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L-Serine-Sensitive Mutants of *Escherichia coli* K-12

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While attempting to isolate D-serine-sensitive mutants of *Escherichia coli* K-12, we found a class of mutants sensitive to low concentrations of L-serine (10 to 25 μg/ml).

Strains of *Escherichia coli* K-12 that cannot form D-serine deaminase do not grow in the presence of D-serine. Mutants unable to form the enzyme can be isolated by standard screening procedures (2). We wished to determine whether regulatory mutants of the *lac* i^s (5) type can occur in the D-serine deaminase system. Thus, we treated *dsdA*$^+$ *dsdC*$^+$/F′*dsdA*$^+$ *dsdC*$^+$ and *dsdA*$^+$ *dsdCx3*/F′*dsdA*$^+$ *dsdCx3* homozygotes, derived from the heterozygous strain EM 20031 (4), with exposure to nitrosoguanidine (1) followed by penicillin selection, and isolated more than 200 apparently D-serine-sensitive mutants from each. However, all of the mutants derived from the inducible homozygote could be induced to form enzyme upon exposure to suboptimal levels of D-serine (50 μg/ml as compared to 500 μg/ml used in penicillin screening), and all of the constitutive homozygotes formed the normal complement of enzyme. Clearly, the sensitivity was not to D-serine.

Commercial D-serine (Nutritional Biochemicals Corp.) is contaminated with 3 to 5% L-serine (Cosloy, *unpublished data*). Several of the mutants were streaked on minimal medium (2) supplemented with 25 μg of L-serine per ml or 500 μg of D-serine per ml. They failed to grow on these media but grew well on unsupplemented minimal plates, indicating that the true defect is abnormal sensitivity to L-serine. We suggest the designation *lss* (L-serine-sensitive) for such mutations. In some cases, D-serine enhanced the L-serine effect, presumably because of its own toxic effect. Two of the *lss* mutants, EM 20033 and EM 20034, together with a similar mutant, EM 20035, isolated from the haploid strain EM 1100 (3), were selected for further study.

The response of the *lss* mutants to various conditions of growth on solid media is presented in Table 1. There are some differences among them. EM 20035 is more sensitive to L-serine than the others; L-threonine at concentration 50 μg/ml is less effective in reversing the inhibition in EM 20033 than in the others. However, it is clear that compounds of the threonine-isoleucine pathway can completely overcome the L-serine effect.

It seemed of interest to determine whether mutations at more than one locus could evoke the *lss* phenotype. Three of the mutations were mapped approximately by standard mating procedures (3), and the mutation of strain EM 20033 was also mapped by P1 transduction (2). All three proved to be at different loci. The *lss* locus of strain EM 20033 is 30 to 40% cotransduced with *lac*. The *lss* locus of strain EM 20034 is transferred at 47 min by Hfr1 (proximal marker *lac*, *ccw* transfer), about the time at which this Hfr transfers markers in the *ilv* region. The *lss* locus of strain EM 20035 is transferred at 5 to 10 min by Hfr6 (terminal marker *lac*, *cw* transfer), about the same time that this Hfr transfers *try*. The data suggest that the first mutation is in one of the *ilv* genes, but it is not obvious what the others may be. It is possible that if more of the mutations had been mapped, more *lss* loci would have been found.

The *lss* mutations do not seem to be unusually common. No attempt was made in the mutant selections to score for mutations at unrelated loci, but the frequency at which *lss* mutations appeared in four independent mutations and selection experiments which we performed was less than that at which *his* and *dsdA* mutations appeared in analogous experiments performed with the same mutagen in the same period. Thus, either the total number of loci at which such mutations may occur is fairly limited, or the probability of such a mutation occurring at most *lss* loci is fairly low.

TABLE 1. *Growth of mutants on minimal media supplemented with* L-*serine and various amino acids of the isoleucine-valine pathway*

Amino acid	Strain					
	EM 20033		EM 20034		EM 20035	
	24 hr[a]	48 hr	24 hr	48 hr	24 hr	48 hr
10 μg of L-ser per ml[b]	+[c]				−	−
25 μg of L-ser per ml	±	+4	−	−	−	−
50 μg of L-ser per ml	−	−	−	−	−	−
50 μg of L-ser + thr per ml	+2	+4	+4	+4	+4	+4
50 μg of L-ser + ile per ml			+4	+4	±	+4
50 μg of L-ser + ilv per ml			+4	+4	+3	+4
50 μg of L-ser + met per ml	−	−	−	−	−	−
50 μg of L-ser + leu per ml			±	+4	±	+4
50 μg of L-ser + pan per ml			−	−	−	−

[a] Refers to the time of incubation at 37 C.

[b] Abbreviations: ile, isoleucine; ilv, isoleucine-valine; leu, leucine; met, methionine; pan, panothenate; ser, serine; thr, threonine.

[c] All growth is recorded on a relative basis, with +4 indicating the amount of growth found on minimal media with no additions.

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