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Glucitol Induction in *Bacillus subtilis* Is Mediated by a Regulatory Factor, GutR

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Expression of the glucitol dehydrogenase gene (*gutB*) is suggested to be regulated both positively and negatively in *Bacillus subtilis*. A mutation in the *gutR* locus results in the constitutive expression of *gutB*. The exact nature of this mutation and the function of *gutR* are still unknown. Cloning and characterization of *gutR* indicated that this gene is located immediately upstream of *gutB* and is transcribed in the opposite direction relative to *gutB*. GutR is suggested to be a 95-kDa protein with a putative helix-turn-helix motif and a nucleotide binding domain at the N-terminal region. At the C-terminal region, a short sequence of GutR shows homology with two proteins, Cyc8 (glucose repression mediator protein) and GsiA (glucose starvation-inducible protein), known to be directly or indirectly involved in catabolite repression. Part of the C-terminal conserved sequence from these proteins shows all the features observed in the tetratricopeptide motif found in many eucaryotic proteins. To study the functional role of *gutR*, chromosomal *gutR* was insertionally inactivated. A total loss of glucitol inducibility was observed. Reintroduction of a functional *gutR* to the GutR-deficient strain through integration at the *amyE* locus restores the inducibility. Therefore, GutR serves as a regulatory factor to modulate glucitol induction. The nature of the *gutR1* mutation was also determined. A single amino acid change (serine-289 to arginine-289) near the putative nucleotide binding motif B in GutR is responsible for the observed phenotype. Possible models for the action of GutR are discussed.

Addition of glucitol to a culture of *Bacillus subtilis* results in the expression of at least two genes, *gutA* and *gutB*, which encode glucitol permease and glucitol dehydrogenase, respectively (4, 32). Characterization of the regulatory region of *gutB* suggests that the expression of *gutB* is subject to both positive and negative regulation (39). A *cis*-acting regulatory element located immediately upstream of the *gutB* promoter is essential for glucitol induction while an imperfect AT-rich inverted repeat sequence located downstream from the promoter negatively regulates expression of *gutB*. A regulatory mutant carrying the *gutR1* mutation has been isolated (11). Both *gutB* and *gutA* are constitutively expressed in this mutant. The *gutR* locus was found by three-factor genetic crosses to be close to *gutB*; the genes in this locus are arranged in the following order: *gutR-gutB-gutA*. To understand the function of *gutR* and the mechanism of glucitol induction, we sought to clone and characterize *gutR*.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. *B. subtilis* 168 (*trpC2*) was used to prepare chromosomal DNA for the cloning of *gutR* and for the routine transformation. *B. subtilis* WB1002 (39) is a *B. subtilis* 168 strain carrying the *gutB-lacZ* transcriptional fusion at the *amyE* locus. The intact *gutB* regulatory region (i.e., including the promoter sequence and both the upstream and downstream sequences flanking the promoter) was used for the construction of this strain. WB1002 was applied to monitor the phenotype of the *gutR1* mutation. *B. subtilis* PG650 (*gutR1 ura3*) was used for the characterization of the *gutR1* mutation (11). *Escherichia coli* DH5α [(φ80 d*lacZ*ΔM15) *endA1 recA1 hsdR17* (r^- m^-) *supE44 thi-1* λ^- *gyrA relA1* F^- Δ(*lacZYA-argF*)*U169*] was the host for the construction of a subgenomic library for the screening of *gutR*. Plasmids pUB18 (36, 37) and pBS (Bluescribe vector from Stratagene) were used for routine subcloning and DNA sequencing in *B. subtilis* and *E. coli*, respectively. Standard competent cells for transformation were prepared as described previously (38). For plasmid isolation, *B. subtilis* was routinely cultured in superrich medium (12). For the determination of the glucitol dehydrogenase activity (23) from various *B. subtilis* strains, cells were cultured in modified superrich medium (39) supplemented with either 2% succinate or 2% glucitol. *B. subtilis* cells carrying pUB18 were plated to tryptose blood agar base (TBAB from Difco) plate containing 10 μg of kanamycin per ml. *E. coli* was cultured on Luria broth plates. For cells carrying the pBS plasmid, ampicillin was added to the medium at a final concentration of 75 μg/ml.

DNA manipulations. Chromosomal DNA preparation, plasmid isolation, Southern and colony hybridization, DNA sequencing, and PCR amplification were performed as described previously (18). Genomic sequencing of the *gutR1* mutation in *B. subtilis* PG650 was performed with the *Taq* polymerase-based mole DNA sequencing system from Promega. Ten micrograms of the purified chromosomal DNA (3.6 fmol) was used in each set of four sequencing reactions with the 25-nucleotide (nt)-long, ^{32}P-end-labeled sequence primer (P3 in Fig. 1). The sequence of this primer corresponds to nt 1142 to 1166 shown in Fig. 2. Sequencing conditions specified by the manufacturer were followed.

Construction of pGUTRM1 to pGUTRM4. These vectors were designed to identify the nature of the *gutR1* mutation. Two PCR primers (P1 and P2 in Fig. 1) were applied to amplify *gutR1* from *B. subtilis* PG650. P1 and P2 primers have the sequence complementary to nt 600 to 617 of the reported *gutB* gene (23) and nt 2841 to 2850 of *gutR* (Fig. 2), respectively. An extra sequence (8 nt) carrying a *Kpn*I site was added to the 5′ end of the P2 primer. The resulting amplified fragment was digested by *Xho*II and *Kpn*I to generate a 2.9-kb fragment

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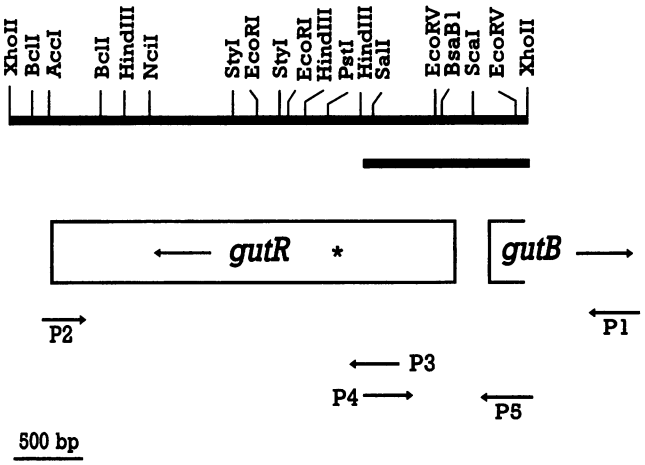

FIG. 1. Physical map of *gutR* and *gutB*. Restriction sites used for subcloning are indicated. The open reading frame for *gutR* is boxed. The transcription direction of *gutR* and *gutB* is shown by arrows. An asterisk indicates the single point mutation (C→A) observed in *gutR1*. The *Bsa*BI site illustrates the location of the insertion of a 1.4-kb erythromycin resistance marker in pGUTR-ERY. The 1-kb *Hin*dIII-*Xho*II fragment used as the hybridization probe for cloning of *gutR* is on the right. P1 to P5 are the PCR or sequencing primers described in the text. The length of these primers is not drawn to scale.

carrying the 5′ end region of *gutB* and the entire structural gene of *gutR1* (Fig. 1). This fragment was ligated to the *Bam*HI- and *Kpn*I-digested pUB18 to generate pGUTRM. Four independent clones named pGUTRM1 to pGUTRM4 were selected for further characterization.

Construction of pIGUTR. This vector was constructed to determine whether a specific single mutation in *gutR* is responsible for the *gutR1* phenotype. A 2.2-kb *Eco*RV-*Kpn*I fragment was obtained from pGUTRM1. This fragment carried a truncated *gut*R1 which missed the *gutR* promoter and the 5′ end region encoding the helix-turn-helix domain of GutR. It was ligated to the *Sma*I-*Kpn*I-digested pBS-ERY to generate pIGUTR. Plasmid pBS-ERY is an *E. coli* pBS plasmid derivative which has a 1.4-kb *Taq*I fragment from pE194 (14). This *Taq*I fragment carries the erythromycin resistance marker and was inserted at the *Acc*I site of pBS.

Construction of pGUTR-ERY and disruption of the chromosomal *gutR*. Plasmid pGUTR-ERY is an integration vector carrying an insertionally inactivated *gutR*. It was constructed to replace the functional *gutR* from the *B. subtilis* chromosome. The 1-kb *Xho*II-*Hin*dIII fragment carrying the 5′ end region of *gutR* (Fig. 1) was isolated from pSDH1 (23) and ligated to the *Bam*HI-*Hin*dIII-digested *E. coli* pBS plasmid to generate pRE378. The unique *Bam*HI site in pRE378 was then inactivated to generate pBRE378. Plasmid pBRE378 has two *Bsa*BI sites. One of them (nt 585 in Fig. 2) is located within *gutR*. Partial digestion of pBRE378 with *Bsa*BI was performed, and the linearized plasmid DNA was ligated with a *Bam*HI linker. Plasmid pBRE378B, which has the *Bam*HI linker inserted at the *Bsa*BI site within *gutR*, was isolated. A 1.4-kb *Bam*HI fragment carrying the erythromycin resistance cassette originally derived from pE194 (14) was ligated to pBRE378B to generate pGUTR-ERY. Plasmid pGUTR-ERY was then linearized by *Eco*RI and transformed to *B. subtilis* 168 by the standard competent cell method. Erythromycin-resistant clones were selected on TBAB plate containing 5 μg of erythromycin per ml and 5 μg of lincomycin per ml. A pair of PCR primers (P4 and P5 in Fig. 1) were designed to amplify the 5′ end region of *gutR* from these erythromycin-resistant clones to confirm the disruption of the *gutR* gene. The P4 primer has the sequence complementary to nt 774 to 793 while the sequence of the P5 primer corresponds to nt 245 to 265 as shown in Fig. 2. The resulting *gutR*-deficient strain was designated WB1101.

Construction of pGUTRAMY and WB1111. pGUTRAMY is an integration vector carrying a functional *gutR* gene in pDH32 (27) which allows the integration of *gutR* at the *amyE* locus. Plasmid pU1 (see Results) carrying the 3.1-kb *Xho*II fragment with the functional *gutR* gene was digested by *Xho*II to release the 3.1-kb insert. This insert was then ligated to the *Bam*HI-digested pDH32 to generate pGUTRAMY. Plasmid pGUTRAMY was then linearized by *Nru*I and transformed to WB1101. Transformed cells were plated to TBAB plates containing 5 μg of chloramphenicol per ml. Transformants were then confirmed to be deficient in α-amylase activity by spotting the cells to the starch-containing TBAB plates. The resulting strain carrying an insertionally inactivated *gutR* at the *gutR* locus and a functional *gutR* at the *amyE* locus was confirmed by PCR. This strain was designated WB1111.

Computer analyses. Two sequence analysis packages, Microgenie (version 6.0 from Beckman) and PC Gene (version 6.7) from Intelligenetics, were used in this study. For the homology search, computation was performed at the National Center for Biotechnology Information with the BLAST network service. To visualize the three-dimensional structure of the EF-Tu–Mg-GDP complex, a Silicon Graphics Indigo workstation with the Insight II program from Biosym Technologies Inc. (San Diego, Calif.) was used.

Nucleotide sequence accession number. The nucleotide sequence reported in this paper has been submitted to GenBank with accession number L19113.

RESULTS

Cloning of the regulatory gene. Since the *gutR* locus was shown to be very close to *gutB* and we had cloned a 5-kb *Hin*dIII fragment carrying both *gutB* and *gutA* (23), chromosomal walking was a logical approach to clone genes located upstream of *gutB*. A 1-kb *Hin*dIII-*Xho*II fragment carrying the 5′ end region of *gutB* was selected as a hybridization probe (Fig. 1). This probe hybridized specifically to a 3.1-kb fragment of *Xho*II-digested *B. subtilis* chromosomal DNA (data not shown). A subgenomic library enriched for 3.1-kb *Xho*II fragments was constructed in *E. coli* DH5α with the Bluescribe plasmid as the cloning vector. Screening of this library through colony hybridization identified seven positive clones, and one of the positive clones, pU1, was used for further characterization.

Characterization of the regulatory gene. The restriction map of the cloned 3.1-kb *Xho*II fragment is shown in Fig. 1. The nucleotide sequence of the entire fragment was determined by sequencing from both strands. An open reading frame (nt 342 to 2831) was found upstream of *gutB*, and the orientation of this gene is opposite to that of *gutB* (Fig. 1). This gene could encode a polypeptide of 829 residues with a deduced molecular weight of 95,076. There is a putative ribosome-binding site (AGGAGGTG) located 7 nt upstream of the translation initiation codon (ATG). Within the open reading frame, two putative catabolite regulatory elements (ATTGAAA at nt 756 to 762 and ATTTAAAG at nt 1933 to 1940), which were similar to that observed in the *gnt* operon, were found (20). Furthermore, at the protein level, a putative helix-turn-helix motif similar to those found in many DNA-binding proteins

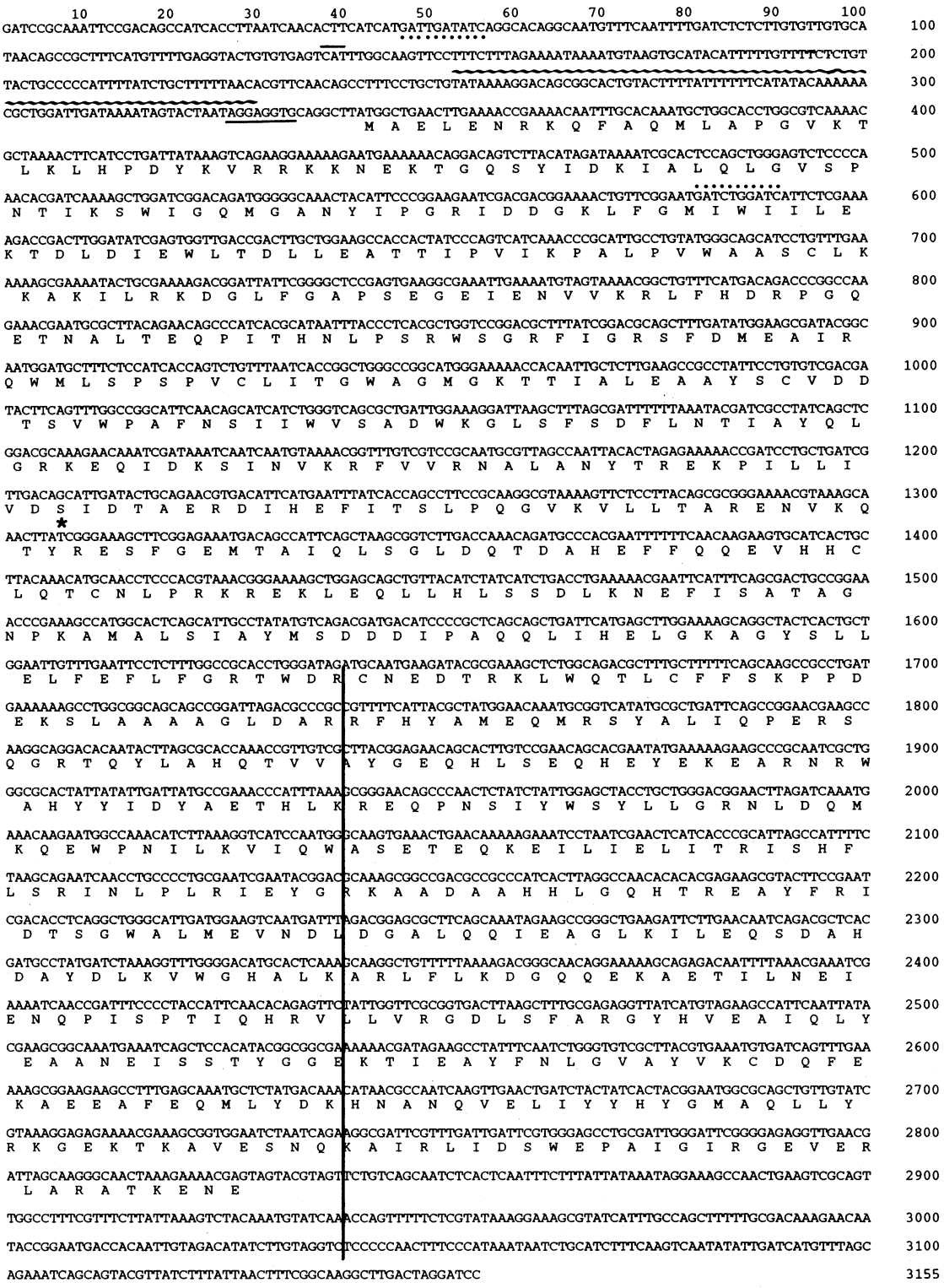

FIG. 2. Nucleotide sequence of *gutR* and the deduced amino acid sequence of its protein product. Sequence of the putative *gutR* ribosome-binding site is underlined. An asterisk marks Ser-289, which is changed to Arg-289 in GutR1. To illustrate the relative location of *gutB* and *gutR*, sequences complementary to the *gutB* translation initiation codon and the regulatory region for glucitol induction are overlined by a straight line and a wavy line, respectively. Sequences for the two *Bsa*BI sites are marked by dotted lines. The *Bsa*BI site overlined indicates the insertion site for a 1.4-kb erythromycin cassette to generate the insertional inactivated *gutR*.

A

Protein	H1	T	H2	Accession number
GutR	42 IDKIALQLGVSPNTIKSWIG 61			
TyrR	483 TRKLAKRLGVSHTAIANKLR 502			P07604
Cro	16 QTKTAKDLGVYQSAINKAIH 35			P03040
NifA	496 QAKAARLLGMTPRQVAYRIQ 515			P03027
NtrC	444 KQEAARLLGWGRNTLTRKLK 463			P06713
FhlA	663 PKGAAQRLGLKRTTLLSRMK 682			P19323
	* **			
CONSENSUS	hn hGh			

B

Protein	Motif A	Motif B	Accession number
GutR	196 CLITGWAGMGKTTIA 210	284 LLIVDSID 291	
Rho	174 GLIVAPPKAGKTMLL 188	261 IILLDSIT 268	P03002
LevR	144 MLLLGPTGSGKSLFA 158	222 ILFMDEIH 229	P23914
GlnQ	30 VVIIGPSGSGKSTLL 44	157 MMLFDGPT 164	P10346
Adk	11 IFVVGGPGSGKGTQC 25	89 GFLIDGYP 96	P00568
Ras	6 LVVVGAGGVGKSALT 20	53 LDILDTAG 60	P01112
EF-Tu	14 VGTIGHVDHGKTTLT 28	76 YAHVDCPG 83	P02990
	* ***	*	
CONSENSUS	hhhh[G/A]XXXXGK[T/S]	hhhhD	

FIG. 3. Comparison of the putative helix-turn-helix (A) and nucleotide binding (B) motifs in GutR with those in other regulatory proteins. Residues that are identical with those in the consensus sequences are marked by asterisks. h and n indicate residues with hydrophobic and nonpolar side chains, respectively. X can be any amino acid. The accession number of each protein in the Swiss Protein Data Bank (version 26) is given.

was found within the N-terminal domain of this protein (25). The alignment of this putative DNA binding motif with other procaryotic DNA-binding proteins is shown in Fig. 3A. Downstream from this motif, there were two sequences that are similar to consensus nucleotide binding motifs A and B, respectively (Fig. 3B). Therefore, this gene product is likely to be a DNA-binding protein with the potential to bind ATP or GTP.

Characterization of the *gutR1* mutation. To determine the nature of the *gutR1* mutation, four independent clones (pGUTRM1 to pGUTRM4) carrying the PCR-amplified *gutR1* fragment were selected for nucleotide sequencing. Only a single point mutation (C→A) was found at nt 1208 after the entire 2.9-kb insert in pGUTRM1 and pGUTRM2 was sequenced (Fig. 4). The same mutation was also observed in pGUTRM3 and pGUTRM4 by sequencing around this target area. Direct genomic sequencing of PG650 chromosomal DNA was also performed. The same point mutation was observed, which confirmed that this observed point mutation was not an

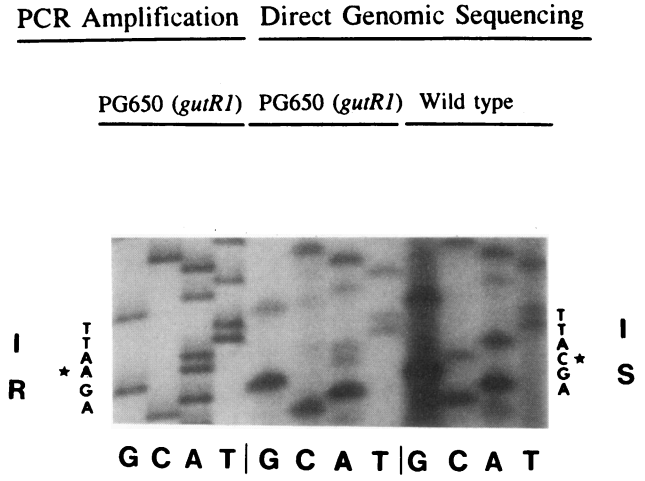

FIG. 4. Determination of the nature of the *gutR1* mutation. PCR-amplified DNA or chromosomal DNA from the *gutR1* strain (PG650) was used as a template for sequencing. An asterisk marks the mutated nucleotide. The change of Ser-289 to Arg-289 is indicated.

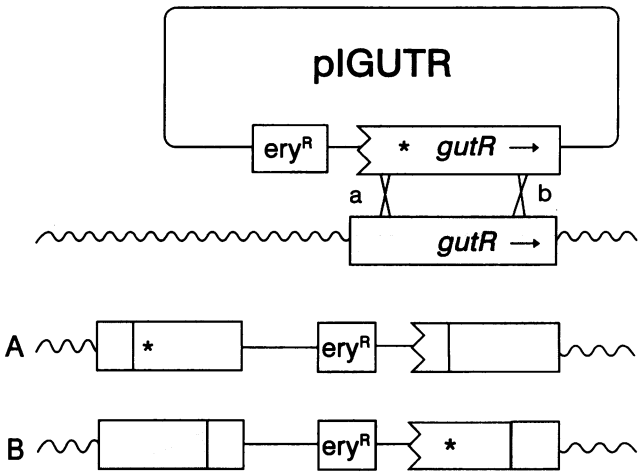

FIG. 5. A single point mutation in *gutR* is responsible for the *gutR1* phenotype. An asterisk indicates the C→A mutation. The wavy line indicates the wild-type *B. subtilis* chromosomal DNA. Jagged lines at the beginning of the boxed region indicate a truncated copy of the gene. A and B represent the resulting chromosomal DNA after integration of pIGUTR at sites a and b, respectively.

artifact generated by PCR. This point mutation resulted in a change of Ser-289 to Arg-289. To determine whether this mutation is responsible for the *gutR1* phenotype, this mutation was introduced into the chromosome via the integration process depicted in Fig. 5. Plasmid pIGUTR (see Materials and Methods for the construction of this plasmid) was introduced into strain WB1002, which carries a *gutB-lacZ* transcriptional fusion integrated at the *amyE* locus. Crossover at site *a* would be expected to generate the *gutR1* phenotype while crossover at site *b* would yield the wild-type *gutR* phenotype. Transformants were selected on TBAB plates containing X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) and erythromycin-lincomycin (without glucitol). A total of 17 blue and 38 white colonies were observed. When these colonies were spotted on X-Gal plates containing 2% glucitol, all colonies turned blue. All these analyses demonstrate that the cloned gene codes for GutR and that a single mutation at nt 1208 is sufficient to generate the *gutR1* phenotype.

Disruption of the chromosomal *gutR* gene. To determine the functional role of *gutR* in regulating the expression of *gutB*, a plasmid, pGUTR-ERY, which has an erythromycin resistance cassette inserted within the 5′ coding region of *gutR* was constructed. The linearized pGUTR-ERY plasmid was introduced into *B. subtilis* 168. Through double-recombination events, the chromosomal *gutR* was replaced by the insertionally inactivated *gutR* to generate strain WB1101. Gene replacement in WB1101 was confirmed by PCR amplification. As shown in Fig. 6, a 0.5-kb *gutR* fragment was amplified from the control strain, *B. subtilis* 168 (lane 2). With the insertion of a 1.4-kb erythromycin cassette within this 0.5-kb fragment, amplification of the insertionally inactivated *gutR* clones (WB1101) should generate a 1.9-kb fragment. This indeed was the case (Fig. 6, lane 3). To determine the effect of inactivating the chromosomal *gutR* on *gutB* expression, glucitol dehydrogenase activity from *B. subtilis* 168 and WB1101 was measured. These cells were cultured in the presence or absence of glucitol. As shown in Table 1, no detectable glucitol dehydrogenase activity could be observed when these cells were cultured in the absence of glucitol. Failure to induce the production of glucitol dehydrogenase was also observed for

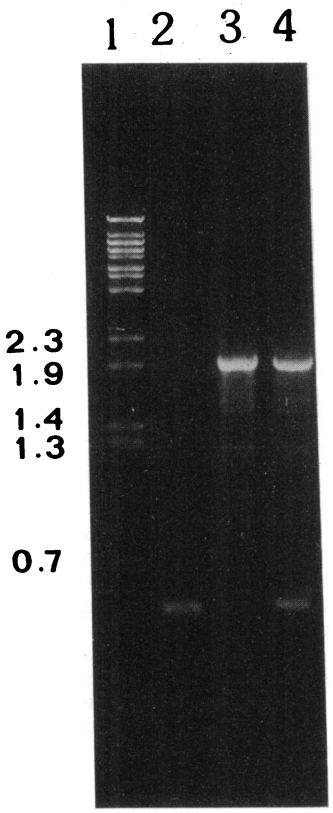

FIG. 6. Inactivation of the chromosomal *gutR* and reintroduction of a functional *gutR* at the *amyE* locus. Chromosomal DNA from wild-type *B. subtilis* 168 (lane 2), WB1101 (lane 3), and WB1111 (lane 4) was used for PCR amplification with a pair of primers (P4 and P5 [Fig. 1]) which annealed to sequences flanking the inserted erythromycin cassette. Lane 1 shows the *Bst*EII-digested lambda DNA as molecular weight markers. Numbers shown on the left are the lengths of fragments in kilobases.

WB1101 even in the presence of glucitol. As a control, *B. subtilis* 168 with a functional chromosomal *gutR* showed an inducible glucitol dehydrogenase specific activity of 93 mU/mg of total cellular protein. These data suggested that GutR might function as a transcriptional activator to mediate the expression of *gutB*.

Restoration of glucitol inducibility in WB1101. The implication that GutR functions as a regulatory factor for glucitol induction is valid only if *gutR* is the only gene affected in the gene disruption experiment. Since structural genes located downstream from *gutR* have not been characterized, it is possible that the insertion in *gutR* has a polar effect on some downstream genes. To demonstrate that disruption of *gutR* itself is responsible for the observed phenotype, an integration plasmid, pGUTRAMY, which carries a functional *gutR* gene in pDH32 was introduced into the *amyE* locus of WB1101 to generate WB1111. The resulting strain shows a deficiency in α-amylase activity. To confirm the introduction of a second copy of *gutR* into the genome of WB1111, PCR amplification with P4 and P5 primers was performed. As shown in Fig. 6, two DNA fragments of 1.9 and 0.5 kb were selectively amplified (lane 4). The longer one derives from the insertionally inactivated *gutR* while the short one derives from the nondisrupted *gutR*. As shown in Table 1, WB1111 regained the glucitol inducibility with the introduction of a functional *gutR* at the *amyE* locus. These data indicate that GutR is the regulatory factor mediating glucitol induction and that the inability to induce glucitol induction in WB1101 is solely due to the absence of functional GutR protein.

TABLE 1. Effect of chromosomal inactivation of *gutR* on *gutB* expression

Strain	Relevant genotype	Carbon source	Glucitol dehydrogenase activity (mU/mg)
168	Wild type	Succinate	0
168	Wild type	Glucitol	93.3 ± 11.5
WB1101	*gutR::ery*	Succinate	0
WB1101	*gutR::ery*	Glucitol	0
WB1111	*gutR::ery amyE::gutR*	Succinate	0
WB1111	*gutR::ery amyE::gutR*	Glucitol	90 ± 10

DISCUSSION

Inducible expression of genes involved in carbohydrate metabolism has been studied in detail for several systems in *B. subtilis* (for reviews, see references 15 and 16). Three general regulatory mechanisms have been demonstrated to regulate the expression of these genes. In the gluconate and xylose systems (8–10), the expression of these genes is regulated by repressors. In the sucrose and possibly the glycerol systems, antitermination is the key regulatory mechanism (3, 13, 30, 33). Recent characterizations of the levanase system illustrate the use of σ^L and a NifA- or NtrC-like regulatory factor, LevR, for controlling expression (6, 19). For the glucitol induction system, the exact regulatory mechanism is unknown but is clearly different from the three mechanisms described above. Characterization of the *gutB* promoter suggests that *gutB* is controlled by a nontypical σ^A-type promoter (39). A 78-bp sequence (Fig. 2) located upstream of this promoter is essential to mediate glucitol induction (39). It is likely that a regulatory protein which binds to this sequence activates transcription. In the present study, we demonstrated that an open reading frame located upstream of *gutB* encodes a regulatory protein controlling glucitol induction (Fig. 1). Characterization of the nature of the *gutR1* mutation confirmed that this regulatory gene indeed is *gutR*. The organization of these *gut* genes in the order *gutR-gutB-gutA* agrees with the previously reported genetic mapping data (11). Although these genes are clustered together, *gutR* is not in the same operon with *gutB* since it is transcribed in the opposite direction relative to *gutB* and *gutA*.

GutR is a relatively large protein with a deduced molecular weight of 95,000. The N-terminal region has a putative helix-turn-helix motif (25) analogous to that found in many other procaryotic DNA-binding proteins (Fig. 3A). Therefore, GutR is likely to be a DNA-binding protein. In fact, in vitro gel retardation experiments demonstrated that the purified GutR protein binds specifically to the upstream regulatory sequence of *gutB* (28).

Downstream from the putative helix-turn-helix domain, GutR was found to have two sequences that matched with the consensus ATP or GTP binding motifs A and B (Fig. 3B). The presence of these motifs suggests that GutR is a nucleotide-binding protein. Several procaryotic transcriptional activators are known to have an ATP binding site, including NifA, NtrC, and TyrR. NtrC interacts with σ^{54}-holoenzyme via DNA looping, and ATP is utilized to convert the RNA polymerase-promoter complex from a closed form to an open form (2, 29, 34). For the case of GutR, it is likely to activate transcription in a different manner. First, computer searches of the Swiss

Protein Data Bank (version 26) failed to detect any significant homology between GutR and other well-characterized transcriptional activators including those in the NtrC family. Furthermore, a nontypical σ^A-type promoter (see the accompanying paper) rather than a σ^L-type promoter was found to be functional in the *gutB* regulatory region. Although the exact nature of this nontypical σ^A-type promoter is still uncertain, this promoter is suggested to have a short spacer sequence of 15 nt as postulated in one of the models. It is tempting to speculate that GutR binds upstream of the promoter and readjusts the local helical structure within the promoter region which leads to the activation of the promoter. In *E. coli*, the mercury resistance operon has a σ^{70}-type promoter with a 19-bp spacer (26). Hg-Mer, a transcriptional regulator, activates this promoter by inducing a local underwinding of the spacer region for about 33° so that the suboptimally phased promoter elements (−10 and −35 regions) can be realigned properly (1, 7, 24). It would be interesting to determine whether GutR can function as a sequence-specific, ATP or GTP-dependent helicase.

Characterization of *gutR1* confirmed that a point mutation resulting in the replacement of Ser-289 by Arg-289 was responsible for the *gutR1* phenotype (i.e., expression of *gutB* in the absence of glucitol). Interestingly, Ser-289 is located within the ATP or GTP binding motif B and just downstream from the highly conserved aspartic acid which stabilizes Mg^{2+} in ATP or GTP. Introduction of a positive charge (from Arg) in this region might reduce the binding of MgATP-MgGTP. However, in the GTP binding motif B of *E. coli* EF-Tu–Mg-GDP complex (17), a cysteine residue that corresponds in position to Ser-289 in GutR is buried in the protein core. Introduction of an arginine residue at this position is likely to induce a conformational change, since it is thermodynamically unfavorable to bury a positive charge into the hydrophobic environment. Therefore, it is possible that GutR exists in two conformations. In the absence of glucitol, GutR is in an inactive form. Binding of glucitol may induce a conformational change of GutR and lead to the activation of the *gutB* transcription. In the case of GutR1, this protein may lock into an active conformation even in the absence of glucitol. This model can explain the phenotypes of WB1101 (the GutR-deficient strain) and the *gutR1* mutant.

Although GutR is suggested to act as a transcriptional activator, other models for the GutR-mediated glucitol induction should not be excluded. GutR may act by inactivating or repressing the synthesis of another protein which directly represses the expression of *gutB*. Glucitol may convert GutR to an active form which shuts off the expression of the direct repressor. Since GutR has a putative nucleotide binding site, GutR may be functionally equivalent to MalK, an ATPase that interacts with the maltose transport proteins (MalF and MalG) to regulate the uptake of maltose in *E. coli* (5). If GutR mediates the uptake of glucitol, inactivation of *gutR* would abolish glucitol induction. This prediction is consistent with the observed data. However, in this case, it would be difficult to explain why GutR can bind to the upstream region of *gutB*.

A search of various data banks indicated that a short C-terminal region of GutR showed certain similarity to both the glucose repression mediator protein from *Saccharomyces cerevisiae* (35) and the GsiA protein (glucose starvation-inducible protein) (21, 22) from *B. subtilis* (Fig. 7A). The only feature in common for all three proteins is their direct or indirect involvement in carbohydrate metabolism. Inactivation of *cyc8* results in release of many glucose-repressible genes from glucose repression in *S. cerevisiae*, while inactivation of *gsiA* allows *B. subtilis* to sporulate efficiently in the presence of

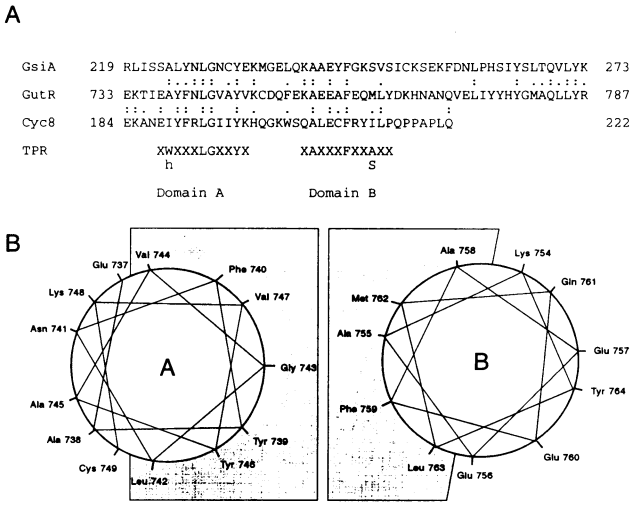

FIG. 7. TPR motif in GutR. (A) Sequence alignment of GutR with *B. subtilis* GsiA and Cyc8 from *S. cerevisiae*. A colon indicates identical residues in the alignment while a dot indicates amino acids with similar properties. TPR represents the consensus sequences of domains A and B of the TPR motif. h represents an amino acid with a hydrophobic side chain while X can be any amino acid. A and S at the ninth position of domain B represent Ala and Ser, respectively. These two are the preferred residues found in this position although other amino acids can also be found at this position. (B) A helical wheel presentation of the two domains in the GutR TPR motif.

glucose. Part of the region in which these proteins share homology shows structural properties similar to that of a novel 34-amino-acid motif designated tetratricopeptide (TPR) motif (31). This TPR motif is predicted to exist as an α-helix and can be divided into two subdomains. Both domains can form amphipathic helices. Indeed, the putative TPR motif in GutR meets all these requirements (Fig. 7). The TPR motif is observed in many eucaryotic proteins and tends to occur repeatedly in the same protein. There are 10 TPR motifs in Cyc8. However, the precise functional roles of the TPR motif are unknown, although it can possibly be involved in protein oligomerization and protein-membrane interaction. Further characterization of the TPR motif in GutR through site-directed mutagenesis may provide insights to determine its structure-function relationships.

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