Alkaline Phosphatase Secretion-Negative Mutant of *Bacillus licheniformis* 749/C

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An alkaline phosphatase secretion-blocked mutant of *Bacillus licheniformis* 749/C was isolated. This mutant had defects in the *phoP* and *phoR* regions of the chromosome. The selection procedure was based on the rationale that *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine can induce mutations of closely linked multiple genes. The malate gene and the *phoP* and *phoR* genes are located at the 260-min position in the *Bacillus subtilis* chromosome; hence, the malate gene could be used as a marker for the mutation of the *phoP* and *phoR* regions of the chromosome. In a two-step selection procedure, strains defective in malate utilization were first selected with the cephalosporin C procedure. Second, these malate-defective strains were further screened in a dye medium to select strains with defects in alkaline phosphatase secretion. One stable mutant (*B. licheniformis* 749/C/NM105) had a total secretion block for alkaline phosphatase and had the following additional characteristics: (i) the amount of alkaline phosphatase synthesized was comparable to that in the wild type; (ii) the alkaline phosphatase was membrane bound; (iii) the mutant strain alkaline phosphatase, in contrast to that of the wild type, could not be extracted with MgCl$_2$, although the amounts of protein extracted from each strain were comparable; (iv) the sodium dodecyl sulfate-polyacrylamide gel pattern of MgCl$_2$-extracted proteins from the mutant strain was different from that of the wild-type proteins; (v) the mutant, unlike the wild type, could not use malate as a sole source of carbon; and (vi) the outside surface of the wall of the mutant cells contained an additional electron-dense layer that was not present on the wild-type cell wall surface.

Secretion is multistep cellular activity (2, 29, 33). The secretory proteins are usually segregated from the cytoplasmic proteins at a membrane site that is common for both biosynthesis and transport across the membrane (27). It is now believed that a unique structural feature of the nascent secretory protein molecule (e.g., the N-terminal hydrophobic amino acid signal sequence) may be recognized by a specific membrane receptor (signal-specific membrane receptor [38-40]). This receptor-nascent secretory protein binding presumably initiates a membrane process, causing the movement of nascent secretory protein molecules across the membrane permeability barrier. In procaryotic cells, the N-terminal signal sequence plays a crucial role in the secretion process (5, 16, 31); however, a variety of other factors are equally important (17).

It has been suggested that there are pluripotent secretion-specific regions in procaryotic cell membranes that might have evolved, by a process of differentiation, into the specific secretory organelles (i.e., endoplasmic reticulum, Golgi bodies, etc.) of eucaryotic cells (2). It is implied in this concept that the plasma membrane of procaryotic cells may have secretion-specific regions containing components required for secretory activity (2, 29, 33). We showed earlier that the secretory enzymes (i.e., penicillinase and alkaline phosphatase) are distributed in a few discrete sites of the plasma membrane of bacilli (10, 30). These specific membrane binding sites could arise from membrane proteins, which we tentatively defined as receptors (30).

Preliminary results on the isolation of membrane receptors for the secreted proteins have been reported for animal cells (22, 24, 37). It is intriguing that the N-terminal signal sequences of the nascent presecretory proteins are not homologous (3). It is likely that the unique structure of a presecretory protein ensures its binding to the putative membrane receptor moiety, although the initial attachment to the membrane may be a nonspecific process dependent only on the hydrophobic nature of the N-terminal signal sequence. The biosynthesis of a secretory protein and the putative receptor could be
integrated if the genes for both of these proteins were coordinately controlled. Thus, the putative receptor gene and the alkaline phosphatase structural or regulatory genes might be closely linked.

Since 1965, several papers have been published on the genetic regulation of alkaline phosphatase in *Bacillus subtilis* (23, 28). The chromosomal map shows one structural gene (*phoS*) located in the 110-min position and the two regulatory genes *phoP* and *phoR* located in the 260-min position. However, the location and functions of these genes are not fully understood (20, 42). The malate utilization character is also located in the 260-min position of the *B. subtilis* chromosome (14). Interspecific variations of these landmark map positions are unlikely. Based on the observation that *N*-methyl-\(N'\)-nitro-\(N\)-nitrosoguanidine (MNNNG) induces a number of closely linked mutations within a short segment of the *Escherichia coli* chromosome, we developed a strategy to select *PhoP* or *PhoR* mutants (4, 13). *Bacillus licheniformis* cells defective in malate utilization were selected after MNNNG mutagenesis. As these mutants were likely to have a mutation within a short segment of the chromosome (i.e., at 260 min), a second selection was made for defects in the *PhoP* or *PhoR* character. Thus, regulatory gene mutants with altered alkaline phosphatase secretion were expected to be isolated.

We report here one stable alkaline phosphatase secretion-blocked mutant, *B. licheniformis* 749/C/NM105. This mutant and the wild-type strain synthesized the same amount of the enzyme; however, the rate of synthesis in the mutant was faster than that in the wild-type cells. The enzymes of both strains were membrane bound, had the same molecular weight, and showed antigenic identity; but the mutant enzyme, unlike the wild type, could not be extracted with high concentrations of magnesium salt.

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**MATERIALS AND METHODS**

**Bacterial strains and media.** *B. licheniformis* 749/C was used in this study. The cells were grown in a low-Pi medium (Difco Laboratories) and salt medium (CH/S medium) and in a minimal medium containing the following (in grams per liter): Tris, 12; \(K_2HPO_4\), 0.87; amino acid mixture: cystine, aspartic acid, asparagine, proline, and glutamine, 0.02 each; biotin, 0.001; cyanocobalamin, 0.001; glucose or maltose, 2; and 10 ml of salt mixture [\(MgSO_4 \cdot 7H_2O\), 1.2 g; \(NH_4)_2SO_4\), 20 g; \(Fe_2SO_4 \cdot 7H_2O\), 0.097 g; \(MnSO_4 \cdot 0.001 g\) in 100 ml of distilled water] per liter. The pH was adjusted to 7.0. The P\(_i\) concentration was 5 mM. For alkaline phosphatase derepression, the P\(_i\) concentration was lowered to less than 0.1 mM. Incubula were prepared by growing a suspension of spores in saline in a high-phosphate medium (CH/S or minimal), and incubula grown for 18 h were used to study growth and enzyme synthesis in minimal or CH/S medium. The cells were grown at 32°C with constant shaking.

**Isolation of mutants.** The bacterial colonies showing altered characteristic for alkaline phosphatase were screened in a low-Pi, minimal medium containing a phosphorylated azo dye (5-bromo-4-chloro-3-iodonyl-phosphate-p-toluidine) at a concentration of 50 \(\mu\)g/ml (32); this dye was colorless and became blue after dephosphorylation. The wild type and mutant strains were maintained on two types of slants, sporulation (9) and low-phosphate minimal-glucose agar. The following screening protocol was used for the isolation of mutants: (i) selection of malate-defective organisms; (ii) selection of organisms with altered alkaline phosphatase characteristics from the malate-defective organisms; and (iii) screening of the malate-defective and alkaline phosphatase-altered organisms for alkaline phosphatase synthesis and secretion in low-Pi, liquid media. *B. licheniformis* 749/C cells were grown for 4 h in a low-Pi, CH/S medium as described earlier (15). These cells were washed and treated with MNNNG (1 mg/ml) as described by Adelberg et al. (1). This treatment killed 99.95% of the cells; the survivors were washed and suspended in a high-Pi, minimal medium containing malate as the sole carbon source. After 3 h of growth at 32°C, 200 \(\mu\)g of cephalosporin C per ml was added, and the cells were grown for a further 14 h. The cephalosporin C-selected cultures were washed and enriched by 18 h of growth in a high-Pi, minimal medium containing glucose. These cells were diluted and plated on a low-Pi, glucose-minimal medium containing the azo dye. Discrete and distinct colonies appeared on these plates after incubation at 32°C for 96 h or longer. The colonies showed a range of color variation (deep blue or white) and were frequently encircled by blue halos of various size. These variations of colony coloration could not be directly correlated with variations in alkaline phosphatase synthesis or secretion. The dye was used to guide our selection of colonies with malate-defective organisms from the selected colonies were maintained on low-Pi, glucose-minimal agar slants. Synthesis and secretion were further examined in low-Pi, CH/S-glucose liquid medium. Growth was assessed by turbidity as measured by a Klett-Summerson colorimeter with a no. 54 filter. Finally, the following species identification tests suggested by Gordon et al. (12) were performed: catalase, Voges-Proskauer, growth in anaerobic agar, growth at 50°C, and growth in 7% sodium chloride.

**Extraction of alkaline phosphatase and SDS-PAGE.** Extractions of the alkaline phosphatase by salt (MgCl\(_2\) or NaCl), trypsin, and Triton X-100 from the mutant and wild-type cells were compared. The cells were harvested when the cell-bound alkaline phosphatase content was highest, which was after 3 h for strain 749/C/NM105 and 6 h for strain 749/C. The harvested cells were washed in 0.01 M Tris-hydrochloride–sodium acetate (TNA buffer) (pH 7.4) (11). The washed cell residues were mixed with 1 M MgCl\(_2\) or NaCl in the same buffer and incubated with constant shaking at 37°C for 30 min. The treated cell suspensions were centrifuged at 40,000 \(\times\) g, and then the supernatants...
and residues were assayed for alkaline phosphatase and protein. A method described earlier (7) was followed for trypsin and Triton X-100 extractions. To increase the accessibility of trypsin to the membrane surface, we used protoplasts for this treatment instead of whole cells.

The salt-extracted material was dialyzed against 0.01 M TNA buffer (pH 7.4) containing 0.5 mM CoCl₂ in the cold (4°C) for 18 h, and the dialysate was concentrated with Aquacide II (Calbiochem). The protein and alkaline phosphatase contents of these concentrated materials were assayed (15, 21); in addition, a sample was subjected to polyacrylamide gel electrophoresis (PAGE) in a 10% sodium dodecyl sulfate (SDS)-polyacrylamide slab gel (19). The protein bands fixed in 15% trichloroacetic acid were stained with 0.1% methanolic Coomassie blue and destained in a 5% methanol-10% acetic acid mixture.

Antigenicity. Membrane-bound enzyme was extracted and purified from washed membrane preparations of B. licheniformis 749/C cells by the procedure used for alkaline phosphatase extraction and purification from B. subtilis SB15 membrane (11). Antibody was prepared against purified membrane-bound enzyme from strain 749/C in rabbits (7, 11). The enzyme from the mutant was extracted with salt. Although only a small amount of enzyme could be extracted, enough crude material was obtained from a large batch of washed cells for a preliminary study of antibody-enzyme interaction. There was no cross-reaction between the B. licheniformis and B. subtilis alkaline phosphatase and anti-alkaline phosphatase antibody.

Electron microscopy. The wild-type and mutant cells grown for 3 h were fixed, dehydrated, embedded, sectioned, and stained for electron microscopic examination (8). Ultrastructural cytochemistry of the alkaline phosphatase was performed by a method described previously (10). The thin sections were examined under a JEM 100C (JEOL, Japan) electron microscope.

Construction of revertants. The method of Koyama et al. (18) was followed to construct revertants of B. licheniformis 749/C/NM105. The inoculum of the mutant strain was grown in CH/S medium. After 18 h of growth, the cells were washed in 0.155 M saline and inoculated into P₇-free CH/S medium to an optical density of 25. After 3.5 h, the cells were again washed in 0.155 M saline and then treated with 1 mg of MNNG per ml for 30 min at 37°C as described above. The treated cells were washed, diluted in 0.155 M saline, and plated on low-P₇ glucose-minimal agar medium containing dye. The colonies were selected on the basis of color variation. Organisms from selected colonies were grown for 4 to 5 h in low-P₇ CH/S medium. Total and secreted alkaline phosphatase levels were determined as described above.

RESULTS

Growth and synthesis and secretion of alkaline phosphatase. Growth of the mutant and wild-type strains was compared in CH/S medium (Fig. 1). The mutant grew much faster and reached the stationary phase much earlier; net growth was 40 to 45% higher than for the wild type. The wild type exhibited poor growth, compared with the mutant, in minimal medium. In fact, growth of the mutant was twofold higher than that of the wild type with glucose as the sole source of carbon (Fig. 2A); however, the mutant did not show any significant growth when malate was used as the sole carbon source (Fig. 2B).

The data on the synthesis and secretion of alkaline phosphatase for the mutant and wild

![FIG. 1. Growth curve of B. licheniformis strains 749/C (●) and 749/C/NM105 (■) in CH/S medium supplemented with salt mixture (see the text).](http://jb.asm.org/)

![FIG. 2. Growth curves of B. licheniformis strains 749/C (○) and 749/C/NM105 in ● in (A) glucose-minimal medium and (B) malate-minimal medium.](http://jb.asm.org/)

Downloaded from http://jb.asm.org/ on October 28, 2017 by guest
type are shown in Fig. 3. The mutant and the wild type synthesized comparable amounts of the enzyme; however, the net amount of enzyme formed varied among different batches of medium. The enzyme yield was highly sensitive to minor variations of the P_{i} concentration in CH/S medium. The striking difference between these two strains was in secretion; the wild-type cells secreted about 59% of the synthesized enzyme, but the amount secreted by the mutant cells was almost undetectable. Furthermore, the characteristics of alkaline phosphatase synthesis in the mutant were significantly different. (i) Synthesis, once started, continued at a very rapid rate, reaching a peak value within 40 to 60 min (Fig. 3). In contrast, in the wild-type culture this rising phase of synthesis continued for several hours (Fig. 3). (ii) After reaching the peak value, the enzyme in the mutant was degraded at a very rapid rate, and nearly 50% of the total enzyme activity was lost within 40 to 60 min (Fig. 3).

By comparing growth (Fig. 1) and alkaline phosphatase synthesis, it was evident that during this period of rapid alkaline phosphatase degradation the mutant culture maintained the logarithmic phase of growth. Such a rapid decline of enzyme activity during the logarithmic phase suggests that a specific control mechanism, rather than any autolytic process, was related to this rapid degradation. In the wild-type cells, however, the enzyme activity declined slowly during the stationary phase of growth (Fig. 3).

As the selection procedure was elaborate, it was important to examine whether the mutant retained the characteristics of typical *B. licheniformis* cells. The key tests suggested by Gordon et al. (12), described above, were all positive, confirming the authenticity of this *Bacillus* species.

Subcellular location of alkaline phosphatase. In strain 749/C cells, 75 to 80% of the alkaline phosphatase was bound to the plasma membrane and cell wall (A. Ghosh, M. Smolensky, S. Vallespere, and B. K. Ghosh, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, J19, p. 83). The particle-bound nature of the enzyme was unchanged in the mutant strain. The log-phase cells of the mutant were treated with lysozyme in an osmotically unsupported medium to effect complete lysis of the cells. This lysate was centrifuged in the cold (4°C) at 40,000 x g, and enzyme activity was assayed in the cytosol and particle fractions. The results showed that about 90% of the alkaline phosphatase was particle bound.

The alkaline phosphatase of bacilli is salt extractable, especially by salts of magnesium (11, 35). To gain insight into the possibility of changes in the nature of the binding of alkaline phosphatase to the membrane, we examined the extraction of the enzyme by different reagents. The data (Table 1) showed a clear and notable contrast between the two strains. Magnesium and sodium salts extracted 86 and 91%, respectively, of the alkaline phosphatase from the wild type, but only 3 and 5%, respectively, from the mutant. It was evident from the data (Table 2) that considerably more cell-bound proteins could be extracted by magnesium or sodium salts from the mutant than from the wild type. The specific absence of alkaline phosphatase in extracts from the mutant was evident from comparing the alkaline phosphatase-specific activities of the magnesium and sodium salt extracts, which were 15- and 19-fold higher, respectively, for the wild type than for the mutant. The amount extracted by Triton X-100 from the wild type was three times higher than that from the mutant.

![FIG. 3. Synthesis (open symbols) and secretion (solid symbols) of alkaline phosphatase (APASE) by the wild-type strain 749/C (○, ○) and mutant strain 749/C/NM105 (■, ■).](http://jb.asm.org/)

**TABLE 1.** Extraction of alkaline phosphatase by MgCl$_2$, NaCl, trypsin, and Triton X-100

<table>
<thead>
<tr>
<th>Strain</th>
<th>MgCl$_2$ (1 M)</th>
<th>NaCl (1 M)</th>
<th>Trypsin (1%)</th>
<th>Triton X-100 (1%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. licheniformis</em> 749/C</td>
<td>86</td>
<td>91</td>
<td>5</td>
<td>45</td>
</tr>
<tr>
<td><em>B. licheniformis</em> 749/C/NM105</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>14</td>
</tr>
</tbody>
</table>

* Note the remarkable differences in the salt extractability of alkaline phosphatase. The data on enzyme activity were consistently within the same range when the experiments were done under comparable conditions.
TABLE 2. Protein extracted from whole cultures by MgCl	extsubscript{2}, NaCl, trypsin, and Triton X-100

<table>
<thead>
<tr>
<th>Strain</th>
<th>Protein extracted (% of total cell protein	extsuperscript{a} with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MgCl	extsubscript{2} (1 M)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>B. licheniformis</td>
<td>1</td>
</tr>
<tr>
<td>749/C</td>
<td></td>
</tr>
<tr>
<td>B. licheniformis</td>
<td>2</td>
</tr>
<tr>
<td>749/C/NM105</td>
<td></td>
</tr>
</tbody>
</table>

	extsuperscript{a} Note that slightly more protein could be extracted by the salts from the mutant (strain 749/C/NM105) than from the wild-type strain.

mutant cells. It should be noted, however, that the Triton X-100-extracted enzyme in these experiments included some membrane-bound particulate enzyme. Centrifugation at 40,000 \( \times \) g failed to precipitate enzyme bound to small membrane particles present in the Triton X-100 extract. However, prolonged centrifugation at 100,000 \( \times \) g showed that Triton X-100-solubilized enzyme, freed of particle-bound enzyme, represented about 20\% of the total cell-bound enzyme. A detailed study on the comparative extractability and properties of purified enzymes from the mutant and wild-type cells is in progress. The data presented in this paper make it clear that the salt extractability of the alkaline phosphatase from the mutant is notably different from that of the wild type. There was also an appreciable difference in the Triton X-100 extractability. In both strains the intact protoplast-bound alkaline phosphatase was not available for trypsin release. A detailed study of the extraction of enzyme from this mutant strain by a variety of detergents is in progress.

SDS-PAGE. The protein profile of the MgCl\textsubscript{2}-extracted material was examined by SDS-PAGE on slab gels (Fig. 4). The protein band corresponding to pure membrane-bound alkaline phosphatase was absent from the extract of the mutant but present in the wild-type cell extract. In addition to this major difference, the gel profiles of both strains differed significantly; most conspicuous were bands A and C, which were only seen in the mutant extract. However, detailed analysis of the gel profile is beyond the scope of this paper. The most important point was that MgCl\textsubscript{2} failed to extract alkaline phosphatase from the mutant strain cell membrane.

Antigenicity. Detailed comparison of the enzymatic properties is beyond the scope of this paper; however, we have presented data on the action of strain 749/C anti-alkaline phosphatase antibody on the mutant strain alkaline phosphatase. The curves for the enzyme-antibody precipitate (Fig. 5B) and for its residual catalytic activity (Fig. 5A) were compared. The crude enzyme preparations from both strains were precipitated with strain 749/C anti-alkaline phosphatase (membrane bound) antibody. The residual catalytic activity of the enzyme from both strains was stimulated. There were quantitative differences in the amount of the precipitates formed and the residual activity stimulation. It was clear that the mutant and wild-type alkaline phosphatases were antigenically similar; a detailed study of the subtle quantitative differences is in progress.

Ultrastructure. It was evident (Fig. 6A) that strain 749/C/NM105 produced endospores characteristic of bacilli. This is important morphological information, supplementary to the species-identifying tests, that the mutant retained the properties of a typical B. licheniformis organism. Both log-phase and stationary-phase cells had a thin electron-dense layer on the outside surface of the cell wall (Fig. 6B). This layer was not detected on the wild-type cell wall surface (Fig. 6C).

Preliminary results from our study on the ultrastructural cytochemistry of alkaline phosphatase showed that the enzyme is bound to the inside surface of the plasma membrane (6D).
Revertants. The alkaline phosphatase synthesis and secretion characteristics of the revertant strains are shown in Table 3. The results showed that strain 749/C/NM105 was totally blocked. In contrast, 67% of the revertant strains secreted significant amounts of alkaline phosphatase. In fact, many of these strains secreted as much or slightly more alkaline phosphatase as the wild-type strain did. This result is generally consistent with the information presented by Koyama et al. (18) and Friedman and Huberman (6) that MNNG may induce reversion by enhancing a DNA repair phenomenon.

DISCUSSION

The basic assumption that led to this study was that the movement of a secretory protein (alkaline phosphatase in this case) through the permeability barrier of a membrane requires a receptor function. The results of the study showed that MNNG mutation produced a secretion-blocked mutant (B. licheniformis 749/C/NM105) with altered membrane-alkaline phosphatase association.

There are three genes for alkaline phosphatase: phoS, phoP, and phoR (14). The phoS gene is located in the 110-min position of the B. subtilis chromosome. This gene regulates the constitutive character of the enzyme (28). The phoR and phoP genes are located in the 260-min position of the B. subtilis chromosome. phoR is not linked to phoP, and its activity regulates the rate of alkaline phosphatase production. Any mutation in this gene causes a slower or faster rate of enzyme synthesis (23). The phoP mutation, on the other hand, produces strains with lowered synthesis rates (23); in addition, the structure of the enzyme molecule appears to be regulated by this gene (41).

B. licheniformis 749/C/NM105 synthesized almost the same amount of enzyme as the wild type did. The enzyme had the same molecular weight, and it was antigenically similar to the wild-type enzyme. Hence, based on these physiological properties, phoP mutation could be ruled out. Enzyme synthesis in the mutant strain was not constitutive; therefore, the possibility of phoS mutation could be eliminated. The synthesis of the enzyme in the mutant, compared with that in the wild type, however, started earlier during growth, and the rate of synthesis was much faster than the rate in the wild type. Furthermore, the amount of enzyme synthesized could not be retained at the peak level in the mutant cells. In spite of rapid cell growth,
FIG. 6. Thin-section electron micrographs of mutant and wild-type cells. (A) Cross-section of a sporulating cell from the mutant strain shows a typical endospore. (B) Mutant strain cell. (C) Wild-type strain cell. (D) Mutant strain cell. Note that the cytochemical reaction product is bound to a few discrete locations on the inside surface of the plasma membrane (arrows). CW, Cell wall; OL, outer layer of cell wall (absent from wild-type strain; cf. panel C); Me, mesosome; FP, forespore. Bars, 0.2 μm.
the enzyme content fell to 50% of the peak value within a very short time. These physiological properties suggest that strain 749/C/NM105 had undergone a phoR gene mutation.

The mutant strain showed three additional changes: (i) alkaline phosphatase secretion was totally blocked; (ii) the enzyme could not be extracted from the cell membrane with magnesium or sodium salt; and (iii) the SDS-PAGE gel profile of the magnesium salt extract of the membrane was different from that of the wild-type cell extract. It could be suggested from these data that the activity of the phoR gene region regulated the rate of alkaline phosphatase secretion in addition to its rate of synthesis. This contention is confirmed by the construction of revertant strains of the mutant with MNNG. The secretion block in strain 749/C/NM105 was removed in 67% of these revertant strains. It has been suggested that with some methylating mutagens, postreplication repair can cope with the lesions responsible for the mutation (6). Thus, the return of the wild-type secretion characteristics to the secretion-blocked mutant after MNNG-induced reversion was likely a result of the lesion and repair of a specific region of the chromosome regulating alkaline phosphatase secretion.

Magnesium salt extractability suggested that the wild-type alkaline phosphatase was an extrinsic (peripheral) membrane protein that might bind to a secretion-specific receptor. The latter, having affinity for the membrane, might have formed a complex with an intrinsic membrane protein(s) embedded through hydrophobic interaction with the membrane interior (34, 36). Thus, a change in the structure of the putative receptor protein brought about by mutation might have caused an alteration in the binding characteristic such that the alkaline phosphatase formed an unusually tenacious complex with the membrane and was resistant to salt extraction. Significant differences in the SDS-PAGE gel profiles of the mutant and wild-type membrane extracts suggested a possible alteration in the protein profile after mutation. Preliminary evidence for the presence of salt-extractable signal recognition protein in the animal secretory cell membrane has been presented (40). It was not possible to infer from our data any interrelationship between the altered alkaline phosphatase binding to the membrane and the secretion block in this mutant. Such a relationship, however, is likely, and study is progressing in this direction. In fact, transformation experiments are in progress, aimed at constructing transformants from B. licheniformis 749/C alkaline phosphatase-negative mutants with B. licheniformis 749/C DNA to produce alkaline phosphatase secretion-positive cells. Further-

more, a similar mutation technique was used to mutagenize B. subtilis SB15 cells that were phenotypically secretion negative. A large number of these mutants secreted substantial amounts of alkaline phosphatase (R. Kumar, A. Ghosh, and B. K. Ghosh, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, H105, p. 123).

Finally, the results of our study are in general agreement with the recently published data of Oliver and Beckwith (25, 26). They demonstrated in E. coli cells a new gene (secA) product that appears to be a 92,000-dalton protein. This product is likely to be involved in cell envelope protein secretion. It may be speculated that such gene products are secretion receptors regulating the movement of protein molecules through the membrane.

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