

Regulation of Nonspecific Acid Phosphatase in *Salmonella*: *phoN* and *phoP* Genes

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Mutations in *Salmonella typhimurium* strains lacking nonspecific acid phosphatase mapped in two unlinked loci. One of these, *phoP*, was cotransducible by phage P22 with *purB*, whereas the second, *phoN*, was cotransducible by phage P1 with *purA*. Mutants with temperature-sensitive nonspecific acid phosphatase activity (measured in whole cells) were also isolated. A *phoN* mutant with thermolabile whole-cell activity was isolated directly from wild-type LT-2. Several other mutants with temperature-sensitive enzyme activity were also isolated as revertants of *phoN* mutants. These data suggest that *phoN* might be a structural locus for nonspecific acid phosphatase. The observation that a mutation resulting in high levels of nonspecific acid phosphatase mapped in *phoP* suggests a possible regulatory role for this locus.

Results from this laboratory have shown that *Salmonella typhimurium* has three periplasmic phosphatases, an acid hexose phosphatase, a cyclic 2',3'-nucleotide phosphodiesterase, and a nonspecific acid phosphatase (6). Cyclic phosphodiesterase and acid hexose phosphatase levels increased when growth was limited by the carbon source in wild-type strains, but not in mutants lacking either adenylate cyclase or cyclic AMP receptor protein, indicating that these enzymes are regulated by the cyclic AMP-catabolite repression system (7).

In contrast, nonspecific acid phosphatase was expressed normally in mutants defective in the cyclic AMP catabolite repression system as well as in a *Salmonella* strain bearing a mutation which caused derepression of *Escherichia coli* alkaline phosphatase (7). The acid phosphatase levels increased when growth was limited by the carbon, nitrogen, or phosphate source (7). These observations suggested that nonspecific acid phosphatase might be controlled by a unique regulatory system. To gain insight into this system, mutants altered in the expression of nonspecific acid phosphatase were isolated. This communication describes the characterization and genetic analysis of these mutants.

MATERIALS AND METHODS

Bacteria and phage. Table 1 lists the genotypes and sources of the strains used in this study. Transduction was done with phage P22int3 (4) or phage P1

grown on *Salmonella* (the generous gift of John Roth).

Chemicals. Sigma Chemical Co. was the source of an α -naphthyl phosphate, tetrazotized *o*-dianisidine (practical grade, ZnCl₂ complex), and 2-deoxygalactose.

Growth media. LC broth, which was used for growth of P1 phage, was prepared by autoclaving a solution containing, per liter, 10 g of tryptone (Difco Laboratories), 10 g of NaCl, and 5 g of yeast extract (Difco). After cooling, 4 ml of 0.5 M CaCl₂, 2.5 ml of 40% (wt/vol) glucose, and 4 ml of 0.25% (wt/vol) thymidine were added. LC bottom and top agar contained 1.5 and 0.6% agar (Difco), respectively. Other media used in this study include N⁻C⁻ and N⁻C⁻P⁻, which are nitrogen- and carbon-free and nitrogen-, carbon-, and phosphorus-free media, respectively. These media were supplemented with nitrogen, carbon, and phosphorus sources, as appropriate. VBCG is a complete minimal glucose medium, and VBC is the same mixture without glucose. These media have been described previously (1). All other media used in this study were as described previously (6).

Mutagenesis. Mutagenesis with diethyl sulfate (DES) was performed by adding 3 drops (a saturating amount) of DES to 5 ml of a stationary-phase culture resuspended in VBCG. After 10 min at 37°C, the culture was diluted 1:100 into VBCG and grown at 37°C until stationary phase was reached.

Mutagenesis with ICR-372 was performed by adding 0.10 ml of nutrient broth, 0.01 ml of a 1-mg/ml solution of ICR-372, and 0.01 ml of a stationary-phase nutrient broth culture to 0.9 ml of VBCG. After growth to stationary phase, the culture was diluted 100-fold into VBCG and was again grown to stationary phase.

Mapping by Hfr crosses. Donors and recipients were grown overnight in nutrient broth. The recipient and donor cultures (0.1 ml each) were spread on minimal glucose medium. The concentration of donors was adjusted by dilution to yield several hundred to

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TABLE 1. Origin and genotype of bacterial strains

Strain	Genotype	Source and/or comments
TA1970	<i>galE hut</i> ⁺	SL3684 × LT-2
TA1972	<i>gal-514 purA1 phoN1</i>	2-Deoxygalactose ^r (P1 ^r) isolate from TA2318
TA2318	<i>purA1 phoN1</i>	From <i>purA1</i> strain of M. Demerec
TA2328	<i>purB12 phoP22</i>	DES mutagenesis of <i>purB12</i>
TA2329	<i>HfrK5 serA13 phoN7</i>	DES mutagenesis of SA535
TA2360	<i>HfrK5 serA13 phoP23</i>	DES mutagenesis of SA535
TA2361	<i>phoN2</i>	ICR-372 mutagenesis of LT- 2
TA2362	<i>phoP12</i>	ICR-372 mutagenesis of LT- 2
TA2367	<i>pho-24</i>	DES mutagenesis of LT-2
TA2374	<i>gal-517 phoP15</i>	From <i>phoP15</i> ; 2-deoxy- galactose ^r (P1 ^r)
TA2375	<i>gal-518 phoN3</i>	From <i>phoN3</i> ; 2-deoxy- galactose ^r (P1 ^r)
TA2376	<i>gal-519 phoN2</i>	From <i>phoN2</i> ; 2-deoxy- galactose ^r (P1 ^r)
TA2377	<i>gal-520 phoN7</i>	From <i>phoN7</i> ; 2-deoxy- galactose ^r (P1 ^r)
TA2379	<i>gal-522 phoN7</i>	From <i>phoN7</i> ; 2-deoxy- galactose ^r (P1 ^r)
TA2382	<i>gal-523 purA1</i>	From <i>purA1</i> ; 2-deoxy- galactose ^r (P1 ^r)
TA2444	<i>phoP13</i>	DES mutagenesis of LT-2
TA2445	<i>phoP14</i>	ICR-372 mutagenesis of LT- 2
TA2446	<i>phoN27</i>	Spontaneous revertant of TA2361
TA2447	<i>phoN28</i>	Spontaneous revertant of <i>phoN4</i>
TA2448	<i>phoN29</i>	Spontaneous revertant of <i>phoN4</i>
TA2449	<i>phoN30 purB12</i>	DES mutagenesis of LT-2 M. Demerec

about 1,000 recombinants per plate. After 2 days at 37°C, the recombinants were stained for nonspecific acid and phosphatase activity.

Transduction with *E. coli* phage P1. The procedures for using phage P1 for genetic analysis of *Salmonella* were based on those of J. Roth (personal communication). *Salmonella* strains require a *galE* mutation for sensitivity to phage P1 (8; E. D. Ornallas, M. Enomoto, and B. A. D. Stocker, Bacteriol. Proc., V238, p. 207, 1971). Mutants of *Salmonella* strains with the *galE* mutation were selected by a positive selection procedure for resistance to 2-deoxygalactose (1). A 0.1-ml amount of an overnight nutrient broth culture was spread on N⁻C⁻ plates previously spread with 0.15 ml of 40% (vol/vol) glycerol, 0.15 ml of 1 M NH₄Cl, 0.10 ml of 40% (wt/vol) 2-deoxygalactose, and 60 μl of 4% D-galactose. After 2 days at 37°C, approximately 200 colonies appeared on the plates. Since these colonies could be either *galE* or *galK*, the colonies were checked for sensitivity to phage C21; only *galE* mutants are sensitive to this phage. Usually 10 to 20% of the 2-deoxygalactose-resistant strains were sensitive to phage C21, and all of these were found to be sensitive to phage P1.

Phage P1 was grown on P1-sensitive donor strains by the confluent lysis method (11). The phage preparation was titrated by diluting into LC broth and

adding 0.10 ml of an overnight culture of indicator bacteria and 0.10 ml of P1 salts (15 mM CaCl₂, 3 mM MgSO₄). After a 30-min incubation at 37°C to permit adsorption, 2 ml of LC top agar was added, and the mixture was poured onto LC plates. After 8 h at 41°C, plaques 0.1 mm in diameter became visible. Incubation at lower temperatures produced smaller plaques (37°C) or no plaques (25°C). Titers of 10¹⁰ plaque-forming units per ml were routinely obtained with this procedure.

Transduction with P1 phage was performed by mixing 10⁸ to 10⁹ plaque-forming units in a solution containing 0.3 ml of LC broth, 0.3 ml of recipients grown in LC broth, and 0.3 ml of P1 salts. After 30 min at 37°C for adsorption, the bacteria were pelleted by centrifugation, resuspended in 0.3 ml of VBC, and plated on selective media. The ratio of transductants to plaque-forming units was about 10⁻⁶.

Transduction with phage P22. All procedures and materials used were as described previously (4).

Acid phosphatase staining procedure. The procedure used was a modification of that developed by Toh-e et al. for use with yeast (12). Solutions of the substrate, α-naphthyl phosphate (5 mg/ml), and tetrazotized o-dianisidine (50 mg/ml) in a 0.6 M sodium acetate buffer, pH 5.5, were prepared within 10 min of use. A 0.1-ml amount of each was then added to a tube containing 2.5 ml of 0.6% agar in 0.5 M sodium acetate, pH 5.5, at 45°C. A pH of 5.5 was used since it is the optimum pH of the *Salmonella* enzyme (14). The soft agar was then poured over an agar plate containing colonies. Viable colonies could be recovered up to 0.5 h after staining by stabbing the desired colony and streaking onto fresh medium. This staining procedure is quite specific for nonspecific acid phosphatase since it alone of the three periplasmic phosphatases hydrolyzes α-naphthyl phosphate at an appreciable rate (14).

Enzyme assays and protein determinations. These were performed as described previously (6).

RESULTS

Isolation of mutants altered in the expression of nonspecific acid phosphatase. Cultures of LT-2 or Hfr strain SA535 which had been grown from single colonies were mutagenized with DES or ICR-372 and grown overnight in VBCG. Dilutions sufficient to yield about 10³ colonies per plate were added to top agar and poured onto N⁻C⁻ plates supplemented with glucose and arginine or with succinate and NH₄⁺. These two media result in high levels of nonspecific acid phosphatase (7) and would be expected to increase the staining differences between wild type and mutants lacking this enzyme. Samples were also poured onto VBCG plates and on nutrient broth plates supplemented with 0.4% glucose and 1 mM sodium phosphate, since these two media result in low levels of nonspecific acid phosphatase (7) and should magnify staining differences between wild type and constitutive mutants.

After sufficient incubation time at 37°C to produce colonies 0.1 to 0.2 mm in diameter, the colonies were stained for acid phosphatase activity. White, nonstaining colonies were visible at frequencies of 10^{-3} for DES-mutagenized cultures and 10^{-4} for ICR-372-mutagenized cultures. Frequencies were independent of the medium used. Nonstaining colonies were picked and restreaked on the same media until pure. The staining characteristics of the nonstaining mutants were found to be the same when grown on all four media listed above regardless of the medium on which they were initially isolated.

Other phenotypes which were observed included constitutive mutants, which stained more rapidly than wild type, especially on nutrient broth plates supplemented with glucose and phosphate. Some of these constitutive colonies grew more slowly than wild type even on nutrient broth. Mutants with partial (weak) staining activity were also observed, as were sectorized colonies with several staining and nonstaining segments. This latter phenotype might be analogous to the sectorized colonies observed when *E. coli* mutator strains were stained for alkaline phosphatase activity (3).

Hfr mapping of mutations in strains missing nonspecific acid phosphatase. Mutant Hfr strains derived from strain SA535 were crossed with a variety of auxotrophic strains on selective media, and the resulting recombinants were stained for nonspecific acid phosphatase activity to measure the frequency of co-recombination. Examination of a number of such mutants revealed that two separate loci were involved in the expression of the nonspecific acid phosphatase. One locus, which mapped at 93 min, was designated *phoN*, and a second locus at 25 min was designated *phoP* (*Salmonella* map units as described in reference 10 were used).

Transductional mapping of mutations in strains missing nonspecific acid phosphatase. Some mutants derived from LT-2 were

cotransducible with the *purB* locus in P22-mediated crosses, and thus their mutations appeared to be in the *phoP* locus (Table 2). Table 2 also shows the results of transductional crosses between various *phoP* mutants and strain TA2328, a *purB phoP* mutant, which indicate that the individual *phoP* mutations are closer than 0.004 to 0.005 recombination units.

The remaining LT-2 mutants missing nonspecific acid phosphatase were not cotransducible with *purB* (<0.5%), and thus their mutations were presumed to be in the *phoN* locus. However, P22-mediated crosses indicated that *phoN* was not cotransducible with *purA* even though these two markers were closely linked in Hfr-mediated crosses. A possible reason for this lack of cotransduction might be that phage P22 contains insufficient DNA to carry the entire *purA* to the *phoN* region. Phage P1 contains twice as much DNA as phage P22 (5, 9), and an attempt was made to demonstrate cotransduction of *phoN* with *purA* in P1-mediated crosses. Strains with mutations presumed to be in the *phoN* region were made P1 sensitive, and P1 lysates prepared from these strains were used to transduce a *purA* mutant, which had also been made P1 sensitive, to prototrophy. Table 3 indicates that *phoN* was about 20% cotransducible with *purA* in P1-mediated crosses. Table 3 also shows that the individual *phoP* mutations are closer than 0.003 to 0.04 recombination units.

Mutations in 49 strains which were missing nonspecific acid phosphatase and were independently isolated from LT-2 were mapped by these techniques. A total of 14 strains contained mutations in the *phoN* locus and the remainder contained mutations in the *phoP* locus.

Transductional mapping of a mutation in a strain constitutive in the expression of nonspecific acid phosphatase. P22-mediated crosses between the constitutive mutant TA2367 and a *purB12* mutant indicated that this muta-

TABLE 2. P22-mediated cotransduction of *purB* and *phoP*

Donor (phage)	Recipient	No. of nonspecific acid phosphatase phenotypes of <i>purB</i> ⁺ transductants			% Cotransduction with <i>purB</i>
		Constitutive	Wild type	Negative	
TA2362	<i>purB12</i>	0	18	273	94
TA2444	<i>purB12</i>	0	13	236	95
TA2445	<i>purB12</i>	0	5	85	94
LT-2	<i>purB12</i>	0	321	0	
TA2362	TA2328 (<i>purB phoP</i>)	0	0	250	
TA2444	TA2328 (<i>purB phoP</i>)	0	0	192	
TA2445	TA2328 (<i>purB phoP</i>)	0	0	213	
LT-2	TA2328 (<i>purB phoP</i>)	0	206	8	96
TA2367	<i>purB12</i>	111	5	0	96
TA2367	TA2328	118	0	6	96

tion mapped in or near the *phoP* region (Table 2). Five other constitutive mutants contained mutations which did not map in either the *phoP* or *phoN* region.

Acid phosphatase levels in the mutants. The staining properties of the mutants were a reliable indication of nonspecific acid phosphatase levels since five *phoP* and five *phoN* mutants that did not stain had less than 5% of wild-type activity when intact cells grown on VBCG were assayed using 5'-AMP as substrate. In addition, mutants having partial staining activity had intermediate nonspecific acid phosphatase activity in whole-cell assays.

Two nonstaining mutants, TA2361 and TA2362, which are representative of the *phoN*

and *phoP* classes, respectively, were grown under a variety of conditions to determine whether any specific condition could induce nonspecific acid phosphatase. Neither mutant expressed detectable activity as a result of any of the starvations used (Table 4). Table 4 also shows that neither mutation affected the level of cyclic phosphodiesterase or acid hexose phosphatase.

When mutants of the two classes were grown in VBCG and the phosphatase activities of whole cells were measured with different substrates uniquely hydrolyzed by nonspecific acid phosphatase, such as β -glycerophosphate, 5'-AMP, or 2'-AMP, the mutants were found to have less than 10% of wild-type activity. In contrast, the mutants retained 80% of wild-type

TABLE 3. *P1*-mediated cotransduction of *purA* and *phoN*

Donor (phage)	Recipient	No. of <i>pho</i> ⁺ /no. of <i>pur</i> ⁺	% Cotransduction
TA1970	TA2382 (<i>pho</i> ⁺ <i>purA1</i>)	0/188	
TA2374	TA2382 (<i>pho</i> ⁺ <i>purA1</i>)	0/103	
TA2375	TA2382 (<i>pho</i> ⁺ <i>purA1</i>)	15/74	20
TA2376	TA2382 (<i>pho</i> ⁺ <i>purA1</i>)	8/30	26
TA2377	TA2382 (<i>pho</i> ⁺ <i>purA1</i>)	31/161	19
TA2379	TA2382 (<i>pho</i> ⁺ <i>purA1</i>)	18/88	20
TA1970 (<i>pho</i> ⁺)	TA1972 (<i>phoN1 purA1</i>)	296/370	20
TA2374 (<i>phoP15</i>)	TA1972 (<i>phoN1 purA1</i>)	100/126	21
TA2375 (<i>phoN3</i>)	TA1972 (<i>phoN1 purA1</i>)	0/23	
TA2376 (<i>phoN2</i>)	TA1972 (<i>phoN1 purA1</i>)	0/107	
TA2377 (<i>phoN7</i>)	TA1972 (<i>phoN1 purA1</i>)	0/300	

TABLE 4. Enzyme assays of *phoP* and *phoN* mutants grown on various media

Strain	Growth medium	Phosphatase sp act ^a (U/ml)		
		Nonspecific acid	Acid hexose	Cyclic phosphodiesterase
TA2361	Nutrient broth + 0.4% glucose	<0.1	3.2	
TA2362	Nutrient broth + 0.4% glucose	<0.1	3.0	
LT-2	Nutrient broth + 0.4% glucose	0.8	3.1	
TA2361	N ⁻ C ⁻ + glucose + NH ₄ Cl	<0.1	8.3	6.3
TA2362	N ⁻ C ⁻ + glucose + NH ₄ Cl	<0.1	8.2	6.2
LT-2	N ⁻ C ⁻ + glucose + NH ₄ Cl	2.5	8.1	6.3
TA2361	N ⁻ C ⁻ + glucose + L-arginine	<0.1	5.6	5.8
TA2362	N ⁻ C ⁻ + glucose + L-arginine	<0.1	5.8	5.8
LT-2	N ⁻ C ⁻ + glucose + L-arginine	7.3	5.2	5.7
TA2361	N ⁻ C ⁻ + citrate + NH ₄ Cl	<0.1	22.1	
TA2362	N ⁻ C ⁻ + citrate + NH ₄ Cl	<0.1	23.4	
LT-2	N ⁻ C ⁻ + citrate + NH ₄ Cl	5.4	23.5	
TA2361	N ⁻ C ⁻ + succinate + NH ₄ Cl	<0.1	78.0	23
TA2362	N ⁻ C ⁻ + succinate + NH ₄ Cl	<0.1	65	26
LT-2	N ⁻ C ⁻ + succinate + NH ₄ Cl	7.3	72	25

^a Strains were grown to an absorbance at 650 nm of 0.4 to 0.7 and assayed for phosphatase activity. There were no noticeable effects of the *pho* mutations on growth rate. Enzyme activity is expressed as units per milliliter of cells of unit absorbance at 650 nm.

activity toward glucose 6-phosphate, which is hydrolyzed by acid hexose phosphatase, and approximately 50% of wild-type activity toward pyrophosphate, which is also hydrolyzed by pyrophosphatase. These data are in good agreement with the phosphatase levels in VBCG-grown cells (7) and with the known specificities of these enzymes (14).

TA2367, a constitutive mutant with a mutation mapping in the *phoP* region, was also examined under different growth conditions to determine the effects of media on the production of nonspecific acid phosphatase. The largest difference (14-fold) between mutant and wild type was observed when cells were grown on rich media, which depresses activity of wild type (Table 5). Table 5 also shows that this mutation had no effect on levels of acid hexose phosphatase.

Tests for crypticity and in vitro complementation. Ward and Glaser (13) and more recently Beacham et al. (2) have described *E. coli* mutants in which certain periplasmic phosphatases appear to be present in low amounts when intact cells are assayed but which have normal activity when the cells are disrupted. Neither the *phoN* nor the *phoP* mutants of *Salmonella* appear to be of this type, since sonic extracts prepared from both mutant classes contained undetectable nonspecific acid phosphatase activity (<1% of wild type) when 5'-AMP was used as substrate.

Experiments were also done in which sonic extracts prepared from both mutant classes were mixed together or with wild-type extracts. In no case was the activity of the mixed extract different from the sum of the activities of its components. Thus, the two types of mutants failed to complement each other's defect in vitro, and neither type of mutant contained a detectable inhibitor of wild-type activity.

Growth characteristics of *phoN* and *phoP* mutants. Preliminary results indicated that TA2361 (*phoN2*) and TA2362 (*phoP12*) were able to use most phosphomonoesters as sources of phosphate as efficiently as LT-2. Included among those compounds were β -glycerophosphate, 5'-AMP, and 2'-AMP, for which no specific transport system has been characterized and which appear to be uniquely hydrolyzed by nonspecific acid phosphatase (13). Phenyl phosphate, *p*-nitrophenyl phosphate, and α -naphthyl phosphate were less efficiently used by the mutants (Table 6), but in no case was the doubling time of the mutant grown with these phosphomonoesters greater than twice that of LT-2.

Isolation of mutants with temperature-sensitive nonspecific acid phosphatase activity. A culture of LT-2 was mutagenized with DES and grown on VBCG at 41°C, and individual colonies were stained for nonspecific acid phosphatase activity. Colonies with partial activity compared with wild type were grown at 30°C and assayed before and after a 10-min

TABLE 6. Growth of *pho* mutants and LT2 on phosphate ester phosphate sources

Phosphate source	Doubling time (h) ^a		
	LT-2	TA2361 (<i>phoN1</i>)	TA2362 (<i>phoP12</i>)
α -Naphthyl phosphate	1.2	2.0	1.9
Phenyl phosphate	1.1	1.7	1.8
<i>p</i> -Nitrophenyl phosphate	1.2	1.4	1.6
Cyanoethyl phosphate	1.2	1.5	1.5
Inorganic phosphate	1.0	1.0	1.1

^a Overnight nutrient broth cultures were inoculated to an absorbance at 650 nm of 0.02 into N⁻C⁻P⁻ medium supplemented with 2% glucose, 10 mM NH₄Cl, and 0.4 mM phosphate source. Doubling times were determined during exponential growth at absorbances at 650 nm of between 0.05 and 0.6.

TABLE 5. Phosphatase levels of TA2367 and LT-2 grown on various media

Strain	Growth medium	Phosphatase sp act ^a	
		Nonspecific acid	Acid hexose
LT-2	Nutrient broth + 0.4% glucose	1.01	4.7
TA2367	Nutrient broth + 0.4% glucose	14.7	4.2
LT-2	N ⁻ C ⁻ + glucose + NH ₄ Cl	2.6	11.2
TA2367	N ⁻ C ⁻ + glucose + NH ₄ Cl	5.2	12.3
LT-2	N ⁻ C ⁻ + glucose + L-proline	11.4	
TA2367	N ⁻ C ⁻ + glucose + L-proline	31.0	
LT-2	N ⁻ C ⁻ + succinate + NH ₄ Cl	7.0	68.8
TA2367	N ⁻ C ⁻ + succinate + NH ₄ Cl	11.7	69.5

^a Strains were grown on the indicated media to an absorbance at 650 nm of 0.5 to 0.7 for assay. Enzyme activity is expressed as units per milliliter of cells of unit absorbance at 650 nm.

incubation at 55°C. One temperature-sensitive mutant was isolated, and the mutation in this strain was located in the *phoN* region. Revertants of *phoN* mutants were isolated by plating *phoN* mutants on media with phenyl phosphate as the sole source of phosphate. Papillae were observed on a light lawn of growth. Several of these revertants were purified. Some of these revertants had only a partial wild-type staining phenotype and a thermolabile nonspecific acid phosphatase activity in whole-cell assays (Fig. 1). The mutations in these revertants were not mapped, but these strains could contain suppressor mutations which are responsible for insertion of abnormal amino acids into nonspecific acid phosphatase, resulting in thermolabile enzymes. None of three isolated *phoP* revertants with partial enzyme activity had a temperature-sensitive enzyme.

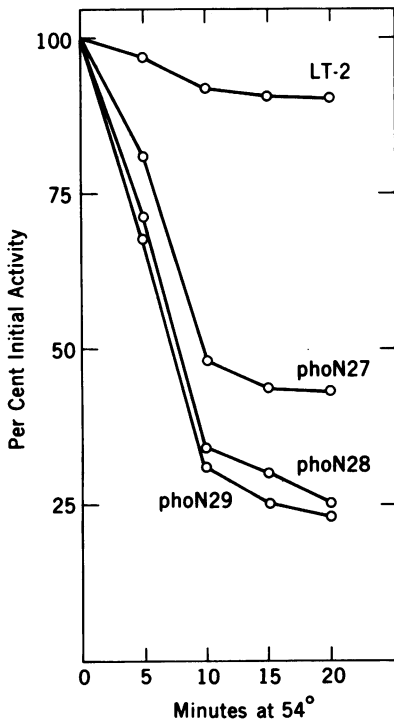


FIG. 1. Thermolability of nonspecific acid phosphatase measured in whole cells. The strains were grown to stationary phase at 30°C on VBCG. Cells were harvested by centrifugation and resuspended in 0.9% saline. The cell suspensions were added in 0.1-ml portions to 0.4 ml of 0.1 M sodium acetate, pH 5.5, and chilled on ice. The 0.5-ml samples were kept on ice or incubated for various times at 54°C. Samples incubated at 54°C were returned to ice for 25 min before enzyme assay. All samples were assayed by the standard procedure.

DISCUSSION

The observation that mutations in either of two unlinked loci led to a loss of nonspecific acid phosphatase activity was unexpected since this enzyme is probably a dimer of identical polypeptides (6).

One of these loci, *phoN*, which is cotransducible by phage P1 with *purA*, appears to be the structural gene. A mutant with temperature-sensitive enzyme activity in whole-cell assays contained a mutation with the *phoN* locus. Revertants with temperature-sensitive nonspecific acid phosphatase were also isolated from *phoN* mutants. There are, however, other possible explanations for these observations. Rigorous proof that *phoN* is the structural locus requires purification and characterization of the modified nonspecific acid phosphatase.

The role of *phoP*, which is cotransducible with *purB* by phage P22, is less clear. Mutations in this locus led to either loss of activity or to constitutive expression of activity; thus, *phoP* might have a regulatory function. Alternatively, *phoP* might encode some processing factor needed to activate acid phosphatase, and mutations might lead to either elimination or overproduction of this factor. In any case, *phoP* mutants were not analogous to the cryptic mutants of Ward and Glaser (13) since nonspecific acid phosphatase activity was not detected in sonic extracts prepared from *phoP* or *phoN* mutants which lacked this activity in whole-cell assays.

The isolation of mutants lacking nonspecific acid phosphatase helps to define the physiological role of this enzyme. The observation that mutants lacking this enzyme usually grow as well as the wild type (strain LT-2) indicates that the enzyme is not essential or even beneficial under most growth conditions. It was especially noteworthy that mutants lacking nonspecific acid phosphatase could utilize 2'-AMP and 5'-AMP as sources of phosphate as efficiently as wild type, even though the mutants had no detectable ability to hydrolyze these compounds. However, hydrolytic activity required to support wild-type growth might not have been significant under the assay conditions used to characterize the nonspecific acid phosphatase described. A second *Salmonella* nonspecific acid phosphatase has been isolated which may have these properties (C. Beck, personal communication). It is also possible that intact nucleoside monophosphates may enter *Salmonella* in amounts sufficient to support normal growth rates.

The role of nonspecific acid phosphatase was apparent only when mutants missing the enzyme

were forced to use certain phosphomonoesters as phosphate sources. Under these conditions *phoP* and *phoN* mutants grew slower than wild-type bacteria. These observations suggest that in wild-type cells, nonspecific acid phosphatase serves as a beneficial, although not essential, scavenging enzyme which facilitates the utilization of various metabolites present in the environment as phosphomonoesters.

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