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Enzymatic Defects in Three Genetic Classes of Serine-Requiring Mutants of *Bacillus pumilus*

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Serine-requiring mutants of *Bacillus pumilus* NRRL B-3275 have been divided into three groups based on the position of the mutant loci on the linkage map of this organism. Representatives of each group were found deficient in enzymatic activities that constitute the phosphorylated pathway for serine biosynthesis. The evidence suggests that the genes coding for the enzymes of the phosphorylated pathway of serine biosynthesis are not clustered in *B. pumilus*.

Serine biosynthesis in several of the major groups of bacteria is mediated by the enzymes of the phosphorylated pathway (2-4, 9-11, 14). The first step in this pathway involves the conversion of 3-phosphoglycerate (PGA) to hydroxypyruvate phosphate (HPAP) by the enzyme phosphoglycerate dehydrogenase. HPAP is converted to serine phosphate (serine-P) by a transaminase, and serine-P is cleaved to free serine through the action of serine phosphate phosphatase (3).

In *Escherichia coli*, the genes coding for the three enzymes of this pathway occur at three widely separated sites on the *E. coli* genetic map (13). A similar situation appears to exist in *Salmonella typhimurium* (12). At present, no information is available on the gene-enzyme relationships in a gram-positive bacterium.

In the present report, evidence is presented that demonstrates that mutations which can be mapped by transduction to three distinct regions of the genome of a strain of *Bacillus pumilus* also represent three biochemically distinguishable classes of defects in the enzyme activities of the phosphorylated pathway.

All mutants used in this study (Table 1) were derived from *B. pumilus* NRRL B-3275. Mutants of the BpB series (i.e., BpB2 through BpB38 in Table 1) were isolated from cultures mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine or ultraviolet irradiation (6). The mutations conferring a requirement for serine in strains not of the BpB series (e.g., strains 1-5-2, 231-3-5, 1-1-4, etc., see Table 1) were of spontaneous origin (5). PBS1-mediated transduction was performed as previously described (6).

For enzyme assays, bacteria were grown and extracts were prepared as described previously (11), with slight modification. One-liter cultures were allowed to grow until the Klett reading reached 140. At this time, samples were plated to verify the serine requirement of each mutant. Plates were checked for revertants at 24 and 48 h. PGA dehydrogenase was assayed by determining the increase in fluorescence due to the formation of acetyl-pyridine-reduced nicotinamide adenine dinucleotide. The assay was modified from the published procedure (11) by substituting bicine buffer (pH 8.5) for phosphate buffer (pH 7.5) and reducing the acetyl-pyridine-nicotinamide adenine dinucleotide concentration to 0.20 mM. The capacity for serine-P formation was assayed by determining the amount of ^{14}C-PGA converted to ^{14}C-serine-P under standard assay conditions with fluoride in the incubation. To assess whether an extract contained hydroxypyruvate-serine-P transaminase, the ability to synthesize serine-P was assayed after supplementation with an extract of *E. coli* K-12 lacking phosphoglycerate dehydrogenase but containing the transaminase. To assay for serine-P phosphatase, the rate of inorganic phosphate release from serine-P was measured colorimetrically (9). Protein was determined by method of Lowry (8).

Genetic mapping studies in *B. pumilus* NRRL B-3275 by PBS1-mediated transduction have demonstrated four linkage groups designated M, N, O, and L (6, 7). Eight independently isolated serine-requiring mutants (Table 1) can be mapped by transduction to one of three of these groups. Mutations designated *serB* are linked to certain markers in group N;

based on two-factor crosses the apparent order of markers in group N is *serB argA1 leu-1 phe-1* (Tables 2 and 3; reference 6). Mutations designated *serC* link to certain markers in group M; based on two-factor crosses the apparent order of markers in group M is *pig serC argO1 met-2 ura-1 cys-1* (Tables 2 and 3; references 7,5). (The genotypic designations of the Ser mutations to which *pig* mutations can be linked were incorrectly cited in portions of reference 5 as *ser-5,-7* [see erratum, J. Bacteriol. 114:462]. The *pig* mutations can be linked to *serC* mutations, not to *serB* mutations.) Mutations designated *serA* link to markers in group O; based on two-factor crosses the apparent order of markers in group O is *lys-1 serA trp-2 ilv-1-ile-1* (Tables 2 and 3; reference 6).

TABLE 1. *Mutants of B. pumilus NRRL B-3275*

Strain designation	Genotype[a]	Reference
BpB2	*lys-1*	6
BpB5	*phe-1*	6
BpB7	*argA1*	6
BpB9	*leu-1 trp-1*	6
BpB10	*trp-2*	6
BpB16	*argO1*	7
BpB18	*met-2*	7
1-5-2	*serB5*	5
231-3-5	*serB6 lys-2*	5
1-1-4	*serB7*	5
231-1-1	*serC2 lys-2*	5
231-1-8	*serC3 lys-2 leu-3*	5
BpB38	*serC4*	This paper
BpB4	*serA1*	6
1-7-1	*serA4 his-3*	5

[a] Assignment of *serA*, *serB*, or *serC* designations to mutations conferring a requirement of serine is based on the map location of the mutation. (references 1, 13, Tables 2 and 3). These loci correspond to genes for the PGA-dehydrogenase, serine-P phosphatase, and serine-P transaminase in *E. coli*.

The enzymatic defects in the three classes of serine-requiring mutants were identified by assaying cell extracts for their capacity to carry out the reactions that constitute the phosphorylated pathway for serine biosynthesis. Strains BpB4 and 1-7-1 do not have an active PGA dehydrogenase (Table 4). Strains 231-1-1 and 231-1-8 contain PGA dehydrogenase and serine-P phosphatase activities, but lack the transaminase and were unable to convert PGA to serine-P unless supplemented with an extract of *E. coli* that contained the transaminase. Strains 1-5-2, 231-3-5, and 1-1-4 form a class of mutants that have only PGA dehydrogenase

TABLE 2. *Linkage values obtained from two-factor transduction crosses of auxotrophs of B. pumilus NRRL B-3275*[a]

Donor	Recipient							
	serB7	*serB5*	*serB6*	*serC2*	*serC3*	*serC4*	*serA4*	*serA1*
argA1	41(171/416)	28(116/416)	35(143/408)	0(0/208)	0(0/208)	0(0/208)	0(0/416)	0(0/206)
leu-1	8(33/416)	5(21/416)	5(21/416)					
phe-1	0(0/208)	0(0/208)	0(0/208)					
argO1	0(0/208)	0(0/208)	0(0/208)	34(71/208)	28(116/416)	30(123/416)	0(0/208)	0(0/416)
met-2				0(0/416)	0(0/416)	0(0/416)		
trp-2	0(0/208)	0(0/208)	0(0/208)	0(0/208)	0(0/208)	0(0/206)	64(262/410)	59(123/208)
lys-1							74(308/416)	

[a] Values within parentheses indicate number of donor-type transductants per total number of transductants examined. Figures outside parentheses indicate the percent of the total number of transductants examined that carry the donor auxotrophic marker (percentage linkage). In reciprocal crosses, *serB7*, *serC2*, and *serA1* were not linked by transduction to the markers *gly-1*, *met-1*, *his-1*, *cys-1* (reference 7; Lovett, unpublished data).

TABLE 3. *Linkage values obtained from two-factor transduction crosses of auxotrophs of B. pumilus NRRL B-3275*[a]

Donor	Recipient						
	argA1	*leu-1*	*phe-1*	*argO1*	*met-2*	*trp-2*	*lys-1*
serB7	44(205/465)	5(21/416)	0(0/416)	0(0/208)		0(0/186)	
serB5	32(67/208)	7(29/416)	0(0/208)	0(0/208)		0(0/208)	
serB6	30(76/252)	3(9/302)	0(0/416)	0(0/208)		0(0/407)	
serC2	0(0/208)			31(64/208)	0(0/416)	0(0/208)	
serC3	0(0/208)			30(62/208)	0(0/415)	0(0/208)	
serC4	0(0/208)			23(95/416)	0(0/208)	0(0/208)	
serA4	0(0/208)			0(0/208)		62(129/208)	
serA1	0(0/208)			0(0/208)		60(124/208)	78(162/208)

[a] See Table 2 for explanation of values.

TABLE 4. *Enzyme activities in extracts of Ser⁻ mutants*

Strain	Genotype	PGA dehydrogenase (nmol/min/mg)	Serine-P formation[a] (nmol/30min/mg)	Serine-P formation[b] (nmol/30min/mg)	Serine-P hydrolysis (nmol/30min/mg)
BpB1	Wild	23.3	299	258	4,770
BpB4	*serA1*	0.4	1	1	2,700
1-7-1	*serA4 his-3*	1.0	6	2	3,750
231-1-1	*serC2 lys-2*	23.9	2	101	4,500
231-1-8	*serC3 lys-2 leu-3*	24.0	4	41	3,990
1-5-2	*serB5*	20.5	1	146	0
231-3-5	*serB6 lys-2*	25.8	3	96	0
1-1-4	*serB7*	27.6	16[c]	75	0

[a] Assays were performed with only the extracts from the designated strain in the incubation. The quantity of serine-P produced in these assays does not represent an initial rate of synthesis.

[b] The extracts from *B. pumilus* strains were supplemented with an extract of *E. coli* which lacked PGA dehydrogenase but contained serine transaminase. Specific activity was based on protein from *B. pumilus* extracts.

[c] Revertants were detected on plates after 48 h.

TABLE 5. *Serine-P formation by biochemical complementation of extracts from Ser⁻ mutants*

Extract		Complementing extract		
Strain	Class	None	Strain 1-1-4 class B	Strain 231-1-1 class C
BpB1	Wild	197[a]	133	
1-7-1	A	6	77	52
1-1-4	B	14		5[b]
1-5-2	B	1		1
231-1-1	C	2	5[b]	

[a] Serine-P formation (nmol per 30 min per assay).

[b] Double entry.

activity. Extracts from these three mutant strains were not capable of degrading serine-P and, on the basis of complementation assays, lacked transaminase activity.

Extracts from wild type and mutants defective in different enzymatic functions were assayed for their ability to complement each other biochemically in the formation of serine-P from PGA (Table 5). Serine-P was formed when either a mutant deficient in transaminase (strain 231-1-1) or a mutant deficient in both transaminase and phosphatase (strain 1-1-4) was complemented with a mutant deficient in PGA dehydrogenase (strain 1-7-1). These data confirm that 1-7-1 has an active transaminase which could not be determined directly or by complementation with the *E. coli* mutant extract.

We conclude that serine auxotrophs which have been designated *serA* have mutations that inactivate the first enzyme in the phosphorylated pathway (PGA dehydrogenase); mutants designated *serC* lack the transaminase, whereas class *serB* mutants lack the last enzyme in the pathway (serine-P phosphatase) as well as the transaminase. Of the mutants tested, none was deficient only in the phosphatase enzyme.

Several alternatives exist for the *serB* class. It is possible that the mutation is in a regulator gene which controls the expression of both the transaminase and phosphatase but has no effect on the dehydrogenase. A multienzyme complex could be present such that inactivation of the phosphatase results in inactivation of the transaminase. The existence of the transaminase and phosphatase as an enzyme complex remains to be tested.

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LITERATURE CITED

1. Demerec, M., E. A. Adelberg, A. J. Clark, and P. E. Hartman. 1966. A proposal for a uniform nomenclature in bacterial genetics. Genetics **54**:61-76.
2. Harder, W., and J. R. Quayle. 1971. The biosynthesis of serine and glycine in Pseudomonas AMI with special reference to growth on carbon sources other than C compounds. Biochem. J. **121**:753-762.
3. Ichihara, A., and D. M. Greenberg. 1957. Further studies on the pathway of serine formation from carbohydrate. J. Biol. Chem. **224**:331-340.

4. Nelson, J. P., and H. B. Naylor. 1971. The synthesis of L-serine by *Micrococcus lysodeikticus*. Can. J. Microbiol. **71**:73-77.
5. Lovett, P. S. 1972. Spontaneous auxotrophic and pigmented mutants occurring at high frequency in *Bacillus pumilus* NRRL B-3275. J. Bacteriol. **112**:977-985.
6. Lovett, P. S., and F. E. Young. 1970. Genetic analysis in *Bacillus pumilus* by PBSI-mediated transduction. J. Bacteriol. **101**:603-608.
7. Lovett, P. S., and F. E. Young. 1971. Linkage groups in *Bacillus pumilus* determined by PBS1-mediated transduction. J. Bacteriol. **106**:697-699.
8. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. S. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
9. Pizer, L. I. 1963. The pathway and control of serine biosynthesis in *Escherichia coli*. J. Biol. Chem. **238**:3934-3944.
10. Pizer, L. I., M. Ponce-de-Leon, and J. Michalka. 1968. Serine biosynthesis and regulation in *Haemophilus influenzae*. J. Bacteriol. **97**:1357-1361.
11. Ponce-de-Leon, M. M., and L. I. Pizer. 1972. Serine biosynthesis and its regulation in *Bacillus subtilis*. J. Bacteriol. **110**:895-904.
12. Sanderson, K. E. 1972. Linkage map of *Salmonella typhimurium*, edition IV. Bacteriol. Rev. **36**:558-586.
13. Taylor, A. L., and C. D. Trotter. 1972. Linkage map of *Escherichia coli* strain K-12. Bacteriol. Rev. **36**:504-524.
14. Umbarger, H. E., M. A. Umbarger, and P. L. M. Siu. 1963. Biosynthesis of serine in *Escherichia coli* and *Salmonella typhimurium*. J. Bacteriol. **85**:1431-1439.