NOTES

Improved Description of the Cell Wall Architecture of the Xylanolytic Eubacterium *Clostridium xylanolyticum*

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The complex cell envelope profile of the anaerobic, spore-forming, xylanolytic eubacterium *Clostridium xylanolyticum* ATCC 49623 (G. M. Rogers and A. A. W. Baecker, *Int. J. Syst. Bacteriol.* 41:140-143, 1991) was investigated in greater detail. Although growing cells of this organism produced a gram-negative staining reaction, electron microscopy of thin sections of cells clearly revealed a gram-positive cell envelope profile. The cell wall consists of a thin peptidoglycan layer with a regularly arranged surface layer outside it. Older cells in the stationary phase may have surface layers on both sides of the peptidoglycan, providing a multilayer thin-section profile. Freeze-etched preparations of whole cells revealed an oblique surface layer lattice ($a = 6.6$ nm; $b = 5.3$ nm; $\gamma \sim 78^\circ$). The results of sodium dodecyl sulfate-polyacrylamide gel electrophoresis of a solubilized whole-cell extract indicated that the molecular mass of the surface layer monomer was approximately 180 kDa. Treatment of the gels with periodic acid-Schiff reagent resulted in a weak, but unambiguously positive staining reaction. Our data indicate that a glycosylated surface layer protein is present on the cell surface of *C. xylanolyticum*.

Biological hydrolysis of hemicelluloses is of considerable interest in forestry and the pulp and paper industry. As a result of a biopulping research program in South Africa, the obligatory anaerobic eubacterium *Clostridium xylanolyticum* was isolated from *Pinus patula* wood chips (8).

The results of light microscopic characterization (spore formation) of an isolate and DNA-DNA hybridization experiments led to taxonomic classification of this organism as a novel *Clostridium* species (8). However, Gram-staining results and the unusual five-layer cell envelope profile ob-

FIG. 1. (a) Electron micrograph of a thin section of a *C. xylanolyticum* cell. A second, artificial S layer (iS) is formed on the inner surface of the peptidoglycan layer (pg). The three-layer wall structure is visible where the cell wall is detached from the peptidoglycan. S, S layer; cm, cytoplasmic membrane. Bar = 50 nm. (b) Freeze-etched and metal-shadowed preparation of intact cells, revealing the regular S-layer lattice and flagella. Bar = 50 nm. (c) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of sodium dodecyl sulfate-solubilized whole cells stained with Coomassie blue. The positions of molecular mass standards are indicated on the left.

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served (8) apparently contradict this conclusion. The divergent observations described above prompted us to investigate the cell envelope profile of *C. xylanolyticum* in greater detail.

Ultrastructural characterizations of many bacteria have shown that their Gram-staining reactions are determined by their cell wall architecture (1–3). Thus, it is not surprising that gram-positive bacteria that have a thin peptidoglycan layer, like several *Clostridium* species (4), do not retain the staining reagent and tend to produce a “misleading” Gram reaction (1). Thin sections of *C. xylanolyticum* (Fig. 1a) show the typical gram-positive cell envelope profile and no outer membrane. Interestingly, a regularly arranged surface layer (S layer) (7, 9, 11) is present as the outermost envelope component. The initial description of *C. xylanolyticum* (8) should have included the statement that the cell wall profile reveals a thin peptidoglycan layer with a regularly arranged S layer outside it. Sleytr and Glauert (10) have reported that cells in the late stationary phase or autolyzed cells can form a second layer from excess S-layer material on the inner side of the peptidoglycan sacculus, providing a multilayer thin-section profile. The five-layer cell envelope structure observed previously in thin sections of *C. xylanolyticum* cells (8) is actually a four-layer structure (the S layer, peptidoglycan, the inner S layer, and cytoplasmic membrane) (Fig. 1a). However, some parts of the cell wall (8) appear to have only three layers and no inner S layer. This phenomenon has also been observed in other S-layer-containing bacteria, such as different *Bacillus* spp. (14, 15) or *Clostridium* spp. (13). Sometimes however, an erroneous conclusion has been drawn, namely, that a new cell wall architecture principle exists in those strains (14). It is obvious that the age of the culture plays an important role in the interpretation of the image obtained from thinly sectioned cells. An analysis of a great number of micrographs of freeze-etched preparations (5) of intact *C. xylanolyticum* cells by optical diffractometry (12) revealed that there is an oblique S-layer lattice ($a = 6.6$ nm; $b = 5.3$ nm; $\gamma \sim 78^\circ$) which covers the bacteria completely (Fig. 1b). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of sodium dodecyl sulfate-solubilized whole cells (5) revealed a high-molecular-weight S-layer band (apparent M_r , approximately 180,000) (Fig. 1c), which upon periodic acid-Schiff staining gave a weak but unambiguously positive staining reaction (data not shown). This observation indicates that an S-layer glycoprotein (6) is present on *C. xylanolyticum* cells. Additional studies will be necessary to determine the biological function and relevance

of the glycosylated S layer of *C. xylanolyticum* in the biodegradation of wood.

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