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Intracellular Peptidoglycan Hydrolases of the Bacterium *Xanthomonas campestris* XL-1

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Abstract—A system of intracellular peptidoglycan hydrolases of *Xanthomonas campestris* XL-1 comprises about 10 enzymes of different localization and substrate specificity. Seven enzymes (A_1-A_7) are localized in cytosol, one enzyme (A_8) in periplasm, and two enzymes (A_9, A_{10}) were found in the fraction of cell walls and membranes. While the culture is entering the logarithmic growth stage from the stationary stage, a change occurs in the activity of the cytosolic enzymes: A_1 significantly increases, and A_5 and A_6 decrease. The spectrum of cytosolic enzymes also depends on the growth medium composition. The enzyme A_7 present in cells secreting extracellular enzymes (medium 5/5) was not found in non-secreting cells (LB medium). Unlike extracellular enzymes, intracellular peptidoglycan hydrolases are primarily acidic proteins. The data indicate that the system of intracellular peptidoglycan hydrolases of *X. campestris* is under complex and strict regulation.

Key words: intracellular peptidoglycan hydrolases, *Xanthomonas campestris*, lysis, autolysis

The cells of all of bacteria contain intracellular bacteriolytic enzymes that play a key role in bacterial growth and division and are necessary for any bacterium [1].

Along with intracellular bacteriolytic enzymes, some bacteria synthesize extracellular bacteriolytic enzymes and secrete them into the surrounding medium [2-6]. In this case, the intracellular bacteriolytic enzymes to be localized in membrane, periplasm, or cell wall [1] and extracellular ones secreted into the surrounding medium are simultaneously synthesized and transferred across the plasmatic membrane to their final location. The regulation of bacteriolytic enzyme synthesis and functioning is still not clearly understood. No data is available on the extracellular and intracellular bacteriolytic enzymes of the same bacterium.

Under conditions of nutritional deficiency the bacterium *X. campestris* secretes into the culture medium several rather well characterized peptidoglycan hydrolases of different substrate specificity [7-10]. A peptidoglycan hydrolase expressing glucosaminidase activity was found in the cytosol of *X. campestris* cells, but not among the extracellular bacteriolytic enzymes [11]. More detailed analysis of the intracellular enzymes from this bacterium was not performed.

Thus, our goal was to study the intracellular autolytic peptidoglycan hydrolases from the bacterium *X. campestris* and to compare them to the previously studied extracellular enzymes.

MATERIALS AND METHODS

Microorganism and growth conditions. The strain *X. campestris* XL-1 was used in this study. Cells were grown on medium 5/5 developed in the Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences (IBPM RAS), containing yeast extract, soybean extract, casein hydrolyzate, and aminopeptide, and on LB medium containing 33 g/liter tryptone and 20 g/liter yeast extract [12]. Growth was conducted in Erlenmeyer flasks on a rotary shaker at 29°C. Cells were harvested by centrifugation at 5000*g* for 20 min and washed three times with 0.01 M Tris-HCl, pH 8.0, with subsequent centrifugation at the same rate.

The cells of exponential (9 h) and stationary (18 h) stages of growth were used in experiments.

Subcellular fractioning. Equal amounts of the cells of exponential and stationary growth stages were taken for the preparation of cell fractions. Periplasmic fraction was prepared by osmotic shock according to the method of Nossal and Heppel [13]. The washed cell pellet was resuspended in one volume of 0.033 M Tris-HCl, pH 8.0, to the final concentration of $2.5 \cdot 10^{10}$ cells/ml. Then one volume of 0.033 M Tris-HCl, pH 8.0, containing 40%

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sucrose and 0.1 mM EDTA was added. The cell suspension was incubated for 10 min at room temperature followed by centrifugation at 18,000*g* for 5 min, and supernatant (periplasm) was collected. Cell pellet was quickly resuspended in two volumes of 0.5 mM $MgCl_2$ and kept in the cold for 5 min. Cells were centrifuged at 18,000*g* for 5 min, and the supernatant containing both periplasmic and cytoplasmic components was collected.

The cells processed with osmotic shock were suspended in 0.05 M Tris-HCl, pH 8.0, frozen to –70°C, and disrupted by extrusion at 9 atm on a press designed in the IBPM RAS. To remove undisrupted cells, the resulting pulp was centrifuged at 8000*g* for 10 min. Then the supernatant was centrifuged at 18,000*g* for 30 min. The pellet contained cell walls and membranes, and the supernatant contained the components of cytosol.

Solubilization of peptidoglycan hydrolases from the cell wall and membrane fraction. Peptidoglycan hydrolases were solubilized from the fraction of cell walls and membranes sequentially with 5 M LiCl and 1% Triton X-100 in 0.05 M Tris-HCl, pH 7.5 [14, 15]. The suspension was incubated for 1 h at 4°C followed by centrifugation at 18,000*g* for 30 min (after the treatment with LiCl) or at 100,000*g* for 30 min (after the treatment with Triton X-100), and the supernatant (extract) was separated from the pellet containing cell walls and membranes.

Electrophoretic analysis of peptidoglycan hydrolases in the fractions of *X. campestris* cells. Electrophoretic analysis was conducted under non-denaturing conditions in 7.5% polyacrylamide gel. The anode system of Ornstein and Davis [16] was used for the separation of acidic and neutral enzymes. The cathode system of Reisfeld [17] was used for the separation of alkaline peptidoglycan hydrolases. After the electrophoresis of proteins in the anode system, polyacrylamide gel was applied on 1% agarose gel in 0.05 M Tris-HCl, pH 8.0, with immobilized *X. campestris* cells (1 mg/ml). After the electrophoresis of proteins in the cathode system, the polyacrylamide gel was washed with 0.05 M Tris-HCl, pH 8.0, for 10-15 min and then applied on the agarose gel with the immobilized *X. campestris* cells. The gels were kept for 24-48 h at 29°C. Zones of *X. campestris* cell lysis appeared in the agarose gel were indicative for the presence of autolytic peptidoglycan hydrolases in corresponding zones of polyacrylamide gel.

Analytical methods. Autolytic activity in cell fractions was determined from their ability to lyse the *X. campestris* cells immobilized in 1% agarose gel in 0.05 M Tris-HCl, pH 8.0, at concentration of 1 mg/ml. Agarose gel (3 mm thick) was filled into Petri dishes, wells (5 mm in diameter) were made in the gel, and the samples tested (20-μl aliquots) were then placed into the wells. The plates were incubated for 24-27 h at 29°C until the zones of lysis appeared.

Glucosaminidase activity was measured using *p*-nitrophenyl-2-acetamido-2-deoxy-β-D-glucopyranoside (NADG) as a substrate [11]. Enzyme sample (0.025-0.1 ml) was added to 0.5 ml of NADG (0.3 mg/ml) in 0.05 M Tris-HCl, pH 8.0, the reaction mixture was brought up to 1 ml with 0.05 M Tris-HCl, pH 8.0, and incubated at 37°C for 5-20 min till the development of yellow color. The reaction tubes were then placed into ice to stop the reaction, and optical density at 400 nm was measured on a Shimadzu UV-160 A spectrophotometer (Japan). The enzyme amount providing the increase in optical density at 400 nm by 0.001 in 1 min (measured in a cuvette with path length of 1 cm) was taken as one unit (U).

Activity of glucose-6-phosphate dehydrogenase was determined from the rate of NADP reduction [18]. The reaction mixture (1 ml) contained 75 mM sodium phosphate buffer, pH 7.5, 5 mM $MgCl_2$, 0.5 mM NADP sodium salt, 10 mM glucose-6-phosphate sodium salt, and 50-200 μl enzyme solution. The reaction was conducted in a temperature-controlled cuvette at 30°C, and the increase in optical density at 340 nm was measured on the Shimadzu UV-160 A spectrophotometer. The enzyme amount providing the reduction of 1 nmol NADP per 1 min was taken as 1 unit.

Activity of lactate dehydrogenase was determined from the rate of NADH oxidation [19]. The reaction mixture (1 ml) contained 50 mM potassium phosphate buffer, pH 7.0, 0.5 mM NADH sodium salt, 5 mM sodium pyruvate, and 50-200 μl enzyme solution. The reaction was conducted in a temperature-controlled cuvette at 30°C, and the decrease in optical density at 340 nm was measured on the Shimadzu UV-160 A spectrophotometer. The enzyme amount providing the oxidation of 1 nmol NADH per 1 min was taken as 1 unit.

Activity of cyclic phosphodiesterase was determined from the rate of *p*-nitrophenol production using bis(*p*-nitrophenyl)phosphate sodium salt as a substrate [20]. The reaction mixture (1 ml) contained 50 mM sodium acetate buffer, pH 6.0, 5 mM $MgCl_2$, 1 mM $CoCl_2$, 50-200 μl enzyme solution, and 1 mg substrate. The reaction mixture was incubated at 37°C for 20 min. The reaction was terminated with 1 ml of 0.1 M NaOH, and the optical density at 400 nm was measured on the Shimadzu UV-160 A spectrophotometer. The enzyme amount producing 1 nmol of *p*-nitrophenol in 20 min was taken as 1 unit.

Protein was determined by the Lowry method [21].

The following chemicals were used in our experiments: Tris, EDTA, bis(*p*-nitrophenyl)phosphate sodium salt, acrylamide, and ammonium persulfate (Serva, Germany); NADP sodium salt, NADH sodium salt, sodium pyruvate, and N,N′-methylene-*bis*-acrylamide (Reanal, Hungary); N,N,N,′N′-tetramethylethylenediamine (Fluka, Switzerland); glucose-6-phosphate sodium salt (Boehringer Mannheim, Germany); *p*-nitrophenyl-2-acetamido-2-deoxy-β-D-glucopyranoside (Koch-Light, England); and other chemicals manufactured in Russia.

RESULTS AND DISCUSSION

The bacterium *X. campestris* growing on rich media, such as the Luria–Bertani (LB) "super" medium [12], does not secrete extracellular bacteriolytic enzymes. But when it grows under nutritional deficiency (modeled, in particular, by the medium 5/5), it secretes into a surrounding medium no less than 20 various proteins including phosphatase, proteases, and several bacteriolytic enzymes [22]. To study the intracellular bacteriolytic enzymes (peptidoglycan hydrolases) from *X. campestris*, we have used the cells grown in medium 5/5 or the LB "super" medium. In both media the culture achieved the middle exponential stage after 9 h of growth and the stationary stage after 18 h of growth.

Equal amounts of cells from the 9- and 18-h cultures were taken for the fractionation. The periplasmic fraction of *X. campestris* cells grown on medium 5/5 was prepared by osmotic shock [13] without lysozyme, the presence of which could hamper the data analysis. We washed the cells with 0.05 M Tris-HCl, pH 8.0, until the bacteriolytic activity became undetectable in the last portion of wash liquid and placed them into a solution containing 40% sucrose and 0.1 mM EDTA. After the incubation at room temperature for 10 min we centrifuged the cell suspension and collected the supernatant. Then we suspended the pellet in 0.05 M $MgCl_2$, kept it in ice bath for 5 min, centrifuged, and collected the supernatant. In both the supernatants we determined the activities of the marker enzymes of periplasm, cyclic phosphodiesterase [20], and cytosol, glucose-6-phosphate dehydrogenase and lactate dehydrogenase [23, 24], to make certain of cell lysis during the osmotic shock (Table 1). The preparation of periplasmic fraction of *E. coli* cells at the stage of treatment with $MgCl_2$ solution is common when employing osmotic shock for cell lysis [13]. In our experiments, 86% of the cyclic phosphodiesterase, the marker enzyme of periplasm, was already found in the supernatant of cells treated with 40% sucrose and 0.1 mM EDTA (Table 1). When the cells were treated with 0.5 mM $MgCl_2$, 20-25% marker cytosolic enzymes and only 14% cyclic phosphodiesterase were found in the supernatant. So, we used the supernatant prepared by the treatment of the cells with sucrose and EDTA as the periplasmic fraction.

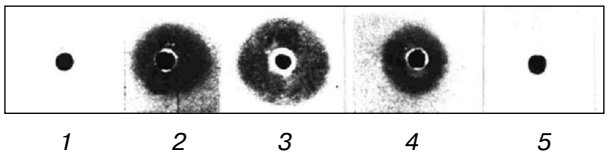

Fig. 1. Autolytic activity of *X. campestris* cell fractions against *X. campestris* cells immobilized in agarose gel. The fractions (20 μl) were placed into wells and incubated at 29°C. *1*) The third portion of wash buffer (0.05 M Tris-HCl, pH 8.0) from the cells; *2*) periplasm; *3*) cytosol; *4*) cell walls and membranes; *5*) the third portion of wash buffer (0.05 M Tris-HCl, pH 8.0) from the cell walls and membranes.

Following the osmotic shock, we disrupted the frozen cells by extrusion using a press. We centrifuged the homogenate and collected the supernatant. This fraction did not contain any components of periplasm and represented the cytosol fraction (Table 1). We washed the pellet three times with 0.05 M Tris-HCl, pH 8.0, to remove completely the soluble lytic activity and used it as a fraction containing cell walls and membranes. Thus, the cytosolic fraction, the periplasmic fraction, and the fraction of cell walls and membranes of *X. campestris* cells were prepared for further experiments. In these fractions we determined autolytic activity and glucosaminidase activity.

Autolytic activity against *X. campestris* cells was detected in all cell fractions (Fig. 1). The activity found in periplasm and cytosol was not due to a contamination by extracellular enzymes (Fig. 1), since we used for fractionation cells completely washed free from extracellular lytic enzymes. The fraction of cell walls and membranes was also washed free of cytosol, because the last portion of wash solution contained no lytic activity (Fig. 1). So we

Table 1. Distribution of cyclic phosphodiesterase, lactate dehydrogenase, and glucose-6-phosphate dehydrogenase activities in soluble fractions of *X. campestris* cells

Cell treatment	Cell fraction	Activity, %		
		lactate dehydrogenase	glucose-6-phosphate dehydrogenase	cyclic phosphodiesterase
0.1 mM EDTA and 40% sucrose	periplasm	0	0	86
0.5 mM $MgCl_2$	cytosol and periplasm	20	25	14
Disruption using a press	cytosol	80	75	0

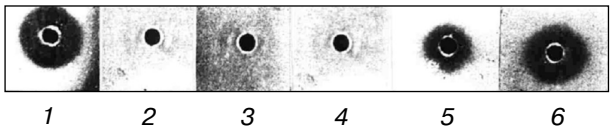

Fig. 2. Autolytic activity in extracts from the fraction of cell walls and membranes after the treatment with various agents. *1*) Cell walls and membranes before the treatment; *2-6*) extracts after the treatment with: *2*) 0.05 M Tris-HCl, pH 8.0; *3*) 0.01 M $MgCl_2$ (20°C); *4*) 0.01 M $MgCl_2$ (37°C); *5*) 5 M LiCl; *6*) 1% Triton X-100.

can deduce that autolytic enzymes are really localized in cell walls and/or membranes. Figure 1 shows the data concerning the cells at the stationary growth stage. The same results were gained with cells at the exponential growth stage.

The method we applied for the detection of autolytic activity is a qualitative one, because both the rate of formation of lysis zone and its size are not in direct ratio to the protein amount. Hence it gives a tentative evaluation of the autolytic activities in various cell fractions considering the size of the lysis zone, the fraction volume, and the quantity of the sample applied to the well. This evaluation suggests that the levels of total autolytic activity in cytosolic and periplasmic fractions are comparable, whereas autolytic activity in the fraction of cell walls and membranes is significantly lower.

Along with the autolytic activity in the cell fractions we have also determined glucosaminidase activity, which we found earlier in cytosol of *X. campestris* cells [11]. It worth noting that, like the total autolytic activity, glucosaminidase activity was found in all of the cell fractions at all growth stages (Table 2). The majority of this activity is concentrated in the cytosol. While the cells are entering the stationary growth stage from the exponential stage, a drastic (more than 2-fold) increase occurs in the total glucosaminidase activity in all of the fractions. It is accompanied by some changes in the distribution of this activity among the cell fractions. The portion of glucosaminidase activity increases from 74.5 ± 1.0 to 84.3 ± 0.3% in cytosol, whereas in periplasm and membranes it decreases from 17 ± 1.8 and 8.5 ± 1.4 to 10.7 ± 0.4 and 5 ± 0.7%, respectively.

Both autolytic and glucosaminidase activities underwent inhibition (by 50-100%) by 10 mM EDTA, 2.5 mM phenylmethylsulfonyl fluoride, or 2.0 mM *p*-chloromercuribenzoate.

In studies on autolytic enzymes we treated the fractions of cell walls and membranes of *X. campestris* sequentially with the following solutions: 0.01 M $MgCl_2$, 5 M LiCl, 1% Triton X-100, and 1% Triton X-100 in the presence of 1 M KCl. We were interested to know how strong the association between these enzymes and cell wall and/or membranes is, as well as how to prepare their solubilized forms. The treated fraction was centrifuged, the supernatant (extract) was collected and assayed for autolytic (Fig. 2) and glucosaminidase activities. Figure 2 indicates that some portion of autolytic activity was only solubilized with the high concentration of LiCl, and another portion by subsequent treatment with Triton X-100. Glucosaminidase activity in this fraction is only solubilized with either Triton X-100 (30-80%) or Triton X-100 in the presence of 1 M KCl (70-80%). A substantial decrease in glucosaminidase activity was therewith observed: by 24% on treatment with 5 M LiCl and by 50-80% on following treatment with 1% Triton X-100. The percentage decrease in activity was calculated as a ratio of activity in extracts plus activity in fractions of cell walls and membranes after the extraction to the initial activity of cell walls and membranes. Thus, the results indicate that autolytic enzymes are firmly bound to cell walls and/or membranes by both ionic and hydrophobic interactions. One can suppose from these data that at least two autolytic enzymes are associated with the fraction of cell walls and membranes, and one of them possesses glucosaminidase activity.

Table 2. Distribution of glucosaminidase activity among cell fractions of *X. campestris* depends on growth stage and culture medium

Fraction	Activity, %			
	exponential stage		stationary stage	
	medium 5/5	medium LB	medium 5/5	medium LB
Periplasm	17.0 ± 1.8	28.5 ± 1.9	10.7 ± 0.4	34.0 ± 0.9
Cytosol	74.5 ± 1.0	66.0 ± 0.9	84.3 ± 0.3	59.0 ± 0.2
Cell walls and membranes	8.5 ± 1.4	5.5 ± 0.9	5.0 ± 0.7	7.0 ± 0.8

Note: The total activity in all three fractions (taken as 100%) was as follows: 4068 U for the cells at the exponential growth stage in medium 5/5; 9595 U for the cells at the stationary growth stage in medium 5/5; 2197 U for the cells at the exponential growth stage in LB medium; and 4701 U for the cells at the stationary growth stage in LB medium.

Different peptidoglycan hydrolases contribute to the autolytic activity of cell fractions. Hence, we sought to determine how many autolytic enzymes are in each fraction of *X. campestris* cells, and to characterize the properties of those enzymes. With this aim the cytosolic and periplasmic proteins, as well as proteins extracted from cell walls and membranes with 1% Triton X-100 and 5 M LiCl, were analyzed by non-denaturing electrophoresis. The anode system of Davis and the cathode system of Reisfeld were used for the separation of acidic/neutral and basic proteins, respectively. After the electrophoresis the polyacrylamide gel was applied on agarose gel containing *X. campestris* cells and incubated at 29°C. Zones of lyzed *X. campestris* cells in the agarose gel indicated the presence of autolytic enzymes in the corresponding polyacrylamide gel bands (Fig. 3).

Six acidic autolytic enzymes (A_1-A_6) with R_f of 0.02, 0.13, 0.38, 0.55, 0.69, and 0.79, respectively, were revealed using the anode system, and one basic enzyme (A_7) with R_f of 0.92 using the cathode system. The enzymes A_1, A_5, A_6, and A_7 possessed maximum activity and produced clear and sharp-cut zones of lysis. The enzyme A_1 coincided in its electrophoretic mobility with the glucosaminidase earlier purified from cytosol [11].

The spectrum and level of cytosolic autolytic enzymes changed in the course of bacterial growth (Fig. 3). In particular, autolytic enzyme A_2 present in cytosol of the cells at the exponential growth stage was not found at the stationary growth stage. The activity of enzyme A_1 significantly increased, whereas the activity of enzymes A_5 and A_6 decreased at the stationary growth stage. Enzyme A_1 was the most active among the cytosolic enzymes in the cells at the stationary growth stage, whereas enzymes A_5 and A_6 were the most active at the exponential growth stage.

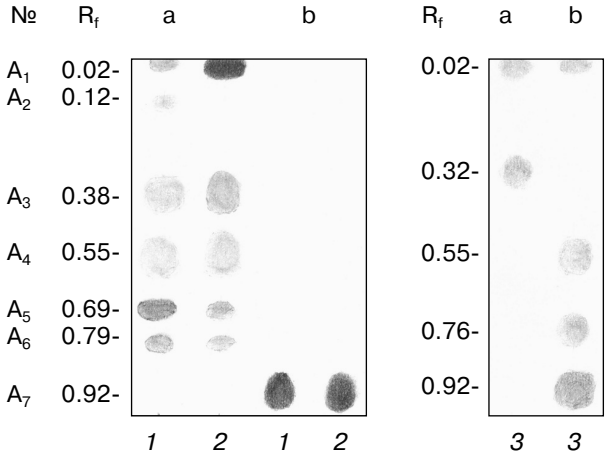

Fig. 3. Electrophoregram of intracellular cytosolic autolytic and extracellular bacteriolytic enzymes of *X. campestris*. The electrophoresis was conducted under non-denaturing conditions in anode (a) or cathode (b) system. Polyacrylamide gel concentration was 7.5%. *1*, *2*) Cytosol (exponential growth stage (*1*) and stationary growth stage (*2*)); *3*) culture liquid of cells from the stationary growth stage; A_1-A_7) cytosolic autolytic enzymes.

A single basic enzyme A_8 with R_f of 0.05 (not shown in Fig. 3) was found in the periplasmic fraction.

We found the acidic autolytic enzyme A_9 with R_f of 0.05 in the extract of cell walls and membranes after treatment with LiCl. Another acidic autolytic enzyme A_{10} with R_f of 0.13 (not shown in the Fig. 3) was found in the Triton extract of cell walls and membranes pretreated with LiCl. So, the electrophoretic analysis of the extracts of cell walls and membranes after the treatment with LiCl and Triton X-100 suggests that two autolytic enzymes are associated with this fraction.

As mentioned above, the data on the electrophoretic analysis of autolytic enzymes were obtained with fractions of cells grown on 5/5 medium. Experiments similar to those described above were further performed on the *X. campestris* cells grown on the LB medium, i.e., when the bacterium does not excrete extracellular enzymes under these conditions. The cells grown on LB medium display the same general principles of distribution and composition of autolytic enzymes among cell fractions with one important exception: A_7, one of the most active cytosolic enzymes in cells grown on 5/5 medium, was not detectable. It is conceivable that this fact is associated with the secretion of extracellular enzymes on medium 5/5. The difference in distribution of glucosaminidase among the fractions of cells grown on LB or 5/5 media is the clearest (Table 2). The portion of glucosaminidase in periplasmic fraction substantially increases on the LB medium. The altered ratio between the activities of cell fractions from the cells of different age is also observed. The glucosaminidase activity in cytosol is virtually unchanged while the cells enter the stationary phase, whereas the portions of glucosaminidase in periplasm and the fraction of cell walls and membranes grow from 28.5 ± 1.9 and 5.5 ± 0.9 to 34 ± 0.9 and 7 ± 0.8%, respectively.

Comparison between intracellular and extracellular enzyme compositions of *X. campestris* by non-denaturing electrophoresis indicated that the extracellular bacteriolytic enzymes are represented preferably by basic proteins whereas the intracellular by acidic proteins (Fig. 3). The difference in properties of these enzymes is probably due to their various mode of action: the activity of extracellular enzymes is targeting to cell wall decomposition from outside, whereas intracellular enzymes act from inside on their own cell wall during its formation.

Note that the cytosolic enzymes A_1 with R_f of 0.02 and A_7 with R_f of 0.92 and two extracellular enzymes possess equal electrophoretic mobilities. These intracellular enzymes may be the precursors or altered molecular forms of corresponding extracellular enzymes. It was mentioned above that the cytosolic enzyme A_7 was not found in cells lacking the excretion of extracellular bacteriolytic enzymes.

Thus, the cells of *X. campestris* have been shown to produce a wide range of intracellular peptidoglycan hydrolases (no less than 10). These enzymes are found in all of the examined cell fractions: periplasm, cytosol, and cell walls and/or membranes. In the course of cell growth changes are observed in both general spectrum of autolytic enzymes and the level of their activity.

As for now, we cannot define what the specificity of any found enzyme is, and whether among the enzymes are those (along with glucosaminidases) with uniform specificity, but associated with different cell fractions. Using glucosaminidase as an example, it can be seen that an enzyme of the same specificity is present in cytosol as well as in periplasm and in the fraction of cell walls and membranes. Detailed studies on the properties of intracellular peptidoglycan hydrolases lie ahead in further investigations.

The presence of a wide range of peptidoglycan hydrolases with different localization in *X. campestris* cells is in agreement with data described elsewhere. It was shown with various bacteria, such as *Escherichia coli*, *Streptococcus pneumonia*, *Enterococcus hirae*, and *Bacillus subtilis*, that multiple peptidoglycan hydrolases with different substrate specificities are usually present in bacterial cells. These enzymes are localized in cytosol and periplasm and are directly bound to peptidoglycan. They may be anchored in the cytoplasmic membrane or linked with lipoteichoic acids of cytoplasmic membranes [1, 25]. Some enzymes are synthesized as precursors and are translocated across the membrane [26]. The major portion of them is localized in periplasm, and only a small amount is bound to peptidoglycan [27]. Other autolytic enzymes are synthesized without N-terminal signal sequence [28]. Another mechanism of export across the membrane might possibly exist for them. The involvement of a specific pore-forming protein is supposed to be necessary for the specific translocation of soluble endolysin of phage λ [29]. Thus, the regulation of peptidoglycan hydrolase action is diverse and very complex. It is still unclear what factors determine the multiplicity of these enzymes and how the functioning of these enzymes depends on the stage of formation of the bacterial cell and its envelope. More detailed studies on the intracellular autolytic enzymes of *X. campestris* and their comparison with extracellular bacteriolytic enzymes excreted by this bacterium will possibly furnish insights into their regulation and functioning.

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