A mutant β-D-glucoside transport system of *Escherichia coli* resistant to catabolite inhibition

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A mutant strain of *Escherichia coli* in which β-glucoside transport is resistant to catabolite inhibition by methyl α-glucoside was characterized. The mutation was probably within the gene, *bglC*, coding for the β-glucoside enzyme II. The mutant organism is shown to transport the β-glucoside substrate, salicin, in preference to methyl α-glucoside or fructose. Salicin also caused inducer exclusion of lactose in the mutant strain.

Escherichia coli *Phosphotransferase* *Sugar transport* *Catabolite inhibition* *Inducer exclusion*

1. INTRODUCTION

Non-catabolizable analogues of glucose, such as 2-deoxyglucose [1], 3-deoxy-3-fluoroglucose [2], methyl α-D-glucoside [3], and 5-thio-glucose [4], powerfully inhibit the growth of *Escherichia coli* on sugars that are taken up via the phosphoenolpyruvate-dependent phosphotransferase (PT) system [5]. This behaviour illustrates the preference for glucose shown by *E.coli* over other sugars; this phenomenon has been termed 'catabolite inhibition' [6,7]. The mechanism of the growth stasis caused by non-catabolizable glucose analogues could be due to the accumulation of the phosphate esters of those analogues (which is known to be toxic; review [8]), or to the higher affinity of those analogues for a component of the PT system required for the uptake both of the analogue and of the competing sugar (review [9]), or to both. Evidence for the former mechanism has been adduced [10]. It is the main purpose of this paper to describe the isolation and properties of a strain of *E.coli* mutated in the system for uptake of β-D-glucosides (*bgl*) that provides evidence for the latter mechanism.

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2. EXPERIMENTAL

2.1. *Organisms*

The organisms were derivatives of *E.coli* K12; their relevant genotypes and the sources from which they were derived are listed in table 1. The abbreviations used to describe genetic markers, not defined in the text, are listed in [11].

The media used for the growth of the organisms, the methods employed for the measurement of growth, the relative utilization of various sugars and the uptake of substrates labelled with ^{14}C, have been described in [3,4]. The genetical methods were as compiled in [12]; P1 phage for generalized transduction was prepared according to [13].

2.2. *Selection of β-D-glucoside-specific regulatory mutants*

A culture of strain CE149 which lacked the PtsM system for the uptake of D-arabinohexoses, but readily took up α-D-glucosides (*umgC*) and β-D-glucosides (*bglR*, *bglC*$^+$), was grown to ca. 0.4 mg dry mass/ml on 5 mM salicin as the sole carbon source. A portion (0.1 ml) was pipetted into medium similar, but also containing 1 mM methyl α-D-glucoside; the resultant mixture was shaken at 37°C for several days. As expected, the presence of the glucose analogue initially inhibited the continued growth of the cells; however, after 72 h, good growth had been achieved. In order to dis-

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Table 1
Strains of *E. coli* used

Organism	Genotype	Source
CE149	F− *umgC ptsM arg bglR bglC*+ *leu thr rpsL*	Laboratory stock
CE156	F− *umgC ptsM arg leu thr rpsL ilvA*::Tn5	Laboratory stock
CE185	F− *umgC ptsM arg leu thr rpsL bglR bglC**	P1.CE162→CE156 Ile+ αMG^R/salicin^R
CE128	*his–gnd*$^{\Delta}$ *thyA ilvD mglP ptsF umgC eda ptsM uhpC pyrC*::Tn10	Laboratory stock
CE162	*his–gnd*^{VS5v} thyA mglP ptsF *umgC uhpC eda ptsM pyrC*::Tn10 *bglR bglC**	This paper
CE187	F− *lac gal glmS pyrC*::Tn10 *umgC ilvA*::Tn5	P1.CBK007→CE166 Nm^R
CE191	f− *lac gal pyrC*::Tn10 *bglR bglC**	P1.CE162→CE187 Ile+ *glmS*+ αMG^R/ salicin^R
CE212	F− *lac gal pyrC*::Tn10 *bglR bglC*+	P1.CE149→CE187 Ile+ *glmS*+ αMG^S/ salicin^S
CBK007	*ilvA*::Tn5 *thy*	C. Berg via M.C. Jones-Mortimer

criminate against *ptsG* mutants, in which the tolerance of methyl α-D-glucoside was due to failure to take up that analogue, a small portion of the culture was grown for 3 generations in medium containing 5 mM glucose as the sole carbon source. Phage P1 was propogated on a portion of this culture and was used to transduce the isoleucine–valine auxotroph CE128, which was also unable to grow upon salicin, to Ilv+. The transductants were screened for their ability to grow on minimal agar plates containing 10 mM salicin as the sole carbon source; Sal+ transductants were screened further for their ability to grow on this medium supplemented with 1 mM methyl α-D-glucoside.

A number of Sal+ Ilv+ transductants, that were also resistant to methyl α-D-glucoside on salicin medium, were purified by isolation of single colonies and were studied further.

2.3. *Spectrophotometric assay of β-glucoside uptake*

Rapidly growing cells were harvested at ca. 0.4 mg dry mass/ml at 10 000 × *g* for 10 min at room temperature and were washed with 50 mM sodium phosphate buffer (pH 7.2). They were resuspended to ca. 0.7 mg dry mass/ml in the same buffer supplemented with chloramphenicol (100 μg/ml). A portion (0.1 ml) of this cell suspension was diluted 10-fold with buffer prewarmed to 37°C, in a cuvette (1 cm light path, 1 ml vol.). The uptake and phosphorylation of β-D-glucosides was detected by the liberation of *p*-nitrophenol from added *p*-nitrophenyl β-D-glucoside; this was measured continuously with a Pye Unicam SP1800 spectrophotometer at 410 nm. Under these conditions, an increase of absorbance of 1.0 unit corresponds to the release of 78.4 nmol *p*-nitrophenol.

3. RESULTS AND DISCUSSION

3.1. *Location of the gene specifying the loss of methyl α-glucoside mediated catabolite inhibition on salicin*

Of 181 Ilv+ transductants selected by the procedure in section 2.2, 56 (31%) were also Salicin+; of these, 7 (12.5% of Sal+, 3.5% of Ilv+) had simultaneously acquired resistance to the inhibitory effect of methyl α-glucoside on salicin medium. Phage P1 was propogated on one of this latter type of transductant (designated CE162) and used to transduce the *ilvA glmS* auxotroph CE187 to Ile+ glmS+; 48 out of 160 such transductants also grew on salicin. All of these 48 Salicin+ Ile+ organisms had inherited the methyl α-glucoside resistance of the donor organism. This very high degree of linkage of the gene that confers tolerance of methyl α-glucoside (see below) is probably due to an alteration in the uptake system specified by *bglC*, which is therefore tentatively designated *bglC**. It has been suggested that the gene order of the *bgl* operon is *bgl B.S.C.R.* [14,15].

One of these Salicin+ Ile+ transductants, designated CE191, was studied further.

3.2. *Effects of glucose and methyl α-glucoside on the utilization of β-glucosides*

Like the wild-type strain CE212, the transductant CE191 was sensitive to methyl α-glucoside when growing on a variety of carbon sources, including fructose (fig.1A): growth was rapidly arrested by addition of the glucose analogue to cultures of the organisms. However, unlike strain CE212, the otherwise isogenic transductant CE191 was unaffected by methyl α-glucoside when salicin was the carbon source (fig.1B). Moreover, the uptake and phosphorylation of *p*-nitrophenyl β-D-glucoside, and consequent liberation of the intensely yellow *p*-nitrophenol by washed suspensions of these organisms, also manifest this

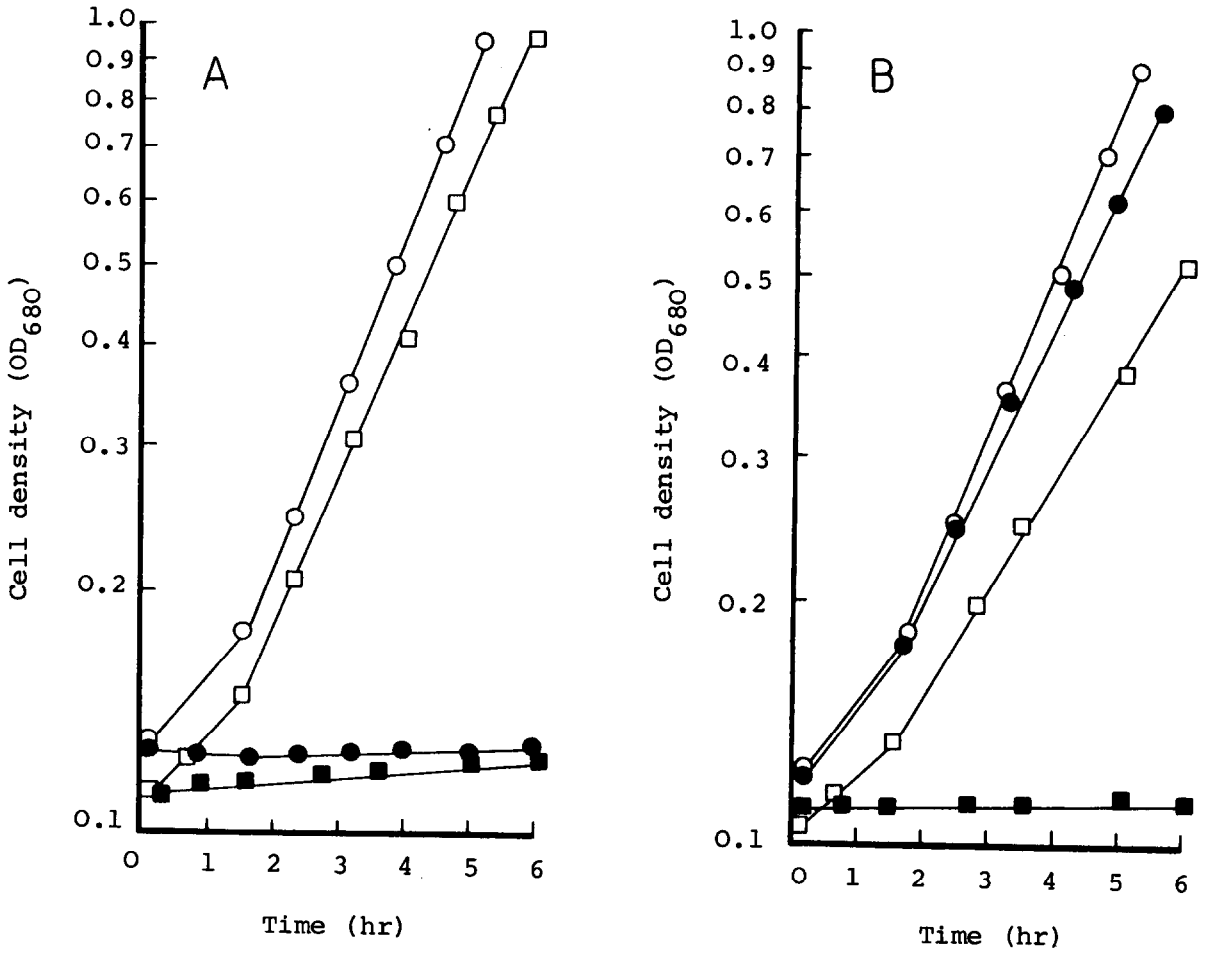

Fig.1. Effect of 1 mM methyl α-glucoside on the growth of *E.coli* strains CE212 (*bglC*$^+$) and CE191 (*bglC**) on 5 mM fructose (A) or 5 mM salicin (B). The cells were grown to mid-exponential phase on the sugar indicated, harvested and resuspended in the same medium with (closed symbols) or without (open symbols) 1 mM methyl α-glucoside; (□,■) strain CE212; (○,●) strain CE191.

difference: unlike strain CE212, the mutant CE191 is virtually unaffected by methyl α-glucoside in this β-glucoside uptake (fig.2). This colorimetric assay measures the simultaneous uptake and phosphorylation of the β-glucoside, which limits the rate of the subsequent cleavage of the *p*-nitrophenyl-β-D-glucoside-6-phosphate; the phospho-β-glucosidases that effect the latter process are not inhibited by a variety of phosphate esters [16,17].

These results therefore imply that the balance of the competition between the utilization of methyl α-glucoside and the β-glucosides, salicin (fig.1B) and *p*-nitrophenyl-β-glucoside (fig.2B), which is normally strongly in favour of glucose and its analogue, has been altered in the mutant, and that this alteration has occurred in the β-glucoside uptake system specified by *bglC*. It is for this reason that the altered gene is designated *bglC**.

Three observations support this conclusion:

(1) Mutants carrying the *bglC** marker take up *p*-nitrophenyl β-glucoside at a somewhat higher rate than wild-type (*bglC*$^+$) cells; in consequence, *bglC**-strains, such as CE191, grow more rapidly on salicin at 37°C (doubling time 1.4 h) than *bglC*$^+$ organisms such as CE212 (doubling time 2.2 h).

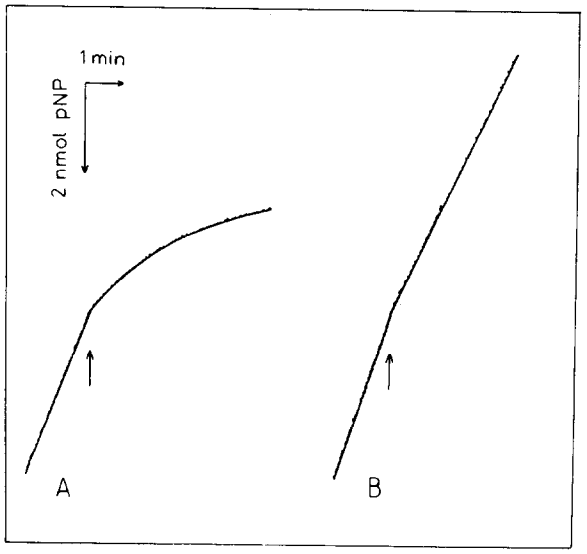

Fig.2. Influence of methyl α-glucoside on the liberation of *p*-nitrophenol from *p*-nitrophenyl β-D-glucoside by washed cell suspensions of strains (A) CE212 (*bglC*$^+$) and (B) CE191 (*bglC**). Cells were grown to mid-exponential phase on 5 mM salicin in supplemented minimal salts medium, harvested and resuspended to A_{680} = 0.1 (in a 1 ml cuvette) in potassium phosphate buffer (pH 7.2) [2,3]; *p*-nitrophenyl β-glucoside was added to 1 mM final conc. This was followed by the addition of methyl α-glucoside to 1 mM, at the time indicated by the arrow. The uptake and phosphorylation of the β-glucoside was measured by the liberation of *p*-nitrophenol at 410 nm.

(2) The uptake of 0.1 mM methyl α-[^{14}C]glucoside which, in the absence of competing sugars, occurs with equal facility and to the same extent in both *umgC* strains, irrespective of whether they were grown on fructose or salicin, is not affected by the addition of fructose to fructose-grown cells. Likewise, the addition of *p*-nitrophenyl β-glucoside to salicin-grown cells of the *bglC*$^+$ strain does not significantly affect the uptake of methyl α-D-[^{14}C]glucoside. However, *p*-nitrophenyl β-glucoside sharply reduces the uptake of methyl α-D-[^{14}C]glucoside by the *bglC** mutant (fig.3).

(3) The specific nature of the β-glucoside mutation in strain CE191, and the manner in which this has altered the preferential utilization of other sugars in respect of β-glucosides, is also shown by the rates at which these substrates, present at the same time, supply carbon to cells growing upon them. Thus, when glucose is added to *bglC*$^+$ cells growing on salicin, only 8% of the subsequent cell carbon is derived from salicin; also with fructose as competing sugar, only 12% of cell carbon is derived from salicin. On the other hand, the *bglC**-mutant CE191 used salicin to >70% in preference to both glucose and fructose; however, the normal balance between fructose and glucose uptake [3] was unchanged by this mutation in the *bgl*-operon (table 2).

Table 2

The effect of the β-glucoside-specific mutation on the preferential utilization of sugars by *E.coli* strain CE191

Sugar mixture (5 mM each)	Ratio of total cell carbon derived from each sugar (%)	
	CE191 (*bglC**)	CE212 (*bglC*$^+$)
Glucose:salicin†	25:75	92:8
Fructose:salicin†	30:70	88:12
Fructose†:glucose	15:85	16:84

Organisms were grown overnight at 37°C on the carbohydrate indicated by †, harvested by centrifugation at room temperature and resuspended (at ca. 0.1 mg dry mass/ml) in a flask containing 10 ml of growth medium with the carbohydrate at 5 mM. When growth at 37°C recommenced, [^{14}C]glucose or [^{14}C]fructose was added (to 5 mM) and the incorporation of radioisotope was measured as described. The ratio of total cell carbon derived from each sugar was determined by comparison of the rate of incorporation of radioisotope in the presence or absence of the unlabelled carbohydrate

The preferential utilization of salicin by the *bglC** strain CE191 applies also to sugars that are not taken up via the PT-system, such as lactose. When wild-type cells, grown on salicin, are placed in medium containing salicin and ^{14}C-labelled lactose, the progressively more rapid incorporation of ^{14}C from the latter sugar into the growing cells shows that, unlike glucose, salicin is not able to prevent the induction of the lactose operon. However, *bglC**-cells continued to use salicin in preference to lactose (fig.4); this effect was reversed by the introduction into the *bglC** mutant of the *iex* allele [18].

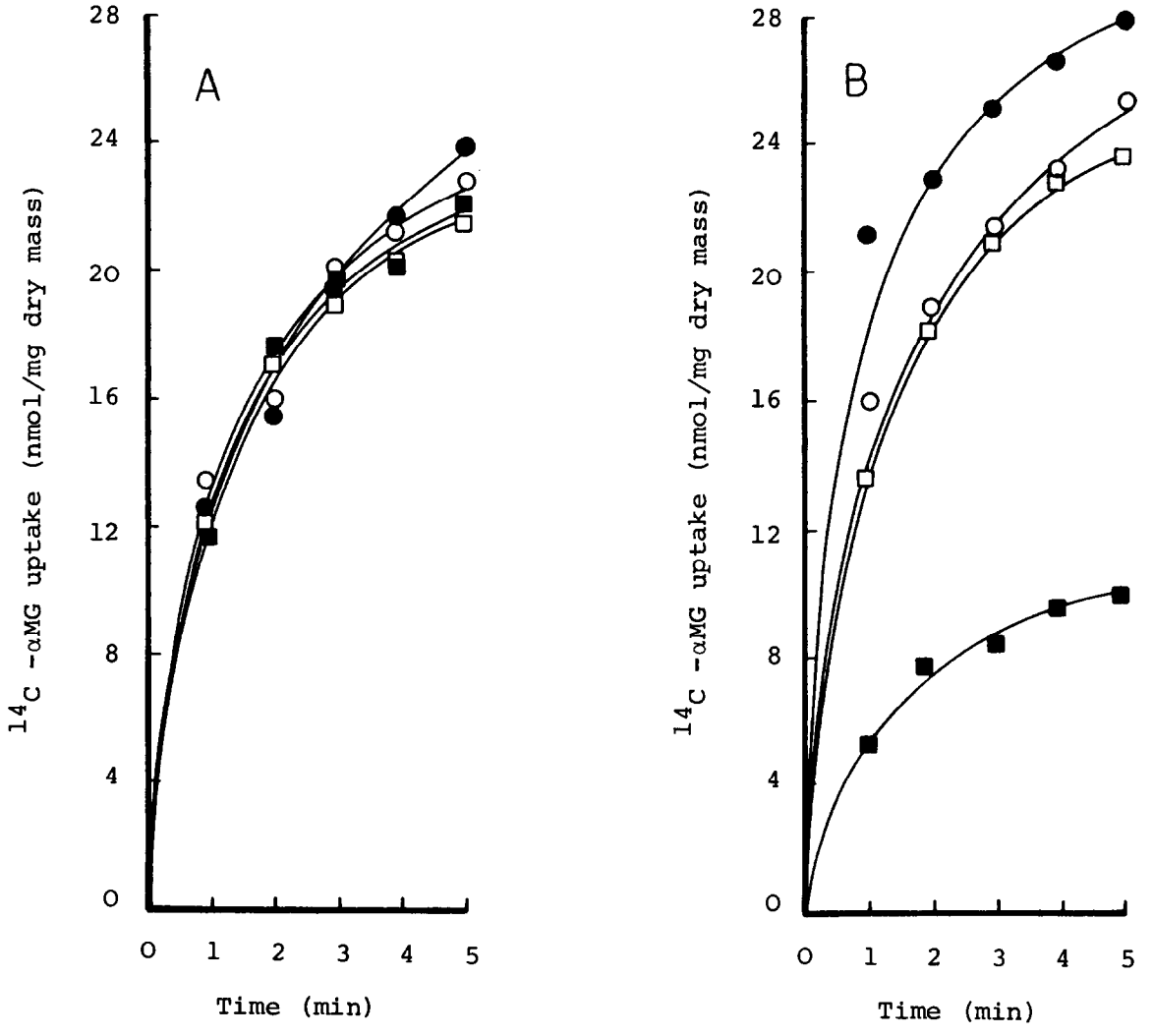

Fig.3. Effect of *p*-nitrophenyl-β-D-glucopyranoside on the uptake of 0.1 mM methyl α-[^{14}C]glucoside by washed whole cell suspensions of *E.coli* strains CE191 (*bglC**) and CE212 (*bglC*$^+$). Cells were grown to mid-exponential phase in 5 mM fructose (A) or 5 mM salicin (B) minimal medium, harvested and resuspended in phosphate buffer to ca. 0.7 mg dry mass/ml. Methyl α-[^{14}C]glucoside uptake was measured in strain CE191 (□,■) and CE212 (○,●) both in the absence (open symbols) and presence (closed symbols) of 1 mM unlabelled fructose (A) or 1 mM unlabelled *p*-nitrophenyl β-glucoside (B).

These results support the view that the *bglC** mutation enhances the ability of the uptake system for β-glucosides to interact with the phosphate, which is transferred from intracellular phosphoenolpyruvate via enzyme I, histidine-containing protein (Hpr) and possibly other factors that are not sugar-specific, to sugars competing for uptake from the medium. The *bglC** mutation also affects the ability of lactose, which is taken up via a proton symport mechanism, to compete with salicin. This effect is reversed by the *iex*-mutation. Therefore, since salicin uptake does not involve the glucose-specific factor III of the PT-system, it is proposed that the diversion of the phosphate flux through the chain of phosphate carriers, to favour the enzyme II for β-glucosides, has occurred at the

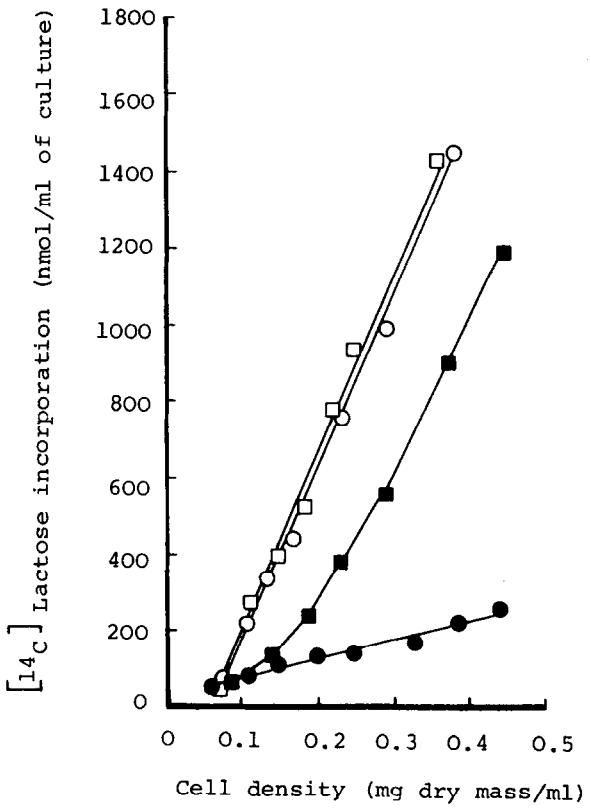

Fig.4. Incorporation of carbon from [^{14}C]lactose by strains of *E.coli* growing on a mixture of 2.5 mM [^{14}C]lactose and 5 mM unlabelled salicin as carbon source: (□,■) CE149 (wild-type *bgl*$^+$); (○,●) CE185 (β-glucoside-specific mutant *bglC**); (open symbols) incorporation of [^{14}C]lactose alone; (closed symbols) incorporation of [^{14}C]lactose in the presence of 5 mM unlabelled salicin. The cells were grown to mid-exponential phase on 5 mM salicin, harvested and resuspended into the above media.

level of Hpr-phosphate [19,20]. This remains to be established.

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