

A Mutant Inducible for Galactitol Utilization in *Escherichia coli* K12

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Galactitol-positive strains of *Escherichia coli* K12 are inhibited by the galactitol analogues L-fucitol and 2-deoxy-D-galactitol, but not by D-fucitol; *Salmonella typhimurium* LT2 is not inhibited by these compounds. Most mutants selected as resistant to either toxic compound are unable to utilize galactitol as carbon source, but a relatively rare class is inducible for the Enzyme II of the galactitol:phosphoenolpyruvate phosphotransferase system, the product of which is D-galactitol 6-phosphate. The lesion in one such mutant maps near *metG* at about min 45 on the *E. coli* genome.

INTRODUCTION

Some, but not all strains, derived from *Escherichia coli* K12 will utilize (*meso*) galactitol as sole carbon source at 30 °C. Galactitol enters cells of *E. coli* by a phosphoenolpyruvate-dependent phosphotransferase mechanism yielding a galactitol phosphate. The galactitol-specific Enzyme II that catalyses this vectorial phosphorylation is specified by the gene *gatA*, and is expressed constitutively (Lengeler, 1975*a, b*; Lengeler & Steinberger, 1978). The *gatA* gene is 53% cotransducible with *metG* (Woodward & Charles, 1980), the exact location of which (about min 45) is unknown (Bachmann & Low, 1980).

While screening a number of compounds for their antibacterial activity we observed that L-fucitol (1-deoxy-D-galactitol) and 2-deoxy-D-galactitol inhibited the growth on glycerol minimal medium of galactitol-positive derivatives of *E. coli* K12. Most mutants selected for resistance to either of the toxic compounds were unable to utilize galactitol as carbon source, but some retained their ability to do so. The properties of one such mutant and the genetic location of the lesion are reported here.

METHODS

The *Escherichia coli* strains used are listed in Table 1. *Salmonella typhimurium* LT2 was obtained from Dr D. A. Smith (University of Birmingham). Strains were grown in Oxoid nutrient broth or in the minimal medium of Ashworth & Kornberg (1966) solidified with 1.5% (w/v) Oxoid agar no. 1 if required. Bacteriophage P1-mediated transduction was performed according to Lennox (1955). Phosphotransferase assays were done by the method of Kornberg & Reeves (1972). L-Fucitol, D-fucitol and 2-deoxy-D-galactitol were prepared by reducing L-fucose, D-fucose and 2-deoxy-D-galactose (all from Sigma) with a small excess of sodium borohydride, and were used as their borate esters. Spontaneous resistant mutants were selected by plating 0.1 ml samples of broth-grown cultures on supplemented minimal medium with 20 mM-glycerol as carbon source and approx. 5 mM inhibitor. Cultures were grown at 30 °C unless otherwise indicated, and cell yields were determined spectrophotometrically at 680 nm.

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Table 1. *Strains of Escherichia coli used*

Strain	Genotype*	Origin or reference
JM559	<i>gat-1 fda^{ts} hisgnd^Δ</i>	Henderson <i>et al.</i> (1977)
JM1761	<i>gat⁺ fda^{ts} hisgnd^Δ</i>	P1.S183-27T × JM559
JM1764	<i>gat-2 fda^{ts} hisgnd^Δ</i>	JM1761 (this paper)
S183-27T	<i>gat⁺ leu his</i>	Robbins (1975); Robbins & Rotman (1975)
SB1803	<i>gat⁺ metG thr leu his pro</i>	Blumenthal (1972)
W6	<i>gat-1 metB</i>	Lederberg <i>et al.</i> (1952); Bachmann (1972)

* The galactitol-negative mutation of strain W6 and its progeny is indicated by the allele number 1, and the galactitol-inducible mutation of strain JM1764 is indicated by the allele number 2.

Table 2. *Galactitol:phosphoenolpyruvate phosphotransferase activity*

Activity, determined at 30 °C by the method of Kornberg & Reeves (1972) in the presence of 4 mM-galactitol, is expressed in nmol min⁻¹ (mg dry mass)⁻¹. The means, standard deviations and numbers of replicate cultures are given.

Strain	Genotype	Carbon source for growth	Activity
JM559	<i>gat-1</i>	Galactitol + lactate	<0.6 (2)
JM1761	<i>gat⁺</i>	Lactate	4.2 (2)
JM1761	<i>gat⁺</i>	Galactitol	4.4 (2)
JM1764	<i>gat-2</i>	Lactate	1.2 ± 0.6 (4)
JM1764	<i>gat-2</i>	Galactitol	3.6 ± 0.5 (4)

RESULTS

Isolation and properties of mutants

Mutants of strain JM1761 (*Gat⁺*) selected on glycerol for resistance to either L-fucitol or 2-deoxy-D-galactitol were always resistant to the other compound. All grew well on glucose at 30 °C (but not at 40 °C because of the temperature-sensitive *fda^{ts}* lesion; Böck & Neidhardt, 1966). The large majority resembled the galactitol Enzyme II-defective strain JM559 in being unable to utilize galactitol as carbon source at 30 °C and in being resistant to inhibition by galactitol during growth on glycerol at 40 °C. One L-fucitol-resistant mutant, strain JM1764, retained the ability to grow on galactitol (10 mM) as sole carbon source at nearly the same rate as the parental strain (mean doubling times 277 min and 246 min, respectively).

Salmonella typhimurium LT2 was not inhibited by either of these galactitol analogues, though it grew with galactitol as sole carbon source. D-Fucitol (6-deoxy-D-galactitol) did not inhibit either *E. coli* or *S. typhimurium*, nor did any strain tested use it as a carbon source.

Assay of galactitol phosphotransferase activity

Galactitol phosphotransferase activity was assayed in strains JM559, JM1761 and JM1764 grown on various carbon sources and rendered permeable with toluene, with the results shown in Table 2. Once a rate of NADH oxidation in the presence of 4 mM-galactitol had been determined, glucose (5 mM) was added to the reaction mixture. This further stimulated the rate of NADH oxidation to 10–20 nmol min⁻¹ (mg dry mass)⁻¹. It is evident from Table 2 that the specific activities of the galactitol phosphotransferase systems in the three mutants differed greatly and that JM559 is *Gat⁻*, JM1764 is *Gat⁺* but inducible, and JM1761 is *Gat⁺* and constitutive.

Genetic location of the lesion in strain JM1764

Bacteriophage P1 grown on strain JM1764 was used to transduce strain JM559 to *Gat⁺*. Of 75 transductants examined 9% were resistant to L-fucitol, as was the donor strain. This

indicates that the lesion is linked, though not closely, to the *gat* locus of strain JM559. This *gat* mutation was mapped by growing bacteriophage P1 on strain JM559 and using it to transduce strain SB1803 to MetG⁺. Of 80 transductants examined 49% were Gat⁻.

DISCUSSION

Since phosphate esters of sugars and hexitols are toxic to cells that accumulate them (reviewed by Ferenci & Kornberg, 1973), analogues of such compounds that are modified in such a way as to prevent catabolism are toxic if they can be taken up and phosphorylated (Kornberg & Smith, 1972; Miles & Pirt, 1973). If the compound enters by a phosphotransferase mechanism the cell may mutate to acquire resistance only by loss of the phosphotransferase system under the conditions of the experiment. This may be achieved in two different ways: either the cell may lose a structural gene for the system, or it may fail to express that gene. The larger class of L-fucitol-resistant mutants, which are indistinguishable in phenotype from strain JM559, are presumably of the former type. Strain JM1764, since it can grow on galactitol as carbon source and can express a galactitol phosphotransferase activity (Table 2), might be a mutant of the latter class.

Like *S. typhimurium* LT2, strain JM1764 was galactitol-positive and resistant to L-fucitol and 2-deoxy-D-galactitol. Lengeler & Steinberger (1978) have shown that *S. typhimurium* LT2, unlike *E. coli* K12, expresses the galactitol phosphotransferase and D-galactitol-6-phosphate dehydrogenase inducibly. This suggested that strain JM1764 might also be inducible for the galactitol phosphotransferase. The results in Table 2 show that this is so. That higher phosphotransferase activity was always obtained when glucose was added as substrate subsequent to galactitol indicates that the galactitol Enzyme II, rather than the phospho-carrier protein HPr or the Enzyme I of the phosphotransferase system, is rate limiting in the assay.

Since L-fucitol and 2-deoxy-D-galactitol are toxic only to strains that express the galactitol phosphotransferase constitutively they cannot be inducers of the phosphotransferase: the inducer thus requires hydroxyl groups at both C-1 and C-2. That D-fucitol (which has no hydroxyl group on C-6 and hence cannot be phosphorylated there) does not inhibit constitutive strains (and is not used as a carbon source) confirms that the product of the phosphotransferase is D-galactitol 6-phosphate rather than D-galactitol 1-phosphate (L-galactitol 6-phosphate).

Strain W6 (Lederberg *et al.*, 1952) and all of its progeny that we have tested (results not shown) are galactitol-negative, so that the *gat-1* mutation in it and its derivative strain JM559 is of some antiquity. The lesion may have arisen by the unintentional selection of faster-growing colonies on partially hydrolysed agar medium above the permissive temperature for growth on galactitol. That the lesion we have isolated in strain JM1764 is located close to the genes *gatA* and *gatD* for the galactitol phosphotransferase and galactitol-6-phosphate dehydrogenase was predicted from what is known of the organization of the very similar operons for the catabolism of mannitol and sorbitol (Lengeler, 1975*a*).

We have not sought to establish the reason for the constitutive expression of the galactitol operon in wild-type cells. The confusing mapping data in this region of the genome (Bachmann & Low, 1980) has deterred any more extensive mapping of the lesion. The observation that *E. coli* K12 strains will mutate to express the galactitol operon inducibly suggests that the prototype strain K12 should be regarded as a constitutive mutant of some ancestral organism.

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REFERENCES

- ASHWORTH, J. M. & KORNBERG, H. L. (1966). The anaplerotic fixation of carbon dioxide by *Escherichia coli*. *Proceedings of the Royal Society B* **165**, 179–188.
- BACHMANN, B. J. (1972). Pedigrees of some mutant strains of *Escherichia coli* K12. *Bacteriological Reviews* **36**, 525–557.
- BACHMANN, B. J. & LOW, K. B. (1980). Linkage map of *Escherichia coli* K12, edition 6. *Microbiological Reviews* **44**, 1–56.
- BLUMENTHAL, T. (1972). P1 transduction: formation of heterogenotes upon cotransduction of bacterial genes with a P2 prophage. *Virology* **47**, 76–93.
- BÖCK, A. & NEIDHARDT, F. C. (1966). Isolation of a mutant of *Escherichia coli* with a temperature-sensitive fructose 1:6-diphosphate aldolase activity. *Journal of Bacteriology* **92**, 470–476.
- FERENCI, T. & KORNBERG, H. L. (1973). The utilisation of fructose by *Escherichia coli*. Properties of a mutant defective in fructose 1-phosphate kinase activity. *Biochemical Journal* **132**, 341–347.
- HENDERSON, P. J. F., GIDDENS, R. A. & JONES-MORTIMER, M. C. (1977). Transport of galactose, glucose and their molecular analogues by *Escherichia coli* K12. BLLD supplementary publication SUP 50074 to *Biochemical Journal* **162**, 309–320.
- KORNBERG, H. L. & REEVES, R. E. (1972). Correlation between hexose transport and phosphotransferase activity in *Escherichia coli*. *Biochemical Journal* **126**, 1241–1243.
- KORNBERG, H. L. & SMITH, J. (1972). Genetic control of glucose uptake by *Escherichia coli*. *FEBS Letters* **20**, 270–272.
- LEDERBERG, J., CAVALLI, L. L. & LEDERBERG, E. M. (1952). Sex compatibility in *Escherichia coli*. *Genetics* **37**, 720–730.
- LENGELER, J. (1975*a*). Mutations affecting transport of the hexitols D-mannitol, D-glucitol and galactitol in *Escherichia coli*: isolation and mapping. *Journal of Bacteriology* **124**, 26–38.
- LENGELER, J. (1975*b*). Nature and properties of hexitol transport systems in *Escherichia coli*. *Journal of Bacteriology* **124**, 39–47.
- LENGELER, J. & STEINBERGER, H. (1978). Analysis of the regulatory mechanisms controlling the synthesis of the hexitol transport systems in *Escherichia coli* K12. *Molecular and General Genetics* **164**, 163–169.
- LENNOX, E. S. (1955). Transduction of linked genetic characters of the host by bacteriophage P1. *Virology* **1**, 190–206.
- MILES, R. J. & PIRT, S. J. (1973). Inhibition by 3-deoxy-3-fluoro-D-glucose of the utilization of lactose and other carbon sources by *Escherichia coli*. *Journal of General Microbiology* **76**, 305–318.
- ROBBINS, A. R. (1975). Regulation of the *Escherichia coli* methylgalactoside transport system by gene *mgID*. *Journal of Bacteriology* **123**, 69–74.
- ROBBINS, A. R. & ROTMAN, B. (1975). Evidence for binding protein-independent substrate translocation by the methylgalactoside transport system of *Escherichia coli* K12. *Proceedings of the National Academy of Sciences of the United States of America* **72**, 423–427.
- WOODWARD, M. J. & CHARLES, H. P. (1980). Genes for ribitol and arabitol utilization, and genes for galactitol utilisation, behave as chromosomal alternatives. *Society for General Microbiology Quarterly* **7**, 82.