Isolation and characterization of catabolite repression-resistant mutants of *Escherichia coli*

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Accepted July 5, 1977

ARMSTRONG, G. D., and H. YAMAZAKI. 1977. Isolation and characterization of catabolite repression-resistant mutants of *Escherichia coli*. Can. J. Microbiol. **23**: 1384–1393.

A method has been developed for the isolation of *Escherichia coli* mutants which are resistant to catabolite repression. The method is based on the fact that a mixture of glucose and gluconate inhibits the development of chemotactic motility in the wild type, but not in the mutants. A motile *E. coli* strain was mutagenized and grown in glucose and gluconate. Mutants which were able to swim into a tube containing a chemotactic attractant (aspartic acid) were isolated. Most of these mutants were able to produce β-galactosidase in the presence of glucose and gluconate and were normal in their ability to degrade adenosine 3',5'-cyclic monophosphate. Some of these mutants were defective in the glucose phosphotransferase system.

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On a mis au point une méthode pour isoler des mutants d'*Escherichia coli* qui sont résistants à la répression catabolique. Cette méthode repose sur la constatation qu'un mélange de glucose et de gluconate inhibe le développement de la mobilité chimiotactique chez le type sauvage mais non chez les mutants. Par mutation on a obtenu une souche mobile d'*E. coli* qui a été cultivée sur du glucose et du gluconate. On a isolé des mutants qui peuvent se déplacer dans un tube contenant une substance attractive chimiotactique (acide aspartique). La plupart de ces mutants peuvent produire de la β-galactosidase en présence de glucose et de gluconate et sont normaux quant à leur capacité de dégrader l'adénosine 3',5'-monophosphate cyclique. Certains de ces mutants ont un système de glucose phosphotransférase défectueux.

[Traduit par le journal]

Introduction

The addition of glucose to an *Escherichia coli* culture causes a transient but strong inhibition of the synthesis of many inducible catabolic enzymes (transient repression), which is followed by a weaker but permanent repression (catabolite repression) (20). Transient repression is related to the transport of glucose via its specific phosphotransferase system, whereas catabolite repression is believed to be caused by some 'catabolite' of this sugar. However, the nature and mode of action of the catabolite are not yet known (20). These questions may be answered by the characterization of mutants which are resistant to catabolite repression. Such mutants will be referred to here as CR^r mutants whereas wild-type strains which are sensitive to catabolite repression, as CR^s.

Catabolite repression has been associated with the ability of some carbon sources (notably glucose) to lower the intracellular concentration of adenosine 3',5'-cyclic monophosphate (cAMP) (9, 11) which is required for the synthesis of inducible catabolic enzymes (26, 27).

Some *E. coli* strains such as Crookes strain and AB257^{PC-1} exhibit the CR^r phenotype because they lack a cAMP-degradative system. Because of this defect, their intracellular cAMP concentration are severalfold higher than the wild type (9, 22). In these strains glucose is able to reduce the cAMP level two- to four-fold as in the CR^s strains, but the reduced level is high enough to support the normal synthesis of inducible catabolic enzymes (9). A D-ribose-positive revertant of *E. coli* B/r strain (1) shows the CR^r phenotype because glucose does not reduce the net synthesis of cAMP in this strain (2). However, how such a reversion causes this phenotype is not yet understood. The *crr* mutants (29) are isolated on the basis of their ability to grow in glycerol in the presence of methyl α-glucoside. They are resistant to phosphotransferase sys-

TABLE 1. Bacterial strains used[a]

Strain	Source	Pertinent characteristics	Derived from	Catabolite repression	References
CSH62	c	$ptsG^+$	Hfr Hayes	CR^s	(21)
HY11	d	—	CSH62	CR^r	This paper
HY12	d	—	CSH62	CR^r	This paper
Crookes	e	Deficient in cAMP phosphodiesterase	—	CR^r	(9)
W1895	f	$ptsG^+$	Hfr Cavalli	CR^s	(10)
W1895D1	f	$ptsG_1$	W1895	CR^r	(10)
LA12	f	$ptsG_2$	Hfr Hayes	CR^r	(18)
X7014-L	f	$purB, ptsG^+, pyrC$	X7014	CR^s	(10)

[a]All strains are E. coli Lac$^+$ (able to utilize lactose), and all except Crookes strain are K-12.
[b]CR^s indicates that glucose or a mixture of glucose and gluconate causes catabolite repression, while CR^r indicates resistance to catabolite repression.
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tem – mediated inhibition of synthesis of catabolic enzymes required for utilization of several carbon sources. It has been proposed (29) that the crr gene specifies a control element of the phosphotransferase system which regulates the activities of adenylate cyclase and the permease proteins.

Other types of E. coli CR^r mutants W1895D1 (14, 30) and LA12 (18) have a defect in the phosphotransferase enzyme II for glucose (10, 12). W1895D1 has been isolated on the basis of its ability to grow in lactose in the presence of methyl α-glucoside (14). Methyl α-glucoside which is transported via the glucose phosphotransferase system in E. coli (16, 17) represses the induction of catabolic enzymes such as β-galactosidase in CR^s strains (10, 25). LA12 has been isolated from a mutagenized culture which was forced to grow for 12 days in glucose and N-acetyllactosamine (whose utilization requires β-galactosidase) as a nitrogen source. These isolation methods which apply selective pressure on growth would specifically enrich for either glucose-transport mutants or mutants specific for the lac operon, and would also yield a population which largely consists of siblings from a limited number of original mutants.

If one wishes to obtain a variety of CR^r mutants, it would be preferable to use a method which is neither based on growth nor specific for a nutritonal ingredient used. Flagellar synthesis in E. coli is subject to catabolite repression (5, 31, 34) and E. coli grown in glucose exhibits greatly reduced chemotactic motility (5). It can then be predicted that CR^r mutant should be

able to develop normal chemotactic motility even when grown in glucose. We describe here a method for the isolation of CR^r mutants based on this prediction as well as preliminary characterizations of some mutants obtained.

Materials and Methods

Chemicals

The following chemicals were obtained from Sigma Co.: isopropyl-β-D-thiogalactoside (IPTG), o-nitrophenyl-β-D-galactoside (ONPG), 5-bromo-4-chloro-3-indolyl-β-D-galactoside ("X-gal"), potassium D-gluconate (gluconate), and methyl α-D-glucoside. [8-^{3}H]cAMP (17.5 Ci/mol (1 Ci = 37 GBq)) was obtained from International Chemical and Nuclear Corp.: methyl-α-[U-^{14}C]D-glucoside (3.8 mCi/mol) from Amersham/Searle Corp.

Media and Plates

Tris maleate minimal medium (TMM) (15) containing thiamine (10 μg/ml), 10^{-3} M phosphate, and an appropriate carbon source was used as a growth medium, unless otherwise specified. Chemotaxis medium (4) consists of 10^{-2} M potassium phosphate (pH 7.0), 10^{-3} M MgSO$_4$, 10^{-4} M ethylenediaminetetraacetate, thiamine (10 μg/ml), and an appropriate carbon source. Chemotaxis agar plates contained 3 g of agar per litre of the chemotaxis medium. Tryptone broth contained per litre 10 g of tryptone, 8 g of NaCl, and 1 g of yeast extract. Tryptone agar plates contained 15 g of agar per litre of tryptone medium. β-Galactosidase indicator plates contained "X-gal" (20 μg/ml), 0.2% glucose, 0.2% gluconate, 0.1% lactose, and thiamine (10 μg/ml) dissolved in the minimal agar (21).

Bacteria and Growth

Bacterial strains used are listed in Table 1. Liquid cultures were prepared by shaking at 35 or 37°C in a gyrotory motion at a speed of 220 rpm. Cell mass density was spectroscopically measured at 500 nm.
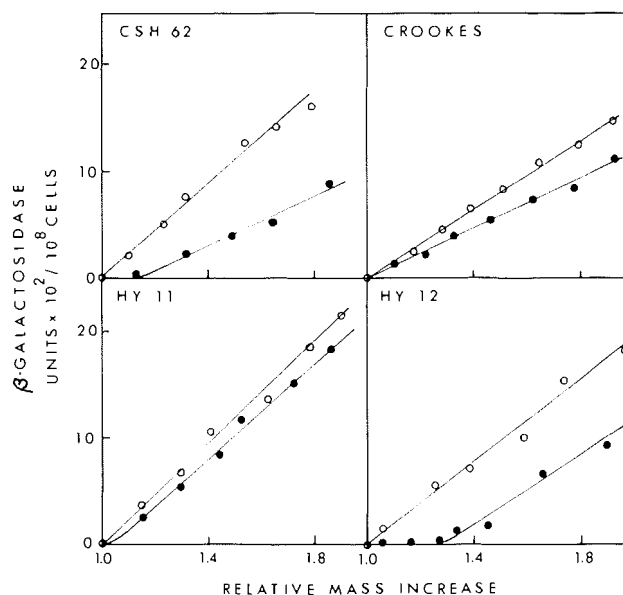
FIG. 1. The effect of glucose on the induction of β-galactosidase in CSH62, Crookes strain, HY11, and HY12. Bacteria were grown at 37°C to log phase in TMM containing 0.4% glycerol as the carbon source. Each culture was divided into two portions. At zero time, IPTG was added to both portions to a final concentration of 1 mM. Glucose solution was immediately added to the first portion (●) to a final concentration of 0.4% and the equivalent volume of H_2O was added to the second portion (○). At various times, samples were removed, and assayed for β-galactosidase activity as described in Materials and Methods. The enzyme activity of each sample divided by the cell density at zero time (ordinate) was plotted against relative mass increase (the cell mass density of sample divided by the zero-time cell mass density). The slope of this plot (known as differential rate) measures the capacity of cells to synthesize the enzyme which is corrected for different growth rates and different initial cell densities (23).

Assay of β-Galactosidase and Tryptophanase

β-Galactosidase was induced by 1 mM IPTG, and assayed by determining o-nitrophenol formed from ONPG as previously described (21). One unit of β-galactosidase is defined as the amount of enzyme liberating 1 μmol of o-nitrophenol per minute at 37°C and pH 7.

Tryptophanase was induced by L-tryptophan (500 μg/ml), and assayed by determining indole formed from tryptophan as described by Pardee and Prestidge (24). The data were corrected for indole accumulated in the induced samples before the enzyme assay. One unit of tryptophanase is defined as the amount of enzyme liberating 1 μmol of indole per minute at 37°C and pH 7.

Assay of Chemotactic Motility

Cells were grown in TMM containing 0.4% glycerol, or a mixture of 0.2% glucose and 0.2% gluconate. Cultures were shaken at 35°C since temperatures higher than 37° tend to inhibit flagellar synthesis (5). The cells were harvested by centrifugation at 10 000 × g and were resuspended into the original volume of chemotaxis medium supplented with the same carbon source, and thiamine (10 μg/ml). The suspension was again centrifuged as above and the supernatant was thoroughly decanted. The pellet was then picked up in a sterile Pasteur pipet and deposited onto the center of a freshly prepared chemo-

taxis agar plate in such a manner that the final bacterial spot settled in a diameter of 3 to 4 mm.

The capillary tubes (31 mm long; 0.2 mm bore) containing the chemotactic attractant aspartic acid were prepared according to the method of Alder (4). Four control tubes (filled with the chemotaxis medium alone) and four attractant tubes (filled with 10^{-3} M aspartic acid in the chemotaxis medium) were alternately and radially placed around the bacterial spot on the chemotaxis agar plate with their open ends 1 cm from the edge of the bacterial spot. These plates were incubated face up at 37°C. At various times thereafter one control tube and one attractant tube were removed; their sealed ends were filed open; the contents of each tube were blown onto tryptone agar plates and spread. The plates were then incubated overnight at 37°C. The difference in the number of bacteria that entered into the attractant tubes and the control tubes were taken as a measure of chemotactic motility.

Assay of cAMP Phosphodiesterase

Cell-free extracts were prepared and cAMP phosphodiesterase in the extracts were assayed by following the hydrolysis of cAMP as previously described (33). The assay mixture contained per millilitre 1 μCi of [³H]cAMP and 10^{-7} mol of cAMP and the cell-free extract. Protein in the extracts was assayed by the Lowry method (19).

TABLE 2. Chemotactic motility after growth in (A) glycerol and (B) a mixture of glucose and gluconate[a]

Swimming period, h	CSH62		Crookes		HY11		HY12	
	−asp	+asp	−asp	+asp	−asp	+asp	−asp	+asp
(A) 6	0	0	0	0	0	0	0	98
(A) 8	0	106	38	286	2	182	16	312
(A) 12	98	t.m.[b]	ca. 700	t.m.[b]	151	t.m.[b]	ca. 600	t.m.[b]
(B) 6	0	0	0	0	0	0	0	0
(B) 8	0	0	3	201	26	403	0	268
(B) 12	6	ca. 1000	t.m.[b]	t.m.[b]	90	t.m.[b]	3	t.m.[b]

[a]Bacteria grown overnight in 0.4% glycerol or a mixture of 0.2% glucose and 0.2% gluconate were concentrated and spotted on chemotaxis agar plates (see Materials and Methods). The bacteria that swam into tubes containing an attractant aspartic acid (+asp) or no aspartic acid (−asp) were spread onto tryptone agar plates and the number of the colonies were counted. The difference between +asp and −asp represents chemotactic motility. Chemotaxis agar plates were made of agar (3 g/ℓ) obtained from Terochem Laboratories Ltd. (Edmonton, Alta), stored at 4°C, and used 1 day after preparation.
[b]t.m. indicates that there were too many cells to be counted. (Their absolute numbers are not important in these cases.)

Assay of Methyl α-D-Glucoside Uptake

Bacteria were grown at 37°C to log phase in TMM containing 0.4% glycerol. Methyl-α-[^{14}C]D-glucoside was added to the culture to final concentrations of 0.1 μCi per millilitre and 100 μg per millilitre. At various times, 100-μl portions were withdrawn from the cultures, and filtered through 25-mm Gelman filters (0.45-μm pore) which had been soaked in the washing medium (TMM containing 0.4% glycerol and 0.01% methyl α-glucoside). The cells collected on the filters were quickly washed with three 1-ml portions of the washing medium, and resuspended into 0.5 ml of 1 N HCl to extract ^{14}C radioactivity from the cells. After 30 min at room temperature, each suspension was homogenized with 10 ml of a detergent-based scintillation fluid. The homogenates were counted in the liquid scintillation counter with a counting efficiency of about 70%.

Results

Characterization of a Parent and a CR[r] Reference Strain

The plan of this work was to isolate catabolite repression-resistant (CR[r]) mutants from a catabolite repression-sensitive (CR[s]) parent on the basis of differential chemotactic motility after growth under the condition of catabolite repression. To test whether a parent strain CSH62 was in fact CR[s], CSH62 and Crookes strain (CR[r] reference) were grown in glycerol which is known to cause minimal catabolite repression (9). β-Galactosidase was induced with IPTG in the presence and absence of glucose. Figure 1 shows that CSH62 was susceptible to both transient and catabolite repression by glucose while Crookes strain exhibited resistance to both repressions.

To test whether CSH62 and Crookes strain possesses chemotactic motility, they were grown in glycerol. The bacteria were then concentrated and deposited onto chemotaxis agar plates. Bacteria possessing chemotactic motility should preferentially swim into a tube containing aspartate, an attractant for E. coli (4). In Table 2 it is shown that after an 8-h swimming period both CSH62 and Crookes strain exhibited chemotactic motility toward aspartate. Beyond 8 h, bacterial movement other than chemotactic motility increased. To verify that chemotactic motility in CSH62 is subject to catabolite repression, CSH62 and Crookes strain (CR[r]) were grown in a mixture of glucose and gluconate which causes more severe catabolite repression than glucose alone (13). In Table 2 it is also shown that during an 8-h swimming period (under the same motility assay conditions as above) no CSH62 cells grown in glucose and gluconate were able to enter the attractant tube indicating that chemotactic motility of CSH62 was suppressed by growth in glucose and gluconate. On the other hand Crookes strain was able to develop nearly normal chemotactic motility during grown in glucose and gluconate.

It should be mentioned here that the rate of bacterial movement on chemotaxis agar plates varied with the type of agar used, freshness of plates, and wetness of bacterial deposits. Therefore the comparison such as shown in Table 2 must be made under as closely controlled conditions as possible.

Isolation of Catabolite Repression-resistant Mutants

To isolate CR[r] mutants, the same experiment

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TABLE 3. Chemotactic motility of mutagenized CSH62 and untreated CSH62 after growth in a mixture of glucose and gluconate[a]

Swimming period, h	Number of bacteria per tube			
	Untreated CSH62		Mutagenized CSH62[b]	
	−asp	+asp	−asp	+asp
2	0	0	0	0
4	0	0	1	90

[a]The experimental conditions are the same as described in Table 2 except that Bactoagar (Difco Laboratories Inc.) was used in chemotaxis agar plates.
[b]CSH62 was mutagenized with N-methyl-N'-nitro-N-nitrosoguanidine according to Adelberg et al. (3). After removing the mutagen, bacteria were grown overnight at 35°C in a mixture of 0.2% glucose and 0.2% gluconate, and assayed for chemotactic motility as described in Materials and Methods.

TABLE 4. Specific activity of cAMP phosphodiesterase in cell-free extracts[a]

Extract from:	Sp. act.[b]
CSH62	1.7
HY11	1.5
HY12	1.9
Crookes strain	0

[a]Cell-free extracts were prepared, filtered through a G-25 Sephadex column, and assayed for cAMP phosphodiesterase activity as previously described (33).
[b]The amounts of cAMP hydrolyzed were plotted against time and the enzyme activity was calculated from the initial linear slope at 2 min of incubation. The specific activity is expressed as nanomoles of cAMP hydrolyzed per minute mg^{-1} protein.

was repeated with a mutagenized CR^s strain. CSH62, after treatment with the mutagenic agent N-methyl-N'-nitro-N-nitrosoguanidine (3), was grown overnight in a mixture of glucose and gluconate. The addition of gluconate would not only intensify catabolite repression but also allow the growth of mutants which have a defect in the glucose phosphotransferase system since the transport of gluconate does not depend on such a transport system (6). In addition, gluconate may permit the growth of mutants which have a defect in glucose catabolism. In Table 3 it is shown that mutagenized CSH62 cultures gave rise to bacteria able to swim into the attractant tube under the conditions which do not allow the cells from the untreated culture to reach the attractant tube. These swimmers are presumed to be mutants which have acquired the ability to form flagella even when grown in glucose and gluconate.

These mutants, however, may include flagellum-specific mutants in which flagellar synthesis only is resistant to catabolite repression while other cAMP-dependent protein synthesis is still sensitive to glucose. Indeed, Silverman and Simon (31) have isolated E. coli mutants which can synthesize flagella in the absence of cAMP or cAMP receptor protein and form flagella even when grown on glucose. We wished here to select against such flagellum-specific mutants and obtain more general types of CR^r mutants. Therefore, the mutants selected on the basis of chemotactic motility were individually tested for the ability to induce β-galactosidase on β-galactosidase indicator plates (which contained glucose and gluconate in addition to inducer).

β-Galactosidase releases deep blue 5-bromo-4-chloro-indigo from X-gal in the plates. Crookes strain (CR^r) colonies appeared as blue whereas CSH62 (CR^s) colonies appeared white after overnight incubation.

Fifty-five of the 60 mutants which were independently isolated on the basis of chemotactic motility developed a blue color as did Crookes strain on the β-galactosidase indicator plates. Since these blue-stained mutants are CR^r in terms of both motility and β-galactosidase formation, it is assumed that they have acquired "general resistance" to catabolite repression. Five mutants which appeared white on the β-galactosidase indicator plates are likely to be flagellum-specific mutants such as flagellar promotor mutants that have lost their dependence on cAMP and cAMP receptor protein for flagellar synthesis.

cAMP Phosphodiesterase Assay

The CR^r phenotype of Crookes strain has been attributed to its high intracellular cAMP concentration which results from the deficiency of cAMP phosphodiesterase activity (9). When the 55 "generally resistant" mutants were assayed for cAMP phosphodiesterase activity, 54 of the 55 mutants possessed normal cAMP phosphodiesterase activity as in their wild-ype parent, CSH62. Table 4 exemplifies this by comparing the enzyme-specific activities of two representative mutants, HY11 and HY12, with that of CSH62. Only one of the 55 mutants possessed a significantly reduced enzyme activity (about 10% of the CSH62 activity).
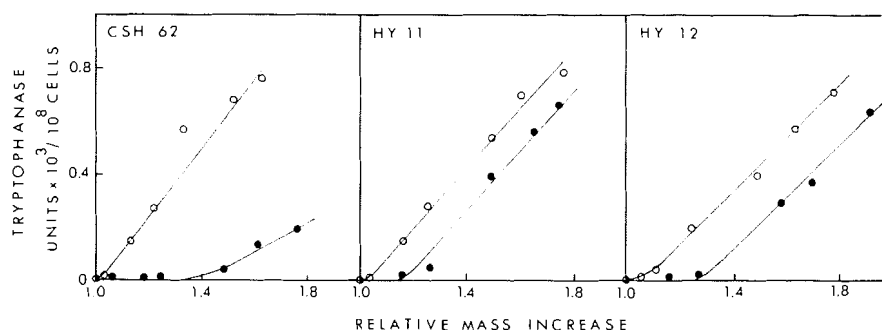
FIG. 2. The effect of glucose on the induction of tryptophanase in CSH62, HY11, and HY12. Bacteria were grown at 37°C to log phase in TMM containing 0.4% glycerol. Each culture was divided into two portions. At zero time, one portion (○) received tryptophan (500 μg/ml), and the second portion (●) received both tryptophan (500 μg/ml) and glucose (0.4%). At various intervals, 1 ml of each portion was removed to determine cell mass density, and two 200-μl samples were also removed to assay tryptophanase activity as described in Materials and Methods. As in Fig. 1, the enzyme activity of each sample divided by the zero-time cell density was plotted against the relative mass increase.

Effect of Glucose on the Kinetics of β-Galactosidase and Tryptophanase Induction

Among these 54 mutants possessing normal activity of cAMP phosphodiesterase, we chose 6 mutants which gave the most intense blue stain on the β-galactosidase indicator plates. These were grown with glycerol as the carbon source, and the effect of glucose on the induction of β-galactosidase was then examined in these liquid cultures. In Fig. 1 it is shown that one of the six mutants (named HY11) exhibited a much shorter period of transient repression than did CSH62 but no catabolite repression, while the other five mutants (represented by HY12) still exhibited marked transient repression but no catabolite repression.

Tryptophanase induction in a wild-type *E. coli* is subject to more severe catabolite repression than β-galactosidase induction (8, 28). In Fig. 2 it is shown that catabolite repression of tryptophanase induction was totally absent in both mutants although their parent CSH62 exhibited greater catabolite repression of tryptophanase induction than that of β-galactosidase induction. In triptophanase induction, transient repression in HY11 was as marked as that in HY12, but significantly shorter in duration than that in CSH62.

Growth in Glucose

CR^r mutants may be hypothetically classified into two groups in terms of glucose utilization: (i) mutants with normal uptake and metabolism of glucose which therefore grow in glucose at

TABLE 5. Doubling times in glycerol, glucose, and a mixture of glucose and gluconate[a]

Strain	Doubling times, min		
	Glycerol	Glucose	Glucose + Gluconate
CSH62	65–70	45–50	45–50
HY11	65–70	120–130	45–50
HY12	65–70	75–80	45–50
W1895	65–70	45–50	—
W1895D1	65–70	85–90	—
LA12	70–75	85–90	—

[a]Bacteria were grown at 37°C in TMM containing either 0.4% glycerol, 0.4% glucose, or a mixture of 0.2% glucose and 0.2% gluconate.

normal rates, and (ii) mutants with impaired uptake or metabolism of glucose which therefore grow in glucose less well. The first group may include mutants which have lost their sensitivity to the catabolite responsible for the repression even though the cells may normally generate the catabolite from glucose. Mutants which poorly generate the catabolite either due to the poor uptake of glucose or to a defect in glucose metabolism would belong to the second group. To characterize further mutants HY11 and HY12, their growth rates in glucose were determined. In Table 5 it is shown that both HY11 and HY12 grow normally in glycerol and in a mixture of glucose and gluconate, but in glucose they grow more slowly than their parent CSH62. Thus both mutants may belong to the second group.

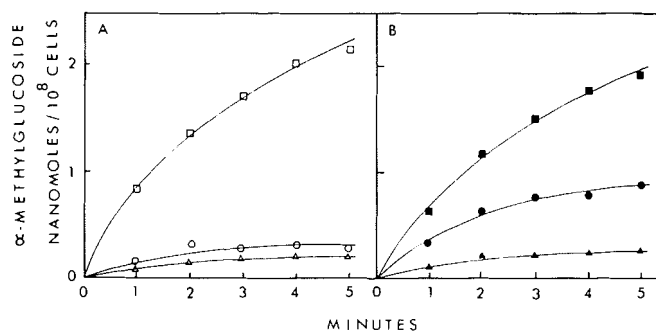Catabolite repression resistant in LA12 ($ptsG_1$) and W1895D1 ($ptsG_2$) has been attri-

FIG. 3. Uptake of methyl α-glucoside by W1895 (*ptsG*$^+$), W1895D1 (*ptsG*), LA12 (*ptsG*), CSH62 (*ptsG*$^+$), HY11, and HY12. Bacteria were grown at 37°C to about 5×10^8 cells per millilitre in TMM containing 0.4% glycerol. At this time (zero time), methyl-α-[^{14}C]glucoside was added to each culture to final concentrations of 0.1 μCi per millilitre and 100 μg per millilitre. At 1-min intervals, 100-μl samples were removed and filtered through a membrane filter. The cells on the filter were washed and assayed for radioactivity as described in Materials and Methods. The data represent the mole quantity of methyl α-glucoside taken up by 10^8 cells. Part A represents W1895 (□), W1895D1 (△), and LA12 (○). Part B compares our mutants HY11 (▲) and HY12 (●) with their parent CSH62 (■).

buted to a defect in the phosphotransferase system for glucose (10). Thus they would also belong to the second type of mutants. As shown in Table 5, they too grow more slowly in glucose than wild-type strains, CSH62 (*ptsG*$^+$) and W1895 (*ptsG*$^+$).

Comparison with the ptsG Mutants

The above results suggest the possibility that HY11 and HY12 may be defective in the phosphotransferase system for glucose (PTSG). To test this, the uptake of methyl α-glucoside was studied. In *E. coli* this compound is transported via PTSG (10), and cannot be metabolized. Previously the uptake of this compound by the *ptsG* strains has been assayed in the absence of carbon sources (10). However, in the present study (Figs. 1 and 2), catabolite repression was determined in glycerol-grown cultures. Therefore, it is relevant to measure the PTSG activity in glycerol. In Fig. 3A it is shown that the uptake of methyl α-glucoside (PTSG activity) is indeed markedly reduced in the *ptsG* reference strains, W1895D1 and LA12, as compared to the wild-type strain W1895. In Fig. 3B it is shown that HY11 exhibited a markedly reduced PTSG activity (about 15% of the activity in its parent, CSH62). HY12, on the other hand, exhibited a moderately reduced PTSG activity (about 50% of the activity in CSH62). These results are consistent with the fact (Table 5) that in glucose HY11 grows more slowly than HY12. Reduced efficiency of glucose transport via PTSG would reduce the extent of

transient repression (20). Therefore, the above results are also consistent with the fact (Fig. 1) that transient repression of β-galactosidase induction is less marked in HY11 than HY12.

The effects of glucose on β-galactosidase induction in the *ptsG* reference strains were also examined (Fig. 4) to compare them with the effects observed in HY11 and HY12. Both LA12 and W1895D1 exhibited no catabolite repression. LA12 exhibited less marked transient repression than did the wild-type strain W1895, whereas W1895D1 exhibited little or no transient repression. The extent of transient repression in LA12 (Fig. 4) was similar to that in HY11 (Fig. 1). This result is consistent with the fact that the PTSG activity in both strains was similarly reduced (Fig. 3).

Preliminary Mapping of CR^r in HY11 and HY12

Preliminary transduction studies were also undertaken to see whether the mutation resulting in the CR^r phenotype in HY11 and HY12 actually maps in the same location as the *ptsG* locus. The *ptsG* locus has been mapped at 24 min between the *purB* (25 min) and *pyrC* loci (23 min) on the *E. coli* K-12 chromosome (7, 10). When phage P1*kC* grown on a *purB*$^+$ *ptsG pyrC*$^+$ donor is used to transduce a *purB ptsG*$^+$ *pyrC* recipient, about 30% of the *purB*$^+$ transductants and 80 to 90% of the *purB*$^+$ *pyrC*$^+$ transductants inherit the *ptsG* mutation (10, 32). In Table 6 it is shown that when P1*kC* grown on HY11 (*purB*$^+$, CR^r, *pyrC*$^+$) or HY12 (*purB*$^+$, CR^r,
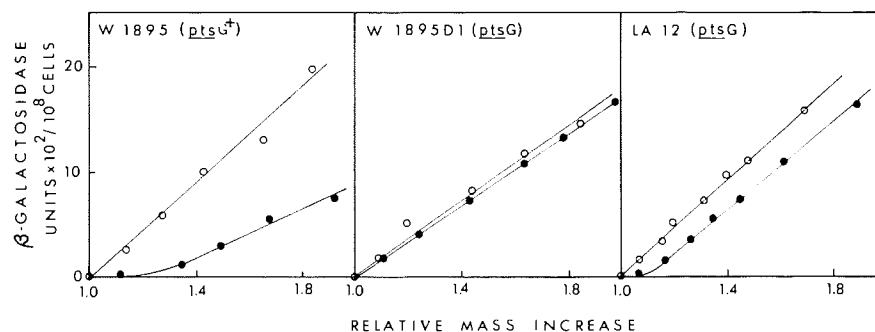
FIG. 4. The effect of glucose on the induction of β-galactosidase in W1895 (*pts*G⁺), W1895D1 (*pts*G), and LA12 (*pts*G). Bacteria were grown at 37°C to log phase in TMM containing 0.4% glycerol and divided into two portions. At zero time, IPTG was added to both portions to a final concentration of 1 mM. Glucose solution was immediately added to the first portion (●) to a final concentration of 0.4% and the equivalent volume of H_2O, to the second portion (○). At various times, samples were withdrawn and assayed for β-galactosidase activity as described in Materials and Methods. As in Fig. 1, the enzyme activity of each sample divided by the zero-time cell density was plotted against the relative mass increase.

TABLE 6. Cotransduction of the catabolite repression-resistance character with the *pur*B and *pyr*C markers[a]

P1*kC* donor	Selected marker	Incidence[b]
HY11 (CR^r)	*pur*B⁺	31/80 (39%)
HY11 (CR^r)	*pur*B⁺, *pyr*C⁺	15/15 (100%)
HY12 (CR^r)	*pur*B⁺	64/133 (48%)
HY12 (CR^r)	*pur*B⁺, *pyr*C⁺	12/12 (100%)
CSH62 (CR^s)	*pur*B⁺	1/166 (0.6%)
CSH62 (CR^s)	*pur*B⁺, *pyr*C⁺	0/23 (0%)

[a]Phage P1*kC* was grown on each of the donor strains (*pur*B⁺, *pyr*C⁺) and the lysate was used to infect the recipient strain X7014-L (*pur*B, *pts*G⁺, *pyr*C) according to the method described by Miller (21). The infected cells were plated onto the β-galactosidase indicator plated with or without uracil (50 μg/ml).

[b]The number of blue-stained colonies/the number of the *pur*B⁺ or *pur*B⁺ *pyr*C⁺ transductants of X7014-L. The blue colonies represent the catabolite repression-resistant strains that produced β-galactosidase in the presence of glucose and gluconate.

*pyr*C⁺) infected the *pur*B *pts*G⁺ recipient, 40–50% of the *pur*B⁺ transductants and all the *pur*B⁺ *pyr*C⁺ transductants inherited the CR^r character of HY11 and HY12. Thus it appears that the CR^r character of HY11 and HY12 is centrally located between the *pur*B and *pyr*C loci as is the *pts*G mutation. All *pur*B⁺ CR^r *pyr*C⁺ transductants grow less well in glucose than CSH62 (*pur*B⁺, *pts*G⁺, *pyr*C⁺), suggesting that the transductants are defective in glucose utilization. However, more detailed genetic and biochemical analysis is required before deciding whether the CR^r mutation in HY11 or HY12 lies in the *pts*G locus.

Discussion

A method has been developed for the isolation of *Escherichia coli* CR^r mutants which are resistant to catabolite repression. The method is based on the fact that a mixture of glucose and gluconate inhibits the development of chemotactic motility in the wild-type catabolite repression-sensitive (CR^s) strains but not in CR^r mutants. The previously available methods select CR^r mutants on the basis of their capacity to grow in lactose (14) or *N*-acetyllactosamine (18) in the presence of methyl α-glucoside (14) or glucose (18). These methods would enrich for lactose-specific mutants as well as the glucose-transport mutants. Since most CR^r mutants might be expected to grow normally in a mixture of glucose and gluconate, the present method would likely yield a variety of CR^r mutants. In fact, 5 of the 60 mutants isolated appear to be CR^r only in terms of the development of chemotactic motility. They are likely flagellum-specific mutants. One of the other 55 mutant is CR^r as a result of its defect in cAMP phosphodiesterase. The remaining 54 are CR^r in terms of both chemotactic motility and induction of β-galactosidase and are presumed to have acquired general resistance to catabolite repression. Some of the mutants which exhibit strong CR^r character (in terms of β-galactosidase induction) appear to be defective in the glucose phosphotransferase system (PTSG). Reduced uptake of glucose via PTSG would reduce not only transient repression but also catabolite repression (due to inefficient generation of the catabolite responsible for the repression). We hope that further charac-

terization of other mutants isolated will reveal types of mutation other than the three described above. Mutants which have lost the capacity to generate the catabolite (responsible for the repression) or the sensitivity to the catabolite will be useful for the elucidation of catabolite repression. We suggest that if one wishes to select against the *pts*G mutants, the present method be modified so that mutagenized cells are grown in glucose without gluconate before selection on the basis of chemotactic motility.

Comparative studies of the *pts*G mutants indicate that the residual PTSG activities of these mutants are approximately correlated with the degrees of transient repression of β-galactosidase in these mutants.

Acknowledgement

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