The *glpT* and *glpQ* genes of the glycerol regulon in *Bacillus subtilis*

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The cloning of the *Bacillus subtilis glpT* and *glpQ* genes and their nucleotide sequences are reported. Analysis of mRNA indicates that *glpT* and *glpQ* constitute one operon which is transcribed from a sigma A type promoter. The steady state amount of *glpTQ* mRNA is increased in cells grown in the presence of glycerol 3-phosphate. The 5' untranslated leader sequence of *glpTQ* mRNA contains an inverted repeat which shows sequence similarity to repeats present in the leader sequences of *glpFK* and *glpD* transcripts. These repeats seem therefore to be essential control elements for all *B. subtilis glp* genes.

Keywords: *Bacillus subtilis*, glycerol regulon, *glpT* gene, *glpQ* gene

INTRODUCTION

Genes of the *B. subtilis glp* regulon are involved in catabolism of glycerol and glycerol 3-phosphate (G3P). The known *glp* genes map at two locations on the bacterial chromosome, 15° and 75°. We have previously reported the cloning of the four *glp* genes located at 75° and their nucleotide sequences (Holmberg & Rutberg, 1989; Holmberg *et al.*, 1990; Beijer *et al.*, 1993). These genes are: *glpP*, encoding a positively acting regulatory protein; *glpF*, encoding a glycerol uptake facilitator; *glpK*, encoding glycerol kinase; and *glpD*, encoding an NAD-independent G3P dehydrogenase. The four genes represent three operons: *glpP*, *glpFK* and *glpD*. *glpFK* and *glpD* are induced by G3P. A conserved inverted repeat is found in the 5' untranslated leader sequences of *glpFK* and *glpD* mRNA, respectively. This repeat has been shown to be a conditional signal for termination of the *glpD* transcript and the GlpP protein is proposed to increase transcription across the inverted repeat in induced cells (Holmberg & Rutberg, 1991, 1992). The activities of, e.g., the *B. subtilis sacPA* and *sacB* operons (Crutz *et al.*, 1990; Débarbouillé *et al.*, 1990) and the *E. coli bgl* system (Houman *et al.*, 1990) are controlled in a way similar to the *glpD* activity. However, the regulatory proteins and their targets in the *sac* and *bgl* systems are not related at the sequence level to GlpP and the *B. subtilis glpD*

inverted repeat, respectively. The *glp* genes are subject to glucose repression but the mechanism is not understood.

The activity of the *Escherichia coli glp* genes is controlled by a repressor protein, the product of the *glpR* gene (Schweizer *et al.*, 1985). Binding of G3P to the repressor decreases its affinity for its operators, leading to induction of the *glp* genes. More than ten *glp* genes have been identified in *E. coli* (Lin, 1987; Truniger *et al.*, 1992). Among these the *glpTQ* operon encodes a G3P permease and a phosphodiesterase which hydrolyses glycerophosphoryl diesters into G3P plus alcohol (Eiglmeier *et al.*, 1987; Tommassen *et al.*, 1991). The permease acts as a G3P-phosphate anionic antiport (Maloney *et al.*, 1990); it may also have a role in phosphate metabolism under some conditions (Brzoska *et al.*, 1987; Larson, 1987). The *B. subtilis glpT* gene maps at 15°. GlpT mutants fail to grow with G3P as a sole carbon source and are defective in uptake of G3P (Lindgren, 1978). GlpP mutants are also defective in uptake of G3P in addition to being non-inducible for the *glpFK* and *glpD* products. Consequently, they cannot grow on either G3P or glycerol as a sole carbon source.

In the present communication we report the cloning of *B. subtilis glpT*. Sequencing of the *glpT* region has revealed a gene downstream of *glpT* which we propose to be *glpQ* on basis of its similarity to *E. coli glpQ*. Analysis of transcripts shows that *glpTQ* is one operon. The 5' untranslated leader sequence of the *glpTQ* mRNA contains an inverted repeat with strong similarity to those present in *glpFK* and *glpD* mRNA. Such a repeat thus seems to be an essential control element for *B. subtilis glp* genes.

Abbreviations: Ap, ampicillin; C230, catechol-2,3-dioxygenase; rbs, ribosome binding site; Cm, chloramphenicol; G3P, glycerol 3-phosphate; Tc, tetracycline.

The EMBL accession number for the nucleotide sequence reported in this paper is Z26522.

Table 1. Bacterial strains and plasmids

Strain/plasmid	Genotype/phenotype	Source/reference
<i>B. subtilis</i>		
BR95	<i>ilvC1 pheA1 trpC2</i>	Our collection
KA6	<i>glpT1</i> (previously referred to as <i>phm2a</i>) <i>pheA1</i>	Lindgren (1978)
<i>E. coli</i>		
MM294	<i>pro thi endA hsr_k hsr_{m+}</i>	Amann <i>et al.</i> (1983)
Plasmids		
pHP13	Cm ^R Em ^R	Haima <i>et al.</i> (1987)
pHV32	Ap ^R Cm ^R Tc ^R	Niaudet <i>et al.</i> (1982)
pBluescript II KS(-)	Ap ^R	Stratagene
pAMB22	Cm ^R Tc ^R XylE ⁻	Zukowski & Miller (1986)
pLUM2	<i>glpT</i> ⁺ Ap ^R Cm ^R Tc ^R	This work
pLUM5	internal <i>glpT</i> fragment, Ap ^R	This work
pLUM620	<i>glpT</i> promoter, XylE ⁺ Cm ^R Tc ^R	This work
pLUM621	<i>glpT</i> promoter, XylE ⁺ Cm ^R Tc ^R	This work

Fig. 1. Schematic representation of the *glpTQ* region of the *B. subtilis* chromosome. The positions of inverted repeats are indicated with hairpin symbols. Arrows indicate direction of transcription. The chromosomal DNA fragment present in each plasmid is shown. The vector parts are: pHV32 in pLUM2; pBluescript II KS(-) in pLUM5; pAMB22 in pLUM620 and pLUM621. C, *Cfr10I*; E, *EcoRI*; EV, *EcoRV*; N, *NaeI*; P, *PstI*; Pv, *PvuII*. Restriction endonuclease sites shown are not necessarily unique. The nucleotide sequence was determined from 600 bp upstream of *glpT* to 800 bp downstream of *glpQ*. Both strands were sequenced and all restriction sites fully overlapped. The nucleotide sequence between EV-Pv is presented in Fig. 2. Plasmid pLUM620 was constructed as follows: a 2.3 kb *SacI*-*KpnI* DNA fragment (including the *NaeI*-*NaeI* fragment and a few extra bp from the cloning cassette of pBluescript) was taken from a plasmid containing the *NaeI* fragment inserted in the *EcoRV* site of pBluescript II KS(-). The *SacI*-*KpnI* fragment was digested with *Cfr10I* and made blunt-end with Klenow enzyme. After digestion with *EcoRI*, the fragment was ligated to pAMB22 that had been cleaved with *EcoRI* and *SmaI*.

METHODS

Strains and plasmids. Bacterial strains and plasmids used in this work are listed in Table 1.

Media. Bacterial strains were kept on Tryptose Blood Agar Base plates. For preparation of plasmid DNA the bacteria were grown in Luria Broth. The minimal salts solution was that of Anagnostopoulos & Spizizen (1961). When cells were kept on minimal plates containing G3P as the sole energy and carbon source, a minimal medium with no phosphate was used (Garen & Levinthal, 1960). When appropriate, the following additions were made: glucose, 5 g l⁻¹ (30 mM); glycerol, 20 mM;

DL- α -G3P, 40 mM; Casamino acids, 5 g l⁻¹. Antibiotics were added to the following concentrations: ampicillin (Ap), 50 mg l⁻¹; chloramphenicol (Cm), 5 mg l⁻¹ (*B. subtilis*) or 12.5 mg l⁻¹ (*E. coli*); tetracycline (Tc), 10 mg l⁻¹.

Transformation. Competent *B. subtilis* cells were prepared as described by Arwert & Venema (1973). *E. coli* cells were made competent as described by Mandel & Higa (1970).

DNA techniques. Chromosomal DNA was prepared essentially as described by Marmur (1961). Plasmid DNA from *E. coli* was prepared using the boiling method of Ausubel *et al.* (1987) (mini preparations) or Qiagen (Diagen; maxi preparations). Plasmid DNA from *B. subtilis* was prepared using Magic Maxipreps (Promega). DNA fragments separated on agarose gels were purified using GeneClean (Bio 101 Inc.).

Cloning procedure. Plasmid pHV32 replicates in *E. coli* but not in *B. subtilis*. It carries a *cat* gene which is expressed in both *E. coli* and *B. subtilis*. Variants of pHV32 which carry fragments of *B. subtilis* chromosomal DNA can transform *B. subtilis* to Cm^R by integrating into the bacterial chromosome by homologous recombination. Plasmid pHV32 carrying *B. subtilis* chromosomal DNA can be regenerated in *E. coli* (Niaudet *et al.*, 1982) by cleaving chromosomal DNA with an appropriate restriction enzyme, followed by ligation and then transformation of *E. coli* selecting for antibiotic resistance.

B. subtilis BR95 DNA and pHV32 were cleaved with *EcoRI*, mixed and ligated. *B. subtilis* KA6 (*glpT1*) was transformed to Cm^R with the ligate. About 0.1% of the Cm^R transformants grew on G3P as a sole carbon and energy source, showing that they had been transformed for the *glpT* marker also. DNA from three such transformants, carrying pHV32, was used to transform *B. subtilis* KA6 to Cm^R. Between 75% and 85% of the Cm^R transformants were also GlpT⁺, showing that pHV32 had integrated close to *glpT*. From two of the three transformants carrying pHV32, variants could be selected which grew in the presence of at least 40 μ g Cm ml⁻¹. This indicates that pHV32 has integrated by the Campbell mode and is flanked by a duplication of the fragment used for integration (Young, 1984). pHV32 and adjacent chromosomal DNA was regenerated from one of these two transformants. Chromosomal DNA from the

transformant was cleaved with different restriction endonucleases, ligated and used to transform *Escherichia coli* MM294 to Ap^R, Cm^R or Tc^R. Plasmid DNA was prepared from the transformants and used to transform *B. subtilis* KA6 selecting for Cm^R. Cm^R transformants were then checked for growth on G3P. Two plasmids generated by *Bgl*II and *Sph*I cleavage, respectively, yielded Cm^R transformants of which 90% grew on G3P and thus carried the wild-type allele of *glpT*. The plasmid called pLUM2 (Fig. 1), obtained with *Bgl*II, was used for further work. It contains the wild-type allele of *glpT1* on an 8.6 kb fragment. Further subcloning showed that the wild-type allele of *glpT1* is located on a 1 kb *Pst*I fragment (pLUM5, Fig. 1).

DNA sequence analysis. Nucleotide sequences were determined by the dideoxy chain-termination method of Sanger *et al.* (1977), using Sequenase version 2.0 (United States Biochemical Corp.) and [α -³⁵S]dATP. Templates used were DNA fragments cloned into pHV32 or pBluescript II KS(−). Primers used were universal or specific for the *B. subtilis* chromosomal DNA being sequenced. Nucleotide sequences were analysed using the GCG Sequence Software Package (Devereux *et al.*, 1984).

Preparation of cell free extracts and assay of catechol-2,3-dioxygenase (C23O). Cell-free extracts were prepared as described by Lindgren & Rutberg (1974). C23O activity was measured as described by Sala-Trepat & Evans (1971).

RNA preparation. Bacteria were grown to OD₆₀₀ = 0.3 at 37 °C on a rotary shaker (200 r.p.m.) in minimal salts solution supplemented with Casamino acids and, when appropriate, Cm. The cultures were then divided into two parts. To one part, G3P (40 mM) was added and to the other part no addition was made. The cultures were further incubated at 37 °C for 1 h and total RNA was then extracted as described by Resnekov *et al.* (1990).

Nuclease S1 mapping. Nuclease S1 mapping was done as described by Sambrook *et al.* (1989). A sample (0.4 µg) of probe labelled at the 5' end was mixed with 40 µg of total RNA. After hybridization overnight at 46 °C, each sample was divided in two and one part was treated with 300 U nuclease S1 ml^{−1} for 1.5 h at 37 °C. Half of each sample was analysed by electrophoresis on a polyacrylamide gel (6%, w/v, acrylamide, 0.3%, w/v, bisacrylamide) containing 7 M urea. A known DNA sequence was used as a size marker.

Northern analysis. Northern blot and hybridization analysis was performed according to the protocols of Amersham. RNA was blotted onto Hybond-N membranes (Amersham). ³²P-labelled probes were synthesized using a random primed DNA labelling kit (Boehringer Mannheim) and [α -³²P]dCTP [> 3000 Ci mmol^{−1} (111 TBq mmol^{−1}); Amersham]. RNA molecular mass markers (Bethesda Research Laboratories) were used to estimate the size of transcripts.

RESULTS AND DISCUSSION

Nucleotide sequence of the *glpT* and *glpQ* genes

The wild-type allele of *glpT1* was cloned on an 8.6 kb fragment of chromosomal *B. subtilis* DNA generating plasmid pLUM2 as described in Methods. DNA sequence analysis of about 3700 bp in pLUM2, including the 1 kb *Pst*I fragment containing the wild-type allele of *glpT1*, revealed three open reading frames (ORFs; Figs 1 and 2). The first ORF, which includes the 1 kb *Pst*I fragment, can encode a protein of 444 amino acids with a molecular mass of 50 kDa. The proposed protein shows 60% identity

with the *E. coli* G3P permease (Fig. 3). This high level of identity with the *E. coli* G3P permease, together with the fact that a mutation which impairs uptake of G3P in *B. subtilis* maps within this ORF, identifies it as *glpT*. Preceding *glpT* by 10 bp is a typical ribosome binding site (rbs; Fig. 2). A hydropathy plot of *B. subtilis* GlpT (not shown) reveals several hydrophobic, potential membrane spanning segments and it is very similar to that reported for *E. coli* GlpT (Eiglmeier *et al.*, 1987). The *E. coli* protein has been proposed to contain 12 membrane spanning segments (Gött & Boos, 1988).

The second ORF starts with an ATG 101 bp downstream of the TAA stop codon of *glpT*. It can encode a protein of 293 amino acids with a molecular mass of 33 kDa. The sequence of the proposed protein shows 37% identity with the *E. coli* GlpQ, a periplasmic glycerophosphoryl diester phosphodiesterase (Fig. 4). The similarity between the two sequences is particularly strong in the amino-terminal region which has been suggested to harbour the catalytic site of *E. coli* GlpQ (Tomassen *et al.*, 1991). The first 26 amino acids may constitute a signal peptide containing three positively charged amino acid residues, a hydrophobic core and a putative cleavage site between two alanines. These features are common among many known *B. subtilis* signal peptides. *B. subtilis* homologues of *E. coli* periplasmic proteins are often lipoproteins but this is not the case for *B. subtilis* GlpQ (Nagarajan, 1993). In *E. coli*, *glpT* and *glpQ* form one operon. In *B. subtilis*, *glpT* and the downstream ORF seem to constitute one operon (see later). From the above facts we propose that the ORF downstream of *B. subtilis glpT* is the *glpQ* gene. *glpQ* is preceded by a possible rbs (Fig. 2).

Downstream of *B. subtilis glpQ*, we have found part of a third open reading frame (corresponding to 286 amino acids). This ORF3 has the opposite orientation relative to *glpT* and *glpQ*. No protein with a sequence similar to the partial sequence of the proposed ORF3 protein has been found in searches of the EMBL data bank (Release 32.0804).

A well-conserved sigma A type (Gross *et al.*, 1992) — 10 sequence, GATAAT, is located 160 bp upstream of the proposed *glpT* ATG start codon. At 15 bp upstream of this sequence a typical —35 sequence, TTTACA, is found. Four bp downstream of the proposed —10 sequence is a sequence where 13/14 bp conform to the 'glucose repression sequence' proposed by Weickert & Chambliss (1990). Similar sequences have been found also upstream of *B. subtilis glpP*, *glpFK* and *glpD*, respectively. A role for these sequences in glucose repression of the *B. subtilis glp* regulon has not been demonstrated, however.

An inverted repeat (position 202–248, Fig. 2) is found between the proposed promoter and the *glpT* coding region. This inverted repeat shows sequence similarity to the previously identified inverted repeats (Fig. 5) in the non-coding regions upstream of *glpD* and *glpFK*, respectively (Holmberg *et al.*, 1990; Beijer *et al.*, 1993). The similarities extend to upstream of the inverted repeats. The importance of the control regions (promoter and

1 ^{EFORV}
 GATATCTAATTCATCCATCTTTAAACAGCCTCCCTCCAGATTTTATGTTACTAGTAAAAAATAATTCATTTAGTAAAGGATAATATTCACCTTC
 101 CTAAGGCCCAATTGAAAAATTACCGGTTCACATTCGCTCCGACTATGATATTTAAGAAAGCGCTATCATGATAAGTTAATAGCTTGGATGGAGATGAG
 201 GAGAGCCAGGAATCCAACCCCTTTTGACAAAGGGTGTTCCTGGCTCTTTTGTGTTTTCGGGAAAGGAGGCACGCCACATCCAGCACAAACAGCAAG
 301 GGGGATAATTATTAATGCTTAATATATTTAAACCGGCTCCCTCATATTGAGAGACTGGATGATTCAAAGATGGATCGGCATACAAACGGCTCAGACTGCA
 401 AGTATTCATTGGTATTTTCATTGGCTACGACGGCTACTACCTGCTCGGAAAAAATTTGCTTTGCCATTCCTTATCTGCAGGAACAGGGATTTTCGAAA
 501 ACAGAACTGGGTTTGGTGGCTGGCGCTGTGTCCATTGCGTACGGCTTCAGCAAAATTCATCATGGGAATGGTGTCTGACCGCTGTAATCCGAGATATTTTC
 601 TGGCGACAGCGCTATTTTATCAGCAATCGTTAATATCTATTCGTTTCAATGCTTGGGTGACTTCGAGCGTCACCATCATGTTTATTTTATGTTTAT
 701 CAACGGATGGTTTCAAGGAATGGGCTGGCGGCTACGCGCAGGACGATGGCCCATGGTTCCTCATAAGTGAACGGCGCACAAAAATGTCGATCTGGAAT
 801 GTCGCCCATACATAGGCGGCGGTATCTGTGCTCGCTGTGCTACTCTTGGGATCGCGATGTTTCGTCACTTGGAAAAGCGTGTTCCTTCCTCCCTGCCATCA
 901 TCGCCATTATCATTTTCATTTTAAATGTTTATGTTTGGTTCGTGATACGCTCAATCTTCGGGACTCCCGCAATGAAGAGTATCGGAATGACTATCCAAA
 1001 ACACGCTTTTAAAAATCAGGAAAAAGAAATTAACAACAAAAAGAAATTCCTTTTCAATATGTGCTGAACAAACAAATTTCTTTGGTACATCGCTTTTGCCAAAT
 1101 GTATTTGTTTATTTCTGTCGATACGCGGTGGTGTGACTGGGCGCTACTTACTTAACAGAGCCAAAGGCTTTTCTCCTGAAGACTACGCTGGTCTGAT
 1201 TTTCTTACGAATACGACGGCATACCGGGAACGATCTGTGCGGCTGGATCAGTGACCGATTTCTTCAAAAGCGCGGGACCGGCAGGCGTTTGTGTTAT
 1301 GGCGGGTGTTCATTTGCTGATTCGCTGTTGCTGTAATCCGCGCGCAATCCGCTCGTGGAACAATATCGCCCTGATCAGCATTTGGCTTCTTAATCTAC
 1401 GGACCTGTTATGCTGATCGGCTTCAAGCAATGACCTAGCACCGAAAAAGCAGCGCGAATCGACCGGTTTGACCGGATTTTTCGGATACATCGCGG
 1501 GATCCGCTTCGCAACCGCAATATGGGCTTTGCTGTTGACCGTTTAACTGGAACGGCGGCTTTATCATGCTGATCTCATCTGCACTTCTTGCCATCGT
 1601 CTCTTGGCTTTGACGTTGAATACAGGAAACCGGCAGAGCATGTTTAAAGAAAGACACATAAAGATTATAGTTTTCACACGCGGTTTACATCCGTT
 1701 TTTTATTATGAAAGACAGGTAGTCTTATCTATAAGGAGGAGAAATCATGAGAAAAATAGAACTAGGCTTGTGTTTCTGCTGGGCTTACTCTC
 1801 ATTTATGTTAAAGCCAGTGTTCGGCAGCGTCAAAAGGAAACCTGCTGTCTACCTGACCGTACCGCAGCAGAGGGGCTTCTGGATATGTGCTT
 1901 GAGCACAGATTCGTCTTACGAAACCGCTCAGAAAAATGAAAGCTGATTTTATGAAATGGATCTGCAATGACAAAGACGGAATTTGATTTGTCATGC
 2001 ATGACGAAAAACTGGACCGCACCAACGGAATGGGTTGGGTGAAAGACCATACGCTCGCAGACATTAAGAAGCTTGACCGCGGTTCTTGGTTTAAATGA
 2101 AGCCTATCCGGAAGAAAGCAAGCGCAGTACGCTCGGCTTAAAGTTCTACATTAGAAGAGGTATTAGACCGTTTCGGAAGCAGCGCAACTACTACATT
 2201 GAAACAAAAATCGCTGACACCTACCCAGGTATGGAAGAAAACTGATCGCTTCTTTGAGAAACATAAAATCTGGAAGAGCAATTAAGCCAGGCCAGG
 2301 TGATCATTCATCGTTACAGAAAGAAAGCCTGGTAAAGTCCATCAATACAGCCAAACCTGCGGACCGTTCAATTTGCTGGAAGCCAAACAAATGGCGTC
 2401 AATGACAGATGCTGCCCTGGAAGAAATCAAAACATACGCTGTGCGTGGGCGCGGATTATAAAGCTTTAAATCAAGAAACGTCGATGATCCGACG
 2501 CACGGCTCTGCTTCTATCTTACACAGTCAATATGAAGCTGATATGCATCGCTGCTTGAATGGGCGGTAACAGGAGTGTTCACCAACTATCTGATC
 2601 TTTTCCACAAAGTAAAAAGGGTTATTAAGCAAAAACTTGGCAGCAATATCGCAAGGTTTCTTCTATATTTTATTTCTTTCGCGCAGCAGCTTCT
 2701 GACTGTCCATTAAAGCAGCTG
 S Q G N L L O
 ORF3

Fig. 2. For legend see facing page.

B.s: MNTFKPAPHIRLDOSKMDAAYVRLRUDVHIGTRIGYAGYYLURKNFAF
E.c: MNTFKPAPHIRLDPAACDPTVRLRNDHIGTRIGYAGYYLURKNFAF

B.s: AIPYVDEGGFSKTEGLVDAASSTAYGFSKFINGVSDRONPRVFLATGL
E.c: AMPYVDEGGFSRGLGFAUSGISTAYGFSKFINGVSDRONPRVFLATGL

B.s: FLSAIVNIFVSMPTSSVTIMFIFMFINGWFGMGWPPCGRTMAHMFSS
E.c: ILAAVYMLFMGFVPMATSSIAVMEVLFLCGWFGMGWPPCGRTMVHMFSS

B.s: ISERGKMSDNNVAHNNGGGLAHVDTLGMAMFVTKSVFFPAIDATII
E.c: OKERGIVSMNCAHNNGGGTPPUFLGMAHNDQHAALYHPAFQATILV

B.s: SFLIYLLVMDTPOSGLPPIEYVAVYKHAFAKNDKELTIRGILROYVL
E.c: ALFAFAMNMDTPOSGLPPIEYVAVYDDYNEKASQELIYAKDUFROYVL

B.s: NNRKLNWYIAHNVFYVFRYGVVDAAPTYTDEARGSPEDSHSYFLYEV
E.c: NNRKLNWYIAHNVFYVFRYGVVDAAPTYTDEARGSPEDSHSYFLYEV

B.s: AGTPGTLCGMHSDRFFKSRAPAGVUFMAQVHTAVVYVUNPAGNPLVD
E.c: AGTPGTLCGMHSDKVRGRNGAGVGFHTLVYIATVYVUNPAGNPLVD

B.s: NTAUINIGFLIYGPVHLIGLDAIHLAPKKAAGTAAGRTGFGYIGGSAAFA
E.c: NICHINIGFLIYGPVHLIGLDAIHLAPKKAAGTAAGRTGFGYIGGSAAFA

B.s: NATMGFVVDKNNNGGRLMUTSSDILAVIFDALTWNTGKRAEHV
E.c: SATMGFVVDKNNNGGRLMUTSSDILAVIFDALTWNTGKRAEHV

E.c: GG

Fig. 3

B.s: HRKNRILAEVLSGLLSFFHYTHVSAASKGNLUSPORTLVAHRGASGVV
E.c: MKLTLKNLSHAIMHSTIDYMGSSAMAADSHERIVLAHRGASGVV

B.s: PEHTILSYETAKKMADETELDLQHTKGNLIVMDEKLORTINGMGWVK
E.c: PEHTILPAKAMAYAQADYLEQDLVHTKODNIVVLDHNYLORVTOVADRFP

B.s: DH-----ITLAOTKMDAGSWFN-----EATPEKAKPQYVGL
E.c: DRARKDGRYYAIDFTLDEIKSLKFTGFDIENGKKVQTYPGRFPMGKSDF

B.s: NVPTLEBVLD-----RFGKHANVYDNRSPOTYPCMEERLITAS--LQK
E.c: RVHTLEBETEFVQGLNHSTGKNIGTYPERKAP-WFHQDEKQIAAKTLEV

B.s: HRLUQKHSKPGQVITDSFSKESQVKV-HQDQNPLOT---VQDL-----
E.c: LKKGTYTGKDDNVYLDQDADELKRIKNELEPKMGMEINLVQITAYTDWN

B.s: --EAKOMAS-----MTDAALEEIKTYAVGAGPDYKALNDE-----
E.c: ETQKQPDGSSWVNYNDWMFKPKAMKQVARYADGIGPDYHMLIETSQPG

B.s: NVYRM-----RSHQCLHPYTVNNE-----ADHHRDLD-----WGVIG
E.c: NIKLTGMVQDAQNNLHVHPYTVRSOKLPEYTPDVNQLDLYNKAGVNG

B.s: VFTNYPD---LHFRYKKGY
E.c: LFTDHPDKAVKFLNKE

Fig. 4

Fig. 3. Comparison between the amino acid sequences of the deduced product of the *B. subtilis glpT* gene (*B.s*) and the *E. coli* GlpT protein (*E.c*). Identical amino acids are boxed. The identity between the two sequences is 60%. When considering conserved amino acid substitutions, the similarity is 80%, as determined with the Bestfit program (Devereux et al., 1984).

Fig. 4. Comparison between the amino acid sequences of the deduced product of the *B. subtilis glpQ* gene (*B.s*) and the *E. coli* GlpQ protein (*E.c*). Identical amino acids are boxed. Dashes represent gaps inserted to optimize the sequence alignment. The identity between the two sequences is 37%. When considering conserved amino acid substitutions, the similarity is 63%, as determined with the Bestfit program (Devereux et al., 1984).

inverted repeat) upstream of *glpD* and *glpFK*, respectively, has been demonstrated (Holmberg & Rutberg, 1991, 1992; Beijer et al., 1993). It seems justified to assume that the inverted repeat upstream of *glpT* is important for control of *glpT* and *glpQ* expression.

A second inverted repeat (position 2636–2665, Fig. 2) is located between *glpQ* and ORF3 and is not homologous to the inverted repeats upstream of *glpT*, *glpFK* and *glpD*, respectively. However, it could function as a transcriptional terminator for transcripts originating both upstream and downstream of the repeat.

Transcription of *glpT* and *glpQ*

The sigma A type promoter preceding *glpT* is located on a 1.3 kb *NaeI* *Cfr101* fragment. This fragment was inserted in front of the promoter-less *xyIE* gene in plasmid pAMB22 to yield pLUM620 (Fig. 1). The identity and

proper orientation of the fragment was verified by sequencing. Cell extracts of BR95/pAMB22 have no measurable C23O activity whereas extracts of BR95/pLUM620 have a measurable C23O activity which is 1.5–4 times higher when the cells are grown in the presence of glycerol or G3P. Glucose represses the C23O activity to a level below that found in extracts of uninduced cells irrespective of whether glycerol or G3P is also present in the growth medium (data not shown). This level of induction is comparable to that seen for induction of *glpFK* but lower than that for *glpD* which has been shown to be about 15-fold (Beijer et al., 1993; Holmberg & Rutberg, 1991). In the following, the promoter driving expression of the *xyIE* gene in pLUM620 will be referred to as the *glpT* promoter.

The 5' end of the transcript initiated from the *glpT* promoter in pLUM620 was mapped using nuclease S1. The probe used was a 346-nt-long *EcoRV*–*XbaI* fragment

Fig. 2. Nucleotide sequence of *glpT*, *glpQ* and part of ORF3 of *B. subtilis*. Both strands were sequenced and all restriction sites fully overlapped. Two potential rbs and a potential promoter sequence are underlined. Nucleotides conforming to the 'glucose repression sequence' are marked with Δ . Inverted repeats are indicated with horizontal arrows. The transcriptional startpoint of *glpTQ* is indicated with a vertical arrow. Left-margin numbers refer to the nucleotide sequence, right margin to amino acid sequences.

Fig. 5. The *glpTQ*, *glpFK* and *glpD* inverted repeats. The inverted repeats are presented as stem-loop structures. Highly conserved regions are boxed.

which extends from upstream of the proposed -35 and -10 sequences of the *glpT* promoter and into the cloning cassette of pAMB22. This fragment (contained in pLUM621; Fig. 1) has a promoter activity similar to that found for pLUM620. The probe was labelled at the 5' end. Since the *Xba*I site originates from the pAMB22 cloning cassette, the experiment will only detect a transcript originating from pLUM620. Total RNA was extracted from BR95/pLUM620 grown with and without G3P. After hybridization with RNA from induced cells and treatment with nuclease S1, a protected fragment of 189 nt was found (Fig. 6). The size of this fragment corresponds to a transcriptional start site at position 160 (Fig. 2).

Fig. 6. Determination of the start of a transcript initiated from the *glpT* promoter. Lane 1, 10 μ g of total RNA from G3P-induced BR95/pLUM620, after nuclease S1 treatment. Lanes 2-5, standard, nucleotide sequence of a known DNA fragment.

The size of the transcript initiated from the *glpT* promoter in BR95 was determined in a Northern blot analysis (Fig. 7). Total RNA was extracted from cultures grown with or without G3P and probed with the 1 kb *Pst*I DNA fragment which is internal for *glpT* (Figs 1 and 2). A transcript of about 2600 nt was detected which corresponds well to the expected size of a *glpTQ* transcript (about 2500 nt). The relative amount of this transcript was about threefold higher in RNA extracts from cultures grown in the presence of G3P.

Fig. 7. Northern analysis of a *glpT*-specific transcript. Lanes 1, 2 and 3, 5, 10 and 20 µg, respectively, of total RNA from uninduced BR95. Lanes 4, 5, and 6, 5, 10 and 20 µg, respectively, of total RNA from G3P-induced BR95. The probe used was a ³²P-labelled internal *glpT* DNA fragment. RNA size markers (bp) are indicated on the left.

In conclusion, the primary structure of proteins involved in glycerol catabolism in *B. subtilis* and *E. coli* is highly conserved. The transcriptional organization of the corresponding genes is also similar; *glpFK*, *glpD* and *glpTQ* are organized in separate operons. In contrast, although the *glp* genes are induced by G3P and repressed by glucose in both organisms, the mechanisms of control seem quite different. This has been observed also for other genes in carbohydrate catabolism in *B. subtilis* and *E. coli* (Steinmetz, 1993).

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