JOURNAL OF BACTERIOLOGY, Aug. 1979, p. 683-685
0021-9193/79/08-0683/03$02.00/0

Vol. 139, No. 2

New Maltose Blu Mutations in *Escherichia coli* K-12

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Received for publication 8 June 1979

Mutations in the genes *pgi*, *pfkA*, and *ptsG* resulted in a maltose Blu phenotype in *Escherichia coli* K-12, bringing the number of known Blu alleles to six. The Blu phenotype, as visualized by staining with iodine vapor, is a convenient mutant isolation technique.

Mutant strains of *Escherichia coli* that accumulate maltodextrin when metabolizing maltose (Fig. 1) form colonies on maltose-containing solid medium that stain blue with iodine. This is the maltose Blu phenotype (1). Three kinds of mutations giving this phenotype have been described. Mutations at *malP* eliminate maltodextrin phosphorylase (Fig. 1) (8), whereas *pgl* mutations, affecting phosphogluconolactonase, evidently lower phosphorylase activity indirectly by causing production of a phosphorylase inhibitor (5, 6). Mutations at *pgm*, the gene for phosphoglucomutase, give a maltose Blu phenotype by blocking metabolism of glucose 1-phosphate, the product of the maltodextrin phosphorylase reaction (Fig. 1), thus causing dextrin accumulation by mass action (1). The *pgm* mutants are also galactose Blu, forming dextrin from accumulated glucose 1-phosphate by a reversal of the phosphorylase reaction when fed galactose (1).

We thought it possible that mutations blocking maltose metabolism at a point beyond the phosphoglucomutase reaction might give a weak maltose Blu phenotype, thus providing a means for isolating or scoring strains with mutations at loci such as *pgi* (phosphoglucose isomerase) or *pfkA* (phosphofructokinase) (Fig. 1). It was reported that a *pgi* mutant was not Blu when grown on a maltose-containing complex medium (6). We tested known *pgi* and *pfkA* mutants (*pgi*, strain DF40 [6]; *pfkA*, strain AM1 [10]) for the Blu phenotype both on complex agar (10 g of tryptone and 4 g of yeast extract per liter in medium 007 [3]) with maltose, and on minimal agar with both glycerol and maltose (medium 007 with 0.2% glycerol and 0.2% maltose). The latter medium supports growth of *pfkA* stains, which cannot grow on maltose as a sole source of carbon, and has been found to be optimal for the Blu phenotype (8). When stained with iodine vapor (9) and inspected against a brightly lighted white background, *pgi* and *pfkA* strains were Blu on glycerol-maltose medium but not on complex medium with maltose. Staining with iodine-iodide solution (8) gave similar results. Maltose concentrations from 0.02 to 1% were tested in agar with 0.2% glycerol, but did not give better staining than 0.2%. *pfkA* strains grow slowly in the presence of maltose, but spraying glycerol-grown colonies with maltose solution or moving glycerol-grown colonies on membrane filters to maltose agar for varying lengths of time before exposure to iodine did not give as intense staining as that obtained with glycerol-maltose agar. In genetic crosses inheritance of *pgi* or *pfkA* markers was scored readily by the Blu phenotype; presence of a *zwf* mutation (Fig. 1) did not affect this scoring.

We tested Blu staining as a method for mutant isolation. A wild-type strain of *E. coli* K-12 was mutagenized with ethyl methane sulfonate (1) and plated to glycerol-maltose agar. Colonies (500 to 1,000 per plate) were stained with iodine vapor, a procedure that causes little cell killing and makes the use of replica plates unnecessary. Twenty independent cultures yielded a number of different Blu isolates (Table 1). We expected *malP*, *pgl*, *pgi*, *pgm*, and *pfkA* mutants and perhaps mutants with other metabolic blocks or with regulatory mutations. Surprisingly, no Blu mutants with the *pgi* or *pfkA* phenotypes were recovered (Table 1). This appeared to be due to an effect of wild-type colonies on dextrin accumulation by nearby mutant colonies. When mixtures of wild-type and known *pgi* or *pfkA* mutant cells were plated to glycerol-maltose agar, only well-isolated mutant colonies showed a Blu phenotype, although crowded colonies from pure cultures of mutant strains gave intense staining.

We were able to isolate *pfkA* mutants with the Blu technique by using ethyl methane sulfonate-mutagenized cultures of a *metB* strain (*metB* cotransduces with *pfkA* [10]) that had been subjected to penicillin counterselection in minimal medium with mannitol (not utilized by *pfkA* strains) and methionine before plating to glycerol-maltose agar with methionine to give

only 100 to 200 colonies per plate. Small, blue-staining colonies (ca. 2% of the total) had the *pfkA* phenotype, including inability to utilize mannitol. When these Blu isolates were transduced to methionine independence with a bacteriophage P1 lysate prepared on a wild-type strain, ca. 20% of transductants had lost the Blu phenotype and were restored to growth on mannitol, confirming the *pfkA* designation.

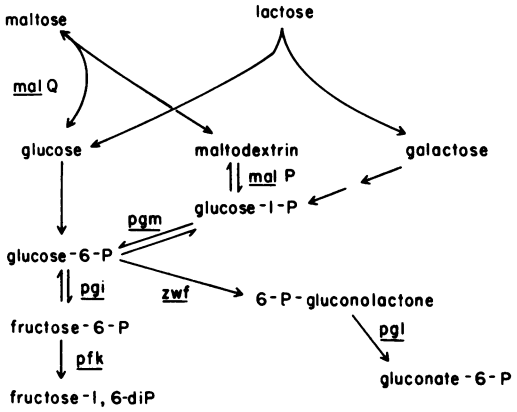

FIG. 1. *Metabolism of maltose in E. coli (1). Enzymatic steps are identified by genetic symbols (2); malQ, amylomaltase; malP, maltodextrin phosphorylase; pgm, phosphoglucomutase; pgi, phosphoglucose isomerase; pfk, phosphofructokinase; zwf, glucose 6-phosphate dehydrogenase; pgl, phosphogluconolactonase.*

A Blu isolate not having any of the expected phenotypes, RR274 (Table 1, line 6), was studied further. It was slightly weak in aerobic growth on glucose and mannose and was completely unable to grow on these sugars anaerobically, although able to ferment glucose 6-phosphate normally. The glucose–mannose anaerobic utilization and Blu characters of this strain were ca. 20% linked by bacteriophage P1 transduction to the *pyrC* locus. (RR274 donor, *pyrC* recipient; 35 of 190 uracil-independent transductants were Blu and unable to utilize glucose or mannose anaerobically.) This map location suggested a mutation at the *ptsG* locus, the gene for a component of the enzyme III^{glc}/IIB′ complex of the phosphoenolpyruvate:sugar phosphotransferase system, which transports glucose and mannose (7). RR274 was confirmed as being unable to transport α-methyl-D-glucopyranoside, a substrate specific for this transport system (7; un-

TABLE 1. *Maltose Blu isolates*

Carbon source utilization[a]								Iodine staining[b]		No. of isolates[c]	Genotype[d]
Glu	Glu(an)	Man	Man(an)	Gal	Mtl	Mal	Gly	Mal	Gal		
+	+	+	+	+	+	+	+	B	W	23	*malP* or *pgl*
+	+	+	+	+	+	+−	+	B	W	3	*mal*?
+	+	+	+	−	+	+	+	B	B	37	*pgm*
+	−	+	+	+	+	+	+	B	B	0	*pgi*
−	−	−	−	+−	−	−	+	B	B	0	*pfkA*
+−	−	+	−	+	+	+	+	B	W	1[e]	*ptsG*
+	+	+	+	+	+	+	+	B	B	11	?
+	+	+	+	+−	+	+	+	B	W	1	?

[a] Use of 0.2% D-glucose (Glu), D-mannose (Man), D-galactose (Gal), D-mannitol (Mtl), maltose (Mal), or glycerol (Gly) as a source of carbon and energy at 37 or 42°C or both. (an) indicates growth on medium with the indicated sugar (0.2%) and 0.01% casein hydrolysate in an anaerobic jar (BBL Microbiology Systems) at 37°C.

[b] Staining on Gly + Mal, Gly + Gal, or Gal minimal agar, at 37 or 42°C or both. B indicates blue staining; W (white) indicates no staining. The symbol B is used for any of a wide variety of strain-specific staining reactions, from intense blue-black through violet to gray. Some strains show staining only at the edges of colonies. Fresh growth gives optimal staining. A number of Blu isolates show stronger growth and less intense staining at 37°C than at 42°C. Some putative *pgm* mutants showed more intense staining on maltose than on galactose, and some had the opposite response. In nearly every case, strains that were Blu on galactose were also Blu on lactose-containing medium. We find that the known *pgm* strain PGM1 (1) stains blue with iodine vapor on lactose-containing minimal medium. This strain has been reported as not being lactose Blu with a different staining procedure (1). No Blu strain was blue staining on media with glucose, mannose, mannitol, or glycerol as a sole source of carbon.

[c] Isolates were from 20 independent ethyl methane sulfonate-mutagenized cultures of K10 (10); differences in phenotype for Blu isolates from the same culture were taken as indicating independent origin.

[d] Putative genotypes, deduced from the indicated carbon source utilization and staining phenotype, with reference to the observed phenotypes of known mutants. A Blu isolate with the indicated pattern of carbon source utilization expected for *pfkA* mutants, but differing from *pfkA* mutants in use of fructose and other carbon sources is not included in the table. Literature references for phenotypes: *mal* (8), *pgl* (6), *pgm* (1), *pgi* (6), *pfkA* (10), *ptsG* (4).

[e] Strain RR274.

published data). A known *ptsG* allele in strain ZSC103L (a lactose-utilizing transductant of ZSC103 [4]) also resulted in a Blu phenotype and complete inability to grow on glucose or mannose anaerobically when it was inherited in transductional crosses repairing *pyrC*. The failure of *ptsG* mutant strains to ferment glucose or mannose was unexpected; a second enzyme II complex acting on these sugars permits their aerobic utilization by *ptsG* mutants (4, 7). Revertants of *ptsG* strains able to use glucose anaerobically include mutants altered at a locus distant from *ptsG* that are affected in growth on a number of carbon sources and are no longer intensely blue staining on maltose agar (Roehl and Vinopal, submitted for publication). This result suggests a regulatory basis for the anaerobic-growth-negative and maltose-Blu phenotypes resulting from *ptsG* mutation.

Blu staining with the iodine vapor technique should be a convenient method for isolation of genetic markers in any microorganism metabolizing maltose by the pathway in Fig. 1.

We thank B. Bachmann and W. Epstein for bacterial strains and K. Kolowsky for assistance. This work was supported by National Science Foundation grant PCM 77-07114.

LITERATURE CITED

1. **Adhya, S., and M. Schwartz.** 1971. Phosphoglucomutase mutants of *Escherichia coli* K-12. J. Bacteriol. **108**:621–626.
2. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**:116–167.
3. **Clark, D. J., and O. Maaloe.** 1967. DNA replication and the division cycle in *Escherichia coli*. J. Mol. Biol. **23**: 99–112.
4. **Curtis, S. J., and W. Epstein.** 1975. Phosphorylation of D-glucose in *Escherichia coli* mutants defective in glucosephosphotransferase, mannosephosphotransferase, and glucokinase. J. Bacteriol. **122**:1189–1199.
5. **Kupor, S. R., and D. G. Fraenkel.** 1972. Glucose metabolism in 6-phosphogluconolactonase mutants of *Escherichia coli*. J. Biol. Chem. **247**:1904–1910.
6. **Kupor, S. R., and D. G. Fraenkel.** 1969. 6-Phosphogluconolactonase mutants of *Escherichia coli* and a maltose blue gene. J. Bacteriol. **100**:1296–1301.
7. **Postma, P. W., and S. Roseman.** 1976. The bacterial phosphoenolpyruvate:sugar phosphotransferase system. Biochim. Biophys. Acta **457**: 213–257.
8. **Schwartz, M.** 1967. Expression phenotypique et localisation genetique de mutations affectant le metabolisme du maltose chez *Escherichia coli* K-12. Ann. Inst. Pasteur (Paris) **112**:673–700.
9. **Steiner, K. E., and J. Preiss.** 1977. Biosynthesis of bacterial glycogen: genetic and allosteric regulation of glycogen biosynthesis in *Salmonella typhimurium* LT-2. J. Bacteriol. **129**:246–253.
10. **Vinopal, R. T., D. Clifton, and D. G. Fraenkel.** 1975. *pfkA* locus of *Escherichia coli*. J. Bacteriol. **122**:1162–1171.