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SHORT COMMUNICATIONS

The Structure of Peptidoglycan from *Lysobacter* sp., a Producer of Extracellular Bacteriolytic Enzymes

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The bacterial cell wall is a multicomponent structure that protects the cell interior and provides for cell interactions with the environment. The main component of the cell wall of eubacteria, peptidoglycan, forms a rigid skeleton, due to which the cell retains its form and integrity and withstands the action of unfavorable external factors [1]. The chemical structure of peptidoglycan is an essential characteristic of bacterial cells.

The investigation was performed with the bacterium *Lysobacter* sp., formerly known as *Xanthomonas campestris*. The bacterium was reclassified based on the computer-aided analysis of the 16S rRNA gene sequences available at the Ribosomal Database Project web site (http://rdp.cme.msu.edu/html). This *Lysobacter* sp., isolated from river water, is able to secrete bacteriolytic enzymes degrading the peptidoglycans of some gram-positive and gram-negative bacteria [2]. These extracellular enzymes are relatively well studied.

Efforts have recently been concentrated on the study of intracellular autolytic enzymes produced by *Lysobacter* sp., whose substrate is the cell wall peptidoglycan of this bacterium [3, 4]. The substrate specificity of these enzymes can be established only if the structure of the peptidoglycan of the producing bacterium is known.

The present work was undertaken to study the chemical structure of peptidoglycan isolated from the cell wall of *Lysobacter* sp.

The strain of *Lysobacter* sp. used in this work was grown on peptone–yeast extract medium at 29°C for 18 h in flasks on a shaker (130–140 rpm).

Cells were harvested by centrifugation at 5000 *g* for 20 min and washed twice with 10 mM Tris–HCl buffer (pH 8.0). Washed cells were suspended in 1 mM Tris–HCl buffer (pH 8.0) and disrupted by subjecting them to three freezing–thawing cycles. The homogenate was mixed with a 4% sodium dodecyl sulfate (SDS) solution to give a final concentration of 2.4% SDS. The mixture was boiled for 30 min and allowed to stand overnight at room temperature. Then the mixture was centrifuged at 20000 *g* for 1 h, and the precipitate, containing cell walls, was used to obtain peptidoglycan. For this, the precipitate was again boiled in 2.4% SDS for 15 min and allowed to stand overnight at room temperature, after which the cell walls were repeatedly washed from SDS with water, digested with pronase (0.1 mg/ml) in 10 mM Tris–HCl buffer (pH 7.5), and incubated at 37°C for 3 h. Then the cell walls were extracted with 0.5 N trichloroacetic acid (TCA) at 4°C for 24 h and at 37°C for 48 h [5–7]. The acid was thoroughly removed from the extract by washing with water, and the extracted peptidoglycan was lyophilized.

The peptidoglycan was hydrolyzed in 4 N HCl at 110°C for 4 h. Amino acids, glucosamine, and muramic acid were determined using a T.399 amino acid analyzer (Microtehna, Czech Republic).

The configuration of diaminopimelic acid was determined by descending paper chromatography [8].

Analysis of the acid hydrolysate of the cell wall peptidoglycan from *Lysobacter* sp. showed that it contains glucosamine, muramic acid, alanine, glutamic acid, and diaminopimelic acid at molar proportions of 1 : 1 : 2 : 1 : 1. The presence of trace amounts of threonine, isoleucine, and aspartic acid in the preparation of peptidoglycan was likely due to its contamination with cell proteins.

In determining the structure of peptidoglycan, it is essential to elucidate the configuration of the component diaminopimelic acid. In bacterial peptidoglycans, this acid may be present in L,L form or *meso*-(L,D) form [1]. The analysis of diaminopimelic acid from the *Lysobacter* sp. peptidoglycan by descending paper chromatography [8] showed that it is in the *meso* form.

The data obtained in this study allow the conclusion to be made that, in accordance with the classification system of Schleifer and Kandler [1], the peptidoglycan isolated from *Lysobacter* sp. can be referred to the A1γ type, which is characterized by the presence of alternating *N*-acetyl glucosamine and *N*-acetyl muramic acid residues in its glycan chain. The muramic acid residue has a tetrapeptide L-alanine–γ-D-glutamic acid–*meso*-diaminopimelic acid–D-alanine. This type of peptidoglycan lacks interpeptide bridges, while the peptide subunits of neighboring glycan chains are linked by bridges between the *meso*-diaminopimelic acid at position 3 of one subunit and the D-alanine at position 4 of another subunit.

A1γ-type peptidoglycan is typical of most of the gram-negative bacteria studied, except for some fuso-

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bacteria [9], *Spirochaeta stenostrepta* [10], *Treponema pallidum* [11], and *Bacteroides asaccharolyticus* [12], whose peptidoglycans have diamino acids other than *meso*-diaminopimelic acid. A1γ-type peptidoglycan was also found in some gram-positive bacteria, such as *Bacillus subtilis* [13] and *Corynebacterium diphtheriae* [14].

To conclude, the peptidoglycan isolated from *Lysobacter* sp. has a structure typical of most gram-negative bacteria.

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