Forespore-Specific Expression of Bacillus subtilis yqfS, Which Encodes Type IV Apurinic/Apyrimidinic Endonuclease, a Component of the Base Excision Repair Pathway

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The temporal and spatial expression of the yqfS gene of Bacillus subtilis, which encodes a type IV apurinic/apyrimidinic endonuclease, was studied. A reporter gene fusion to the yqfS opening reading frame revealed that this gene is not transcribed during vegetative growth but is transcribed during the last steps of the sporulation process and is localized to the developing forespore compartment. In agreement with these results, yqfS mRNAs were mainly detected by both Northern blotting and reverse transcription-PCR, during the last steps of sporulation. The expression pattern of the yqfS-lacZ fusion suggested that yqfS may be an additional member of the ErG regulon. A primer extension product mapped the transcriptional start site of yqfS, 54 to 55 bp upstream of translation start codon of yqfS. Such an extension product was obtained from RNA samples of sporulating cells but not from those of vegetatively growing cells. Inspection of the nucleotide sequence lying upstream of the in vivo-mapped transcriptional yqfS start site revealed the presence of a sequence with good homology to promoters preceding genes of the αC regulon. Although yqfS expression was temporally regulated, neither oxidative damage (after either treatment with paraquat or hydrogen peroxide) nor mitomycin C treatment induced the transcription of this gene.

Endogenous and environmental factors such as reactive oxygen species, UV light, and chemical carcinogens alter the chemical structure of DNA bases, producing lesions that are substrates for a myriad of DNA glycosylases of the base excision repair (BER) pathway (27). The apurinic/apyrimidinic (AP) sites generated not only by the action of DNA glycosylases but also by the spontaneous depurination and depyrimidination of DNA (29, 30) are inherently toxic and highly mutagenic and thus should be rapidly processed and eliminated (31). The first catalytic event during the repair of AP sites is carried out by AP endonucleases, which cleave the DNA backbone immediately 5' of an AP site, generating a 5' deoxyribose-phosphate group and a 3' deoxyribose-hydroxyl group. AP endonucleases have been classified into two families, namely, ExoIII and type IV AP endonucleases (3, 13), and these enzymes have been conserved across the species of the three domains of life (23).

Dormant spores of Bacillus subtilis are more resistant than their vegetatively growing counterparts to several chemical substances, including acids, bases, alkylating agents, and oxidizing agents (reviewed in references 40, 41, and 58). The existence of core coats, the low permeability of spores to hydrophilic compounds, and the protection of spore DNA from damage by its saturation with α/β-type small acid-soluble proteins (SASPs) account for this resistance (reviewed in references 40, 56, and 58). It has been demonstrated that α/β-type SASPs slow DNA depurination-depyrimidination, as well as hydroxyl radical-induced DNA backbone cleavage, thus contributing to spore resistance to heat and oxidizing agents (reviewed in references 40 and 58). α/β-type SASPs bind to spore DNA and are in part responsible of the strong resistance of B. subtilis spores to UV light (reviewed in references 40, 41, and 58); however, these DNA-binding proteins do not confer protection to DNA against base alkylation (55).

The genome of B. subtilis (26) possesses genes that potentially encode ExoIII and type IV AP endonucleases, namely, exoA and yqfS, whose products show a high level of homology to ExoIII and type IV AP endonucleases, respectively. Although the enzymology of B. subtilis ExoA has been studied in detail (53), nothing has been reported regarding the mechanisms that control its expression during growth and sporulation of B. subtilis.

The expression of DNA repair systems in the gram-positive spore-forming bacterium B. subtilis has been shown to be differentially regulated during growth and differentiation (4, 11, 32, 34), as well as during spor germination and outgrowth (54). DNA lesions acquired during unpredictable periods of B. subtilis spores dormancy must be necessarily corrected during germination by spore-specific expressed DNA repair systems (reviewed in references 40 and 58). The best example studied thus far is the correction of the UV-C induced spore photoproduct (5-thyminil-5,6-dihydrothymine) through both the specific spore photoprodut lyase protein (SplB) and the general excision-repair system (UVR) (reviewed in references 40 and 41). However, during unpredicted periods of spore dormancy B. subtilis spores could potentially accumulate, in addition to spore photoprodut (SP), different types of DNA lesions, such as strand brakes, cyclobutane pyrimidine dimers (CPDs), chemically altered bases, and AP sites that could affect essential functions such as transcription and replication during ger-
B. subtilis strain 168 was employed for this study. The strains used in this study are shown in Table 1. Plasmids used in this work are listed in Table 2. The complete yqfS gene was amplified by PCR with genomic DNA from B. subtilis 168 as a template and the oligonucleotide primers 5'-GCGAAATTCGGCGTTGAA CGAAGAAGGTTAAGCC-3' (forward) and 5'-CGGGATCCGGCGCGTTGA GTAGCGAACC-3' (reverse). The primers were designed to insert EcoRI and BamHI sites (underlined). Amplification was performed on 0.1 µg of chromosomal DNA by using an MJ Research (Watertown, Mass.) Minicycler according to the instructions of the supplier.

**TABLE 1.** Strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Genotype and/or phenotype</th>
<th>Source (reference)</th>
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<tr>
<td><strong>Strains</strong></td>
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<tr>
<td>B. subtilis 168</td>
<td>trpC2</td>
<td>Laboratory stock</td>
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<td>WN118</td>
<td>sigGΔ1 trpC2</td>
<td>Wayne Nicholson</td>
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<td>PERM317</td>
<td>trpC2 ygfS-lacZ; Cm'</td>
<td>This study</td>
</tr>
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<td>PERM336</td>
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<td>This study</td>
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<tr>
<td>YB3000</td>
<td>metB5 trpC2 xin-1 sigB amyE (deleted for spb) pCCR202 (recA-lacZ at amyE); Cm'</td>
<td>R. E. Yasbin</td>
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<td><strong>E. coli</strong></td>
<td>e14 (McrA-ΔΔmcrCB-hsdSMR-mrr) 171 endA1 supE44 thi-1 lacY1 recB recC sbcC uvaC::Tn5 (Kan') urac [F' proAB lacZD15 lacZM15 Tn10 (Tet')]</td>
<td>Stratagene</td>
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<td>E. coli SURE, pUC18; Amp' Te'</td>
<td>This study</td>
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<td>PERM253</td>
<td>E. coli SURE, pPERM253; Amp' Te'</td>
<td>This study</td>
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<td>XL1-Blue</td>
<td>recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lacF' proAB lacPZΔ M15 Tn10 (Tet')</td>
<td>Stratagene</td>
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<td>E. coli XL1-Blue, pPERM253; Amp'</td>
<td>This study</td>
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<tr>
<td>XL10-Gold Kan</td>
<td>Tet' Δ(mcrA) 183, Δ(mcrCB-hsdSMR-mrr); 173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lacH' [F' proAB lacPZΔM15 Tn10 (Tet') Tn5 (Kan')]</td>
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<td>Integrational lacZ fusion vector; Cm'</td>
<td>W. Nicholson (17)</td>
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<td>Multisite E. coli cloning vector</td>
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<td>pPERM253</td>
<td>ygfS gene cloned in pUC18</td>
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<td>514-bp EcoRI/Nael fragment of ygfS from pPERM253 cloned in pJF751</td>
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<tr>
<td>pPERM348</td>
<td>ygfS ORF cloned into the BamHI site of PQE-30</td>
<td>This study</td>
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* Cm', chloramphenicol resistant; Amp', ampicillin resistant; Te', tetracycline resistant.

mination (40). Although the expression of splB in the forespore compartment by σ54 RNA polymerase has been widely substantiated (44, 45), few data exist in the literature concerning the expression of other specific or general DNA repair systems in the forespore compartment.

As mentioned above, in the genome of B. subtilis exists an open reading frame (ORF), ygfS, whose predicted product shows 53% homology with the type IV AP endonuclease of Escherichia coli. We describe here the expression of the cloned ygfS gene of B. subtilis from an IPTG (isopropyl-β-D-thiogalactopyranoside)-inducible promoter in E. coli. Our results demonstrate that a His6-YqfS purified enzyme is able to process the cleavage of abasic sites in the DNA. In addition, our results demonstrated that the expression of ygfS is forespore specific but was not induced by the stress imposed by superoxide radicals, by hydrogen peroxide, or by the DNA-damaging agent mitomycin C.

**MATERIALS AND METHODS**

Bacterial strains, plasmids, and growth conditions. B. subtilis and E. coli strains used in the present study are shown in Table 1. Plasmids used in this work are listed in Table 2. The complete ygfS gene was amplified by PCR with genomic DNA from B. subtilis 168 as a template and the oligonucleotide primers 5'-GCGAAATTCGGCGTTGAA CGAAGAAGGTTAAGCC-3' (forward) and 5'-CGGGATCCGGCGCGTTGA GTAGCGAACC-3' (reverse). The primers were designed to insert EcoRI and BamHI sites (underlined). Amplification was performed on 0.1 µg of chromosomal DNA by using an MJ Research (Watertown, Mass.) Minicycler according to the instructions of the supplier.

**Cloning of ygfS and construction and integration of a ygfS-lacZ gene fusion.** The complete ygfS gene was amplified by PCR with genomic DNA from B. subtilis 168 as a template and the oligonucleotide primers 5'-GCGAAATTCGGCGTTGAA CGAAGAAGGTTAAGCC-3' (forward) and 5'-CGGGATCCGGCGCGTTGA GTAGCGAACC-3' (reverse). The primers were designed to insert EcoRI and BamHI sites (underlined). Amplification was performed on 0.1 µg of chromosomal DNA by using an MJ Research (Watertown, Mass.) Minicycler according to the instructions of the supplier.

**Purification of His6-YqfS and substrates for AP endonuclease activity.** E. coli PERM348 containing plasmid pPERM348 (Table 1) was grown in 50 ml of LB medium, supplemented with ampicillin (100 µg/ml) at 37°C to an OD of 0.6 at 600 nm (OD600) of 0.5. Expression of ygfS was induced during 4 h at 37°C by the addition of IPTG to 0.5 mM. Cells were collected by centrifugation and washed two times with 10 ml of 50 mM Tris-HCl (pH 7.5)–300 mM NaCl (buffer A). The cells were disrupted in 10 ml of the same buffer containing lysozyme (10 mg/ml) for 30 min at 37°C. The cell homogenate was subjected to centrifugation to eliminate un-disrupted cells and cell debris, and the supernatant was applied to a 5-ml nickel-nitrilotriacetic acid-agarose column previously equilibrated with buffer A. The column was washed with 50 ml of buffer A containing 10 mM imidazole plus 50
ml of buffer A containing 20 mM imidazole, and the protein bound to the resin was eluted with 15 ml of buffer A containing 100 mM imidazole; 2-ml fractions were collected during this last step. Aliquots (15 μl) of the cell homogenate, the flowthrough, and the bound fractions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Two types of substrates were prepared to assay AP endonuclease activity of His6-YqfS, namely, pBluescript (Stratagene), which was partially depurinated after a previously described protocol (28) and a 5′-end-radiolabeled double-stranded 19-mer nucleotide containing a single abasic site (20).

The endonuclease activity against His6-YqfS against pBluescript containing AP sites (AP-pB) was determined in a mixture reaction of 25 μl containing 600 ng of purified His6-YqfS and 100 ng of substrate in 50 mM Tris-HCl (pH 7.5) containing 1 mM dithiothreitol. The reactions were incubated at 37°C for 30 min and analyzed by electrophoresis on a 1% agarose gel and then subjected to autoradiography.

Cell growth and enzymatic assays. B. subtilis strains carrying the yqfS-lacZ fusion were grown and allowed to sporulate in liquid DSM. Samples of 1.5 ml were collected during vegetative growth and throughout sporulation. Cells were washed with 0.1 M Tris-HCl (pH 7.5) and processed for determination of β-galactosidase (42) and glucose dehydrogenase (GDH) activities (19, 42). The β-galactosidase activities were determined in cell extracts obtained from mother cells and forespore fractions prepared according to a previously described protocol (35). Northern blot and primer extension experiments. The total RNA for both Northern blotting experiments and mapping of the 5′ end of yqfS was isolated as previously described (35). Northern blots were performed with RNA samples isolated from strains B. subtilis 168 and WN118 (sigG mutant). RNA samples (20 μg) were separated by electrophoresis through 1% agarose-formamide gel and transferred to a high-bond nylon membrane. The membrane containing the transferred RNA was hybridized at 70°C with a 1,181-bp EcoRI-BamHI fragment from pPERM253 containing the entire yqfS sequence. The probe was labeled by random priming with [α-32P]dCTP by using the Rediprime II DNA labeling system according to the instructions of the provider (Amersham Biosciences, Buckinghamshire, England). Detection of hybrids was performed by autoradiography exposing the membrane to Kodak X-Omat films.

The 5′ end of yqfS was mapped by primer extension (37) of yqfS transcripts produced during sporulation. To this end, total RNA was isolated from vegetative and sporulating cells of B. subtilis PERM317. In order to obtain the maximum amount of yqfS transcripts during sporulation, we monitored the expression of β-galactosidase activity directed by the yqfS-lacZ fusion in this strain. The total RNA (40 μg from each sample) was hybridized with the 20-mer oligonucleotide 5′-CGGCGCGTATTTGTGCGTG-3′, which was complementary to the yqfS mRNA from nucleotides 106 to 124 downstream from the putative yqfS translational start codon. The oligonucleotide was labeled on its 5′ end with [γ-32P]ATP and T4 polynucleotide kinase. The primer was extended with Moloney murine leukemia virus reverse transcriptase, and the extended products were separated by electrophoresis through a 6% polyacrylamide DNA sequencing gel.

The position of the extended products was determined by running a sequencing reaction generated with the same 20-base primer and a 1,978-bp PCR product (PCR RS) extending from 247 bp upstream of the yqfR start codon to 416 bp downstream of the start codon of yqfS (Fig. 1).

RT-PCR experiments. Total RNA from vegetative or sporulating B. subtilis 168 cells, grown in DSM, was isolated by using the TRI reagent (Molecular Research Center, Inc.). Reverse transcription-PCRs (RT-PCRs) were performed with the RNA samples and a Master Amp RT-PCR kit (Epicentre Technologies) according to the instructions of the provider. The primers used for RT-PCRs were 5′-CCTGTGCTGAGAATAGGC-3′ (forward) and 5′-CGGCGCGTATTTGTGCGTG-3′ (reverse) to generate a 132-bp RT-PCR product extending from 4 bp upstream from the start codon of yqfS to 128 bp downstream of this point (Fig. 1). As a control, in each experiment, the absence of chromosomal DNA in the RNA samples was assessed by mounting PCR products with Vent DNA polymerase (New England Biolabs) and the set of primers described above.

RESULTS

Cloning of yqfS. The existence of a type IV AP endonuclease in the genome of B. subtilis was investigated by using the primary structure of E. coli Nfo (51) as a query to search against the database of National Center for Biotechnology Information with a Gapped BLAST program (2). As described in Materials and Methods, this approach was used to retrieve a gene termed yqfS from the genome of B. subtilis (26). Analysis of the yqfS primary structure revealed an ORF of 891 bp with enough information for the synthesis of a predicted protein of 31 kDa. Amino acid alignments showed that YqfS possesses homologies of 53, 52, and 32% with E. coli Nfo (51),
Although the radiolabeled 20-bp-mer was also cleaved by *E. coli* Nfo (Fig. 3A, lane 7), it was observed that a fraction of the partially depurinated plasmid (CCC) to the open circular form (OC) due to single-strand breaks performed by the His6-YqfS protein (lane 3). As shown in Fig. 2 (lane 4), the nondepurinated plasmid (U-pB) was not a substrate for the His6-YqfS protein. Essentially, different amounts of the His6-tagged protein were incubated with 510 nM of this AP substrate. The products of the reaction analyzed on a denaturing acrylamide gel revealed that the endonucleolytic activity of YqfS at the AP site was dependent on the concentration of the enzyme used (Fig. 3A, lanes 2 to 6). To better evaluate this conclusion, these results were analyzed by densitometry, thereby corroborating that cleavage of the AP substrate by His6-YqfS is concentration dependent (Fig. 3B).

FIG. 2. Endonuclease activity of His6-YqfS against a plasmid containing AP sites. Aliquots (600 ng) of His6-YqfS were incubated with 100 ng of either untreated (U-pB [lane 4]) or AP-containing sites (AP-pB [lane 3]) of pBluescript. Lane 1, AP sites-containing plasmid incubated with 50 mM Tris-HCl (pH 7.5)–300 mM NaCl; lane 2, untreated plasmid incubated with 50 mM Tris-HCl (pH 7.5)–300 mM NaCl. The reactions were incubated at 37°C for 30 min and then analyzed by electrophoresis on a 1% agarose gel stained with ethidium bromide.

FIG. 3. Endonuclease activity of His6-YqfS against a double-stranded 19-mer containing a single AP site. (A) A total of 510 nmol of 5’-end-radiolabeled double-stranded 19-mer nucleotide containing a single AP site was incubated for 30 min at 37°C with different concentrations of His6-YqfS. The reactions were separated on a 20% denaturing acrylamide gel and then subjected to autoradiography. Lane 1, no enzyme; lanes 2 to 6, 0.3, 0.6, 1.2, 2.4, and 3.6 μg of His6-YqfS, respectively; lane 7, 2 U of *E. coli* Nfo. Radioactively labeled cleaved (C) and uncleaved (U) strands are as indicated. (B) Densitometry of the experiment shown in panel A; the percentage of uncleaved substrate was plotted as a function of the amount of His6-YqfS added to the reaction.

Saccharomyces cerevisiae Apn1 (46), and Thermotoga maritima endonuclease IV (20), respectively.

**Purification and enzymatic activity of YqfS.** The His6-YqfS protein synthesized in *E. coli* was purified to homogeneity by metal chelate affinity chromatography, yielding a 36-kDa protein (data not shown).

To corroborate the predicted AP endonuclease activity of YqfS, two enzymatic assays were performed. First, the His6-YqfS pure enzyme was incubated with a partially depurinated plasmid DNA as a substrate (AP-pB). The results presented in Fig. 2 reveal the conversion of the double-stranded 19-mer nucleotide containing a single AP site. The reactions were incubated at 37°C for 30 min and then analyzed by electrophoresis on a 1% agarose gel stained with ethidium bromide.

The cell extracts used to determine β-galactosidase activity
were also assayed for GDH activity, an enzyme encoded by the stage III, forespore-specific \textit{gdh} gene (19, 39). The results shown in Fig. 4 revealed that the expression patterns of the \textit{yqfS-lacZ} fusion and the GDH activity followed essentially identical kinetics, strongly indicating that \textit{yqfS} gene expression is activated in the forespore compartment during the last steps of sporulation.

To further support this contention, Northern blot experiments were performed with total RNA isolated from cells of strain \textit{B. subtilis} PERM168 collected before and after the onset of sporulation. The results (Fig. 5A) indicated that \textit{yqfS} mRNA appeared as a 2.3-kb band during sporulation stages T$_5$ through T$_9$, observing a major hybridization signal at T$_7$. As shown in Fig. 5A, no signal was detected in the blot with RNA isolated from cells growing exponentially, supporting the conclusion that \textit{yqfS} expression is sporulation specific. Moreover, RT-PCR experiments resulted in the major amplification of a \textit{yqfS} product when total RNA isolated from sporulating cells was used as a template. Figure 5B shows that the RT-PCR product of \textit{yqfS} (132 bp) was more abundant with RNA samples of the step T$_7$ of sporulation.

\textbf{$\sigma^G$ dependence of \textit{yqfS} expression.} The expression of forespore specific genes in \textit{B. subtilis} is carried out through the sequential action of two temporally expressed RNA polymerases containing either $\sigma^E$ or $\sigma^G$ factors (21, 43). However, as shown above, the expression pattern of the \textit{yqfS-lacZ} fusion was very similar to the $\sigma^G$-dependent \textit{gdh} gene, suggesting that \textit{yqfS} is under the control of $\sigma^G$-containing RNA polymerase. This notion was directly tested by two different approaches. First, the \textit{yqfS-lacZ} fusion was introduced by transformation into competent cells of \textit{B. subtilis} WN126 harboring a deletion of the $\sigma^G$ gene, an \textit{spo} mutant in which sporulation is arrested during stage III (24, 60). The resulting strain, \textit{B. subtilis} PERM336, grown in DSM expressed very low levels of \textit{yqfS}-directed $\beta$-galactosidase activity during both vegetative- and stationary-growth phases (data not shown). Consistent with this result, the levels of GDH in this strain were almost zero (data not shown).

In a second approach, Northern blot experiments were performed with RNA isolated from vegetative and stationary cells of \textit{B. subtilis} sigG$\Delta$I grown in liquid DSM. The results shown in Fig. 6A revealed the lack of \textit{yqfS} mRNAs in this sigG mutant genetic background, since no hybridization signal was detected during both exponential-growth-phase and stationary-growth-phase cells. Such a result was also confirmed by RT-PCR experiments, which failed to amplify the 132-bp \textit{yqfS} fragment from RNA samples isolated before and after the onset of sporulation (Fig. 6B). Taken together, these results are consistent with \textit{yqfS} expression being dependent on $\sigma^G$ RNA polymerase.

\textbf{Mapping the transcriptional start site of \textit{yqfS}.} The genetic organization of the \textit{yqfS} locus reveals that this gene is flanked upstream by \textit{yqfR}, which encodes a putative RNA helicase, and downstream by \textit{yqfU}, which encodes a protein of unknown function (Fig. 1). The existence of only one potential transcriptional terminator until the end of \textit{yqfU} suggests that the three genes could be cotranscribed as a polycistronic message. To investigate this possibility, primer extension analysis was performed to map the 5' ends of the mRNAs originating from upstream from the \textit{yqfS} coding sequence. Experiments were carried out with total RNA isolated from \textit{B. subtilis} PERM317 harboring the \textit{yqfS-lacZ} fusion. Cells used to isolate RNA were harvested during both vegetative growth and the T$_7$ sporulation stage, the time of maximum expression of the \textit{yqfS-lacZ} fusion. The results shown in Fig. 7 (lane 2) revealed the synthesis of a major extension product located 54 to 55 bp upstream of translation start codon of \textit{yqfS}. Such an extension product was obtained only in experiments performed with...
with a 32P-labeled 1,181-bp fragment encompassing the entire 5' region of the start site of transcription (strain WN118). (A) B. subtilis WN118 was grown in liquid DSM. Total RNA was isolated (34) from either vegetative (lane 1) or sporulating (stage T7; lane 2) B. subtilis PERM317 cells grown in DSM. Primer extension was performed as described in Materials and Methods. The asterisk indicates the position of the primer extension product in the DNA sequence lying upstream of yqfS (Fig. 7, lane 1). Inspection of the nucleotide sequences lying upstream of the in vivo mapped transcriptional start site revealed the existence of sequences with good homology to promoters preceding genes of the σ35 region, which possessed three of the four absolutely conserved bases present on sigG promoters (Fig. 8). On the other hand, the −10 region conserved three of the four absolutely conserved residues observed in such σ35 promoters. However, it was found that the −10 and −35 regions were separated by 16 bp instead of the reported 17 to 18 bp for the σ35 consensus sequence (Fig. 8).

**FIG. 7.** Primer extension analysis for mapping the transcriptional start site of yqfS. Total RNA was isolated (34) from either vegetative (lane 1) or sporulating (stage T7; lane 2) B. subtilis PERM317 cells grown in DSM. Primer extension was performed as described in Materials and Methods. The asterisk indicates the position of the primer extension product in the DNA sequence lying upstream of yqfS (see Fig. 1). The 5' end of the yqfS transcript was determined by running a DNA sequencing ladder generated with the same primer (lanes G, A, T, and C) and was labeled with an arrowhead.

**FIG. 8.** Comparison of the consensus E. coli promoter sequence (top line) with the putative promoter sequence lying upstream of yqfS (bottom line). Absolutely conserved (boldface) or highly conserved (underlined) bases in E. coli-type promoters (21, 43). The position of the mapped transcriptional start site of yqfS is indicated with an asterisk.

**FIG. 9.** Lack of induction of a yqfS-lacZ fusion by oxidative stress or during the SOS response. In E. coli, the expression of the type IV AP endonuclease nfo gene is induced by generators of superoxide radicals, such as paraquat (8). On the other hand, B. subtilis responds to H2O2 stress displaying an adaptive response that induces the expression of genes such as katA (catalase), ahpCF (alkyl hydroperoxide reductase), mrgA, and the hemA operon (1, 4, 7, 9, 10, 14). We therefore investigated whether the yqfS gene in B. subtilis is also induced by the oxidative stress imposed by either superoxide radicals or hydrogen peroxide. To this end, the strain B. subtilis PERM317 containing the yqfS-lacZ fusion, integrated into the yqfS locus, was grown in LB medium to the mid-exponential phase and treated with paraquat (10 μM) or hydrogen peroxide (200 μM). The results (Fig. 9A) revealed that, at the concentrations

**FIG. 6.** Northern blot (A) and RT-PCR analysis (B) of yqfS transcription during vegetative growth and sporulation of B. subtilis sigGΔ1 (strain WN118). (A) B. subtilis WN118 was grown in liquid DSM. Total RNA was isolated (35) from either vegetative (WT, lane 1) or sporulating (stage T7; lane 2) B. subtilis PERM317 cells. RNA samples (1 μg) were separated on agarose-formaldehyde gels (lower panel) and transferred to nylon membranes. The membrane was hybridized with a 32P-labeled 1,181-bp fragment encompassing the entire yqfS sequence as described in Materials and Methods. (B) RNA samples (1 μg) isolated at the times indicated (in hours) from a B. subtilis sigGΔ1 DSM culture were processed for RT-PCR analysis as described in Materials and Methods. The asterisk indicates the position of the primer extension product in the DNA sequence lying upstream of yqfS. (see yqfS end of the yqfS transcript was determined by running a DNA sequencing ladder generated with the same primer (lanes G, A, T, and C) and was labeled with an arrowhead.

**FIG. 9A**. Lack of induction of a yqfS-lacZ fusion by paraquat, H2O2, or mitomycin. B. subtilis PERM317 was grown to an OD600 of 0.5 in either minimal Spizizen medium (A) or LB medium (B). The culture made in minimal Spizizen medium was divided into three subcultures; one (labeled “0”) was left untreated, and the other two were treated with either paraquat (PQ; 10 μM) or H2O2 (200 μM). The LB culture was treated in the same manner except that mitomycin C (MC; 0.5 μg/ml) was added to the culture. (C) B. subtilis YB3000 was grown in LB medium to an OD600 of 0.5; at this point, the culture was equally divided, and mitomycin C (0.5 μg/ml) was added to one of the subcultures. In all cases, the β-galactosidase activity was determined with cell samples collected 2 h after the addition of the inducers.
tested, neither paraquat nor \( \text{H}_2\text{O}_2 \) was capable of inducing the expression of the \( yqfS-lacZ \) fusion.

Several \( B. \text{subtilis} \) genes involved in DNA repair, such as \( uvr \) components and \( \text{recA} \), have been shown to be inducible not only by DNA damage but also by the physiological state of competence (32, 34, 48). These genes (\( \text{din} \)) are part of a global response which in \( B. \text{subtilis} \) is called the SOS response (33). In order to determine whether the type IV AP-endonuclease gene of \( B. \text{subtilis} \) is a component of the \( B. \text{subtilis} \) SOS regulon, the strain containing the \( yqfS-lacZ \) fusion was grown to exponential phase and then treated with mitomycin C to a final concentration of 0.5 \( \mu \text{g/ml} \). As shown in Fig. 9B, mitomycin C induced the \( \beta \)-galactosidase levels of the strain \( B. \text{subtilis} \) PERM317 only 1.2 times above the levels expressed by the untreated control. In contrast with this result, when \( B. \text{subtilis} \) YB3000 containing a \( \text{recA-lacZ} \) fusion was treated with mitomycin (Fig. 9C), the levels of \( \beta \)-galactosidase activity increased 35 times.

**DISCUSSION**

\( B. \text{subtilis} \) has been studied extensively as a paradigm for bacterial differentiation and development. Spores produced by this organism prevent or dramatically slow the DNA damage inflicted by oxidative stress, UV light, heat and desiccation (reviewed in references 40 and 58). However, during long periods of dormancy spores accumulate potentially lethal and mutagenic DNA damage such as SP, strand brakes, CPDs, chemically altered bases, and AP sites that could affect transcription and replication during germination (40, 56). Therefore, it is of interest to determine how the many DNA repair systems present are regulated by \( B. \text{subtilis} \), especially in relation to the sporulation and germination processes.

Thus, the \( yqfS \) ORF was cloned, and the product of this gene was isolated and tested for its enzymatic activity. The results presented in Fig. 2 and 3 clearly indicate that this protein has AP endonuclease activity. Having established the nature of the product of the \( yqfS \) gene, we wanted to determine the mechanism(s) that control the expression of this gene. Our data demonstrate that there is temporal and spatial expression of the \( yqfS \) gene. Specifically, \( \beta \)-galactosidase activity for a \( yqfS-lacZ \) reveals that this gene is not apparently transcribed during vegetative growth but is transcribed during stages of the sporulation process (Fig. 4). Northern blot and RT-PCR experiments (Fig. 5) confirmed a major abundance of \( yqfS \) messengers during stages of the sporulation process of the strain \( B. \text{subtilis} \) PERM317. These results suggested that \( yqfS \) expression is temporally activated and confined to the forespore compartment in accordance with a pattern similar to that described for stage III, forespore-specific genes (57). This suggestion was further supported not only by cell fractionation experiments, which demonstrated that \( yqfS \) expression occurs inside of the spore, but also by the observation that the kinetics of GDH synthesis, a stage III, forespore-specific marker, are indistinguishable from those observed for the \( yqfS-lacZ \) fusion (Fig. 4). These results strongly support the idea that the synthesis of the \( YqfS \) protein occurs during the last stages of the sporulation process and is packaged in the spore.

The forespore-specific expression of the \( yqfS-lacZ \) fusion during the last steps of \( B. \text{subtilis} \) sporulation suggested that the transcription of \( yqfS \) is carried out by RNA polymerase containing the \( \sigma^G \) factor (Fig. 4). However, gene expression inside of the forespore occurs by the sequential action of two RNA polymerases containing either the \( \sigma^F \) or \( \sigma^G \) factors (21, 25).

Therefore, we could not rule out a possible transcription of \( yqfS \) by RNA polymerase \( \sigma^F \). This point was addressed by measuring the levels of expression of the \( yqfS-lacZ \) fusion introduced into a \( B. \text{subtilis} \) strain lacking the \( \text{sigG} \) gene (Table 1). The results showed that \( yqfS \)-directed \( \beta \)-galactosidase activity is almost null in this genetic background, as is the synthesis of GDH activity (data not shown). In agreement with this observation, both Northern blot and RT-PCR experiments performed with total RNA isolated during vegetative and stationary growth of the strain \( B. \text{subtilis} \) \( \text{sigG} \) demonstrated the absence of \( yqfS \) messengers in this mutant strain (Fig. 6).

Taken collectively, these results strongly suggest that \( yqfS \) expression occurs inside of the spore by the action of \( \sigma^G \)-containing RNA polymerase.

Forespore-specific expressed genes such as \( \text{sspA-E} \), \( \text{splA-splB} \), \( \text{gdh} \), \( \text{ger} \), and \( \text{spoVA} \), among others, are representative of the \( \sigma^G \) regulon (15, 16, 19, 21, 25, 44, 45, 57). Experimental evidence has demonstrated that these genes possess specific promoters that are exclusively transcribed by \( \sigma^G \) containing RNA polymerase (15, 39, 43, 44, 47). The results described above suggest that \( yqfS \) might be a new member of this regulon.

This conclusion was strongly supported by the in vivo mapping of the transcriptional start site of \( yqfS \) (Fig. 7). A major extension product initiating 54 to 55 bp upstream of the putative \( yqfS \) start codon was amplified from RNA samples isolated from sporulating but not from vegetatively growing cells (Fig. 7).

Inspection of the sequences preceding the \( yqfS \) transcriptional start site revealed the existence of a promoter with homology to the consensus sequence of \( \sigma^G \) promoters (21, 43). Although the −10 region of the putative \( yqfS \) promoter shows a low level of homology, the −35 region almost perfectly matched the consensus of \( \sigma^G \) promoters (Fig. 8). One possible solution with the designation of this putative \( \sigma^G \) promoter is the spacing between the −35 and −10 regions. However, as mentioned above, our data support the hypothesis that the \( yqfS \) gene is transcribed by a \( \sigma^G \)-containing RNA polymerase.

The \( yqfS \) region in the \( B. \text{subtilis} \) chromosome shows the existence of a set of three genes located in the same orientation, in the following order: \( yqfR \), \( yqfS \), and \( yqfU \) (Fig. 1). The lack of putative transcriptional terminators downstream of \( yqfR \) and \( yqfS \) suggests that the three genes are transcribed as a polycistronic unit. However, the primer extension experiments described above, together with the identification of a 2.3-kb \( yqfS \) messenger, indicate that \( yqfS \) is cotranscribed with \( yqfU \) as a bicistronic mRNA from the putative \( yqfS \) promoter just described.

Expression of the two major AP endonucleases is differentially regulated in \( E. \text{coli} \). Whereas \( \text{exoIII} \) is constitutively expressed, the \( \text{nfo} \) gene is inducible by oxidative stress. Chemical compounds such as paraquat and menadione, which generate superoxide radicals, induce a 10- to 20-fold increase in the level of \( \text{Nfo} \) (8). The lack of induction in the levels of expression of the \( yqfS-lacZ \) fusion after the treatment of \( B. \text{subtilis} \) PERM317 with paraquat (Fig. 9) revealed that in \( B. \text{subtilis} \) the \( yqfS \) gene is not regulated by the oxidative stress imposed by superoxide radicals.
In *B. subtilis* the adaptive response to H$_2$O$_2$ stress is subjected to negative regulation by the repressor PerR, a Fur homolog (6). Treatment of *B. subtilis* PERM317 with H$_2$O$_2$ did not change the levels of expression of the yqfS-lacZ fusion (Fig. 9), suggesting that yqfS is not regulated by PerR. Consistent with these results, no cis-acting DNA sequences similar to those present in *perR* boxes (22) were observed around the putative promoter of yqfS.

Analysis of the upstream regions of yqfS also revealed the absence of dinR-like boxes (11, 62). This observation is in agreement with the lack of induction of the yqfS-lacZ fusion after the treatment of *B. subtilis* PERM317 with the DNA-damaging agent mitomycin (Fig. 9).

Taking all of these results together, we conclude that although in *E. coli* the expression of nfo is linked to the oxidative stress generated by superoxide radicals (8), in *B. subtilis* the regulation of yqfS expression occurs in a temporal and forespore-specific manner and appears to be part of the σ$^E$ regulon. In addition, the lack of induction of β-galactosidase in the yqfS-lacZ fusion strains after treatment by either hydrogen peroxide or the DNA-damaging agent mitomycin revealed that yqfS is not under the control of the PerR or SOS regulons.

Despite the existence of spore mechanisms that prevent or alter DNA insults, potentially lethal and mutagenic damage accumulates in DNA during long-term storage of spores in the laboratory (40, 58) and during the exposure of these spores to environmental stresses, particularly solar radiation (40, 41, 59, 61). Interestingly, artificial and solar UV radiation induce the formation of CP, CPDs, and strand breaks but not of AP sites in *B. subtilis* spore DNA (59). It remains to be investigated whether AP sites are generated during germination of *B. subtilis* spores either spontaneously or promoted by oxidative stress or through the action of DNA glycosylases during the elimination of chemically modified bases (18). Moreover, depending on their chemical structure, single-strand breaks generated on spore DNA could be processed as well by YqfS during germination, since it has been well established that type IV AP endonucleases are able to remove phosphoglycolaldehyde, phosphate, deoxyribose-5-phosphate, and 4-hydroxy-2-pentenal from the 3′ terminus of duplex DNA (18). Therefore, as an obligatory step for the correction of the different types of DNA damage processed by the BER pathway, YqfS may play an important role in the repair of DNA damage inflicted on *B. subtilis* during either spore dormancy or germination.

In conclusion, we provide here for the first time evidence that an important component of the BER system of *B. subtilis*, namely, the yqfS gene, is specifically expressed inside of the spores during the final developmental stages. Thus, together with the SpIB, UVR, and Rec systems, YqfS could be part of the DNA repair proteins that increase the survival potential of *B. subtilis* spores.

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