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NOTES

Scanning Electron Microscopy of *Thermoplasma acidophilum*

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The scanning electron microscope was utilized to observe the morphology of the thermophilic, acidophilic mycoplasma, *Thermoplasma acidophilum*. Upon examination of the surface morphology, the size and shape of this unusual mycoplasma revealed its similarity to the other mycoplasmas that have been investigated.

Thermoplasma acidophilum, the obligate thermophilic, acidophilic member of the *Mycoplasmatales* grows optimally at 59 C and pH 2 (2). Because of the extreme conditions required for growth of this mycoplasma, cultures growing on solid media are difficult to obtain. Belly et al. (1) have shown that colonies growing on agar have the typical mycoplasma colonial appearance. However, due to the difficulty involved in growing this organism on solid media, no scanning electron microscopy has been done to determine if this unusual mycoplasma, *T. acidophilum*, resembles other members of the *Mycoplasmatales*. Klainer and Pollack (4) and Kammer et al. (3) have used the scanning electron microscope to study the morphology of growth phases of *Mycoplasma pneumonia*, *Mycoplasma hyorhinis*, *Mycoplasma gallisepticum*, *Acholeplasma laidlawii* A, *Acholeplasma laidlawii* B, *Acholeplasma granularum* and T-strains. The present study was undertaken to determine, by means of scanning electron microscopy, the morphology of *Thermoplasma acidophilum* and to compare its morphology with that of the other members of the *Mycoplasmatales* studied by this method.

Thermoplasma acidophilum Darland et al. (isolate 122-1 B2; originally deposited as ATCC 25905) was grown for 48 h at 59 C in liquid medium (pH 2) as previously described (5), concentrated by centrifugation in a tabletop Sorvall SS-1 centrifuge for 15 min at 9,000 rpm, and suspended in 3 ml of fresh medium. A loopful of the concentrated suspension was used to inoculate agar plates which were prepared by mixing equal volumes of sterile 5.6% (wt/vol) Ionagar no. 2 (Consolidated Laboratories, Inc., Chicago, Ill.) and sterile liquid medium (5), yielding a final agar concentration of 2.8%. All solutions were cooled to 45 C before mixing. Plates were maintained in a sealed, moist container and incubated at 60 C for 72 h.

Colonies were prepared as follows: agar blocks supporting cells were cut from the media, fixed in vapors of 4% (wt/vol) unbuffered aqueous osmium tetroxide overnight under refrigeration, and placed on glass cover slips and mounted on specimen stubs. The agar blocks were air dried, painted on the edges with silver conductive paint, and metal coated with palladium-gold (40:60; Ladd Research Industries, Burlington, Vt.) on the rotary stage of a vacuum evaporator model VE 10 (Varian/vacuum division, Portland, Ore.). Specimens were examined with a Cambridge Stereoscan (type 96113 Mark 2A) scanning electron microscope (Cambridge Instruments Company, Ltd., London, England) operating at 10 kV with the stage angle kept constant at 45°. Specimens were photographed with P/N 55 film (Polaroid Corporation, Cambridge, Mass.).

Scanning electron microscopy of *T. acidophilum* on an agar surface revealed aggregates of tightly packed spherical, as well as individual, cells. The cells had a size range of 0.5 to 1.9 μm. We measured 100 cells in one micrograph, and the average size was 1 μm. In Fig. 1 the individual cells of *T. acidophilum* are visible. Many are arranged in clumps. The variety in the size of the cells is seen in Fig. 2. Most cells are spherical in shape, but some cells have collapsed, as indicated by indentations on their surfaces. In Fig. 3, these indentations are seen more clearly (arrows). Some cells are

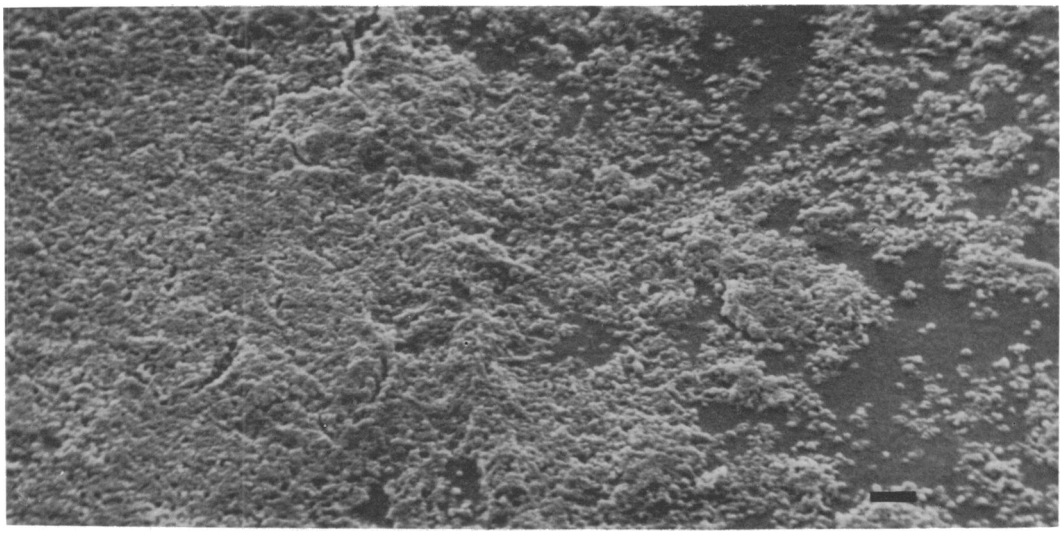

FIG. 1. *Scanning electron micrograph of T. acidophilum growing along a streak on Ionagar. Individual cells are visible in some areas, but most cells are in clumps or in large masses. (Bar equals 10 μm.)*

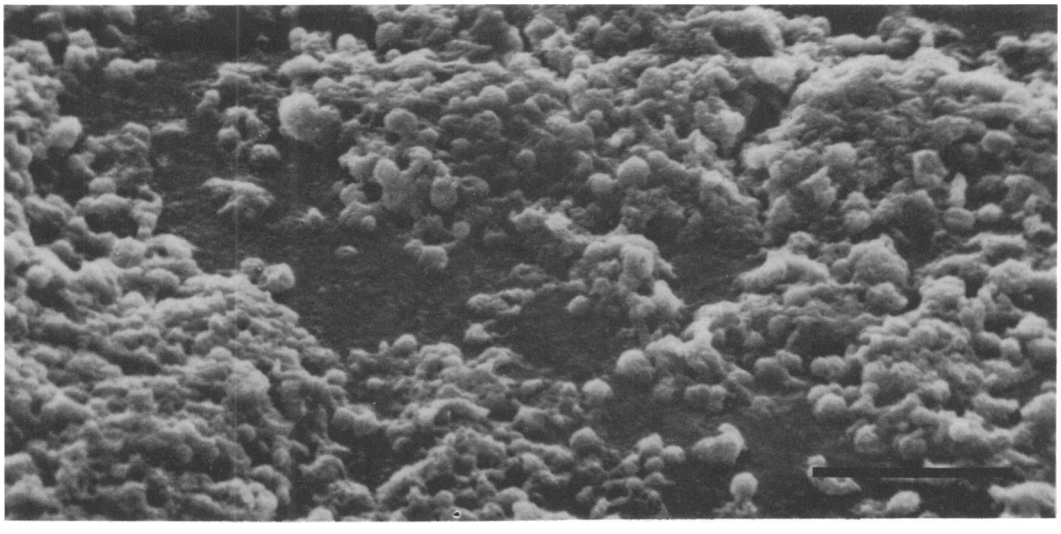

FIG. 2. *Scanning electron micrograph of T. acidophilum. The individual cells are mostly spherical in shape and range in size from 0.5 to 1.9 μm. (Bar equals 10 μm.)*

almost completely collapsed in this field. Several large forms, about 5 μm in diameter, were found in this study. One of these is seen in Fig. 4. Many cells around this large form are collapsed in a "donut-like" shape. This large form is shown at higher magnification in Fig. 5. Difficulty in growing *T. acidophilum* on solid medium has hindered morphological studies comparing this microorganism with other members of the *Mycoplasmatales*. Brock and his colleagues (1) succeeded in growing colonies on solid media for light microscopy, which showed that *T. acidophilum* appeared to produce colonies similar to the colonies of other *Mycoplasmatales*, but the limitations inherent in the system made it desirable to study *T. acidophilum* by other methods. Transmission electron microscopy of thin sections (2) of *T. acidophilum* reveal the double-track membrane and lack of cell wall, typical of other members of the *Mycoplasmatales*. However, scanning electron microscopy permits observation of whole

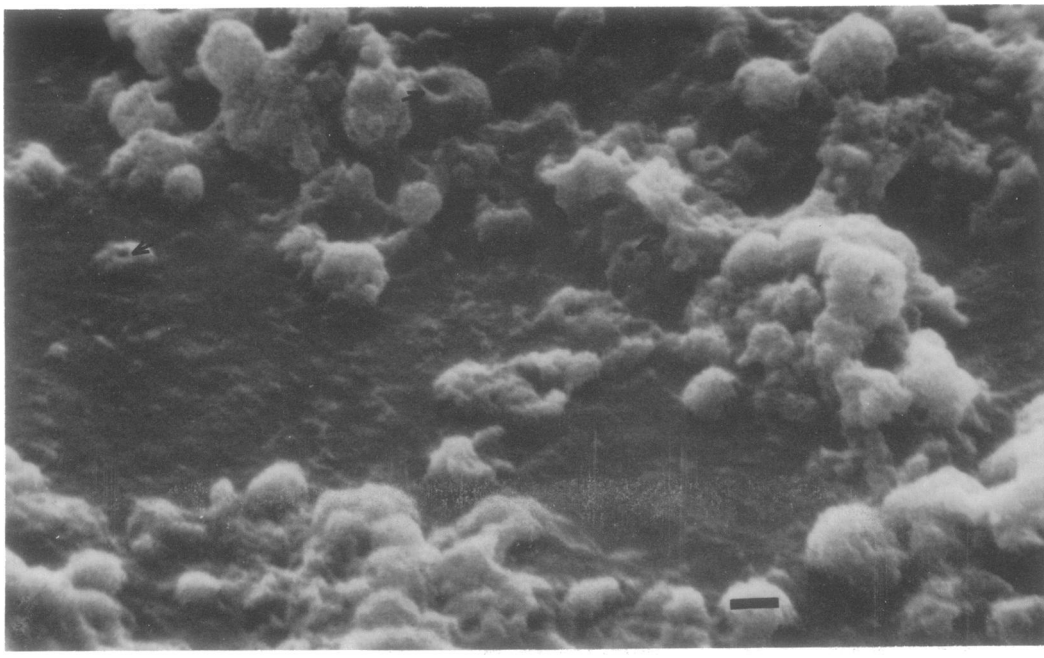

FIG. 3. *Scanning electron micrograph of T. acidophilum. Most cells are spherical; some cells have indentations, indicated by arrows. (Bar equals 1 μm.)*

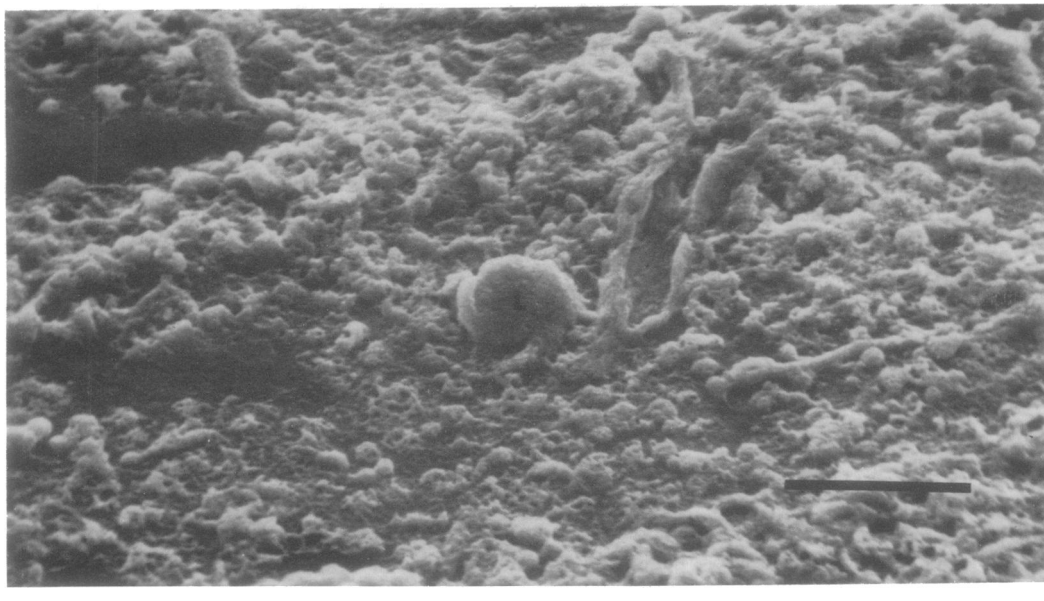

FIG. 4. *Scanning electron micrograph of T. acidophilum. A large form (L) is seen in this figure. Many of the other cells in this field are collapsed. (Bar equals 10 μm.)*

cells at high magnifications in three-dimensional perspective. Our results with scanning electron microscopy show this unusual mycoplasma, *T. acidophilum*, to be similar in morphology to other mycoplasmas (3, 4) studied by this method.

Cells of *T. acidophilum* are polymorphic and demonstrate great diversity in size, with a range in our study of 0.5 to 1.9 μm, not including the obvious large forms which measured approximately 5 μm in diameter. Measurement of 100 cells in one scanning electron microscope field

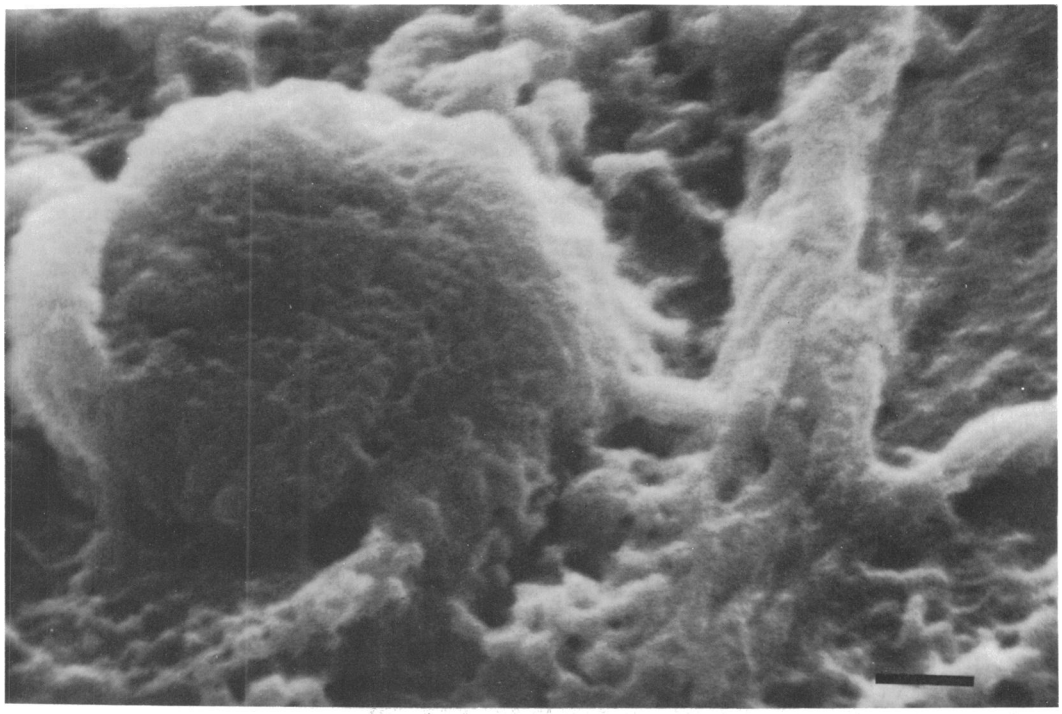

FIG. 5. *Scanning electron micrograph of T. acidophilum. A higher magnification of the large form shown in Fig. 4. (Bar equals 1 μm.)*

gave an average size of 1 μm. Unfortunately, many of the cells were clumped in such a way that precise measurement was difficult. We measured cells with edges which were readily discernible. A more precise evaluation of size and shape could be achieved by critical-point drying of cells at dilutions where individual cells would be visible in the scanning electron microscope. The large form seen in Fig. 4 and 5 is similar to that seen in *Acholeplasma laidlawii* (4). This is, to our knowledge, the first report of a large form in *T. acidophilum*. There is some suggestion from our micrographs that the large form is made up of 1-μm subunits. Further investigation must be made to clearly visualize the subunits, if they exist. It could be informative to examine ultrathin sections of such a body in the transmission electron microscope. Eventually, one might expect to observe intermediate forms in cultures. The cells of *T. acidophilum* tend to occur in irregular clumps or aggregates, as do other members of the *Mycoplasmatales*. *T. acidophilum* is shown to have the imbricate surface texture that is characteristic of cells which lack cell walls. This result further indicates the similarity of *T. acidophilum* to other mycoplasmas.

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