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Map Location of the *ssd* Mutation in *Escherichia coli* K-12

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A pleiotropic mutation at the *ssd* locus was mapped at 86 min near *rha*. A mutation at the *ssd* locus resulted in elevated L-serine deaminase activity, inability to grow with succinate as the carbon source, and inability to grow under anaerobic conditions.

In our attempts to transduce the *wyb* mutation (5) to various strains of *Escherichia coli* K-12, we have isolated an interesting strain, strain VE2-2, with a phenotype similar but not identical to that of the donor strain, MS845 (4). Strain VE2-2 carries a pleiotropic mutation resulting in high L-serine deaminase (L-SD) levels and an inability to grow with succinate as the carbon source or with glucose anaerobically.

Strain MS845 has been previously shown to have high levels of L-SD (5). It is also unable to grow anaerobically in filled screw-cap tubes. It cannot use succinate aerobically; however, its parent, strain W4977, also uses succinate poorly, as does a revertant, strain POR 17.

The major difference between the two strains is that strain VE2-2 has L-leucyl, -L-phenylalanyl-tRNA protein transferase, an activity which is totally lacking in strain MS845. We report here the map location of the mutation in strain VE2-2 at 86 min between *rha* and *metB* (2). We name this locus *ssd* (succinate-nonutilizing [*s*], high serine [*s*] deaminase [*d*]).

The transfer of the *wyb* mutation is made difficult by the fact that there is no obvious selective advantage to the mutated state. We hypothesized that a high level of L-SD might facilitate the use of serine as the carbon and nitrogen source.

For testing this, strain CU1008, which has an *ilvA* deletion, was transduced with phage P1cm grown on strain MS845 and plated on minimal medium with serine (2 mg/ml), isoleucine (20 μg/ml), and valine (20 μg/ml). Transductants were isolated after 7 to 10 days from two of eight transduction experiments. In six other cases, no colonies appeared on any plates within 3 weeks. Strain VE2-2 (*ilvA*) is 1 of about 100 transductants from one of the successful experiments.

Spontaneous revertants to succinate utilization were isolated readily by plating strain VE2-2 with succinate as the sole carbon and energy source. Of 15 independent succinate-utilizing revertants, all showed low L-SD levels and grew anaerobically. It seems then that high levels of L-SD and an inability to use glucose anaerobically and succinate aerobically are all characteristics of a single mutation, here called *ssd*.

The *ssd* locus has been located between *rha* and *metB* by the following crosses. Of 80 methionine-independent transductants of strain MN-2 (*metB his*) transduced with a phage grown on strain VE2-2, 36 were unable to use succinate.

Similarly, of 96 methionine-independent transductants of strain P4X (*metB*), 12 were unable to use succinate. Five succinate-deficient transductants were assayed for L-SD activity and showed the elevated values associated with the *ssd* phenotype: 0.09, 0.13, 0.14, 0.18, and 0.21 μmol of pyruvate per 35-min assay (5). This compares with three succinate-metabolizing transductants (0.02, 0.02, 0.03 μmol of pyruvate per 35-min assay) and the parent, P4X (0.03 μmol of pyruvate per 35-min assay). It is clear that the mutation transferred in this cross determines high L-SD activity and inability to use succinate.

This map location was confirmed by transducing a *glpK* mutant of strain CU1008 with strain VE2-2. Of 74 glycerol-positive transductants, 35 were succinate negative. In similar crosses to *glpK* hosts, 150 of 450 glycerol-positive transductants were succinate deficient, suggesting that *ssd* is about 0.6 min from *glpK*.

For locating the mutation more closely, strain MN-8 (*rha metB*) was transduced with strain VE2-2, and transductants were selected for both characteristics. Among 198 methionine-independent transductants, *ssd* and *rha* were linked in 179 (Table 1).

Although this indicates that *ssd* is very near *rha*, the reciprocal selection for rhamnose-positive strains produced no succinate-negative strains when it was done in the usual way. However, growth on rhamnose is quite slow, and there is a heavy selection against the succinate-negative phenotype, even on glucose, but especially on rhamnose. When the rhamnose-positive transductants were streaked for single colonies from transduction plates directly on glucose minimal medium and several colonies screened for each colony studied, many succi-

TABLE 1. *metB*$^+$ *transductants in a three-point cross (rha ssd*$^+$ *metB) transduced with strain VE2-2 (rha*$^+$ *ssd metB*$^+$*)*

Utilization of:		No. of transductants isolated	% of total[a]
Rhamnose	Succinate		
+	−	122	62
+	+	4	2
−	−	15	8
−	+	57	29

[a] Values are rounded to the nearest whole numeral.

nate-negative clones were found. We thus can make a definite assignment of *ssd* at 86 min, close to *rha* and linked to *metB* and *glpK*.

On the basis of its map location, one might consider that *ssd* could be an isolate of either of two mutations affecting glycolysis in *E. coli* K-12, *tpi*, affecting triose phosphate isomerase (1), or *pfk*, affecting phosphofructokinase (2), or of a mutation conferring colicin resistance and impairing the transport of some amino acids (3).

Strains carrying the *tpi* and *pfk* mutations do not grow with glucose as the carbon and energy source, so that they could not be assayed for L-SD in the usual way. Instead, an *ssd* transductant of strain MN-2 was assayed for phosphofructokinase and shown to be identical to its parent in activity.

Similarly, strain VE2-2 was assayed for triose phosphate isomerase and again had the same activity as its parent strain.

It seems then that *ssd* is different from *tpi* and *pfk*. However, preliminary experiments indicate that the *ssd* mutation may be at the same locus as the Plate mutation (3).

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