RESEARCH LETTER

Enhanced expression of the *bgl* operon of *Escherichia coli* in the stationary phase

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Abstract

The *bgl* operon is silent and uninducible in wild-type strains of *Escherichia coli* and requires mutational activation for optimal expression. We show that transcription from the wild-type and the activated *bgl* promoter exhibits a growth phase-dependent enhancement that is highest in the stationary phase. We have assessed the effect of mutations in *rpoS*, *crl*, *hns*, *leuO* and *bglJ*, known to regulate *bgl* expression, on the growth phase-dependent increase in *bgl* activity. These studies show that this increase is greater in the absence of wild-type *rpoS* and/or *crl*. Our studies also indicate that while BglJ has a moderate effect on the expression of the *bgl* operon in the stationary phase in the absence of *rpoS*/*crl*, the modest increase in LeuO concentration in the stationary phase is insufficient to affect transcription from the *bgl* promoter. Measurements of the fitness of strains carrying the wild type or a null allele of *crl* showed that, while the strain deleted for *crl* exhibited a growth advantage over the crl^+ strain in an $rpoS^+$ background, it showed a low-level disadvantage in the presence of an *rpoS* allele that results in attenuated RpoS expression. Possible physiological implications of these results are discussed.

Introduction

The *bgl* operon, responsible for the uptake and utilization of aryl-β-glucosides, salicin and arbutin, is maintained in a silent and uninducible state in most wild-type strains of *Escherichia coli*, resulting in a Bgl^- phenotype. Bgl^+ mutants arise in the population spontaneously at a detectable frequency (Prasad & Schaefler, 1974; Reynolds *et al*., 1981, 1986; Schnetz & Rak, 1992). Sequences that include DNA structural elements as well as sites for the binding of the global repressor H-NS are responsible for silencing of the *bgl* operon and activation is the result of disruption of these negative elements (Lopilato & Wright, 1990; Schnetz, 1995; Singh *et al*., 1995; Schnetz & Wang, 1996; Mukerji & Mahadevan, 1997a). Apart from *cis*-acting mutations in the *bgl* locus, several *trans*-acting mutations that result in the inactivation of H-NS, alteration of DNA supercoiling and overexpression of BglJ or LeuO, have also been shown to activate the *bgl* operon (Defez & de Felice, 1981; Di Nardo *et al*., 1982; Giel *et al*., 1996; Ueguchi *et al*., 1998; Madhusudan *et al*., 2005). Once activated, *bgl* expression is specifically regulated by aryl-β-glucosides involving a mechanism of transcriptional antitermination (Mahadevan & Wright, 1987; Schnetz & Rak, 1988; Amster-Choder *et al*., 1989; Amster-Choder & Wright, 1990, 1992) and is subject to catabolite control (Gorke & Rak, 1999; Gulati & Mahadevan, 2000). Although silent under laboratory conditions, this operon may be expressed under specific physiological conditions, conferring a selective advantage to the organism (Mukerji & Mahadevan, 1997b). The observation that in a pathogenic isolate of *E. coli*, the wild-type *bgl* promoter is active *in vivo*, i.e., in mouse liver but not *in vitro* (Khan & Isaacson, 1998) supports this hypothesis.

For enteric bacteria such as the *E. coli*, the intestinal environment allows a comfortable existence most of the time. However, outside the host and even in nonintestinal environments within the host such as those encountered during infection, they may experience low nutrient levels, variation in pH, temperature and osmolarity and competition from other microorganisms. The expression profile and regulation of many genes might vary under such conditions. Even during growth in a nutrient-rich medium, bacteria experience somewhat similar stress as they enter the stationary phase. In an attempt to examine the expression

of the *bgl* operon under such stress, we have assessed and compared the activity of the wild-type and activated *bgl* promoter at different phases of the growth. The possible factors likely to be involved in regulating the *bgl* promoter under such conditions have also been considered.

The stationary phase-specific sigma factor RpoS (Lange & Hengge-Aronis, 1991) and the stationary phase factor Crl that is involved in the regulation of many genes of the RpoS regulon have been shown to negatively regulate *bgl* expression (Dole *et al.*, 2002; Schnetz, 2002). We have investigated the effect of *rpoS*/*crl* on *bgl* expression at different phases of growth and in strains carrying mutations in different genes that are known to affect *bgl* expression. Our results show a modest effect of BglJ in stationary phase-specific enhancement of *bgl* expression in the absence of negative regulation by RpoS/Crl and no role for LeuO in the enhancement.

Materials and methods

Bacterial strains and growth media

The bacterial strains and plasmids used in this study are listed in Table 1. Cells were grown with aeration in Luria–Bertani (LB) medium (HiMedia) at 37 °C. Antibiotics were added at the following concentrations: tetracycline (15 μg mL^{-1}), chloramphenicol (15 μg mL^{-1}), ampicillin (100 μg mL^{-1}) and kanamycin (50 μg mL^{-1}). P1 transductions were performed as described by Miller (1992).

Construction of *bglJ*, *leuO* and *crl* deletion strains

The *bglJ* and *leuO* deletion strains were created using the procedure of Datsenko & Wanner (2000). Hybrid primers with 36 nucleotide extension homologous to the *bglJ* gene (region spanning −182 to +523) were designed for the template plasmid pKD3 carrying the chloramphenicol resistance gene that is flanked by FLP recognition target (FRT) sites (forward primer: 5′-aac ttg cct tct gtt tat gga gat gcc gca gaa tgg gtg tag gct gga gct gct tcg-3′; reverse primer: 5′-tag gga tgc aac aca tta ctt gtt tcg caa tgc cgc cat atg aat atc ctc ctt a-3′). The PCR product thus generated was used to replace the *bglJ* gene in the strain BGL1 using the helper plasmid pKD46 expressing the phage λ-*red* recombinase, with chloramphenicol resistance serving as the selectable marker. The presence of the deletion was confirmed using PCR analysis. Similarly, hybrid primers with 36 nucleotide extension homologous to the *leuO* gene (region spanning −167 to +768) were designed for the template plasmid pKD3 (forward primer: 5′-cca cgg caa tgg att ctg ttt tta tca gaa ccc gta gtg tag gct gga gct gct tcg-3′; reverse primer: 5′-cga cca aat gcg ttt gcg aca cca cgc taa gta cgc cat atg aat atc ctc ctt a-3′). The PCR product thus generated was used to replace the *leuO* gene in the strain BGL1 expressing the helper plasmid pKD46. The presence of the deletion was confirmed using PCR analysis. To create the double deletion, the antibiotic resistance gene was eliminated from Δ*leuO*::*cat* using a helper plasmid pCP20 that encodes the FLP recombinase, leaving an 80 bp scar. The deletion was

Table 1. Bacterial strains and plasmids used in this study

Strain/plasmid	Relevant genotype or phenotype	References or sources
BGL1	CSH26 Δ*crl*λ(Φ*bgl-lacZ*) Bgl$^-$ Crl$^-$	Ueguchi *et al.* (1996)
BGL1H	BGL1 *hns*::*amp* Bgl$^+$ Crl$^-$	This study
BGL1R	BGL1 *rpoS359*::Tn*10* Bgl$^-$ Crl$^-$	This study
BGL1-S	BGL1 *bglR*::IS*1*, Bgl$^+$ Crl$^-$	This study
BGL1-SR	BGL1-S *rpoS359*::Tn*10* Bgl$^+$ Crl$^-$	This study
BGL1-SJ	BGL1-S Δ*bglJ*::*cat* Bgl$^+$ Crl$^-$	This study
BGL1-SL	BGL1-S Δ*leuO*::*cat* Bgl$^+$ Crl$^-$	This study
BGL1-SLJ	BGL1-S Δ*leuO* Δ*bglJ*::*cat*	This study
CU305	BGL1 LeuO$^+$ Bgl$^+$ Crl$^-$	Ueguchi *et al.* (1998)
CU305H	CU305 *hns*::*amp* LeuO$^+$ Bgl$^+$ Crl$^-$	This study
RV	F$^-$Δ*lacX74 thi bglR*^{0} Bgl$^-$ Crl$^+$	Moorthy & Mahadevan (2002)
RVCrl	RVΔ*crl*::*kan* Bgl$^-$ Crl$^-$	This study
MM1	RV *tna*::Tn*10* Bgl$^-$ Crl$^+$	Moorthy & Mahadevan (2002)
ZK819	ZK126 *rpoS819* Str^R Bgl$^-$ Crl$^+$	Zambrano *et al.* (1993)
ZK819Crl	ZK819Δ*crl*::*kan* Bgl$^-$ Crl$^-$	This study
ZK819 Tn*10*	ZK819 *tna*::Tn*10* Bgl$^-$ Crl$^+$	Madan *et al.* (2005)
DY330	W3110 Δ*lacU*169*gal*490 [λc1857 Δ(*cro-bioA*)]	Lee *et al.* (2001)
pKESK19	*crl* gene clone under *tet* promoter in pBR322	Schnetz (2002)
pCP20	Plasmid expressing FLP recombinase	Cherepanov & Wackernagel (1995)
pKD3	Template plasmid carrying *cat* cassette flanked by FRT repeats	Datsenko & Wanner (2000)
pKD4	Template plasmid carrying *kan* cassette flanked by FRT repeats	Datsenko & Wanner (2000)
pKD46	Red recombinase expression plasmid	Datsenko & Wanner (2000)

confirmed using PCR analysis. The Δ*bglJ*::*cat* mutation was introduced into the strain by P1 transduction and was confirmed using PCR analysis. For constructing a deletion of the *crl* locus, hybrid primers with a 36-nucleotide extension homologous to the *crl* gene (region spanning −26 to +438) were designed for the template plasmid pKD4 carrying the kanamycin resistance gene that is flanked by FRT sites (forward primer: 5′-cag ttg cat cac aac agg aga tag caa tga cgt tac gtg tag gct gga gct gct tcg-3′; reverse primer: 5′-aat att gcc gga tgt gat gca tcc ggc aca ttt cac cat atg aat atc ctc ctt a-3′). The PCR product thus generated was used to replace the *crl* gene in the strain DY330. The presence of the deletion was confirmed using PCR analysis and Δ*crl*::*kan* was moved to different strains by P1 transduction.

β-Galactosidase assays

Measurements of β-galactosidase activities were carried out as described by Miller (1992). Bacterial strains were grown overnight in LB medium at 37 °C and were used to inoculate 20-mL LB medium with 7 mM salicin as an inducer. Cells were harvested at different phases of growth, and assays for β-galactosidase activity were performed. The results presented are an average of at least three independent experiments.

Competition assays

Cultures were grown in 3 mL of LB broth in glass test tubes under aeration in a New Brunswick shaker. Mixed cultures were started after 24 h of growth by transferring appropriate volumes of the two cultures in a fresh tube with a total final volume of 3 mL. The titres of the cultures were determined by plating different dilutions on LB plates containing the appropriate antibiotics.

Results

Activity of the *bgl* promoter increases in the stationary phase

BGL1, a lambda lysogen carrying a *lacZ* transcriptional fusion to the wild-type *bgl* promoter at the *attB* site and a wild-type *bgl* operon at its original chromosomal location (Ueguchi *et al*., 1996), was used to measure the expression of the *bgl* promoter in terms of β-galactosidase activity inducible by salicin. The *bgl* promoter activity increased across the growth phase and there was about a fourfold enhancement in the activity in the stationary phase as compared with that in the early exponential phase (Fig. 1a). When BGL1-S, a derivative of BGL1 in which the *bgl* operon at its normal chromosomal locus is activated by an IS*1* insertion while the *bgl* promoter fused with *lacZ* is wild type, was used for similar assays, there was over 10-fold

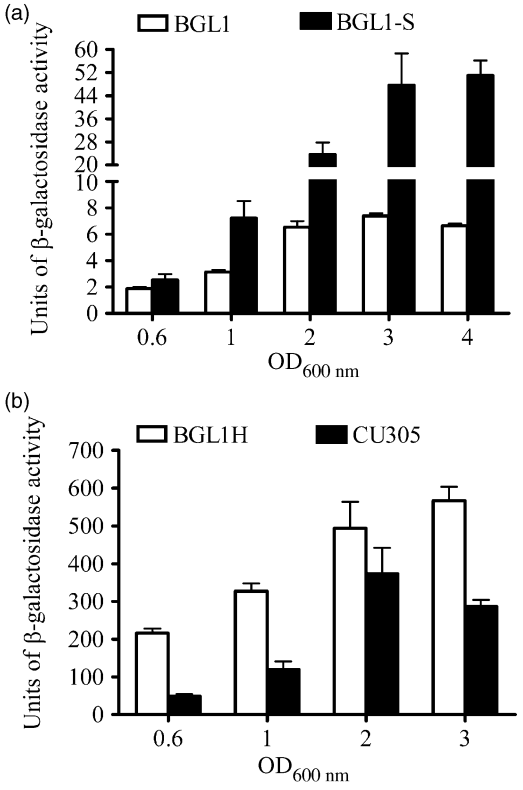

Fig. 1. Activity of the chromosomal *bglR*^{0}–*lacZ* fusion in (a) BGL1 and BGL1-S (b) BGL1H (*hns*) and CU305 (LeuO^{+}).

higher activity in stationary phase compared with that in early exponential phase (Fig. 1a). In this strain, higher levels of BglG produced by the activated *bgl* operon lead to amplification of the transcriptional signal from the *lacZ* fusion. When a mutant allele of *hns* was introduced into BGL1 by means of P1 transduction, leading to activation of both copies of the *bgl* promoter, there was about a threefold further increase in the activity of the *bgl* promoter in the stationary phase as compared with the early exponential phase (Fig. 1b). Similarly, CU305, a derivative of BGL1 in which both copies of the *bgl* promoter are activated by the overexpression of LeuO (Ueguchi *et al*., 1998), showed close to a sixfold increase in *bgl* activity in the stationary phase as compared with the early exponential phase (Fig. 1b). The growth rates in LB medium of all the strains used were comparable (data not shown).

The *bgl* operon is negatively regulated by RpoS and Crl

Although wild type with respect to *rpo*S, the reporter strain, BGL1, a derivative of CSH26, carries a deletion in *crl*. In an attempt to study the effect of RpoS and Crl on *bgl* expression in the stationary phase, the different reporter strains

described above were transformed with the plasmid pKESK19 that carries a copy of the wild-type *crl* gene under the control of a constitutive promoter (Schnetz, 2002). These transformants were used for the measurement of *bgl* promoter activity at different phases of growth as described above. In the presence of wild-type RpoS and Crl, there was a reduction of *bgl* expression in all the strains tested compared with the expression in the absence of either (Figs 2a, b and 3a, b). However, there was a modest stationary phase-dependent increase in *bgl* activity even in the presence of RpoS and Crl in all the strains tested (Figs 2a, b and 3a, b). In the earlier reports, the negative effect of RpoS on *bgl* expression has been shown in the exponential phase of growth (Dole *et al.*, 2002; Schnetz, 2002). In this study, the effect of RpoS and Crl has been studied at different growth phases of growth, using strains carrying different alleles of *bgl*.

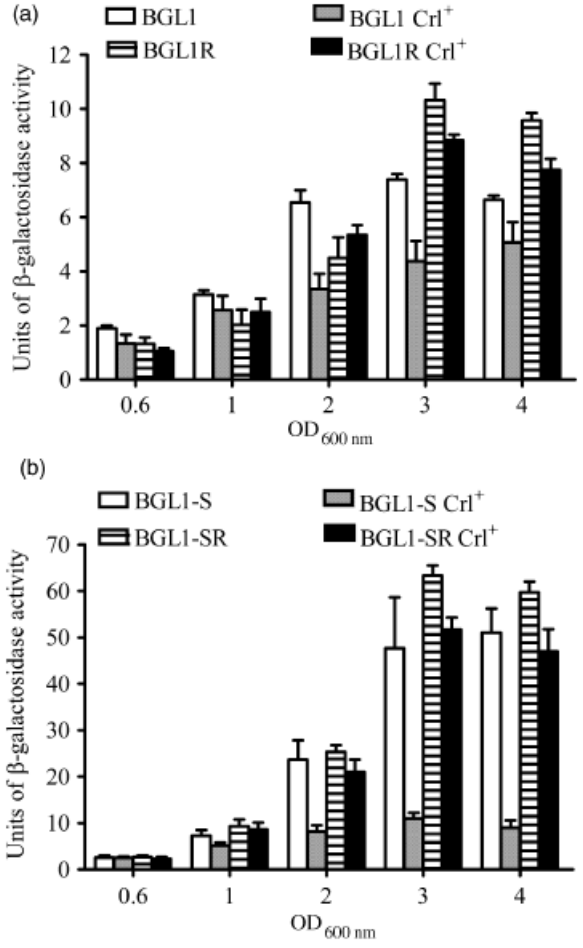

Fig. 2. Activity of the chromosomal *bglR*^{O}*–lacZ* fusion in (a) BGL1 and (b) BGL1-S in the presence or absence of RpoS and/or Crl (introduced on a plasmid).

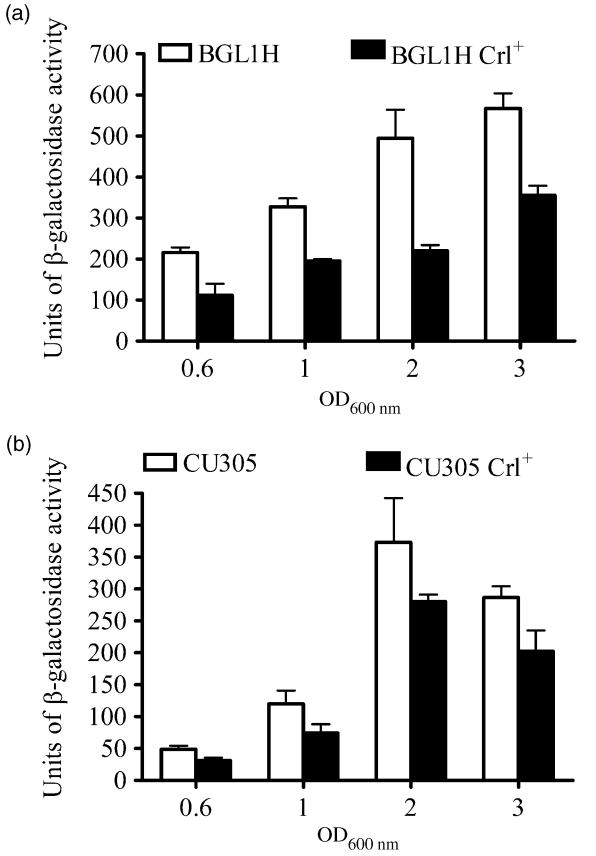

Fig. 3. Activity of the chromosomal *bglR*^{O}*–lacZ* fusion in (a) BGL1H and (b) CU305 in the absence or presence of Crl (carried on a plasmid).

Role of BglJ and LeuO in the regulation of the *bgl* operon

To identify the possible factors responsible for the growth phase-dependent enhancement in *bgl* promoter activity, independent deletions of *bglJ* and *leuO* were created in BGL1 using the λ-*red* recombination system (Datsenko & Wanner, 2000) and the *bgl* promoter activity was measured as described earlier. Deletion of *bglJ* had a moderate effect (less than twofold) on the expression of the *bgl* operon in the absence of Crl (Fig. 4a). However, in the presence of Crl, i.e. under the negative influence of RpoS, BglJ had no effect on the regulation of the *bgl* operon (Fig. 4b). In contrast, *leuO* deletion had no significant effect on the growth phase-dependent increase in the *bgl* promoter activity in the presence or absence of Crl (Fig. 4a and b). The *bgl* promoter activity in the strain carrying a *leuO bglJ* double deletion was similar to that of a strain carrying a deletion of *bglJ*. Deletion of *bglJ* or *leu*O in the presence or absence of Crl did not have any significant effect on *bgl* expression in the *hns* mutant where expression is already high (data not shown). The *bgl* promoter activity in a double mutant carrying a loss of function mutation in *hns* and a *leuO* mutation leading to its

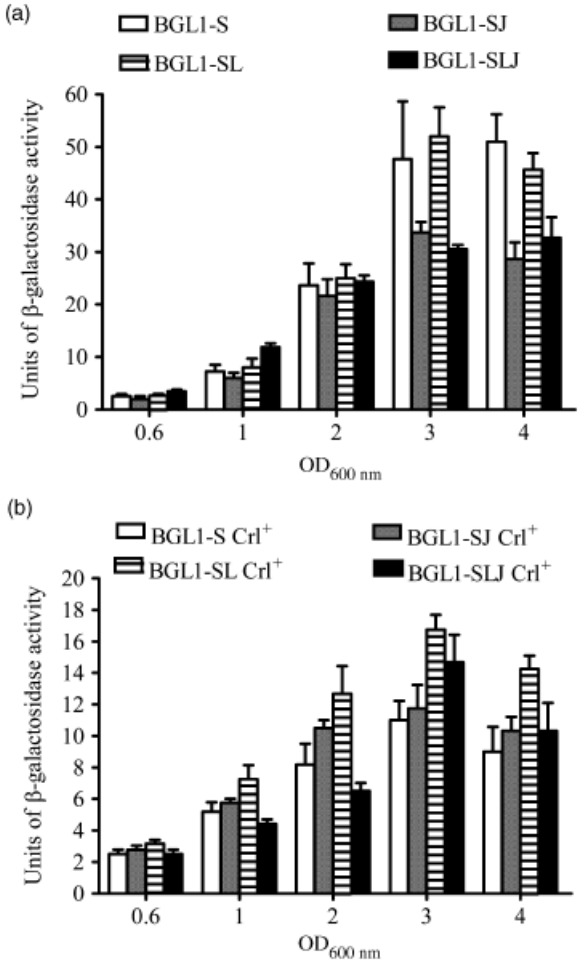

Fig. 4. Activity of the chromosomal *bglR*^{0}–*lacZ* fusion in BGL1-S with *bglJ* and/or *leuO* deletion in (a) the absence of Crl and (b) the presence of Crl (carried on a plasmid).

overexpression is similar to that of a strain with only the *hns* mutation with no further enhancement (data not shown).

Strains carrying *crl* deletion show a growth advantage in stationary phase (GASP) phenotype over the wild-type strain

Because *crl* mutations lead to an enhancement of *bgl* expression and activation of the *bgl* operon has been shown to confer a GASP phenotype (Madan *et al*., 2005), it is likely that mutations in *crl* confer a fitness advantage in the stationary phase. To determine this, a deletion of the *crl* locus was created using the λ-*red* recombination system as described above. The deletion was moved to the laboratory strain RV (Moorthy & Mahadevan, 2002), which is wild type for *rpoS* and *bgl*, as well as to ZK819, which is wild type for *bgl* and carries the *rpoS819* allele resulting in attenuated expression of *rpoS*. The *rpoS 819* allele has a 46 bp duplication at the 3′ end, resulting in a frame-shift that replaces the last four amino acids of RpoS with 39 new residues (Zambrano *et al*., 1993; Madan *et al*., 2005). RVCrl was competed against the isogenic MM1, which is wild type for *crl*. One-day-old cultures of the two strains grown in LB were mixed at a ratio of 1 : 1000 and 1000 : 1, and the populations were monitored by a viable count assay. Each competition was performed at least four times. Results from representative experiments are shown in Fig. 5a. The *crl* deletion strain increased in number when present in minority and showed a distinct GASP phenotype over the *crl*$^{+}$ strain. Similarly, ZK819Crl was competed against ZK819 Tn*10*, which is wild type for *crl*, and representative figures are shown (Fig. 5b). The results indicate that the strain having a mutant *rpoS819* allele and a deletion of *crl* has a disadvantage over the *crl*$^{+}$ strain carrying the *rpoS819* allele, when present in minority.

Discussion

The results described above show that expression of the normally silent *bgl* operon of *E. coli* is growth phase-dependent and enhances by nearly fourfold in the stationary phase as compared with that in the early exponential phase. In addition, the expression from different activated alleles of *bgl* was also found to be the highest in the stationary phase. These results, taken together with the earlier findings that the expression of the wild-type *bgl* operon increases by about 22-fold under anaerobic conditions of growth (Lopilato & Wright, 1990) and that the wild-type *bgl* operon is derepressed under the *in vivo* conditions of mouse liver (Khan & Isaacson, 1998), suggest that there could be specific physiological conditions under which the expression of the apparently silent *bgl* operon is enhanced. The results presented here are consistent with the microarray data that show an increase in the expression of *bglG* (2.54-fold) and *bglB* (1.48-fold) in the stationary phase in the strain MG1655 grown in LB (Selinger *et al*., 2000). Interestingly, the expression of other β-glucosidase genes has also been shown to be enhanced in the stationary phase in microarray data (*bglA*: 10.44-fold; *ascF*: 3.07-fold; *ascG*; 3.47-fold; *celD*: 2.39-fold; *celC*: 2.86-fold) (Selinger *et al*., 2000). However, these results need to be validated and their physiological significance needs to be ascertained.

It is not clear as to how bacteria benefit by increasing expression from the wild-type *bgl* promoter by fourfold in the stationary phase because this increase is not sufficient to enable β-glucoside utilization. In nature, however, bacteria encounter several kinds of stress and there might be a cumulative effect resulting in appreciable enhancement in the wild-type *bgl* promoter activity to enable the organism to utilize β-glucosides. Alternatively, a modest increase in *bgl* activity might be involved in some other cellular function. For example, the transport mechanism used by the

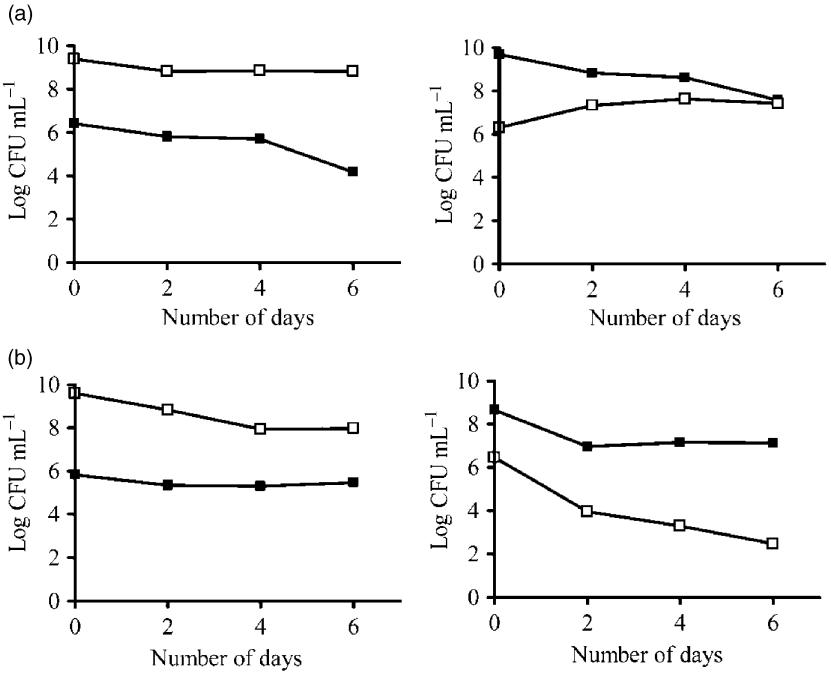

Fig. 5. Competition experiment between (a) RVCrl (□) and MM1 (■) and (b) ZK819Crl (□) and ZK819 Tn*10* (■). One-day-old cultures of the two strains were mixed in ratios of 1 : 1000 and 1000 : 1, and viable counts were taken at regular time intervals.

bgl system could be involved in the uptake of other molecules available under conditions of stress. Another possibility is that the BglG–BglF-signalling system associated with the induction of the operon by β-glucosides may regulate additional target genes involved in stress regulation. Regulation of gene expression associated with pathogenesis has been shown to be mediated by homologues of the BglG–BglF signalling system in *Listeria monocytogenes* (Brehm *et al.*, 1999).

Bgl$^+$ mutants have been shown to exhibit a GASP phenotype over the wild-type Bgl$^-$ counterpart when they carry an *rpoS* allele that results in attenuated RpoS expression (Madan *et al.*, 2005). Although the expression of the *bgl* operon is modestly enhanced in the stationary phase, a further enhancement by means of a genetic change is likely to give it a definite advantage over a strain having a wild-type copy of the *bgl* operon and such mutants are expected to be selected in the stationary phase.

It has been shown that RpoS, along with Crl, negatively regulates the *bgl* operon (Dole *et al.*, 2002; Schnetz, 2002). The results reported above have confirmed a similar effect throughout the growth phase and in strains carrying different alleles of *bgl*. Thus, it appears that this negative regulation is a general phenomenon, independent of the factors involved in the silencing and activation of the *bgl* operon. Even in the presence of this negative regulation, there is a modest enhancement of *bgl* expression in the stationary phase. When the negative effect of RpoS and Crl is abrogated, a distinct enhancement of *bgl* expression is seen in the stationary phase.

The most likely candidate responsible for the stationary phase-specific enhancement of *bgl* expression is the nucleoid protein H-NS, as its role in the negative regulation of *bgl* expression is well documented. H-NS mutations are also pleiotropic and its levels are known to go down during stationary phase (Azam *et al.*, 1999). However, the stationary phase-specific enhancement in *bgl* expression is seen even in the absence of functional H-NS, both in the presence and in the absence of Crl. Therefore, H-NS is unlikely to be responsible for the enhancement.

Long-term cultures of *E. coli* are known to accumulate large deletions spanning several kilo bases of the chromosome. Deletions spanning 19 kb of sequences located between 5.2 and 5.9 min on the *E. coli* chromosome confer a selective advantage in stab culture thought to be related to the loss of the *crl* gene (Faure *et al.*, 2004). The results presented above indicate that a strain carrying a *crl* deletion has a growth advantage over the *crl*$^+$ strain in the stationary phase. This is similar to reports where *crl* deletion has been shown to increase the competitive fitness of *Salmonella* in the stationary phase (Robbe-Saule *et al.*, 2007). Alleles of *rpoS* with attenuated expression are also known to confer a competitive advantage to strains during the stationary phase (Zambrano *et al.*, 1993). Crl has been shown to be an important factor for modulating the activity of RpoS and is known to be involved in the regulation of a subset of the *rpoS* regulon (Pratt & Silhavy, 1998; Typas *et al.*, 2007). Crl binds to σ^s and enhance its transcriptional activity by facilitating the $E\sigma^s$ holoenzyme formation (Bougdour *et al.*, 2004; Typas *et al.*, 2007). Hence, the effect of *crl*

deletion is likely to be similar to having an attenuated *rpoS* allele by reducing the levels of $E\sigma^s$ holoenzyme. In addition, *crl* deletion may also be advantageous as a result of the enhancement of *bgl* expression. However, when combined with an *rpoS* allele that leads to attenuated RpoS expression, the effect of the mutation in *crl* is likely to have a drastic effect on RpoS activity, resulting in a compromised stress response.

Polymorphism in *rpo*S is common in *E. coli* and *Salmonella typhimurium*. Different alleles of *rpoS* are known to be advantageous under different environmental conditions. Mutations in *rpo*S are known to accumulate in stationary-phase batch cultures and in steady-state glucose-limited chemostat populations of *E. coli* (Notley-McRobb *et al*., 2002, 2003; King *et al*., 2006). Wild-type alleles of *rpoS* have an advantage at acidic pH (Farrell & Finkel, 2003), while loss of *rpoS* is under positive selection in batch cultures (Chen *et al*., 2004). Null alleles of *rpoS* have a distinct competitive advantage in chemostats (Notley-McRobb & Ferenci, 2000; Notley-McRobb *et al*., 2002; Ferenci, 2003). While *rpoS* function is known to be a disadvantage during colonization of mouse intestine (Krogfelt *et al*., 2000), *rpoS* null mutants are outcompeted by wild-type strains and have more acid sensitivity (Vulic & Kolter, 2002; Farrell & Finkel, 2003; Richard & Foster, 2003; Finkel, 2006). Hence, under varying environmental conditions, different levels of expression of RpoS, modulated by different *rpoS* alleles, are advantageous. There are several genes in the *rpoS-crl* regulon and the altered expression of many of these genes, as a result of different *rpoS/crl* alleles, is likely to contribute to the fitness of the strains under different environmental conditions. The results described above show that *bgl* is one such system.

Overexpression of LeuO leads to activation of the *bgl* operon (Ueguchi *et al*., 1998). There are specific conditions, such as stationary phase and branched chain amino acid starvation, in which the expression of LeuO is known to be enhanced (Fang *et al*., 2000; Majumder *et al*., 2001). Hence, it is conceivable that under these conditions the expression of the *bgl* operon is affected. However, in this study, although we observed an increase in the expression of the *bgl* operon in the stationary phase, we found no correlation between this increase and the increased expression of *leuO*, at least under the conditions tested. It might be interesting to test the expression of the *bgl* operon under other physiological conditions, such as starvation for branched chain amino acids. Similarly, overexpression of BglJ has been shown to activate the *bgl* operon (Giel *et al*., 1996). A recent study indicated that the expression of BglJ is enhanced in glucose-limited continuous cultures (Franchini & Egli, 2006), suggesting its involvement under starvation conditions. Our study shows a modest effect of *bglJ* in the regulation of the *bgl* operon in the absence of the negative regulation by RpoS/Crl.

The studies reported above underscore the fact that although insights into the mechanistic details of the role of various regulators may be gained by experimenting with cells growing in rich media, it would be physiologically more relevant to study gene expression under a combination of stresses as would occur in nature. These studies also highlight the complex interactions between different global regulators in the stationary phase and the care needed to evaluate carefully the different genotypes of both laboratory strains as well as natural isolates before interpreting the effects of specific mutations.

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