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Ultrastructure of Lipopolysaccharide Isolated from *Thermoplasma acidophilum*

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The fine structure of lipopolysaccharide isolated from *Thermoplasma acidophilum* was examined by electron microscopy. Negative staining of the lipopolysaccharide revealed long, ribbon-like structures with some branching. The average width of the lipopolysaccharide ribbons was 5 nm. Treatment of the lipopolysaccharide with 0.5% sodium dodecyl sulfate resulted in the dissociation of the ribbon-like structures to spherical- and vesicular-shaped particles and some short, rodlike structures. Results suggest that the lipopolysaccharide from *T. acidophilum* is morphologically similar to lipopolysaccharide isolated from gram-negative bacteria.

The electron microscopic appearance of lipopolysaccharides (LPS) isolated from a variety of microorganisms has revealed a striking similarity of structures (1-4, 6, 8-10, 12-18, 20, 22) ranging from flat, linear ribbons to spherical structures, depending on the method of LPS extraction and subsequent treatment.

The present report concerns a study of the fine structure of the LPS isolated from an acidophilic, thermophilic mycoplasma, *Thermoplasma acidophilum*. The chemical characterization of this LPS has been previously described (11).

MATERIALS AND METHODS

Microorganisms and isolation of LPS. *T. acidophilum* (isolate 122-1B2; ATCC 25905) was grown at 59 C (pH 2.0) and harvested as previously described (7, 19). After lyophilization of the cells, lipids were extracted (7) and the residue was subjected to the phenol-water extraction procedure (21). LPS was recovered from the dialyzed aqueous phase and lyophilized (11).

Electron microscopy. Lyophilized LPS was dissolved in deionized water (5 mg/ml) or 0.5% sodium dodecyl sulfate and examined by electron microscopy after negative staining. For negative staining, an equal volume of LPS preparation and 2% phosphotungstic acid were mixed, a drop of the mixture was placed on a Formvar-coated, 300-mesh copper grid, and excess material was removed with filter paper. The grid was then air-dried before use. All of the electron micrographs were made with a Philips 200 electron microscope at an accelerating voltage of 80 kV.

RESULTS

Figures 1 and 2 show the appearance of a negatively stained LPS isolated from *T. acidophilum*. Long ribbons of material with an average diameter of 5 nm were the predominant morphological forms observed. There appeared to be little branching of the ribbons; however, a few branched structures were noted.

Figure 3 shows negatively stained *T. acidophilum* LPS after treatment with 0.5% sodium dodecyl sulfate. The long ribbons appeared to be dissociated into very short rodlike pieces in a few instances but appeared more frequently as spherical or vesicular forms, ranging in size from approximately 30 to 100 nm.

DISCUSSION

The LPS from *T. acidophilum* has been shown to be a polymer composed of mannose (man), glucose (glc), and glycerol and 40-carbon alkane in a molar ratio of 24:1:1:2, with a monomeric weight of approximately 5,300 (11). This new type of LPS has the tentative structure [man *p*-(1 $\xrightarrow{\alpha}$ 2)-man *p*-(1 $\xrightarrow{\alpha}$ 2)-man *p*-(1 $\xrightarrow{\alpha}$ 3)]$_8$-glc *p*-glycerol diether. The polymer is obtained in 96% purity in the aqueous phase of the phenol-water extraction. The major contaminants are protein, less than 4%, and phosphorus, approximately 0.2%, one-half of which is accounted for as nucleic acid phosphorus (11).

The typical LPS found in gram-negative bacteria is a complex molecule composed of repeating units of oligosaccharide attached to a core of heptose, 2-keto-3-deoxyoctonate, and lipid A (5).

The electron microscopic examination of negatively stained *T. acidophilum* LPS showed ribbon-like structures approximately 5 nm in width. LPS from strains of *Escherichia coli* (2, 8, 10, 12, 14, 17, 20), *Salmonella typhimurium*

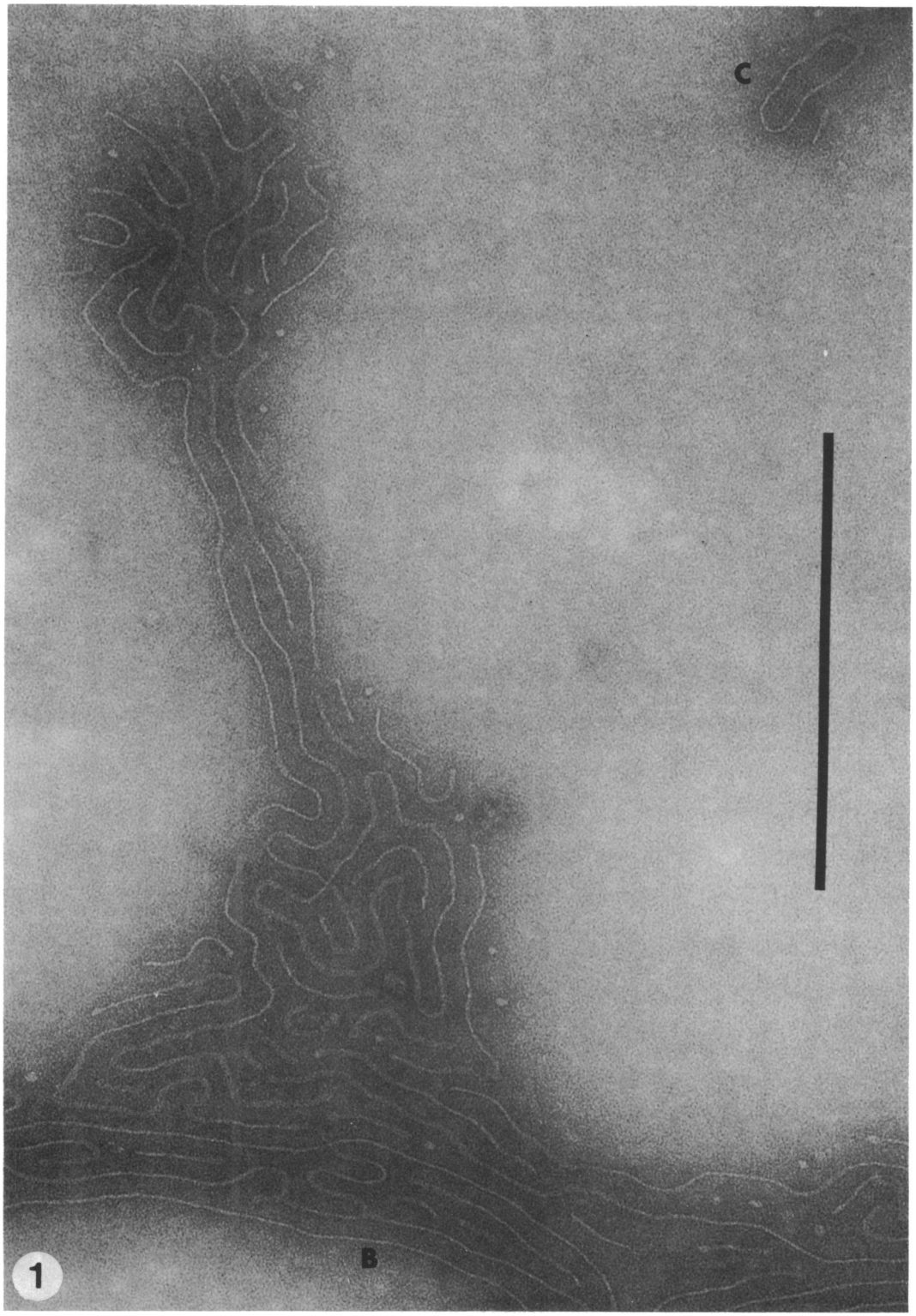

FIG. 1. *LPS (negatively stained) from T. acidophilum. Note circular strand (C) and branching (B). Bar represents 0.5 μm.*

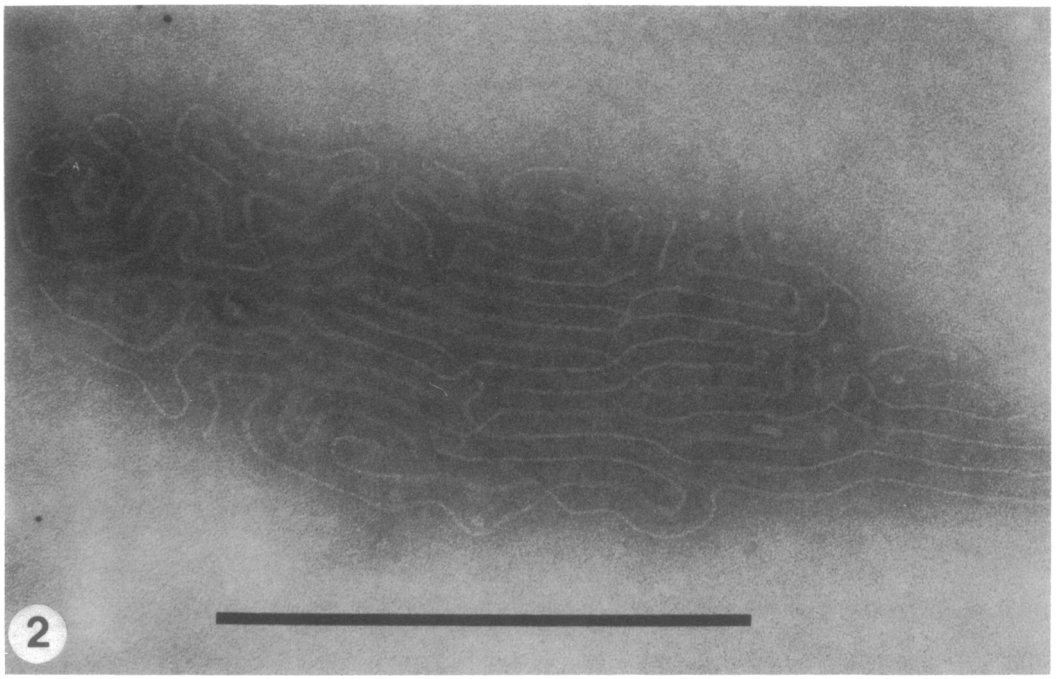

FIG. 2. *LPS (negatively stained) from T. acidophilum. Note increase in overlap of strands. Bar represents 0.5 μm.*

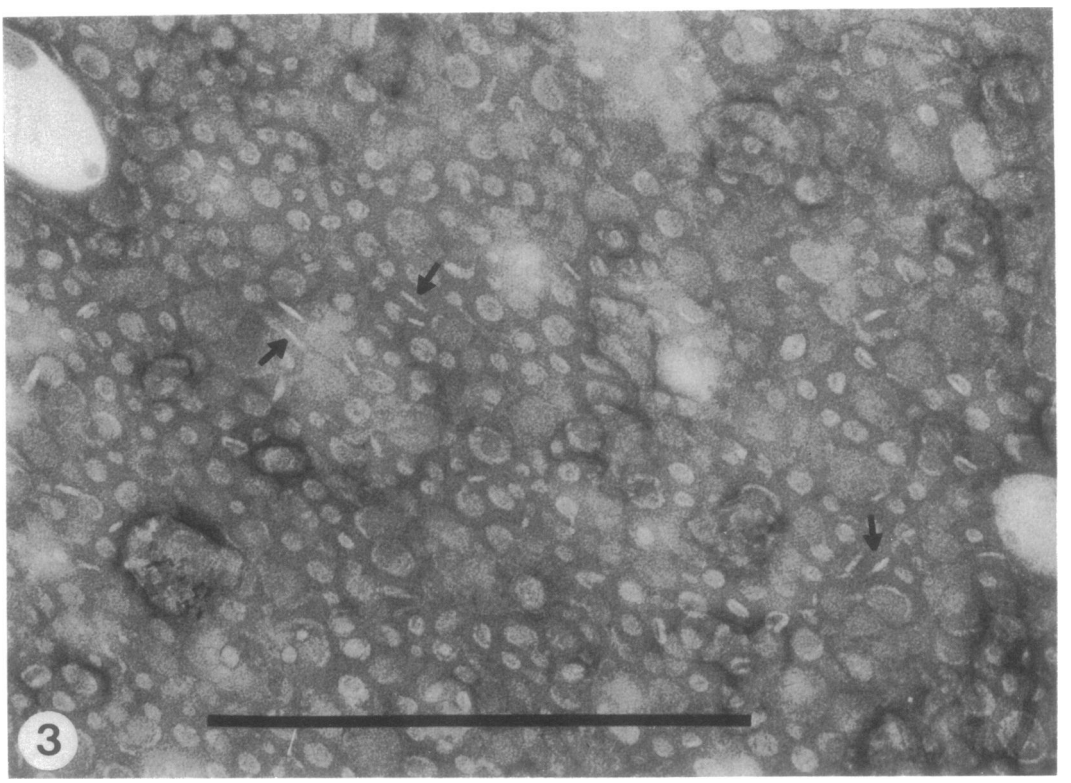

FIG. 3. *LPS (negatively stained) from T. acidophilum after treatment with 0.5% sodium dodecyl sulfate showing short rodlike pieces (unlabeled arrows) and spherical or vesicular forms. Bar represents 0.5 μm.*

(8, 16, 18), *Treponema pallidum* (6), *Bordetella pertussis* (14), and *Yersinia pestis* (4) appear to have quite similar morphological structures after negative staining and examination by electron microscopy. Measurements of negatively stained LPS from *T. acidophilum* show close agreement with measurements of LPS from these other microorganisms.

Treatment of *T. acidophilum* LPS with 0.5% sodium dodecyl sulfate results in dissociation of the ribbon-like structures into spherical- and vesicular-shaped particles. The intact LPS, in water, is highly aggregated, exhibiting an apparent molecular weight greater than 1,200,000. The detergent alters the aggregation of the LPS, possibly by micelle formation, yielding material which exhibits permeation chromatographic behavior indicative of a molecular weight of 67,000 (11). This dissociation by a surfactant also occurs with LPS from other microorganisms. A variety of surfactants have been used to dissociate LPS from a number of different microorganisms (1, 4, 8, 13, 14, 17). The resulting morphological structures resemble those of sodium dodecyl sulfate-dissociated LPS from *T. acidophilum*.

Thus, even though LPS from *T. acidophilum* is chemically distinct from the typical gram-negative bacterial LPS, there is a great similarity in morphological structures of the two types of LPS.

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