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The Effect of the *lacY* Gene on the Induction of IPTG Inducible Promoters, Studied in *Escherichia coli* and *Pseudomonas fluorescens*

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Abstract. The role of the *Escherichia coli lacY* gene product (the lactose permease) in the induction of isopropyl-β-D-thiogalactopyranoside (IPTG) inducible promoters was studied in *E. coli* and *P. fluorescens*. This was done by comparing strains containing a *lacIPOZYA* chromosomal insert with newly constructed strains containing inserts without the *lacY* gene (*lacIPOZ*). The lactose operon inserts were introduced as single-copy chromosomal inserts to eliminate differences in expression caused by differences in copy number. Comparison between the two types of inserts showed that the lactose permease was essential to allow growth on lactose by both bacteria and that the lactose permease plays an important role in transporting the inducer IPTG across the membrane of *P. fluorescens*. The use of a functional lactose permease allows expression of β-galactosidase to increase more than fivefold from a wild-type *lac* promoter in *P. fluorescens* SS1001. We suggest that an increase in the rate of protein synthesis from *lac*-type promoters could be enhanced if an active lactose permease is present as well.

Regulation of the *E. coli* lactose operon is one of the best studied areas in molecular biology. This knowledge has been used to construct several different *lac*-type promoters [1, 9, 24]. Common to most of these *lac*-type promoter systems is the regulatory system from the *E. coli* lactose operon. Most of these systems contain the *lacI* gene, supplier of the lac repressor, which prevents transcription from the promoter itself by binding to the lac operator region situated next to the promoter. mRNA transcription starts when the repressor is removed from the operator by an inducer. The most commonly used inducer for induction of these *lac*-type promoter systems is isopropyl-thio-β-D-galactoside (IPTG). In opposition to lactose (also an inducer of these systems if altered by β-galactosidase [18]), IPTG is not a substrate for β-galactosidase (the product of the *lacZ* gene) and is, therefore, not metabolized.

Lac-type promoters have been used for synthesizing a large variety of proteins in several Gram-negative bacteria including *E. coli* [6, 17, 21, 23, 29], *Pseudomonas putida* [6, 19, 31], *Myxococcus xanthus* [25], *Synechococcus* sp. PCC 7942 [13], *Rhizobium melotti* [6], and *Agrobacterium tumefaciens* [6].

For efficient transcription from these promoters, the IPTG must enter the cells efficiently. This gratuitous inducer is very expensive and may in high concentrations interfere with cell growth. Owing to the cost and possible toxic effect of IPTG, it would be advantageous to use less inducer to obtain the same level of transcription. Jensen et al. have shown that the *E. coli* lactose permease (the *lacY* gene product) takes part in transporting IPTG across the cell membrane [20]. However, owing to other mechanisms of transport (or diffusion), the contribution of the lactose permease has been reported not to be significant in *E. coli*, and induction in this species can easily be obtained without the lactose permease [3]. Consequently, the lactose permease has been left out in virtually all studies that have used *lac*-type promoters in species other than *E. coli* (in all studies referred to above). Since the reasons for the unconstrained uptake of IPTG in *E. coli* are not very clear, we set out to examine whether the lactose permease contribution could be greater when inducing other strains that in most cases show significantly lower gene expression from *lac*-type promoters than *E. coli* does.

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Table 1. Strain list

Strain	Species	Relevant genotypes or characteristics	References
CSH26	*E. coli*	Δ(*lac-pro*) *thi*	28
LH5.4–5.7	*E. coli*	CSH 26::*lacIPOZYA-kn-res* lac+ Km^R	15
LH4.1–4.4	*P. fluorescens*	SS1001::*lacIPOZYA-kn-res* lac+ Km^R	15
LH7.1–7.4	*P. fluorescens*	SS1001::*lacIPOZ-Kn-res* lac− Km^R	This study
LH8.1–8.4	*E. coli*	CSH 26::*lacIPOZ-kn-res* lac− Km^R	This study
MT102-PIR	*E. coli*	MT102::*pir-kn* π+	15
NF1815	*E. coli*	*leu thi* Sm^R	N. Fiil
SS1001	*P. fluorescens*	lac− gal+ Cm^R	34

Table 2. Plasmid list

Plasmid	Replicon	Reference	Markers
pLH3	p15A	15	Cm^R, *lacIPOZYA*
pLH4	R6K	15	Ap^R, Km^R, *lacIPOZYA*
pLH5	p15A	This study	Km^R, *lacIPOZ*
pLH6	R6K	This study	Ap^R, Km^R, *lacIPOZ*
RK600	pMB1	21	Cm^R, RP4*tra*+
pLOW1	p15A	15	Cm^R
pLOW2	p15A	15	Km^R
pUT-Kn-res	R6K	Sternberg, C., Pers. com., 15	Ap^R, Km^R

This report presents insertion vectors that can be used to answer this question in various Gram-negative bacteria, and it provides proof that in *Pseudomonas fluorescens*, at least, the lactose permease of *E. coli* contributes to enhance induction of an IPTG-inducible promoter.

Materials and Methods

Bacterial strains and plasmids. See description of all bacterial strains and plasmids used in Tables 1 and 2 respectively.

Media and growth conditions. LB broth [32] and minimal media [7] containing appropriate carbon sources (0.2 or 0.4% wt/vol) were used for growing *E. coli* and *P. fluorescens* strains. Antibiotics were added at the following concentrations; ampicillin (100 µg/ml), chloramphenicol (30 µg/ml) and kanamycin (20 µg/ml). Proline (50 µg/ml) and thiamine (2.5 µg/ml) were also added when *E. coli* strains were grown in minimal media.

E. coli strains were grown at 37°C, and *P. fluorescens* strains were grown at 30°C unless otherwise stated. Growth media were autoclaved. Antibiotics, carbon sources, and amino acids were sterilized by filtration (0.2 µm pore size, cellulose acetate, MFS 25 disposable syringe filter units, Micro Filtration Systems, CA) and added to the media after autoclavation. Solid media was made by adding 2% Bacto-Agar (Difco) to LB media or 2% Agar Noble (Difco) to minimal media.

Conjugations and recombinant DNA techniques. Cultures of donor, recipient, and helper strains were grown overnight in LB broth [32], containing relevant antibiotics to prevent plasmid loss. Cells were washed twice in LB medium without antibiotics. 50 µl of each culture was deposited on a cellulose nitrate filter (pore size, 0.2 µm; diameter, 47mm; Micro Filtration Systems). Filters were placed on LB agar plates, and filter matings were incubated overnight at 30°C. After incubation, filters were transferred to 9 ml minimal salt medium, vortexed to harvest cells from the filter, and dilutions were plated on appropriate transconjugant-selective media. Plasmid DNA extraction, restriction enzyme digests, ligations, transformations, agarose electrophoresis, and other standard recombinant DNA techniques were carried out by standard procedures as described by Sambrook et al. [32]. All enzymes were purchased from GIBCO BRL, Life Technologies.

DNA hybridizations. Chromosomal DNA was extracted from transconjugant strains by the method described by Grimberg et al. [14]. 2µg of chromosomal DNA from each strain was digested with 10 U of *Kpn*I for 3 h and separated by agarose gel electrophoresis [0.5% agarose (Sigma) in TAE buffer] at 1 V/cm for 15 h. DNA was transferred onto Nylon membranes (Boehringer Mannheim) with a 2016 Vacugen vacuum blotting unit (LKB, Sweden). Nonradioactive hybridizations were performed with an 825-bp *lacZ* probe labeled with dioxygenin by use of the DIG DNA labeling and detection kit (Boehringer Mannheim, cat. no. 1093657). This probe has previously been described by Hansen et al. [15]. Hybridizations and detections were carried out as suggested by the manufacturers.

Cloning of the *lacY*$^+$ and *lacY*$^-$ insertion fragments. The $lacY^+$ insertion fragment has previously been described by Hansen et al. [15]. It is a 12-kb fragment encompassing both the regulatory region with the lac repressor (*lacI*), the promoter region(*Plac*), the operator (*O*), and the three structural genes encoding enzymes used for lactose catabolism (*lacZ, Y* and *A*). The $lacY^-$ construct was made by inserting a 4.9-kb *Pst*I-*Dra*I fragment from pLH3 encompassing only the *lacIOP* and Z region into pLOW2 after this vector had been opened by a *Pst*I-*Hinc*II double digest. This plasmid is called pLH5. Both the *lacY*$^+$ and *lacY*$^-$ fragments were excised from pLH3 and pLH5 respectively as *Not*I fragments and inserted into the unique *Not*I site of pUT-kn-res (a mini-Tn5 delivery vector). This gave rise to pLH4 and pLH6, which could now be used to insert *lacIPOZYA* and *lacIPOZ* into the chromosome of a variety of Gram-negative bacteria including *E. coli* and *P. fluorescens.*

Insertion of the *lacY*$^+$ and *lacY*$^-$ fragments into the chromosome of *E. coli* and *P. fluorescens* strains. The two lac-insertion fragments described above were inserted into the chromosome of *E. coli* CSH26 and *P. fluorescens* SS1001 by a well-described mini-Tn5 delivery system [10, 15, 16]. The principle of the system is to clone any DNA sequence into the unique *Not*I site of one of numerous mini-Tn*5* suicide vectors. Next, you transfer this suicide vector into a recipient of choice by conjugation. After conjugation, the cloned DNA sequence will integrate on the chromosome of the recipient together with an antibiotic resistance marker. Insertion of the *lacIPOZYA* construct into *E. coli* CSH26 and *P. fluorescens* has previously been described by Hansen et al. [15]. Insertion of the *lacIPOZ* construct into the chromosome of *E. coli* CSH26 was obtained by performing tri-parental filter matings between NF1815/RK600 (a helper strain used to mobilize the transfer of pLH6), MT102-pir/pLH6 (the donor strain with delivery plasmid), and CSH26. Insertion of the *lacIPOZ* construct into the chromosome of *P. fluorescens* SS1001 was carried out by a tri-parental filtermating between NF1815/RK600, MT102-pir/pLH6, and SS1001. The CSH26::*lacIPOZ* clones were selected on minimal media containing glucose, proline, thiamine, and kanamycin; the SS1001::*lacIPOZ* clones were selected on minimal media containing only glucose and kanamycin.

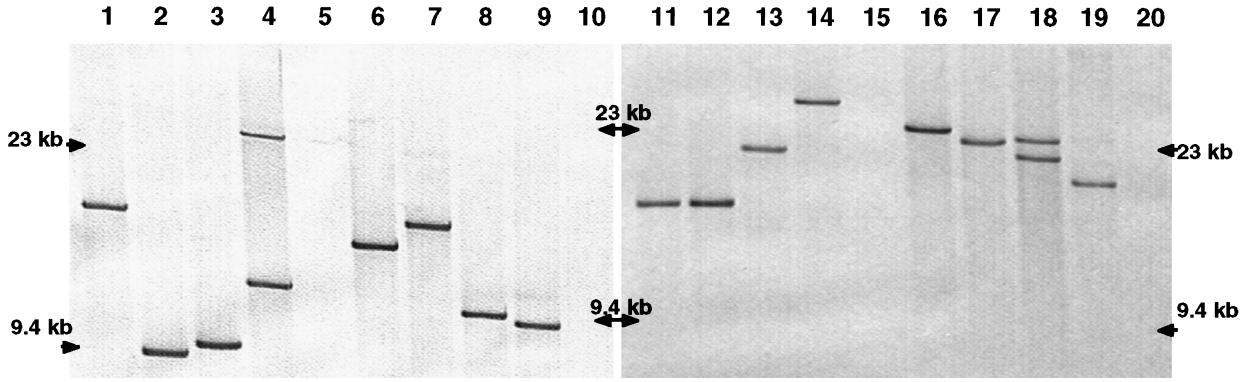

Fig. 1. Visualization of chromosomal inserts of lac-fragments in *E. coli* CSH26 and *P. fluorescens* SS1001 clones. Chromosomal DNA from all clones was prepared [14]; 2µg DNA from each strain was digested with 10 U *kpn*I for 3 h. DNA was separated by agarose gel electrophoresis on 0.5% wt/vol agarose gels. DNA was blotted onto Nylon membranes. Hybridization was performed with an 825-bp *lacZ* probe labeled with dioxygenin. Lanes 1 through 4, DNA from *P. fluorescens* LH7.1 through LH7.4. Lanes 6 through 9, DNA from *E. coli* LH8.1 through LH8.4. Lanes 11 through 14, DNA from *P. fluorescens* LH4.1 through LH4.4. Lanes 16 through 19, DNA from *E. coli* LH5.4 through 5.7. Lanes 5 and 15, DNA from *P. fluorescens* SS1001. Lanes 10 and 20, DNA from *E. coli* CSH26.

After selection, the helper plasmid RK600 was cured from the *E. coli* clones (RK600 can not replicate in Pseudomonas) by continuous growth in LB broth for 24 h at 42°C without antibiotics.

Verification of *lacZ* activity by plating on X-gal plates. The substrate 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) is a substrate for the *lacZ* gene product β-galactosidase. When cleaved, it forms a blue dye easily visible in colonies of strains containing β-galactosidase [3]. In this way it is possible to see whether the clones have received the *lacZ* gene. All clones were streaked out on LB agar plates containing IPTG (1 mM) and X-gal (50 µg/ml). Plates were incubated at 30°C, and blue colonies were detected.

Growth experiments. All strains were examined for growth on lactose minimal media by the following procedure. Strains were inoculated from the freezer in 10 ml LB broth and grown overnight. Cells were washed in a salt solution [33] and then inoculated into minimal medium [7] containing 0.2% lactose. All *E. coli* strains were also supplemented with 50 µg/ml proline and 2.5 µg/ml thiamine. Growth was then monitored by measuring the OD_{600} in a spectrophotometer (Ultrospec 2000, Pharmacia Biotech, Cambridge, England).

β-galactosidase assays. Strains for β-galactosidase assays were grown overnight in minimal media containing 0.4% glycerol. In addition, *E. coli* strains were supplemented with proline and thiamine. ON cultures were diluted to an optical density at 600 nm(OD_{600}) of approximately 0.005 in the same medium. During exponential growth, strains were induced with 0.1 mM isopropyl-β-D-thiogalactopyranoside(IPTG), and 500-µl samples were taken at the times indicated below. Cells were permeabilized by adding 10 µl of toluene to each sample and vortexing for 10 s. Toluene was evaporated by incubating samples for 40 min at 37°C. β-galactosidase activity was measured as described by Miller [27]. One β-galactosidase unit equals one Miller unit × OD_{600} at sample time. The differential rate of β-galactosidase synthesis (DRS) was defined by Monod as $\Delta Z/\Delta B$ [8], meaning the change in β-galactosidase activity relative to growth. In this study the DRS is calculated as the slope of units β-galactosidase plotted against OD_{600} (units/OD_{600}) of an induced culture. In addition to the IPTG concentration mentioned above, induction with 0.01mM and 1mM IPTG was also examined.

Testing for lactose permease activity in *E. coli* by incubation on melibiose plates at 42°C. The sugar melibiose can be utilized by *E. coli* as a growth substrate at 42°C only if the strain has a functional lactose permease, the *lacY* gene product [28], the reason being that the melibiose permease is inactivated at this temperature. However, melibiose can be transported into the cells by the lactose permease. This enabled us to test whether the *E. coli* clones constructed in this study had a functioning lactose permease. All clones tested were inoculated in LB broth and grown overnight. Cells were washed twice in Winogradsky medium and streaked on a minimal medium agar plate containing melibiose(0.2%), proline, and thiamine. The plate was incubated overnight at 42°C, and lactose permease activity was visually confirmed by appearance of growth.

Results and Discussion

We wanted to test whether the lactose permease was a significant contributor to the uptake of IPTG in Gram-negative bacteria. If so, the amount of protein produced from genes induced by IPTG would be expected to increase in strains containing a functioning lactose permease. This question is of great importance because IPTG-inducible promoters (*lac*-type promoters) are widely used to express proteins in Gram-negative bacteria other than *E. coli* (as described in the introduction). In order to examine this question, we constructed a series of clones of *E. coli* CSH26 and *P. fluorescens* SS1001. Half of these clones had all the lactose operon genes *lacIPOZYA* (*lacY*$^+$) inserted into the chromosome; the other half had a truncated insert of *lacIPOZ* (*lacY*$^-$). First we wanted to establish the fact that the copy number in all the clones was the same and to verify that the genes(*lacZ* and *lacY*) were functioning in the strains used. Next, we proceeded to induce the clones with relatively low concentrations of IPTG to examine the difference (if any) in β-galactosidase production.

Verification of lac inserts in *E. coli* CSH26 and *P. fluorescens* SS1001. Four clones of *E. coli* CSH26 plus

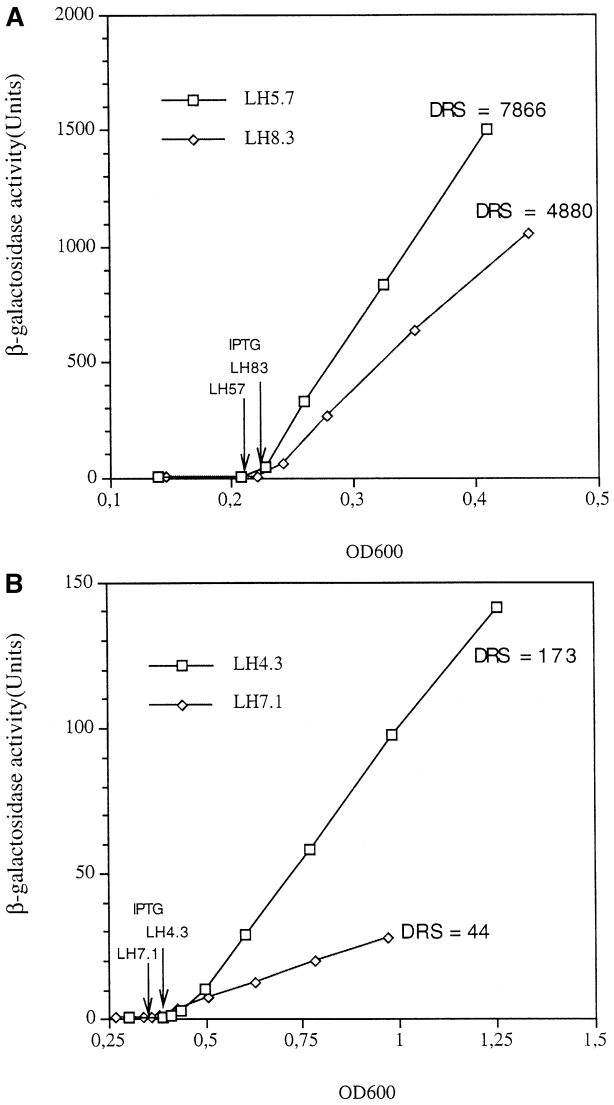

Fig. 2. Induction experiment to measure β-galactosidase expression from *Plac* in *E. coli* and *P. fluorescens* clones with or without the lactose permease (representative clones only). At the optical density indicated by arrows, IPTG was added to a final concentration of 0.1 mM. β-Galactosidase activity was measured as described in Materials and Methods, and the differential rate of synthesis (DRS) was determined as the slope of the induced curve. Fig. 2A: *E. coli* clones; Fig. 2B: *P. fluorescens* clones (se also Table 3 for DRS values for all clones assayed).

four clones of *P. fluorescens* SS1001 containing the *lacIPOZYA* inserts were isolated. These strains were designated LH5.4–5.7 and LH4.1–4.4 respectively [15]. In addition, four clones of CSH26 and SS1001 containing the *lacIPOZ* inserts were isolated and designated LH8.1–8.4 and LH7.1–7.4 respectively (Table 1). All clones were analyzed for chromosomal inserts by Southern blot analysis. This was done to ensure that the lac fragments were integrated in the chromosome, that they differed in integration sites, and to ensure that the lac inserts only integrated once per clone. Figure 1 shows the chromosomal digests of all 16 strains plus CSH26 and SS1001 hybridized to an 825-bp *lacZ* probe. As seen in Fig. 1, all selected clones contain one band (exceptions in lane 4 and 18) corresponding to one insert per clone. Chromosomal integration also seems to have occurred in different places for each clone. The two control strains CSH26 and SS1001 showed no hybridization to the *lacZ* probe. This confirms that all integrations were successful.

We tested all clones for β-galactosidase activity to ensure that the *lacZ* gene was functioning. This was done by streaking colonies on LB plates containing X-gal. All clones gave blue colonies on this medium, confirming the presence of β-galactosidase. To ensure that the *lacIPOZYA* clones contained a functioning lactose permease and that the *lacIPOZ* clones did not, all *E. coli* clones were spread-plated on minimal medium supplemented with melibiose, proline, and thiamine. The plates were incubated overnight at 42°C. Only clones LH5.4–5.7 showed growth on this medium, corresponding to the absence of the *lacY* gene from clones LH8.1–8.4. Such a test could not be performed in the *Pseudomonas* clones since they could not grow at temperatures above 37°C.

The role of *lacY* in clones growing on lactose. All clones were tested for growth on lactose to further verify the function of both genes in the strains used. The *lacIPOZYA* clones of both CSH26 and SS1001 were able to utilize lactose as a carbon source. When growing at 37°C in minimal medium supplemented with lactose as the only carbon source, the CSH26 clones had a doubling time of approximately 50 min. The SS1001 clones had a doubling time of approximately 150 min in the same medium at 30°C. None of the *lacIPOZ* clones were able to grow on this medium. Several studies have shown that strains of a variety of Gram-negative bacteria are able to utilize lactose as a sole carbon source when supplied with both the *lacY* and the *lacZ* genes [2, 11, 12, 22, 26, 30, 34]. Furthermore, the study made by Drahos et al. [11] showed that strains of *P. fluorescens* were unable to grow on lactose minimal medium unless they were provided with the *lacY* gene. However, the Drahos et al. study did not include the *lac* promoter. In addition to showing that the *E. coli* lactose permease was functioning in this particular *P. fluorescens* strain, the present study confirms that the lactose permease is necessary for transporting lactose into all clones tested. These observations suggest that little or no lactose enters the *P. fluorescens* cells by other transport systems or diffusion. The requirement of the lactose permease for growth on lactose is a well-known phenomenon in *E. coli* [3].

Table 3. Different tests described in this study

E. coli clones									
Clone	5.4	5.5	5.6[a]	5.7	8.1	8.2	8.3	8.4	CSH26
Growth on lactose	+	+	+	+	−	−	−	−	−
Melibiose, 42°C	+	+	+	+	−	−	−	−	−
X-gal blue	+	+	+	+	+	+	+	+	−
DRS[c] (0.1 mM IPTG)	10517	7713	12140	7866	5845	4842	4880	6507	0
DRS mean	8699 ± 1576				5519 ± 806				n.d.[b]
P. fluorescens clones									
Clone	4.1	4.2	4.3	4.4	7.1	7.2	7.3	7.4[a]	SS
Growth on lactose	+	+	+	+	−	−	−	−	
Melibiose, 42°C	n.d.[b]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	r
X-gal blue	+	+	+	+	+	+	+	+	
DRS[c] (0.1 mM IPTG)	260	235	173	n.d.	44	57	27	68	
DRS mean	223 ± 45				43 ± 15				r

[a] Owing to double inserts, these strains are ignored when calculating mean values.
[b] n.d.: not determined owing to lack of growth or activity.
[c] DRS: Differential rate of β-galactosidase synthesis.

The role of *lacY* in the uptake of lac-operon inducer. To study whether the lactose permease delivered any significant contribution to the transport of IPTG across the membrane of both *E. coli* and *P. fluorescens*, we performed induction experiments on all 16 clones and on the two control strains CSH26 and SS1001. All clones were grown in glycerol minimal medium. During exponential growth, 0.1 mM IPTG was added to cultures, and growth was monitored throughout the experiment. Samples were taken before and after induction to monitor β-galactosidase activity in both uninduced(basal level) and induced states (see Fig. 2 for representative clones). As seen in Table 3, the two control strains had no detectable β-galactosidase activity. All clones, however, showed a basal level of β-galactosidase activity. Basal levels for the *E. coli* clones were between 5 and 8 Miller units. The *P. fluorescens* clones had a lower basal level at 1–3 Miller Units. Differential β-galactosidase synthesis rates for all clones measured are shown in Table 3. The differential rate of β-galactosidase synthesis in *E. coli* was 1.5-fold higher in the *lacIPOZYA* clones than the *lacIPOZ* clones. Although statistically significant (Students *t*-test, $p < 0.05$), this difference may not be of importance when trying to increase production of proteins using *lac*-type promoters. In the case of the SS1001 clones, however, the rate of β-galactosidase synthesis was increased fivefold in induced clones that included the *lacY* gene compared with the clones that did not. Consequently, the lactose permease is a very important contributor in transporting IPTG across the *P. fluorescens* membrane. The increased accumulation of IPTG inside the cells containing the lactose permease can be of great value when expressing proteins from promoters that are induced by IPTG. This could be of importance when using IPTG as an inducer in heterogeneous environments such as soil, where it is often nessesarry to add high concentrations of IPTG to achieve sufficient induction [5].

The β-galactosidase activity levels of clones LH5.6 and LH7.4 showed clearly that they produced more β-galactosidase than the other clones of similar types (Table 3). This corresponds very well with the results from the Southern blot above, which showed double integration for these two clones.

Induction in the *P. fluorescens* clones was also tested with the IPTG concentrations 0.01 mM and 1 mM. With the high IPTG concentration, both the *lacIPOZYA* and the *lacIPOZ* clones yielded high levels of induction. The fivefold difference in DRS seen at 0.1 mM IPTG decreased to approximately 1.5. This is probably owing to increased IPTG diffusion across the bacterial membrane, decreasing the contribution of the lactose permease in IPTG uptake. Addition of 0.01 mM IPTG showed no significant induction in either clone (Table 4). The importance of the *lacY* gene in IPTG induction is emphasised by the fact that the average DRS in the *lacIPOZYA* clones induced with 0.1 mM IPTG is comparable to the average DRS of the *lacIPOZ* clones induced with 1 mM IPTG (Table 4). In an additional experiment we added 0.2% lactose to cells of LH4.4 and LH7.4 (see

Table 4. Mean differential rate of β-galactosidase synthesis (DRS) at different IPTG concentrations in *P. fluorescens* clones

IPTG concentration (mM)	Mean DRS LH4.1–4.4 *lacIPOZYA*	Mean DRS LH7.1–7.3 *lacIPOZ*	DRS increase *LacY*$^+$/*lacY*$^-$
0.01	<10	<10	n.d.[a]
0.1	223	43	5.2
1	319	212	1.5

[a] n.d.: not determined owing to low enzyme activities.

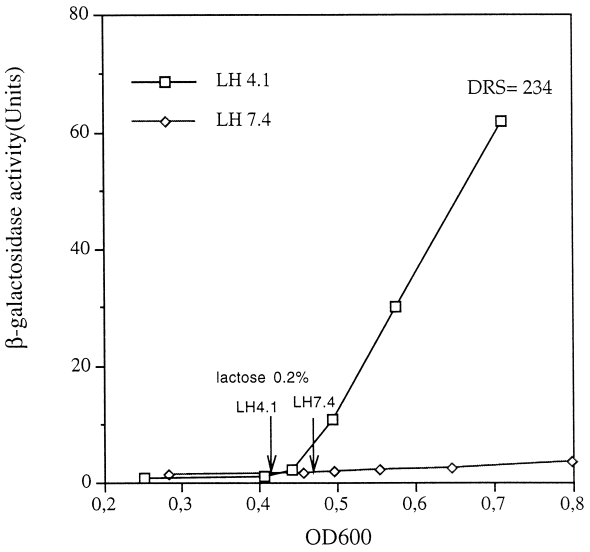

Fig. 3. Induction experiment to measure *lacZ* expression from *Plac* in *P. fluorescens* clones with (LH4.1) or without (LH7.4) the lactose permease. At the optical density indicated by arrows, lactose was added to a final concentration of 0.2%. β-galactosidase activity was measured as described in Materials and Methods, and the differential rate of synthesis (DRS) was determined as the slope of the induced curve.

Fig. 3). The figure shows that there was a rapid increase in β-galactosidase activity in LH4.4 after induction with lactose, while no increase was observed in LH7.4(*lacY*$^-$). Actually, the rate of β-galactosidase synthesis was comparable to that of the same strain induced with IPTG (compare Fig. 3 and Table 3). This could be of significant economic value since the cost of lactose is only a fraction of the cost of IPTG (even if IPTG is a gratuitous inducer and lactose is not).

The role of the lactose permease in transporting IPTG across the bacterial membrane has not been considered to be very important [4] even though it has been shown to contribute to IPTG transport and alter the induction pattern in *E. coli* when induced with low concentrations of IPTG [20]. This is probably owing to the relatively small difference in β-galactosidase production when *lacY*$^+$ and *lacY*$^-$ strains of *E. coli* are induced with IPTG, as shown in the present study. However, as genetic manipulations of bacteria have evolved to include species of Gram-negative bacteria other than *E. coli*, it becomes increasingly important to consider how inducers are transported into the cells of these species. This study clearly shows that there is indeed a significant difference in protein production from *lac*-type promotors induced with IPTG. If the *lacY* gene is present in *P. fluorescens* SS1001, the rate of β-galactosidase production increases more than fivefold when induced with 0.1 mM IPTG compared with clones without the *lacY* gene. Since this increase can be due only to an increased concentration of IPTG inside the cells, the presence of *lacY* can also enhance protein production when other proteins are produced from any promoter that uses IPTG as the inducing element. As stated in the introduction, *lac*-type promoters are widely used in cloning of many Gram-negative bacteria. The results of this study suggest that this induction system can become even more powerful than it is at present.

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