Comparison of the Phosphatases of *Lysobacter enzymogenes* with Those of Related Bacteria

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*Lysobacter enzymogenes* ATCC 29487 (UASM 495) produces an outer-membrane-associated phosphatase and an excreted phosphatase. The cell-associated enzyme was compared to phosphatases of nine other Gram-negative gliding bacteria and to that of *Escherichia coli*. The other three species of the genus *Lysobacter* also produce a particulate, cell-associated phosphatase. Antiserum prepared against the phosphatase from the outer membrane of *L. enzymogenes* effectively precipitated the phosphatases of two other *L. enzymogenes* strains and the enzymes of *L. antibioticus*, *L. brunescens* and *L. gummosus*. Some inhibition of the enzyme by the antiserum also was observed. No significant reaction could be detected between the antiserum and the cell-associated phosphatases of species of *Cytophaga johnsonae*, *C. compacta*, *Myxococcus xanthus*, *E. coli* and the excreted phosphatase of *L. enzymogenes*. The results indicate that the four species of the genus *Lysobacter* are closely related despite their physiological differences and that the outer-membrane-associated phosphatases of these organisms have different structural characteristics than the phosphatases of the other Gram-negative bacteria that were used. Furthermore, differences in the amino acid compositions of the cell-associated and the excreted phosphatase of *L. enzymogenes* confirm the immunological results and are in agreement with the physical and chemical differences noted between the two enzymes.

INTRODUCTION

Species of the genus *Lysobacter* are non-fruiting, gliding bacteria of the class Flexibacteriae (Reichenbach, 1981). They are noted for the many extracellular products they excrete, such as enzymes, polysaccharides and antibiotics (Christensen & Cook, 1978; Reichenbach & Dworkin, 1981) and are important in biodegradation. A number of the extracellular enzymes produced by *L. enzymogenes* have been studied, including proteinases (Whitaker, 1970), and nucleases (von Tigerstrom, 1980, 1981). In addition, this organism produces two phosphatases (von Tigerstrom, 1983, 1984). One of the phosphatases remains associated with the cells and is located in the outer membrane (von Tigerstrom & Stelmaschuk, 1985), whereas the other is excreted into the medium (von Tigerstrom, 1984). Both enzymes have relatively broad substrate specificities. The cell-associated phosphatase is a hydrophobic membrane protein with a molecular mass of 69 kDa and is most active at pH 8·5. It is inhibited by EDTA and is less heat stable when inorganic phosphate is present (von Tigerstrom & Stelmaschuk, 1986). On the other hand, the excreted phosphatase is a 25 kDa, hydrophilic protein with a pH optimum of 7·5. It is not inhibited by EDTA and is more heat stable in the presence of inorganic phosphate (von Tigerstrom, 1984; von Tigerstrom & Stelmaschuk, 1986). Thus it is clear that the two enzymes differ in many important properties and that both have some unusual characteristics.

We wanted to extend the comparison of these two phosphatases by carrying out amino acid analyses of the *L. enzymogenes* phosphatases. We also wanted to compare the outer-membrane-associated phosphatase of *L. enzymogenes* to cell-associated phosphatases of other gliding bacteria. Antiserum was prepared against the cell-associated phosphatase of this organism and
used to determine the immunological relationship of the outer-membrane-associated phosphatase of *L. enzymogenes* to the excreted enzyme and to cell-associated phosphatase of other *Lysobacter* species and other gliding bacteria. The results of these experiments are the subject of this paper.

**METHODS**

Organisms, media, preparation of enzymes sources and enzyme assays. *Lysobacter enzymogenes* ATCC 29487 (UASM 495), *L. antibioticus* ATCC 29479, *L. brunescens* ATCC 29486, *L. gummosus* ATCC 29489, *Cytophaga johnsonae* 405 and *C. compacta* 15D were obtained from Dr F. D. Cook, Department of Soil Science, Faculty of Agriculture, University of Alberta, Edmonton, Canada. *Escherichia coli* C6F1, constituted for alkaline phosphatase production, was from the culture collection of the Department of Microbiology, University of Alberta, Edmonton, Canada, and the other organisms were purchased from the American Type Culture Collection.

All organisms were grown aerobically in liquid media as previously described (von Tigerstrom, 1980) and they were harvested during the stationary phase. For maximum production of phosphatase, 0-8% (w/v) peptone broth was used as the medium. As indicated below, some organisms also were grown in 2% (w/v) casitone or in 0-8% (w/v) tryptone, 0-2% (w/v) yeast extract, 2 mM-MgCl₂ with or without the addition of 0-2% (w/v) starch. The culture supernatants served as a source of the excreted phosphatase. As a source of cell-associated phosphatase the cells were suspended in 10 mM-Tris/HCl, 50 mM-NaCl, 1-5 mM-MgCl₂ pH 7-4 (buffer 1) and cell-free extracts were prepared as previously described (von Tigerstrom & Stelmaschuk, 1986).

*L. enzymogenes* ATCC 29487 was used to prepare purified cell-associated phosphatase (von Tigerstrom & Stelmaschuk, 1986) and excreted phosphatase (von Tigerstrom, 1984). The purified cell-associated phosphatase was used to prepare the antiserum.

The assays of the cell-associated phosphatase at pH 8-5 and the excreted phosphatase at pH 7-5 with *p*-nitrophenyl phosphate (Sigma) as the substrate have been reported (von Tigerstrom, 1984). The enzyme activity found in cell-free extracts of washed cells is termed cell-associated, the activity found in the culture supernatant is termed excreted.

Preparation of antiserum, immune inhibition and precipitation. Each male rabbit was injected with 400 µg of cell-associated phosphatase from *L. enzymogenes* ATCC 29487 in complete Freund’s adjuvant. This was repeated after 28 d with 500 µg phosphatase in incomplete Freund’s adjuvant. The animals were bled at day 41 to obtain the antiserum. The antiserum was assayed by immunodiffusion, where a single precipitin band was obtained against the phosphatase, and by the tube assays described below.

The immune inhibition and precipitation were done as follows: the enzyme solution (200 µl) containing up to 1-5 units phosphatase ml⁻¹ in buffer 1 with 0-2% Zwittergent 3-14 (Calbiochem-Behring) was combined with 50 µl antiserum or a dilution of the serum in buffer 1 containing 10% (v/v) normal rabbit serum and 0-2% Zwittergent 3-14. Incubation was at 37°C for 60 min. Normal rabbit serum was used as the control. The phosphatase activity of this preparation was determined before centrifugation to measure the extent of inhibition and, after centrifugation in a Beckman Microfuge for 1 min, the resulting supernatant was assayed to determine the extent of inhibition plus precipitation. The preparations usually were diluted up to 20-fold with buffer 1 containing 0-2% Zwittergent 3-14 just before the phosphatase assays were done.

A double-antibody method (Midgley & Hepburn, 1980) with goat anti-rabbit IgG serum was used to improve the immune precipitation. Four parts of phosphatase were reacted with one part of diluted anti-phosphatase serum at 37°C for 45 min. Four parts of goat anti-rabbit IgG serum were then added and incubation at 37°C was continued for 30 min and then at 0°C for 60 min. The preparations were centrifuged as above and the phosphatase activities in the supernatants were determined. The two antiserum dilutions used for the experiments (1/50 and 1/1000) were selected from a precipitation curve employing nine different dilutions ranging from 1/12.5 to 1/3200.

These immune precipitations allowed detection of approximately 0-5 ng phosphatase since routine enzyme assays were done with 5-6 ng of the enzyme (von Tigerstrom & Stelmaschuk, 1986) and 10% of this could easily be detected.

Other methods. Protein determinations were carried out by the Lowry method or by the method of Warburg & Christian (1941). Excess detergent was removed from the cell-associated phosphatase by centrifugation of the enzyme through a glycerol gradient using an SW40 Ti rotor at 202000 gₑₑₑ, for 24 h. The glycerol was then removed by dialysis. The amino acid analyses of samples hydrolysed for 24 h were done in the Department of Biochemistry, University of Alberta, Edmonton, Canada.

**RESULTS AND DISCUSSION**

Growth of the organisms and influence of the medium on phosphatase production

*Lysobacter enzymogenes* usually was grown in tryptone or peptone broth for the production of phosphatases (von Tigerstrom, 1983, 1984). However, using 16 different types of tryptone and
peptone from different suppliers, we found that the specific activities of the extracellular enzymes of *L. enzymogenes* varied greatly. For example, a 20-fold variation was observed in the excreted phosphatase and there was even a fourfold variation in this enzyme using different lots of tryptone from the same supplier. It had been established previously that the inorganic phosphate and Mg\(^{2+}\) concentrations greatly affected the production of this enzyme (von Tigerstrom, 1983), but this was not the sole reason for the observed differences.

The production of the cell-associated phosphatase of *L. enzymogenes* also was affected by the composition of the medium, but to a lesser degree. It is important to note that the antiserum prepared against purified cell-associated phosphatase from *L. enzymogenes* grown in peptone broth reacted equally well with the enzyme when the organism was grown in different media. Thus, the quantity of enzyme, not the type of enzyme, was affected by using different media.

Two of the organisms used in this study could not be grown in tryptone or peptone broth. *L. brunescens* autolysed after very limited growth to produce a clear, brownish solution. This could only be partially overcome by the addition of 2 mM-MgCl\(_2\). Therefore, this organism was grown, with a doubling time of about 4 h, in the complex medium containing tryptone, yeast extract, MgCl\(_2\) and starch described in Methods. *Myxococcus xanthus* grew very slowly, if at all, in peptone or tryptone broth. Therefore, 2% (w/v) casitone, the main component of medium 220 (American Type Culture Collection, 1982) recommended for the cultivation of this organism, was used. Both these media support good growth of *L. enzymogenes*, but they severely limit the production of most of the extracellular enzymes.

### Phosphatase activities of the organisms

Table 1 shows the activities of the cell-associated and excreted phosphatases from ten different myxobacteria and, for comparison, from *Escherichia coli C4F1*. The three strains of *L. enzymogenes* had very different activities for both enzymes although they were grown under identical conditions. The three cytophagas had high cell-associated, but very low excreted phosphatase activities (<0.2% of the total activity). All three strains of *L. enzymogenes* and *L. antibioticus* had high excreted activities. It had been shown earlier that the excreted enzyme of *L. enzymogenes* is very different from the cell-associated phosphatase (von Tigerstrom, 1984). This also seems to be the case for *L. antibioticus* since its excreted enzyme was more active at pH 7.5 than at pH 8.5 and was not affected by EDTA. The cell-associated phosphatase activity was low in *M. xanthus* and also in the *Lysobacter* species when they were grown in more complex medium than peptone broth.

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**Table 1. Phosphatase activities of the organisms**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Cell-associated</th>
<th>Excreted</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. enzymogenes</em> 29487</td>
<td>620 (100)</td>
<td>160 (100)</td>
</tr>
<tr>
<td><em>L. enzymogenes</em> 27796</td>
<td>140 (23)</td>
<td>130 (81)</td>
</tr>
<tr>
<td><em>L. enzymogenes</em> 29488</td>
<td>530 (86)</td>
<td>440 (275)</td>
</tr>
<tr>
<td><em>L. antibioticus</em> 29479</td>
<td>19 (2.6)</td>
<td>130 (80)</td>
</tr>
<tr>
<td><em>C. johnsonae</em> 405</td>
<td>850 (137)</td>
<td>2 (1.2)</td>
</tr>
<tr>
<td><em>C. johnsonae</em> 17061</td>
<td>1600 (256)</td>
<td>3 (1.9)</td>
</tr>
<tr>
<td><em>C. compacta</em> 15D</td>
<td>1100 (176)</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td><em>M. xanthus</em> 25232a</td>
<td>4.4 (0.6)</td>
<td>~0 (~0)</td>
</tr>
<tr>
<td><em>E. coli</em> C4F1</td>
<td>570 (92)</td>
<td>31 (19)</td>
</tr>
<tr>
<td><em>L. enzymogenes</em> 29487b</td>
<td>54 (8.7)</td>
<td></td>
</tr>
<tr>
<td><em>L. antibioticus</em> 29479b</td>
<td>8.3 (1.3)</td>
<td></td>
</tr>
<tr>
<td><em>L. brunescens</em> 29486c</td>
<td>14 (2.2)</td>
<td></td>
</tr>
<tr>
<td><em>L. gummosus</em> 29489b</td>
<td>89 (14)</td>
<td></td>
</tr>
</tbody>
</table>

The medium was 0.8% (w/v) peptone except when casitone, tryptone/yeast extract/MgCl\(_2\) or tryptone/yeast extract/MgCl\(_2\)/starch\(^b\) were used, as described in Methods. The numbers in parentheses refer to percentages of *L. enzymogenes* 29487 activities.
Table 2. Sedimentation of cell-associated phosphatase at 48000 g

The organisms were grown as indicated in Table 1 and cell-free extracts were prepared. The activities were determined in the cell-free extracts and in the supernatants and pellets after 60 min centrifugation of the cell-free extract at 48000 g. The activity in the cell-free extract was given a value of 100%.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Phosphatase sedimented at 48000 g from cell-free extracts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. antibioticus</td>
<td>81</td>
</tr>
<tr>
<td>L. brunescens</td>
<td>26</td>
</tr>
<tr>
<td>L. enzymogenes</td>
<td>80</td>
</tr>
<tr>
<td>L. gummosus</td>
<td>90</td>
</tr>
<tr>
<td>C. johnsonae</td>
<td>8</td>
</tr>
</tbody>
</table>

Location of the cell-associated phosphatase

The cell-associated phosphatase of *L. enzymogenes* is a particulate enzyme present in the outer membrane (von Tigerstrom & Stelmaschuk, 1985). A number of proteins are known to be associated with the outer membrane of *E. coli* and other Gram-negative organisms (Osborn & Wu, 1980; Lugtenberg & van Alphen, 1983), but the outer membrane is generally devoid of hydrolytic enzymes (Lugtenberg & van Alphen, 1983). Therefore, the location of the phosphatase in *L. enzymogenes* is unusual. It was of interest to determine whether the cell-associated phosphatases of the other *Lysobacter* species and of *C. johnsonae* also were particulate in nature. Cell-free extracts of the organisms were centrifuged at 48000 g and the amounts of enzyme sedimented were estimated (Table 2). *L. antibioticus* and *L. gummosus* also have particulate cell-associated phosphatases indicating that the enzymes are associated with cell envelope components. In *L. brunescens*, only 26% of the phosphatase was sedimented and it is not clear whether the enzyme from this organism is less hydrophobic or whether the greater solubility is a reflection of the high autolytic activity of this organism. The low activity of the enzyme in these cells makes it difficult to obtain decisive results.

The *Cytophaga* phosphatase is a soluble enzyme in the cell-free extract and may be similar in nature and location to the *E. coli* periplasmic alkaline phosphatase (Neu & Heppel, 1965; Reid & Wilson, 1971). The small amounts of excreted phosphatase activity for the cytophagas and *E. coli*, indicated in Table 1, might result from some leakage of periplasmic enzymes under the culture conditions. Since these activities were so low, their identities were not established.

Immunological experiments

The inhibition and immune precipitation of the cell-associated phosphatases from four *Lysobacter* species by anti-phosphatase serum were determined. The antiserum dilutions used were selected from a precipitation curve prepared with the purified *L. enzymogenes* cell-associated phosphatase. An antiserum dilution of 1/50 was chosen because it was approximately an eightfold excess of the antiserum required for complete precipitation of the enzyme. The dilution of 1/1000 was chosen because it precipitated about 50% of the enzyme, representing the midpoint of the precipitation curve. The cell-free extracts containing the enzymes were combined with antiserum at these two different concentrations and the resulting enzyme activities were determined before centrifugation, and in the supernatant solution after centrifugation, as described in Methods. The purified cell-associated phosphatase from *L. enzymogenes* 29487 was used for comparison. The results in Table 3 demonstrate inhibition of the phosphatases by the immunoglobulin. They also indicate the amount of enzyme precipitated under these conditions.

Only slight inhibition, but extensive precipitation, was observed with the crude and the purified homologous enzyme and with the phosphatase from *L. gummosus*. The weak inhibition was not unexpected since the interaction between enzyme and the immunoglobulin may cause little steric hindrance at the active site, especially for an enzyme with a low-molecular-mass substrate (Cinader, 1963). The phosphatases of *L. antibioticus* and *L. brunescens* were not inhibited and only a small amount of precipitation was detected with the enzyme from *L.*
Table 3. Inhibition and precipitation of cell-associated phosphatases by antiserum

The antiserum was prepared against the cell-associated enzyme from *L. enzymogenes* and the immune assay was carried out as described in Methods. Values are means of two determinations ± the deviation from the mean.

<table>
<thead>
<tr>
<th>Enzyme source</th>
<th>Percentage inhibition by antiserum diluted:</th>
<th>Percentage inhibition and percentage activity precipitated by antiserum diluted:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/50</td>
<td>1/100</td>
</tr>
<tr>
<td><em>L. enzymogenes</em> 29487</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purified enzyme</td>
<td>12.8 (± 1.4)</td>
<td>8.8 (± 3.5)</td>
</tr>
<tr>
<td>Cell extract</td>
<td>8.9 (± 3.0)</td>
<td>14.6 (± 2.2)</td>
</tr>
<tr>
<td>Cell extracts of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. antibioticus</em> 29479</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. brunescens</em> 29486</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. gummosus</em> 29489</td>
<td>8.7 (± 1.8)</td>
<td>13.3 (± 0.5)</td>
</tr>
</tbody>
</table>

- No inhibition or precipitation.

Table 4. Precipitation of phosphatases by the double antibody method

Enzyme preparations were reacted with rabbit anti-phosphatase and goat anti-rabbit IgG serum as described in Methods. The values are the mean of two determinations ± the deviation from the mean.

<table>
<thead>
<tr>
<th>Enzyme source</th>
<th>Percentage activity precipitated by antiserum diluted:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/50</td>
</tr>
<tr>
<td><em>L. enzymogenes</em> 29487</td>
<td></td>
</tr>
<tr>
<td>Cell-associated enzyme, purified</td>
<td>99.0 (± 0.0)</td>
</tr>
<tr>
<td>Cell extract</td>
<td>98.1 (± 2.0)</td>
</tr>
<tr>
<td>Excreted enzyme, purified</td>
<td>0</td>
</tr>
<tr>
<td>Cell extracts of:</td>
<td></td>
</tr>
<tr>
<td><em>L. enzymogenes</em> 27796</td>
<td>90</td>
</tr>
<tr>
<td><em>L. enzymogenes</em> 29488</td>
<td>96</td>
</tr>
<tr>
<td><em>L. antibioticus</em> 29479</td>
<td>68.5 (± 0.2)</td>
</tr>
<tr>
<td><em>L. brunescens</em> 29486</td>
<td>61.8 (± 1.0)</td>
</tr>
<tr>
<td><em>L. gummosus</em> 29489</td>
<td>93.5 (± 1.1)</td>
</tr>
<tr>
<td><em>C. johnsonae</em> 405</td>
<td>0</td>
</tr>
<tr>
<td><em>C. johnsonae</em> 17061</td>
<td>0</td>
</tr>
<tr>
<td><em>C. compacta</em> 15D</td>
<td>0</td>
</tr>
<tr>
<td><em>M. xanthus</em> 25232</td>
<td>3</td>
</tr>
<tr>
<td><em>E. coli</em> C4F1</td>
<td>5</td>
</tr>
</tbody>
</table>

*brunescens*. These results suggest that the phosphatases of *L. enzymogenes* and *L. gummosus* are more closely related immunologically than the enzymes of *L. antibioticus* and *L. brunescens*. However, some immune complex was formed with the enzyme from *L. brunescens*, and the phosphatase in the cell-free extract from *L. antibioticus* was so low that any immune complex formed may not have been removed by centrifugation.

We attempted to increase the sensitivity of the assay by removing the immunoglobulin and the immune complexes with protein A agarose. This was not successful since protein A agarose alone reduced the activity of the cell-associated phosphatase by 60%. However, we were successful in removing rabbit immunoglobulin and immune complexes with goat anti-rabbit IgG antiserum (Table 4). The purified and crude homologous enzyme reacted well. So did the cell-associated phosphatases from the two other strains of *L. enzymogenes* and from *L. gummosus*, again indicating a close antigenic relationship. A definite, but more distant, relationship seems to exist between the enzymes of *L. enzymogenes* and those of *L. antibioticus*.
Table 5. Amino acid analyses of the cell-associated and the excreted phosphatase of Lysobacter enzymogenes

The analyses were obtained after 24 h hydrolysis. Values for Cys and Trp were not obtained.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Cell-associated phosphatase</th>
<th>Excreted phosphatase</th>
<th>Amino acid</th>
<th>Cell-associated phosphatase</th>
<th>Excreted phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp (and Asn)</td>
<td>10.2</td>
<td>18.4</td>
<td>Met</td>
<td>2.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Thr</td>
<td>8.1</td>
<td>7.2</td>
<td>Ile</td>
<td>2.9</td>
<td>2.9</td>
</tr>
<tr>
<td>Ser</td>
<td>5.5</td>
<td>9.4</td>
<td>Leu</td>
<td>7.4</td>
<td>5.9</td>
</tr>
<tr>
<td>Glu (and Gln)</td>
<td>10.4</td>
<td>6.6</td>
<td>Tyr</td>
<td>2.3</td>
<td>5.1</td>
</tr>
<tr>
<td>Pro</td>
<td>6.1</td>
<td>4.1</td>
<td>Phe</td>
<td>2.1</td>
<td>3.1</td>
</tr>
<tr>
<td>Gly</td>
<td>11.5</td>
<td>13.8</td>
<td>His</td>
<td>2.7</td>
<td>2.3</td>
</tr>
<tr>
<td>Ala</td>
<td>12.5</td>
<td>11.1</td>
<td>Lys</td>
<td>4.7</td>
<td>2.3</td>
</tr>
<tr>
<td>Val</td>
<td>5.3</td>
<td>5.0</td>
<td>Arg</td>
<td>5.9</td>
<td>2.2</td>
</tr>
</tbody>
</table>

and L. brunescens. Interestingly, the cell-associated phosphatase of L. enzymogenes had little or no immunological relationship to the phosphatases of the other myxobacteria or to that of E. coli.

The genus Lysobacter comprising the four species used here was established in 1978, based primarily on cell and colonial morphologies, physiological and nutritional characteristics and DNA G + C contents (Christensen & Cook, 1978). However, until now, no immunological comparisons of the organisms or of their products have been done. The results obtained here confirm that the four Lysobacter species and the different strains of L. enzymogenes are more closely related to each other than to the other Gram-negative organisms tested. An antiserum against a common enzyme, such as the cell-associated phosphatase, may be a useful tool in further taxonomic work concerning the myxobacteria.

Although the cell-associated phosphatases of all the Lysobacter reacted well with the antiserum, no reaction was obtained with the excreted phosphatase of L. enzymogenes (Table 4). The amino acid compositions of the cell-associated phosphatase and the excreted phosphatase from the same organism were determined (Table 5). There were variations in the amounts of several of the amino acids, especially Asp, Ser, Met, Tyr, Lys and Arg. These differences, and the physical and chemical differences between the two enzymes noted earlier, lead to the conclusion that the enzymes are products of different genes.

It also is clear from the amino acid compositions that the two enzymes have very similar numbers of non-polar amino acids (about 47%). These results and the average hydrophobicities of 0.909 and 0.819 for cell-associated and excreted enzyme, respectively (Cantor & Schimmel, 1980), do not reflect the more hydrophobic nature of the cell-associated enzyme. Therefore, the tertiary structure of the enzymes must account for the observed differences in the solubilities and locations of these phosphatases.

I would like to thank Dr G. W. Stemke for assistance and advice in the production of the antiserum and for the supply of goat anti-rabbit IgG serum. I also thank M. Nattriss of the Department of Biochemistry for carrying out the amino acid analyses. This work was supported by the Natural Sciences and Engineering Research Council of Canada.

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Phosphatases of Lysobacter enzymogenes


