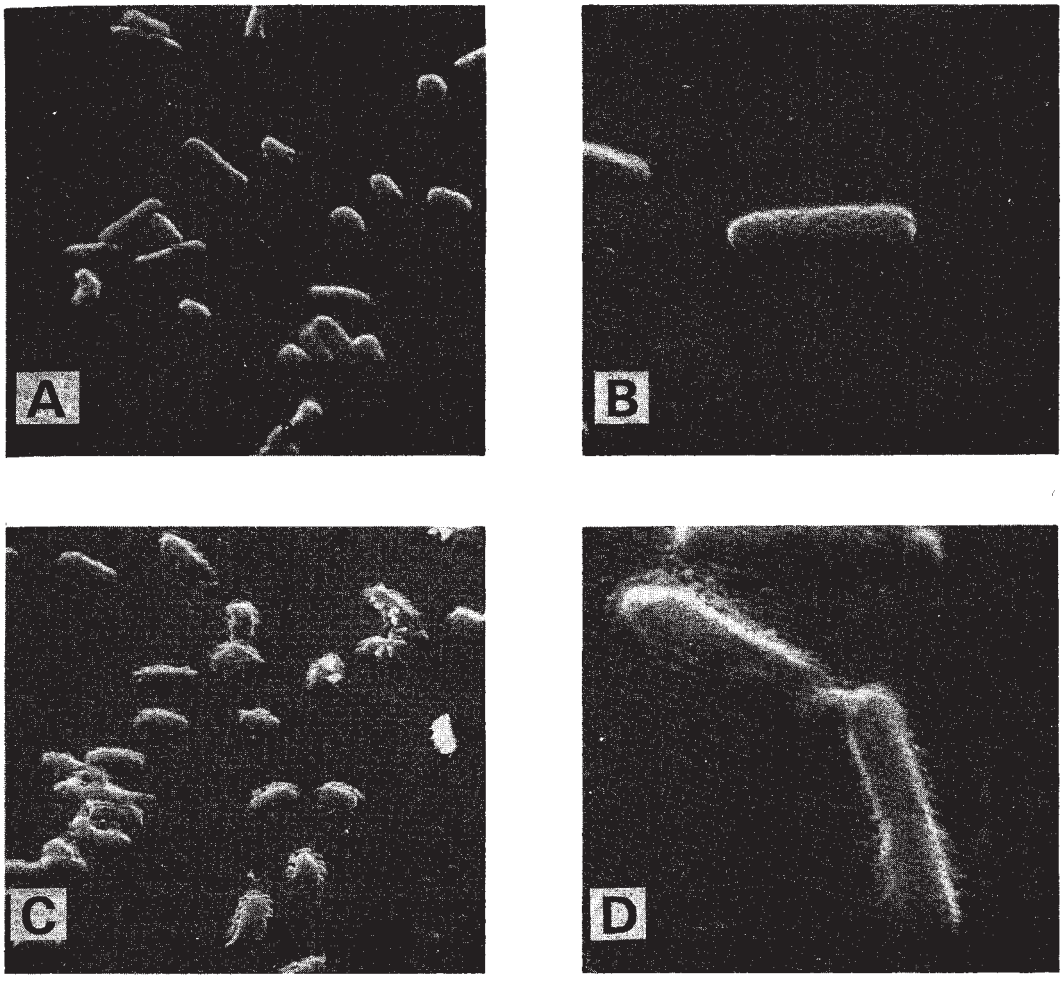


FIG. 2. *Escherichia coli* ATCC 4157 (UC 48) - Escherich strain - produces smooth and rough forms (ATCC Cat. 12th ed.).
a., b. smooth cells
c., d. rough cells

304 SYMPOSIUM: CLASSIFICATION OF MICROORGANISMS OF INDUSTRIAL IMPORTANCE

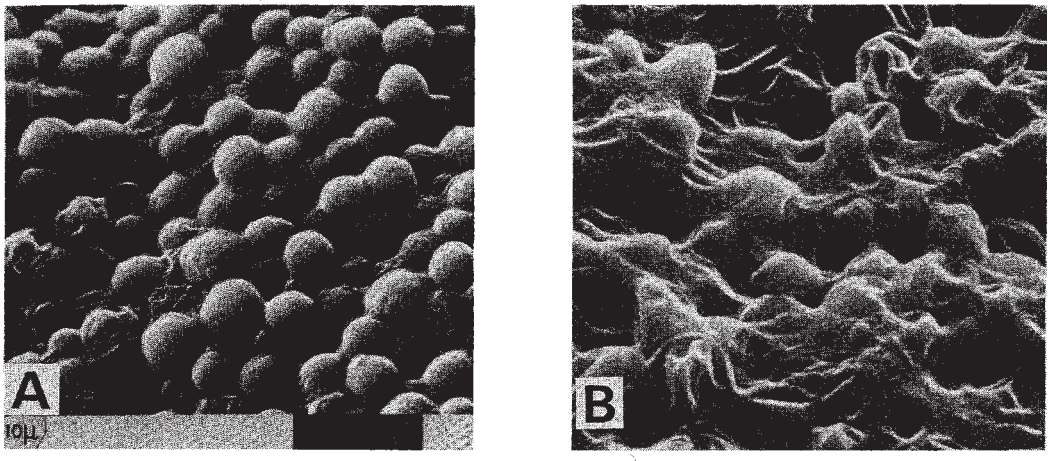
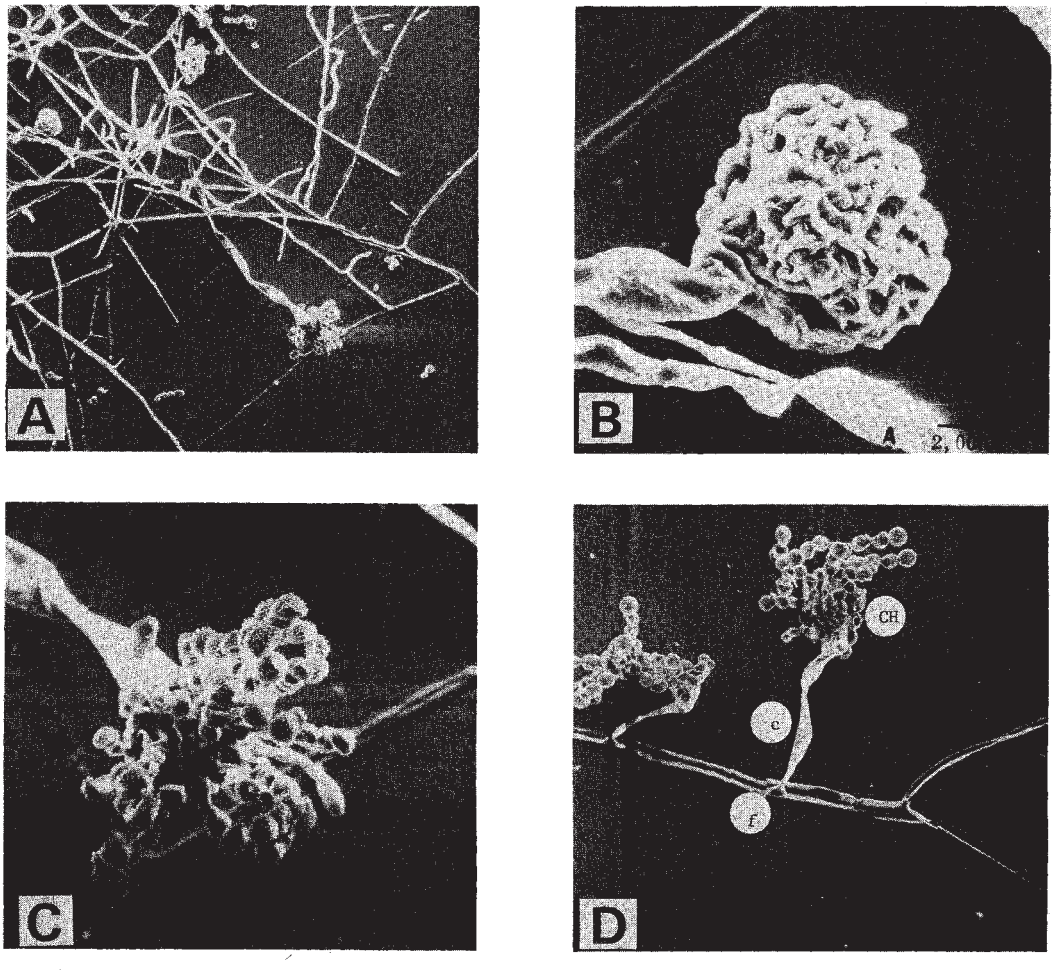


FIG. 3. *Saccharomyces cerevisiae.*
a. UC 7189 = parent
b. UC 7188 = mutant



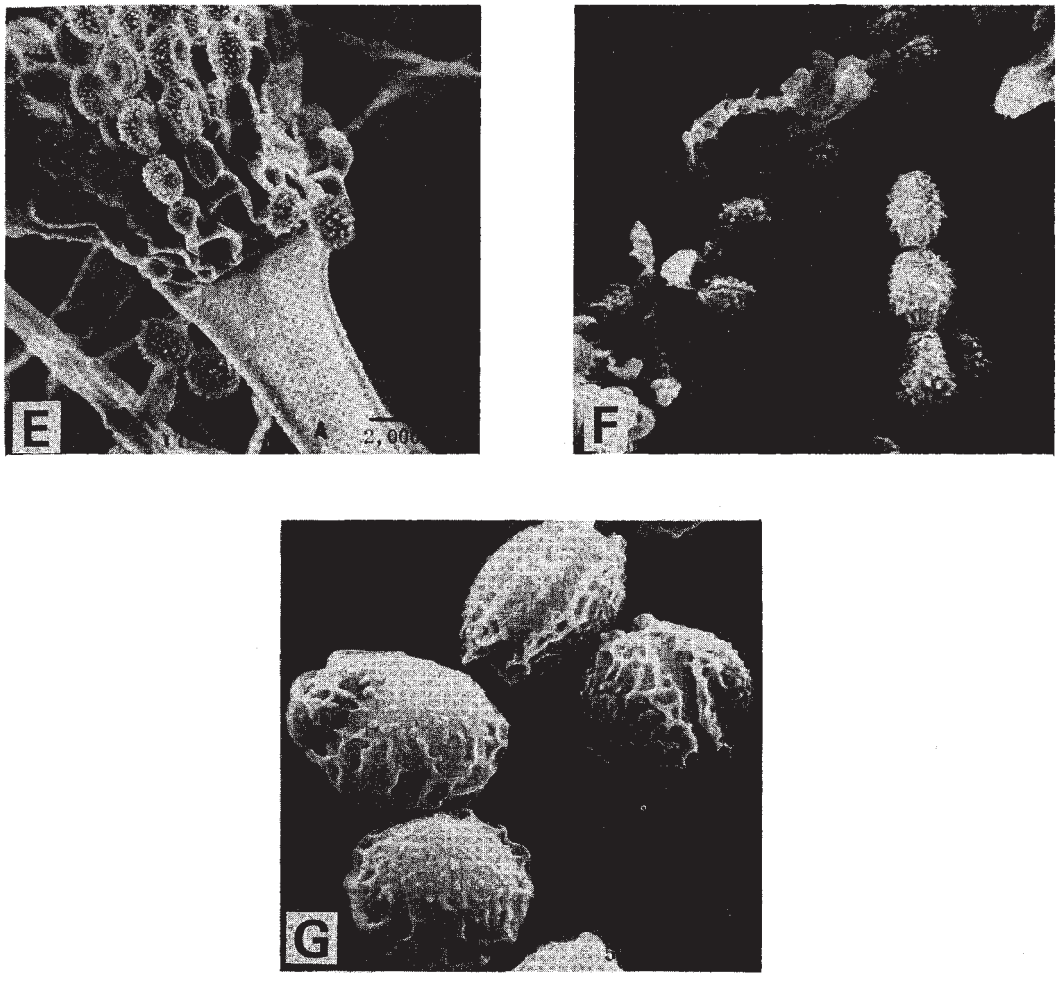


FIG. 4. *Aspergillus echinulatus* LSHTM BB-190 (UC 4403).

a. Spatial arrangement of vegetative and aerial growth and developing conidial heads
b. Immature conidial head
c. Mature conidial head
d. Foot cell (f) - Conidiophore (c) - Conidial Head (CH)
e. Warty conidiophore and echinulate conidia
f. Detached conidia showing echinulate surface
g. Lenticular ascospores showing surface pattern and equatorial ridge

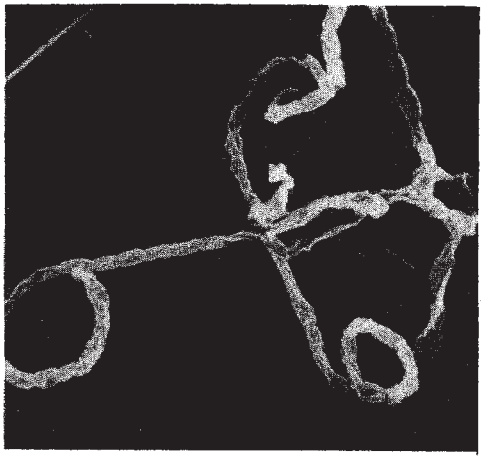


FIG. 5. *Streptomyces caelestis* UC 2011 (smooth spores).

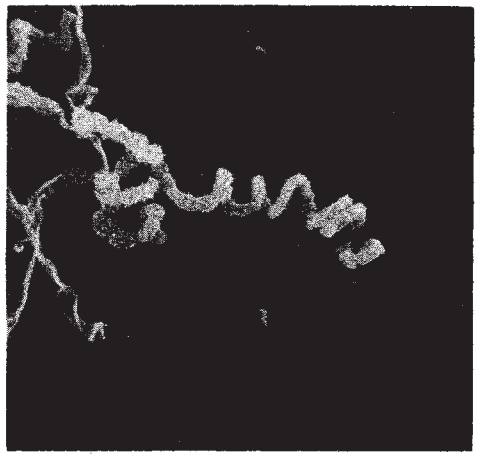


FIG. 6. *Streptomyces chartreusis* UC 2012 (spiny spores).

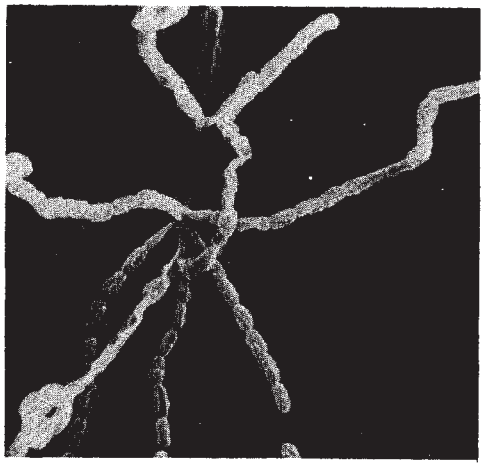


FIG. 7. *Streptomyces viridochromogenes* UC 2324 (spiny spores).

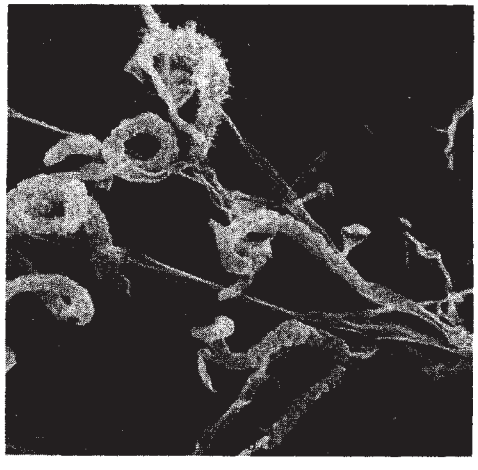


FIG. 8. *Streptomyces lomondensis* var. *lomondensis* UC 5355 (spores with fine hairs).

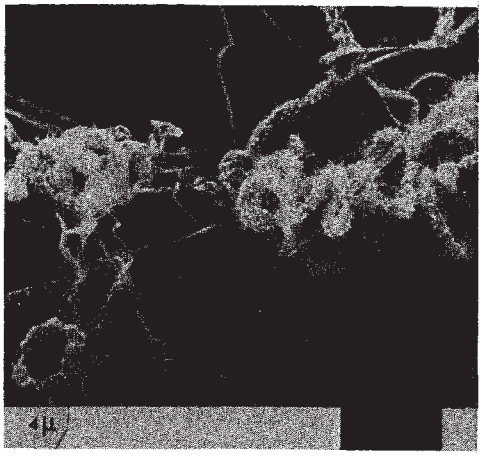


FIG. 9. *Streptomyces vellosus* UC 5656 (spores with long hairs).

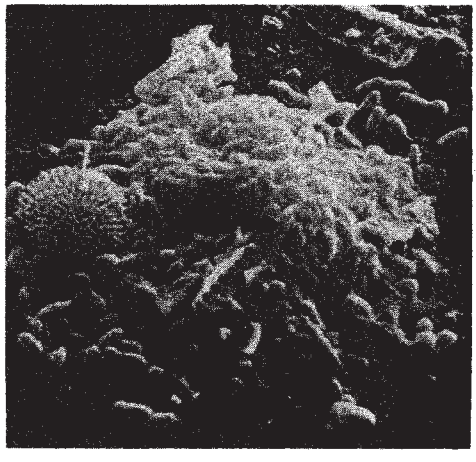


FIG. 10. Example of materials analysis. Bacterial cells and diatoms.

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