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Salmonella Locus Affecting Phosphoenolpyruvate Synthase Activity Identified by a Deletion Analysis

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Strain *leu-4017*, derived from *Salmonella typhimurium* LT2, cannot utilize acetate, pyruvate, or citric acid cycle intermediates as sole sources of carbon. The mutation in this strain extends from the A cistron of the leucine operon to some point between *leu* and *azi*, presumably deleting one or more loci involved in the utilization of these compounds. One of these loci is required for phosphoenolpyruvate synthase activity.

The order of loci near *leu* on the genome of *Salmonella typhimurium* is *serB*, *thr*, *ara*, *leu-DCBAO*, *azi*, and *argD*. *ara* and *leu* loci are sufficiently near one another so that they are jointly transduced by P22 phage at a frequency of ca. 50%. Joint transduction of *leu* and *azi* mediated by P22 phage occurs at a low frequency (ca. 2%; T. Klopotowski, *personal communication*). A search was initiated for loci lying between *leu* and *azi*. Five leucine auxotrophs with deletions extending from the leucine operon in the direction of *azi* (reference 2, Fig. 1) were tested for the ability to utilize a number of compounds as sole sources of carbon. The compounds were selected from a group described by Gutnick et al. (4) and were tested by the auxanographic procedure used by them. For the study reported here, the medium was supplemented with 50 μg of L-leucine per ml.

These mutants could be divided into two groups on the basis of their growth responses toward certain carbon sources (Table 1). Strains *leu-447*, *leu-4046*, *leu-5071*, and *leu-5111* behaved like the parent strain in being able to utilize the compounds listed in Table 1 as a sole source of carbon. Strain *leu-4017*, on the other hand, did not utilize any of them well. It is likely that the phenotype of strain *leu-4017* (leucine requirement, inability to utilize compounds in Table 1) is the result of a single mutational event because the mutation arose spontaneously. This conclusion is supported by the results of a transductional analysis: three *leu*$^+$ recombinants (selected on mineral-glucose medium) from a cross between strain *leu-4017* and phage grown on the wild-type strain could grow on all of the compounds listed in Table 1. Thus, *leu-4017* probably extends sufficiently far into the *leu-azi* region to delete one or more genes necessary for the utilization of these compounds (Fig. 1). Sites *leu-447*, *leu-4046*, *leu-5071*, and *leu-5111*, on the other hand, presumably do not extend as far as *leu-4017*.

Strain *leu-4017* did not utilize the following compounds for growth (Table 1): acetate or laurate; pyruvate or compounds that give rise to pyruvate (alanine, cysteine, lactate, serine); citric acid cycle intermediates or compounds that give rise to them (citrate, fumarate, isocitrate, malate, oxalacetate, proline, propionate, succinate). It is important to note that only those compounds are listed in Table 1 for which some difference was observed between the parent and the deletion strains. A number of other compounds were tested that did not differentiate between parent and mutant strains. Thus, strain *leu-4017* could utilize the following types of compounds: mono- and disaccharides (D-glucose, D-mannose, D-fructose, maltose, melibiose, and trehalose); sugar derivatives (D-glucosamine, *N*-acetyl-D-glucosamine, D-glucarate, D-gluconolactone, D-mannitol, galactitol, D-sorbitol, L-fucose, and L-rhamnose); compounds directly related to glycolytic intermediates (dihydroxyacetone, DL-glyceric acid, glycerol); ribose and related compounds (adenosine, cytidine, guanosine, inosine, and uridine); and deoxyribose and thymidine. Apparently, this strain has intact glycolytic and pentose phosphate pathways.

The fact that strain *leu-4017* can utilize glycerate but not pyruvate for growth suggested that it might lack phosphoenolpyruvate (PEP) synthase activity. Parent and mutant cells were grown in a minimal salts solution (2) containing

0.5% glycerol and 50 μg of L-leucine per ml. Extracts prepared by sonic oscillation were assayed for PEP synthase activity by measuring an adenosine triphosphate-dependent decrease in pyruvate by the method of Cooper and Kornberg (3). Extracts prepared from the parent had a PEP synthase specific activity of 0.056 μmoles per min per mg of protein; extracts from strain *leu-4017* had less than 10% of the parent activity. Brice and Kornberg described mutants of *Escherichia coli* in which a genetic alteration near *aroD* resulted in a loss of PEP synthase activity (1). Because of the close similarity between the genetic maps of *S. typhimurium* and *E. coli* (5, 6), it is probable that the locus described by Brice and Kornberg and that described here have different functions. Perhaps one locus is a structural gene and the other a regulatory gene.

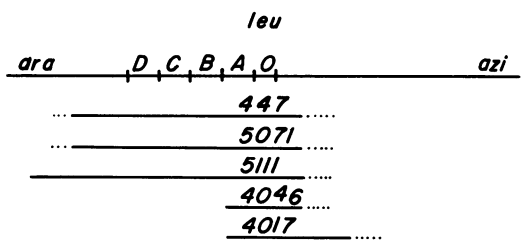

FIG. 1. *Extent of several deletions that enter the leucine operon. Most of the relationships above were deduced from the results of transduction experiments (2).*

The inability of strain *leu-4017* to utilize citric acid cycle intermediates and acetate is probably not due to a lack of PEP synthase activity because the synthase-deficient mutants of Brice and Kornberg (1) can utilize these compounds for growth. The inability to utilize citric acid cycle intermediates and acetate might be due to one or more of the following: (i) lack of a functional citric acid cycle, (ii) permeability barrier, (iii) inability to convert citric acid cycle intermediates via oxalacetate to glycolytic intermediates. With glucose as carbon source, strain *leu-4017* grows as fast as the parent, both under aerobic and anaerobic conditions. This suggests that it has a functional citric acid cycle. The fact that strain *leu-4017* does not require glutamate for growth also indicates that at least part of the citric acid cycle must be functioning.

In summary, the deletion in strain *leu-4017* extends into the *leu-azi* region affecting one or more genes which are involved in the utilization of acetate, pyruvate, and citric acid cycle intermediates as sole carbon sources. One of these genes is required for PEP synthase activity. The list of carbon and nitrogen sources compiled by Gutnick et al. (4) should be useful in identifying other loci in cases where strains carrying appropriate deletions are available.

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TABLE 1. *Comparison of growth response between parent and deletion strains*

Compound	Growth response exhibited by strains[a]					
	Parent[b]	*leu-4017*	*leu-447*	*leu-4046*	*leu-5071*	*leu-5111*
Acetate, sodium	b	0	b	b	b	b
L-Alanine	b	0	b	b	b	b
Citrate, sodium	a	0	a	a	a	a
L-cysteine	b	d	b	c	c	c
Fumaric acid	a	c	a	b	a	a
DL-Isocitrate, sodium	a	d	b	a	b	b
Lactic acid	a	0	a	a	a	a
Lauric acid	b	0	c	b	c	b
L-Malic acid	b	0	b	b	b	b
Oxalacetic acid	a	d	a	a	a	a
L-Proline	a	c	b	b	b	b
Propionate, sodium	a	0	b	b	b	b
Pyruvate, sodium	b	0	c	c	c	b
L-Serine	b	0	b	b	b	c
D-Serine	b	0	b	b	b	b
Succinate, sodium	b	0	b	b	b	b

[a] Determined by an auxanographic technique described previously (4). Symbols: a, heavy growth; b, medium growth; c, light growth; d, growth barely detectable; 0, no growth.

[b] Parent was *S. typhimurium* LT2 *ara-9 gal-205*. The leucine auxotrophs were all derived from this parent and were either of spontaneous origin or induced with nitrous acid.

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