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BglR Protein, Which Belongs to the BglG Family of Transcriptional Antiterminators, Is Involved in β-Glucoside Utilization in *Lactococcus lactis*

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A fragment of the *Lactococcus lactis* chromosome containing an open reading frame of 265 codons, denoted *bglR*, has been characterized. The polypeptide encoded by *bglR* shares 36 to 30% sequence identity with a family of regulatory proteins including ArbG from *Erwinia chrysanthemi*, BglG from *Escherichia coli*, and SacT and SacY from *Bacillus subtilis*. These regulatory proteins are involved in positive control of the utilization of different sugars by transcription antitermination. For some of these regulatory proteins it has been demonstrated that antitermination is exerted by binding to a conserved RNA sequence, partially overlapping the transcription terminator and thus preventing transcription termination. Upstream of *bglR*, we identified a transcription terminator whose 5′ end was overlapped by a 32-bp sequence, highly homologous to the RNA-binding site that is conserved in other regulatory systems. Constitutive expression of *bglR* in *E. coli* increased the expression of a *bglG*::*lacZ* transcriptional fusion. The fact that the expression of BglG is autoregulated in *E. coli* suggests that BglG and BglR are functionally equivalent. In *L. lactis*, we observed that (i) the expression of a *bglR*::*lacZ* fusion is increased by β-glucoside sugars, (ii) disruption of *bglR* impairs growth on some β-glucosides, and (iii) the expression of *bglR* is positively autoregulated. Because of these structural and functional similarities between BglR and the transcription antiterminators of the BglG family, we propose that BglR may be the lactococcal counterpart of the *E. coli* BglG regulator of β-glucoside utilization.

The *bgl* operon of *Escherichia coli* is involved in the utilization of β-glucoside sugars (25, 36). This operon is cryptic in wild-type strains but can be rendered functional by a variety of spontaneous mutations (30, 31, 35). When functional, this operon is inducible by β-glucosides. This control is exerted through transcription antitermination mediated by the BglG protein (3, 22, 25, 33, 34). Transcription initiates constitutively at the *bgl* promoter, but in the absence of β-glucosides, most transcripts terminate at a ρ-independent terminator located in the leader region, immediately upstream of the *bglG* gene. In the presence of β-glucosides, the BglG protein binds to a specific sequence of the mRNA, overlapping part of the transcription terminator, thus preventing RNA polymerase from terminating. The binding of BglG is modulated by its phosphorylation by EII^{Bgl}, an enzyme of the phosphoenolpyruvate:β-glucoside phosphotransferase system, which is involved in uptake and phosphorylation of β-glucosides (36). In the absence of β-glucosides, $P\sim EII^{Bgl}$ phosphorylates BglG, which thus becomes unable to bind to RNA and act as an antiterminator (3, 4). If β-glucosides are present, they are phosphorylated by $P\sim EII^{Bgl}$ and P~BglG is dephosphorylated. The protein can thus bind to RNA, which results in transcription antitermination.

Based on a strong conservation of the amino acid sequences of the regulatory proteins and of their putative RNA targets, partially overlapping a transcription terminator, a similar regulatory model has been proposed for several other genetic systems. These systems include the control of the *Bacillus subtilis* levansucrase *sacB* gene by SacY (7, 13), of the *B. subtilis* *sacPA* operon by SacT (6, 14, 39), of *B. subtilis* β-glucan utilization by *licT* (40), of *Lactobacillus casei* lactose utilization (1), and of the *Erwinia chrysanthemi* β-glucoside phosphotransferase gene *arbF* by ArbG (15).

In this article, we describe the *Lactococcus lactis bglR* gene, whose product shares homology with the BglG family of antiterminators. *bglR* is preceded by a transcription terminator partially overlapped by the conserved target sequence of the BglG antiterminators. Constitutive expression of *bglR* in *E. coli* increased the expression of a *bglG*::*lacZ* transcriptional fusion. The fact that the expression of BglG is autoregulated in *E. coli* suggests that BglG and BglR are functionally related. In *L. lactis*, the expression of *bglR* is positively controlled by β-glucosides and its disruption results in a deficiency in β-glucoside utilization. Taken together, these results indicate that BglR is a lactococcal counterpart of the *E. coli* BglG regulator of β-glucoside utilization.

MATERIALS AND METHODS

Bacterial strains and media. *L. lactis* subsp. *lactis* IL1403 (11) and derivatives, *E. coli* TG1 (18) and MA152 (24), and *B. subtilis* MT119 (41) were grown as previously described (8). Growth on various carbohydrates in a chemically defined broth (28, 29, 38), supplemented with the appropriate sugar at 1% final concentration, was monitored. The medium was inoculated with 1% of a culture grown overnight and then washed twice.

DNA manipulations. Plasmids and chromosomal DNAs were extracted as previously described (8). *E. coli* and *B. subtilis* strains were transformed according to the standard

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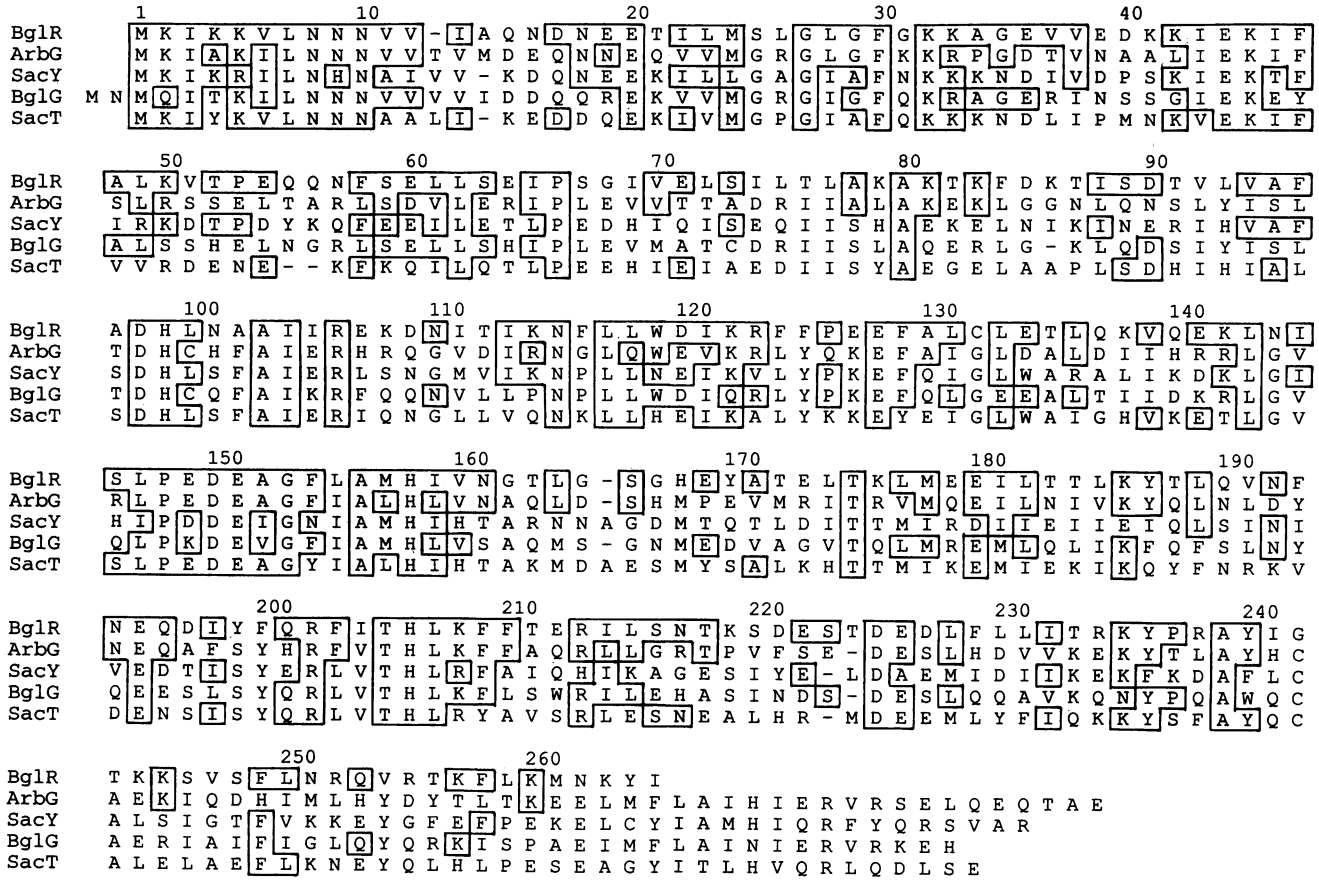

FIG. 1. Amino acid sequence homology between the product of *bglR* and regulatory proteins in the BglG family. Abbreviations: BglR, product of *L. lactis bglR*; ArbG, *E. chrysanthemi* ArbG (15); SacY, *B. subtilis* SacY (44); BglG, *E. coli* BglG (36); SacT, *B. subtilis* SacT (16). Gaps have been inserted to maximize homology. Identical residues to those of BglR are boxed.

procedure using $CaCl_2$ (32) and the modified protocol (10) of Anagnostopoulos and Spizizen (5), respectively. *L. lactis* was transformed by an electroporation technique (20). All other molecular techniques were done as described by Sambrook et al. (32).

DNA sequencing and analysis. An *Xba*I-*Pst*I 2.9-kb DNA fragment from plasmid pIL341 (8), encompassing a part of *trpB*, *trpA*, and the downstream sequence was cloned in both orientations in plasmid pBluescript (Stratagene), giving rise to plasmids pIL439 and pIL440, respectively. Nested deletions in the insert carried on these plasmids were produced by using exonuclease III and mung bean nuclease (Stratagene), and the nucleotide sequence was determined as described before (8). The DNA and protein sequences were analyzed with the GCG software package (17), CLUSTAL software (19), and BLAST software (2).

Measurement of β-galactosidase activity. *L. lactis* cells were grown to late exponential phase in M17 medium containing the appropriate sugar, and β-galactosidase activity was determined as previously described (42). *E. coli* cells were grown in Luria-Bertani broth (26) to stationary phase, and β-galactosidase activity was determined essentially as described by Miller (27). β-Galactosidase activities were expressed in nanomoles of *o*-nitrophenol released per minute per microgram of protein.

Nucleotide sequence accession number. The GenBank, EMBL, and DDBJ nucleotide sequence accession number is L27422.

RESULTS AND DISCUSSION

Identification of the *bglR* gene. The nucleotide sequence of an 821-bp DNA region immediately downstream of the *L. lactis trpA* gene has been determined. Analysis of this sequence revealed the presence of an open reading frame 265 codons long. The putative product of this open reading frame is homologous to the family of regulatory proteins including ArbG (15), BglG (24, 36), SacT (14), and SacY (44), with percent identities of 36, 34, 31, and 30, respectively (Fig. 1). The lactococcal gene was named *bglR*.

A putative transcription terminator and a BglG-binding site are present upstream of *bglR*. A region of dyad symmetry followed by a stretch of T's, resembling ρ-independent transcription terminators, was found upstream of *bglR* (Fig. 2). Upstream of this putative terminator and partially overlapping it, we identified a sequence which is conserved at the same relative position in all known terminators controlled by the regulatory proteins of the BglG family (Fig. 2). This sequence has been shown in *E. coli* to encode an RNA with the potential to bind BglG (22).

BglR can compensate for the absence of BglG in *E. coli*. Because of the homology between BglR and BglG, we specu-

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111 GGATAGTGATTATTAAGTTAAGCTAGACCTTATCAAACCTATAGTTATAGGGGGATAAGGTCTATTTTT 179
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FIG. 2. Putative transcription terminator and BglG antiterminator RNA-binding site upstream of *bglR*. Numbers refer to sequence L27422. The *bglR* gene starts at nucleotide 197. Sequences able to form a putative transcription terminator are underlined with arrows. Asterisks indicate highly conserved nucleotides upstream of transcription terminators controlled by regulators in the BglG family (14–16, 22, 33).

lated that BglR could functionally replace BglG in *E. coli*. To test this possibility, *E. coli* MA152 (24), containing a *bglG-lacZ* transcriptional fusion, was used. In this strain, constitutive expression of *bglG* results in activation of the expression of *lacZ*. We constructed plasmid pIL1139, which expresses *bglR* under the control of a strong, constitutive promoter. This was done by cloning a PCR-amplified DNA fragment containing *bglR* (coordinates 197 to 995 of sequence L27422) with *Nru*I and *Sal*I sites added at the 5′ and 3′ ends, respectively, into the corresponding restriction sites of expression vector pGKV259 (43). Plasmids pGKV259 and pIL1139 were each introduced into strain MA152, and β-galactosidase activity was measured in each strain. *lacZ* expression was low in the absence of *bglR* (1.7 β-galactosidase units) and was increased significantly by the presence of *bglR* (19.1 β-galactosidase units), demonstrating that *bglR* affects the expression of *bglG* in *E. coli*. This indicates that BglR may have a function similar to that of regulators of the BglG family.

Expression of *bglR* is induced by β-glucoside sugars. Since all known proteins of the BglG family are involved in the regulation of sugar utilization and since the expression of most of these proteins responds to the presence of specific sugars, the effects of various sugars on the expression of *bglR* were examined. A *bglR::lacZ* fusion was constructed by inserting a 1,277-nucleotide *Eco*RV-*Rsa*I fragment of the *trpA-bglR* region of the IL1403 chromosome, made blunt by treatment with T4 polymerase, at the *Sma*I site of plasmid pMC1871 (37). A *Pst*I cassette carrying *bglR::lacZ* was then inserted at the unique *Pst*I site of pE194 (21), which does not replicate in *L. lactis* IL1403 (12). This delivery plasmid was introduced into IL1403 by electroporation, Em^r transformants were selected, and the presence of a single copy of the *bglR::lacZ* fusion in the chromosome of one transformant, designated IL3677, was confirmed by Southern hybridization. Strain IL3677 was grown in M17 medium containing 1% of various sugars to the mid-exponential growth phase, and β-galactosidase activity was measured (Fig. 3). All sugars able to support growth of *L. lactis* IL1403 were tested. The highest β-galactosidase activity was induced by the β-glucoside sugars esculin, arbutin, salicin, and cellobiose. Growth in glucose resulted in the lowest β-galactosidase activity.

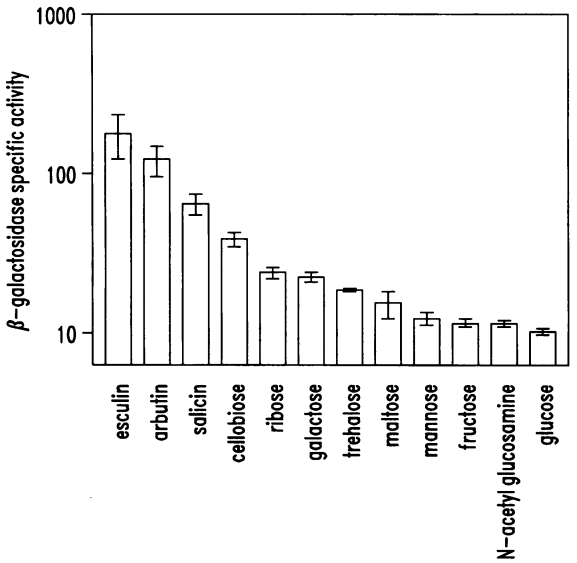

FIG. 3. Effects of various sugars on expression of *bglR*. *L. lactis* IL3677 which carries a *bglR::lacZ* chromosomal fusion was grown in M17 medium containing the indicated sugars (1%) to mid-log phase, and the β-galactosidase specific activity was measured. β-galactosidase values are the means of at least three independent assays ± standard deviations.

***bglR* is required for β-glucoside utilization.** Since *bglR*, like *E. coli bglG*, is controlled by β-glucoside sugars, we suspected that BglR might be involved in regulating β-glucoside utilization. To test this hypothesis, *bglR* was disrupted by insertion of a 10-nucleotide *Hin*dIII linker at the unique *Swa*I site and replacement of the wild-type gene by the disrupted gene, using a recently described strategy (9, 23). Clones containing the disrupted *bglR* were distinguished from those containing the wild-type *bglR* by comparing the profiles of their *Hin*dIII-cleaved DNAs hybridized with the *bglR* DNA. In the presence of salicin or arbutin, growth of the mutant was severely impaired compared with growth of the wild-type strain (Fig. 4). In the presence of cellobiose, the early growth of the mutant was slightly stimulated, but later, both strains grew at essentially the same rate. Both strains grew similarly in the presence of glucose. Growth of the wild-type *L. lactis* strain on β-glucosides is multiphasic, suggesting that different pathways are used sequentially for the utilization of these sugars. Our data indicate that *bglR* is required for the utilization of β-glucosides through at least some of these pathways.

Expression of *bglR* is autoregulated. The presence, upstream of *bglR*, of a sequence resembling the BglG-binding RNA site and partially overlapping a putative transcription terminator suggests that BglR could control the transcription of its own gene. To test this hypothesis, plasmid pGKV259 (the expression vector alone) and plasmid pIL1139 (expressing constitutively *bglR*) were each introduced into strain IL3677, and the expression of the *bglR::lacZ* fusion was measured. Constitutive expression of *bglR* resulted in two- to threefold increased synthesis of β-galactosidase in the presence of β-glucoside sugars (data not shown), indicating that *bglR* is autoregulated.

Conclusions. The *bglR*-encoded protein is homologous to regulatory proteins of the BglG family. All previously described members of this family control the utilization of sugars (1, 13, 15, 16, 36, 39, 40). Disruption of *bglR* revealed that its product is required for maximal growth on arbutin and salicin as sole carbon sources.

The conservation of protein sequence and biological function between BglR and the members of the BglG family, the presence of a conserved regulatory sequence upstream of *bglR*, and the observation that *bglR* is autoregulated indicate that *bglR* probably acts through a regulatory mechanism similar to that mediated by BglG. This hypothesis is supported by the observation that the expression of *bglR* stimulates expression of a *bglG::lacZ* fusion in *E. coli*.

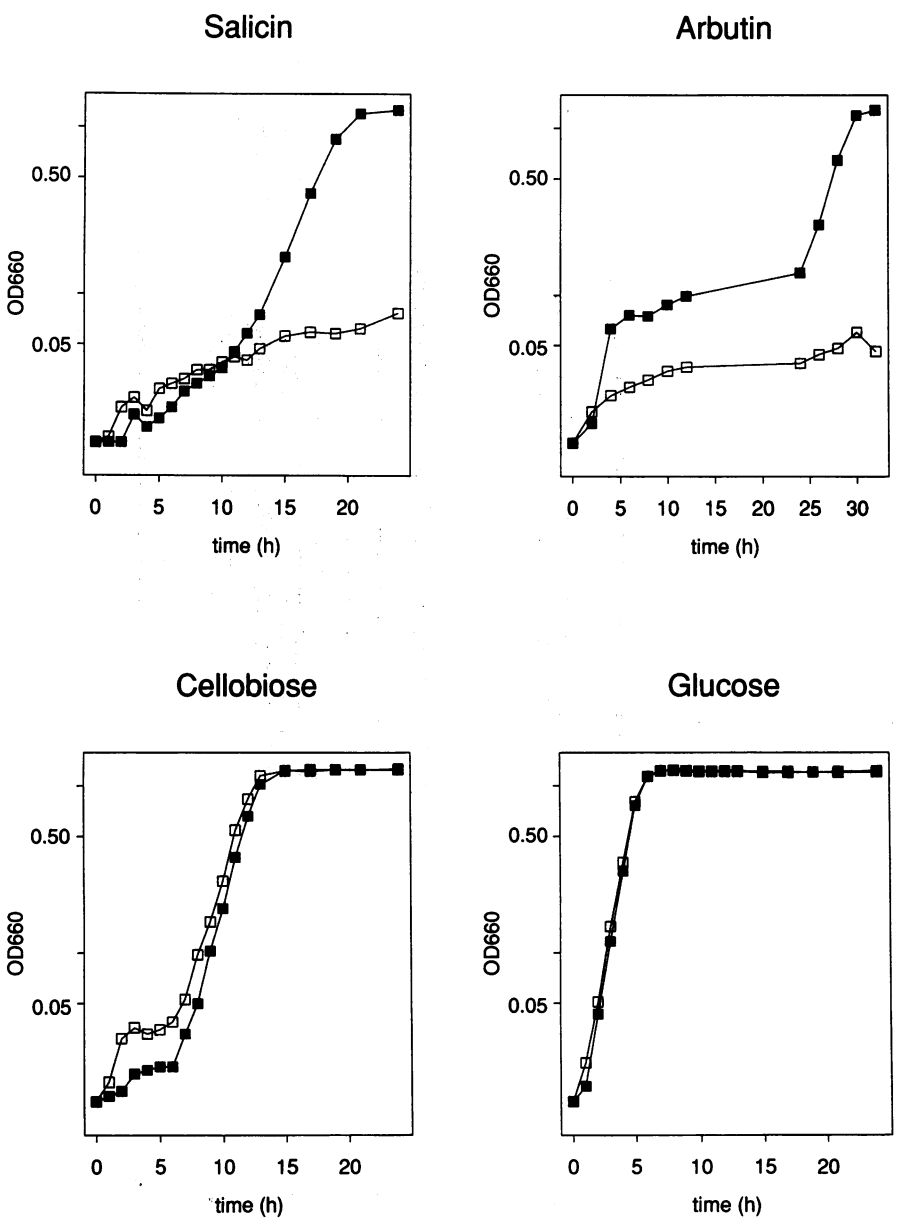

FIG. 4. Effect of *bglR* disruption on the utilization of β-glucosides by *L. lactis*. IL1403 (solid squares) and its *bglR*-disrupted derivative IL3990 (open squares) were grown in chemically defined medium supplemented with 1% salicin, arbutin, cellobiose, or glucose. The optical density at 660 nm (OD_{660}) was measured at the indicated time intervals.

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