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Clustered Genes for Galactose Metabolism from *Streptococcus mutans* Cloned in *Escherichia coli*

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DNA cloned into *Escherichia coli* from a serotype *c* strain of *Streptococcus mutans* allowed a *galKTE* mutant to utilize galactose for growth. However, the DNA does not appear to encode enzymes of the Leloir pathway used by *E. coli*, but rather appears to encode enzymes of the tagatose phosphate pathway.

In the course of shotgun cloning experiments to demonstrate that genes of the cariogenic bacterium *Streptococcus mutans* PS14 (serotype *c*) would be functionally expressed in *Escherichia coli* K-12, we constructed, with restriction endonucleases *Hin*dIII and *Bam*HI, chimeric plasmids that complemented a *galKTE* deletion (6). *E. coli*, like most bacteria, metabolizes galactose via the Leloir pathway: D-galactose → D-galactose 1-phosphate → UDP-D-galactose → UDP-D-glucose → D-glucose 1-phosphate. However, in *S. mutans* another pathway besides the Leloir pathway can be found (7). Galactose is phophorylated during phosphoenolpyruvate-dependent phosphotransferase transport of galactose or lactose (3) and is then metabolized through the tagatose phosphate pathway first elucidated in *Staphylococcus aureus* by Bissett and Anderson (1) and demonstrated for *S. mutans* by Hamilton and Lebtag (7): D-galactose 6-phosphate → D-tagatose 6-phosphate → tagatose 1,6-diphosphate → D-glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Although it is apparently possible for *E. coli* to make trace amounts of intracellular galactose 6-phosphate (18), presumably by phosphoenolpyruvate-dependent phosphorylation of galactose during membrane translocation (9, 15), there is no known physiological role for galactose 6-phosphate in *E. coli* (18). Since phosphoenolpyruvate-dependent phosphotransferase components of gram-negative and gram-positive bacteria are poor at in vitro functional complementation (17), we expected that our clones would consist of clustered *S. mutans* genes for the metabolism of galactose via the Leloir pathway. Surprisingly, that does not appear to be the case.

Using restriction endonuclease digestion, agarose gel or agarose-polyacrylamide gel electrophoresis, ligation and transformation, and selection on antibiotic-containing media as detailed previously (8, 8a), we made physical maps of several chimeric plasmids and their subclones (Fig. 1). The Gal$^+$ phenotype, as tested on minimal medium (5) plus auxotrophic supplements and 0.5% galactose as a carbon source, was expressed by several subclones, including both orientations in the vector plasmid pBR322 (14) for the 5.06-kilobase (kb) insert and both orientations in the vector plasmid pACYC184 (4) for the 3.28-kb insert. We concluded from results with the 3.28-kb insert that genes encoding no more than 120,000 daltons of protein product were sufficient for expression of the Gal$^+$ phenotype. Minicell analysis, performed as previously described (8), indicated that at least three major proteins of approximate sizes 40,000, 16,000, and 14,000 daltons were encoded by the 3.28-kb insert (data not shown).

However, the expression of the Gal$^+$ phenotype was not optimal in our clones. When strain χ1849 (8), an *E. coli* K-12 strain with the Δ(*galuvrB*)*47* mutation (which deletes the *galK*, *-T*, and *-E* genes), contained a recombinant plasmid, pYA501, carrying the 5.06-kb insert, its doubling time on galactose minimal medium was 6.5 to 8.5 h, whereas on glucose minimal medium the doubling time was about 3 h.

Several lines of evidence demonstrated that the cloned *S. mutans* galactose utilization DNA did not contain genes for the Leloir pathway. The first line of evidence was complementation data (Table 1). The plasmid pYA501 was not able to complement either single *galE* or single *galU* mutations for growth on galactose minimal medium. However, this was presumably due to the toxic accumulation of galactose 1-phosphate

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and UDP-galactose (20) since pYA501 would complement *galE* and *galU* when additional mutations earlier in the pathway were introduced (i.e., strains χ2420 and χ2456 in Table 1). Such results implied that pYA501 did not specify enzymes analogous to the *galE* and *galU* gene products, UDP-galactose 4-epimerase (EC 2.7.7.12) and glucose 1-phosphate uridylyl transferase (EC 2.7.7.10).

The second line of evidence was from indirect testing for cell surface galactose moieties which are synthesized from Leloir pathway intermediates. For instance, an *E. coli* strain with a *galKTE* deletion lacks galactose in the lipopolysaccharide core, due to absence of UDP-galactose synthesis, and the strain becomes resistant to bacteriophage U3 (19) and sensitive to bacteriophage C21 (2, 16); in the case of strain χ1849, this phage sensitivity phenotype was not affected by introducing pYA501. Also, when tested at both 30 and 42°C in the presence of Mg^{2+} and cycloserine (12), there was no evidence of colanic acid exopolysaccharide biosynthesis (which requires UDP-galactose) by strain χ1849 containing one of the Gal^+ recombinant plasmids.

Third, when the presence of the Leloir pathway enzymes was tested directly, using assays described by Postma (13) and Kundig and Roseman (10), we found inducible levels of galactokinase (EC 2.7.1.6), galactose 1-phosphate uridylyl transferase, and UDP-galactose 4-epimerase in the parental strain χ1846; but no detectable activity in strain χ1849 (the *galKTE* deletion derivative of strain χ1846) or in strain χ1849 which contained the recombinant plasmid pYA501 (data not shown).

We have preliminary evidence that we have cloned the enzymes for the tagatose 6-phosphate pathway rather than Leloir pathway enzymes. Crude enzyme extracts from strain χ1849(pYA501) were able to metabolize galactose 6-phosphate to triose phosphates in the presence of ATP, as measured by a glyceraldehyde 3-phosphate dehydrogenase-coupled reaction (unpublished data), and showed galactose 6-phosphate isomerase activity (V. L. Crow and T. Thomas, personal communication), whereas strain χ1849 lacked both of these abilities. Lengeler (11) has presented evidence that intermediates in galactitol metabolism in *E. coli* include D-tagatose 6-phosphate and D-tagatose 1,6-diphosphate and that *E. coli* K-12 strains have a low tagatose 6-phosphate kinase (*pfkA* or *-B* gene product) activity and a temperature-sensitive tagatose 1,6-diphosphate aldolase (*kba* gene product) activity. Presumably, these activities, especially if enhanced by a temperature-resistant mutation in the *kba* gene, could complement a cloned galactose 6-phosphate isomerase gene, thus constituting a tagatose phosphate pathway. However, we believe that multiple clustered genes are necessary for the observed Gal^+ phenotype in our clones since the range of sites where transposon mutagenesis will produce *gal* mutations includes both the left and the right *Hin*dIII/*Pst*I fragments of the 3.28-kb insert (Fig. 1), whereas transposition into the middle *Pst*I/*Pst*I portion of the 3.28-kb insert produces a leaky *gal* mutation (unpublished data). Besides performing a more detailed genetic characterization of this presumed gene cluster, we plan to further investigate the basis for the slow growth of strain χ1849 containing plasmid pYA501 on galactose as the sole carbon and energy source.

TABLE 1. Leloir pathway complementation by galactose utilization recombinant plasmids

Bacterial strain[a]	Relevant genotype	Ability of pYA501 transformant to grow on minimal medium with:	
		Glucose	Galactose
χ4	*galT2*	+	+
χ934	*galK4*	+	+
χ2419	*galE17*	+	−
χ1038	*galK2 galT22*	+	+
χ2420	*galK14 galE15*	+	+
χ2316	Δ(*galU*)*183*	+	−
χ1849	Δ(*gal-uvrB*)*47*	+	+
χ2456	Δ(*gal-chlD*)*69* Δ(*galU*)*183*	+	+

[a] All strains were from this laboratory.

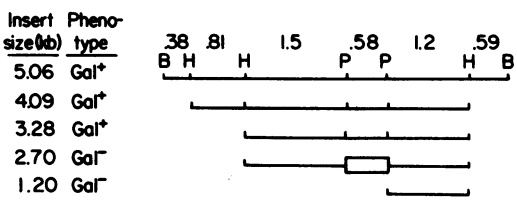

FIG. 1. *S. mutans* galactose utilization genes cloned in *E. coli*. The *S. mutans* DNA inserts cloned in various plasmid vectors (see text) are shown. The sizes of the inserts (in kilobases) with sites for restriction endonucleases *Bam*HI (B), *Hin*dIII (H), and *Pst*I (P) as well as the phenotype the inserts confer when present in a Δ(*gal-uvrB*) *E. coli* strain are indicated. The open box represents a deletion of 0.58 kb in the 2.70-kb insert.

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