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Use of the Scanning Electron Microscope for Viewing Bacteria in Soil

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Scanning electron microscopy was used for viewing *Bacillus cereus* and *Staphylococcus aureus* in three different soils. Both organisms were detected in the test soils at an approximate concentration of 10^7 cells per gram of soil; theoretically, the minimal number of microorganisms required for detection with the scanning electron microscope technique was between 10^7 and 10^{10} cells per gram of soil. Because the concentration of cells was critical, the use of scanning electron microscopy as an extraterrestrial life detection instrument would be limited with soils containing more than 10^7 bacteria per gram of soil.

This study was undertaken in order to determine the sensitivity of the scanning electron microscope in the detection of microorganisms or other biological material, such as protein, in soil and, in doing so, to evaluate the possible application of this technique to extraterrestrial life detection.

Gray (1) used scanning electron microscopy to view soil organisms in situ; this technique was superior to previous techniques because of greater magnification, less distortion of the specimen, and less disruption of the soil environment. The same technique was utilized by Williams and Davies (3) for viewing several genera of the order *Actinomycetales*.

MATERIALS AND METHODS

Bacterial cultures. *Bacillus cereus* isolated from a California desert soil and *Staphylococcus aureus* ATCC 12411 were used in these studies. *B. cereus* spores were obtained by growing the organism on the surface of thermoacidurans agar modified (Difco) for 6 days at 37 C. *S. aureus* stock culture was prepared by growing the organism on the surface of Trypticase Soy Agar (BBL) for 24 hr at 37 C. The cultures were harvested in chilled 0.025 M phosphate buffer (*p*H 7.0) and were washed seven times before being finally suspended in the buffer. Stock cultures were stored at 5 C. Portions of the *B. cereus* stock culture were heat-shocked at 80 C for 10 min on the day of use.

Soils. The three soils used provided substrates with different physical and chemical properties. The textural classification of the desert (entisol) soil was clay, whereas the prairie (mollisol) and tree (alfisol) soils were classified as silt loams, with a higher clay content in the prairie soil.

Sample preparation and viewing. To view bacterial cells in soil, soil containing a known concentration of bacteria per gram of soil was fixed to the viewing platform with Krylon as adhesive, or soil previously fixed to the platform was inoculated with a known number of bacteria. After the film had air-dried, the specimen was coated with gold-palladium alloy, to an approximate thickness of 500 A, by means of vacuum evaporation. The metal coating minimizes the accumulation of surface charges that would decrease resolution. The platform was then placed in the scanning chamber of the microscope (Cambridge Instrument Co., Ltd., London, England). After the chamber was evacuated to approximately 10^{-5} torr, the specimen was scanned with an electron beam. The electrons liberated from the surface of the specimen were detected by a scintillator-photomultiplier system. The resulting image was formed on a cathode-ray tube. Photographs were taken with a Polaroid camera (Dallmeyer f/2.8 lens with a 1:0.7 objective-image ratio).

An excellent discussion of the theory, operation, and application of the scanning electron microscope has been published by Oatley, Nixon, and Pease (2).

RESULTS

The selection of *B. cereus* and *S. aureus* for pure culture studies in the three different soils allowed better controlled experiments: (i) the organisms represented two morphological types, (ii) the soils differed in their individual physicochemical properties, and (iii) the addition of a known concentration of a specific organism provided a more reliable method of determining the sensitivity of the instrument, rather than using different media and culture techniques for maximal recovery of indigenous bacterial flora.

Theoretically, the minimal number of microorganisms required for detection with the scanning electron microscope, assuming a 1-μ sphere,

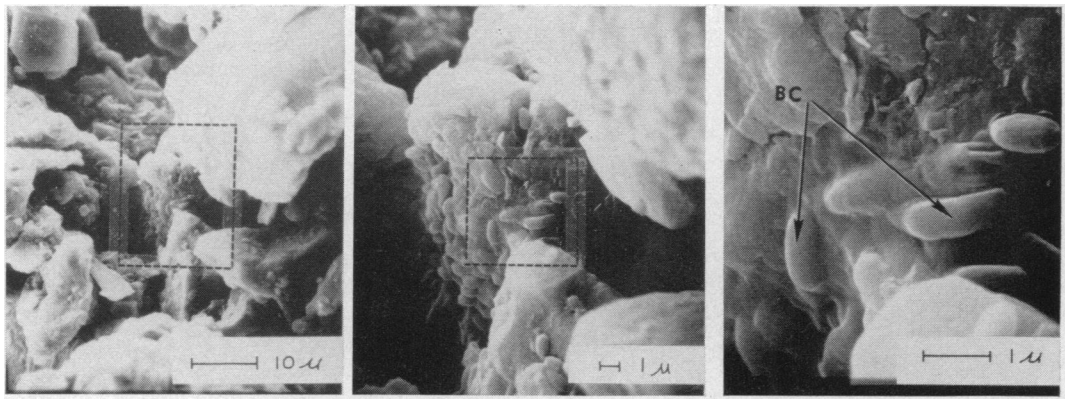

FIG. 1–3. *Micrographs of tree soil inoculated with Bacillus cereus. Area enclosed by dotted line is the field of view at next higher magnification. Figure 1, × 1,550; Fig. 2, × 4,700; Fig. 3, × 15,500.*

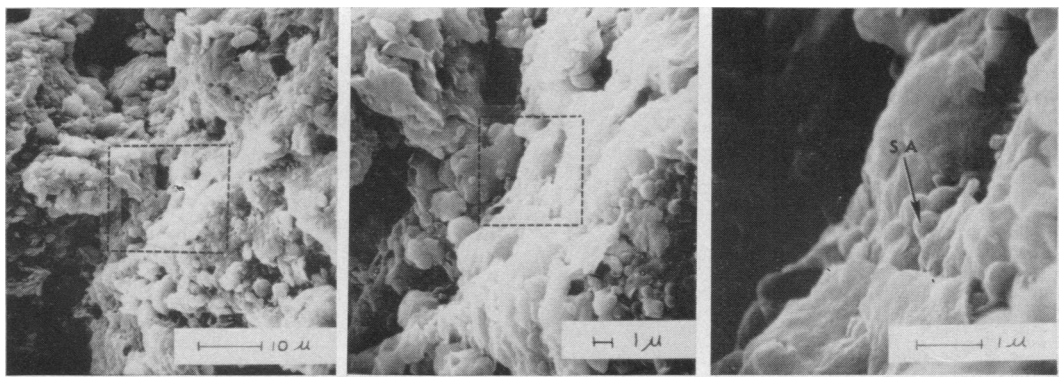

FIG. 4–6. *Micrographs of desert soil inoculated with Staphylococcus aureus. Area enclosed by dotted line is the field of view at next higher magnification. Figure 4, × 1,550; Fig. 5, × 4,600; Fig. 6, × 15,500.*

was between 10^7 and 10^{10} cells per gram of soil. *B. cereus* and *S. aureus* were detectable in the three test soils at an approximate concentration of 10^7 cells per gram of soil. The best results were obtained when the specimen was scanned at 1,000 × magnification. Suspected areas were observed at higher magnifications.

Figures 1–3 are scanning electron micrographs of different magnifications of *B. cereus* in tree soil. Approximately 10^9 cells per gram of soil were present. The same clarity and depth of field were noticed with this organism in desert and prairie soils. There was a tendency toward nonuniform distribution of *B. cereus* that was also apparent with *S. aureus*. Similar distribution also occurred in soils inoculated with a small number of *B. cereus* and *S. aureus* cells, homogeneously dispersed and incubated to allow the organisms to multiply.

Nonuniform distribution of bacteria in soils can be regarded as a natural phenomenon, i.e., the establishment of bacterial clones is dependent upon microenvironments: availability of nutrients, gaseous exchange, and the physicochemical properties of the soil.

Figures 4–6 are micrographs of different magnifications of desert soil inoculated with *S. aureus*. Approximately 10^9 cells per gram of soil were present. It was sometimes difficult to distinguish *S. aureus* from soil particles at lower magnifications (Fig. 4). Similar results were obtained with prairie and tree soils inoculated with this organism.

DISCUSSION

Scanning electron microscopy can be used for viewing microorganisms in soil. However, the concentration of cells is critical. This would limit the use of this technique as an extraterrestrial life-detection instrument with soils containing

more than 10^7 bacteria per gram of soil. This does not preclude its use to examine localization of bacteria around root hairs, as suggested by Gray (1), or to view surface details and spatial relationships of microorganisms, as reported by Williams and Davies (3).

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