Cloning and Sequencing of a 27.8-kb Nucleotide Sequence of the 79°–81° Region of the Bacillus subtilis Genome Containing the sspE Locus

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Abstract

The nucleotide sequence of a 27830-bp DNA segment in the 79°–81° region of the Bacillus subtilis genome has been determined. This region contains 29 complete ORFs including the sspE gene, which encodes a small acid-soluble spore protein gamma and locates on the one side terminal of our assigned region. A homology search for the products deduced from the 29 ORFs revealed that nine of them exhibit significant similarity to known proteins, e.g. proteins involved in an iron uptake system, a multidrug resistance protein, a chloramphenicol resistance protein, epoxide hydrolase, adenine glycosylase, and a glucose-1-dehydrogenase homolog.

Key words: Bacillus subtilis; genome sequencing; 79°–81° region; sspE locus

As part of the Bacillus subtilis genome cooperative project, we are now responsible for the sequencing of an approximately 142-kb chromosomal region between 69° (XEXT11) and 81° (sspE). In our first report of this project,1 we reported the nucleotide sequence and gene organization of a 12.4-kb region, from the Not I site of XEXT23, containing the deduced operons of the sugar phosphotransferase system and ABC transport system. In this report, we describe the nucleotide sequence and gene organization of a 27.8 kb region containing the sspE locus.3

1. Cloning and Sequencing of a 27.8-kb Region between 79° and 81° of the B. subtilis Genome

A 12.3-kb insert from the Not I site of the phage 4-1 containing a part of NEXT23 was sequenced as described previously, and a neighboring 9.4 kb region (AflII-inv) cloned by the inverse PCR method was also sequenced (submitted for publication). To amplify the next region, we produced primers AflII-F [38mer, 5'TGTAAAACGACGGCCAGTGGTTTAGTGTCATCAACCGC3'; the underlining indicates the M13 sequence; the 3' end of the primer corresponds to position 20 (base) in Fig. 1] and sspE-K [38mer, 5'CAGGAAACAGCTATGACCTTTGACTCATTCTG-3'; the underlining indicates the M13 sequence; the 3' end corresponds to position 27810]. The amplified fragment was partially digested with DXasel and then blunt-ended with T4 polymerase, and the fragments were separated by agarose (1.2%, w/v) gel electrophoresis to obtain 700 ~ 1500 bp fragments. The latter were ligated to Sma I-digested and alkaline phosphatase-treated pUC118 DNA. E. coli JM109 cells were transformed with the ligation mixture to obtain a randomly overlapping library for sequencing. The sequencing reaction was performed using Dye Primer Cycle Sequencing Kit (Applied Biosystems). DNA sequencer type 373A (Applied Biosystems) was used. We determined the sequences of both DNA strands. For gap-filling and confirmation of the species of uncertain bases, the dye terminator sequencing method was adopted. The DXA sequences obtained were assembled using an Inherit Auto Assembler software (Applied Biosystems). DNA sequencer type 373A (Applied Biosystems) was used. We determined the sequences of both DNA strands. For gap-filling and confirmation of the species of uncertain bases, the dye terminator sequencing method was adopted. The DNA sequences obtained were assembled using an Inherit Auto Assembler software (Applied Biosystems), and further analyzed for the location of possible open reading frames (ORFs) using Gene Works (IntelliGenetics, Inc.). The amino acid sequences of the putative products of identified ORFs were examined as to their similarity to sequences reported in a non-redundant protein sequence data bank using the FASTA e-mail server at the Human Genome Sequencing Center.
Figure 1. Physical map and gene organization of the 79°–81° region. A physical map of the sequenced 27.8-kb region from 79°–81° region of the *B. subtilis* chromosome. The *NotI* site of NEXT47 and the movement of the replication fork are indicated. The deduced 29 ORFs, and their transcriptional and translational directions are indicated by arrows and arrowheads. Furthermore, these ORFs are colored according to function: red, identified; blue, suggested; yellow, unknown but conserved in other organisms; white, unknown and unique (FASTA optimized score < 220). The deduced rho-independent terminators are denoted by asterisks before numbers: 1, ΔG = -27.5 kcal/mol; 2, -30.6 kcal/mol; 3, -24.6 kcal/mol; 4, -35.1 kcal/mol; 5, -21.2 kcal/mol; 6, -22.4 kcal/mol; 7, -28.0 kcal/mol; 8, -17.5 kcal/mol; 9, -22.6 kcal/mol; 10, -22.7 kcal/mol; 11, -32.8 kcal/mol. *S* and *E* indicate *SalI* and *EcoRI* sites, respectively.
Table 1. Putative ORFs in the 27.8-kb sequence at region between \( yfiQ \) and \( sspE \).

| ORF | Endpoints (nucleotides) | Size of product (aa/kDa) | SD consensus sequence (upper case) and initiation codon (bold) |
|-----|------------------------|--------------------------|---------------------------------------------------------------|
| \( yfiQ \) | 417/1502 | 362/41.0 | AAAGGAGGagtctgtaatg |
| \( yfiR \) | 2158/1544 | 205/23.7 | AAGCAGGAGTatctg |
| \( yfiS \) | 3377/2127 | 417/44.4 | tcAGGAGGagacttcatg |
| \( yfiT \) | 3501/4034 | 178/20.7 | AAGGAGGacacagtcatg |
| \( yfiU \) | 5590/4037 | 518/54.9 | AAAGGAGGagacttcatg |
| \( yfiV \) | 6183/5704 | 180/18.2 | tgAGGAGGTGATgaatag |
| \( yfiW \) | 6355/7128 | 258/29.0 | AAGGAGGagacttcatg |
| \( yfiX \) | 7094/8923 | 610/69.1 | AAGGAGGagacttcatg |
| \( yfiY \) | 9921/8047 | 326/36.3 | ttgAGGAGGaaagatg |
| \( yfiZ \) | 10052/11050 | 333/35.1 | ctAGGAGGaagattgtcatg |
| \( yfhA \) | 11050/12078 | 343/36.0 | gAAAGGAGGagacattcatg |
| \( yfhB \) | 12196/13074 | 293/32.1 | AAGGAGGagacattcatg |
| \( yfhC \) | 13165/13791 | 194/22.5 | ttGAGGAGGaagattgtcatg |
| \( yfhD \) | 13799/13791 | 63/7.3 | ttGAGGAGGaagattgtcatg |
| \( yfhE \) | 14156/14049 | 36/4.3 | gAAAGGAGGagacattcatg |
| \( yfhF \) | 15122/14214 | 303/33.9 | tgAGGAGGagacattcatg |
| \( yfhG \) | 15211/16002 | 264/31.0 | AAGGAGGagacattcatg |
| \( yfhH \) | 16007/16318 | 104/12.0 | gAAAGGAGGagacattcatg |
| \( yfhI \) | 16458/17654 | 399/41.7 | AAGGAGGagacattcatg |
| \( yfhJ \) | 17967/18233 | 89/10.5 | AAGGAGGagacattcatg |
| \( yfhK \) | 18381/18996 | 172/18.7 | cAGGAGGagacattcatg |
| \( yfhL \) | 18984/19313 | 110/12.0 | AAGGAGGagacattcatg |
| \( yfhM \) | 19303/20160 | 286/32.8 | cAAAGGAGGagacattcatg |
| \( yfhN \) | 20396/21382 | 329/37.7 | cAAAGGAGGagacattcatg |
| \( yfhO \) | 21582/24038 | 819/93.8 | ggtGAGGagacattcatg |
| \( yfhP \) | 25017/24037 | 327/37.1 | cAAAGGAGGagacattcatg |
| \( yfhQ \) | 25233/26339 | 369/42.0 | ttGAGGAGGagacattcatg |
| \( yfhR \) | 26056/27405 | 250/27.1 | cAAAGGAGGagacattcatg |
| \( sspE \) | 27477/27728 | 84/9.3 | cgtGAGGAGGagacattcatg |

\( \) indicates the transcriptional direction of the ORF.

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2. Genes Found in the 79°–81° Region and their Features

As shown in Fig. 1, a computer analysis of the 27830-bp sequence revealed 29 complete ORFs. Among them, 26 start with ATG, 1 with GTG, and 2 with TTG (Table 1). Nine ORFs are transcribed and translated in the same orientation from 81° to 79°, and the other 20 in the opposite orientation, which are identical to that of the movement of the replication fork (Fig. 1).

3. Homology of the ORFs in the 79°–81° Region

The aa sequences of the putative products of the identified ORFs were examined as to their similarity to sequences in a non-redundant protein sequence data bank. The aa sequence of YfiY exhibits high similarity to those of the iron transport proteins of \( E. chrysanthemi \) (CbrB)\(^5\) and \( B. subtilis \) (FeuA and FeuB)\(^7,6\) (Table 2). The aa sequence of YfiZ exhibits high similarity to those of the iron transport proteins of \( E. chrysanthemi \) (CbrB)\(^5\) and \( B. subtilis \) (FeuB)\(^7,6\) (Table 2). The aa sequence of YfhA exhibits high similarity to those of the iron transport proteins of \( B. subtilis \) (FeuB)\(^7,6\) \( E. chrysanthemi \) (CbrC)\(^5\) and \( E. coli \) (FeC)\(^6\) (Table 2). These results suggest the putative products of three ORFs (\( yfiY \), \( yfiZ \) and \( yfhA \)) are included in the iron uptake system. In \( E. chrysanthemi \), the iron uptake system included the ABC transporter family consists of an iron binding protein (CbrA), two integral membrane proteins (CbrB and CbrC), and an ATP-binding protein (CbrD).\(^5\) Recently, \( fhuDBGC \) genes involved in an iron uptake system were reported in \( B. subtilis \)\(^7,6\) and these gene products may also belong to the ABC transporter family. However, in the case of YfiY, YfiZ and YfhA, there was no protein corresponding to the ATP-binding one such as CbrD or FhuC. The absence of a gene encoding the ATP-binding protein suggests that it may be located elsewhere on the \( B. subtilis \) genome or the YfiY-Z-YfhA system may transport an iron by using a different energy source.
Table 2. Similarity of predicted ORF products to known proteins.

| Product | Similar protein in database | Identity observed | Fasta score |
|---------|-----------------------------|-------------------|-------------|
| YfQ     | Intercellular adhesion protein (icaC) of *Staphylococcus epidermidis* [gp:SEU43366.3, gi:1161382] | 26.2% in 340 residues | 457 |
| YfS     | Hypothetical protein of *Synechocystis* sp. [gp:SYCSRJG.42, gi:1001821] | 25.8% in 356 residues | 321 |
|         | Putative transporter of *Mycobacterium smegmatis* [gp:MSU46844.4, gi:1197634] | 26.9% in 305 residues | 282 |
|         | Nickel resistance protein (nreB) of *Alcaligenes xylosoxidans* [gp:APANREA.4, gi:468280] | 21.7% in 391 residues | 277 |
|         | Multidrug resistance protein 2 (bmrZ) of *B. subtilis* [sp:BMR2_BACSU] | 22.2% in 400 residues | 264 |
| YfU     | Hypothetical transport protein of *Streptomyces violaceoruber* [gp:STUI4299.4, gi:763513] | 23.0% in 526 residues | 500 |
|         | Tetracycline resistance protein homolog (act) of *Streptomyces coelicolor* [pir:B40046] | 24.4% in 476 residues | 498 |
|         | Tetracenomycin C resistance and export protein (tcmA) of *Streptomyces glaucescens* [sp:TCMA_STRGA] | 22.9% in 503 residues | 473 |
| YfY     | cbrA gene product of *Erwinia chrysanthemi* [pir:S54820] | 31.3% in 268 residues | 447 |
|         | Iron uptake system binding protein precursor (feuA) of *B. subtilis* [sp:FEUA_BACSU] | 26.5% in 294 residues | 314 |
|         | Ferrichrome binding protein precursor (fhuD) of *B. subtilis* [sp:FEUD_BACSU] | 27.5% in 302 residues | 244 |
| YfZ     | cbrB gene product of *Erwinia chrysanthemi* [pir:S54821] | 49.6% in 280 residues | 735 |
|         | fhuB gene product of *B. subtilis* [gp:BSHUBG.2, gi:1070013] | 42.3% in 324 residues | 702 |
|         | Iron uptake system protein (feuB) of *B. subtilis* [sp:FEUB_BACSU] | 35.0% in 326 residues | 635 |
| YfhA    | fhuG gene product of *B. subtilis* [gp:BSHUBG.3, gi:1070014] | 40.0% in 335 residues | 706 |
|         | cbrC gene product of *Erwinia chrysanthemi* [pir:S54822] | 40.7% in 327 residues | 653 |
| YfhB    | (fecD) dicitrate transport protein of *E. coli* [pir:QHECDZ] | 37.3% in 330 residues | 540 |
|         | phzF gene product of *Pseudomonas fluorescens* [sp:PHZFIA.8, gi:1045018] | 26.8% in 287 residues | 242 |
| YfhC    | Cell division inhibitor homolog (sulA) of *H. influenzae* [gp:HEAHI1208, gi:1007092] | 30.1% in 302 residues | 424 |
| YfhF    | Chloramphenicol resistance protein (cmlV) of *Streptomyces venezuelae* [sp:SVU09991.1, gi:498887] | 32.7% in 395 residues | 717 |
|         | Protein AraJ precursor of *E. coli* [sp:ARAJ_ECOLI] | 29.5% in 366 residues | 598 |
|         | Chloramphenicol resistance protein of *Rhodococcus fasciens* [pir:S25183] | 27.9% in 366 residues | 585 |
| YfhN    | Soluble epoxide hydrolase (AtsEH) of *Arabidopsis thaliana* [gp:ATHATSEH.1, gi:11099600] | 33.5% in 206 residues | 379 |
|         | Epoxide hydrolase of *Solanum tuberosum* [gp:STU0294.1, gi:407938] | 31.8% in 195 residues | 336 |
|         | Soluble epoxide hydrolase (seh) of *Rattus norvegicus* [sp:HYES_RAT] | 32.8% in 192 residues | 332 |
| YfhO    | Hypothetical protein of *Synechocystis* sp. [gp:SYCSRLLH.56, gi:1001347] | 43.9% in 301 residues | 745 |
| YfhQ    | A/G specific adenine glycosylase (mutY) of *H. influenzae* [sp: MUT_HAIN] | 33.4% in 311 residues | 607 |
|         | A/G specific adenine glycosylase (mutY) of *Salmonella typhimurium* [sp: MUT_SALTY] | 37.7% in 268 residues | 596 |
|         | A/G specific adenine glycosylase (mutY) of *E. coli* [sp: MUT_ECOLI] | 38.0% in 271 residues | 593 |
| YfhR    | Short-chain alcohol dehydrogenase of *Picea abies* [pir:S34678] | 32.7% in 251 residues | 364 |
|         | 3-oxoacyl-[acyl-carrier protein] reductase of *Cuphea lanceolata* [sp:FABG_CUPLA] | 29.8% in 242 residues | 345 |
|         | Glucose 1-dehydrogenase II (gdhII) of *B. megaterium* [sp:DHG2_BACME] | 28.7% in 247 residues | 313 |
|         | Glucose 1-dehydrogenase A (gdhA) of *B. megaterium* [sp:DHG_A_BACME] | 27.5% in 247 residues | 305 |
| SspE    | Small acid-soluble spore protein (sspE) of *B. subtilis* [sp:SASG_BACSU] | 100% in 84 residues | 318 |

gi, gp, pir, and sp indicate the NCBI, GenBank Protein, PIR, and Swiss Prot databases, respectively.
The aa sequence of YfQ exhibits some similarity to that of the intercellular adhesion protein (IcaC) of *Staphylococcus epidermidis* (Table 2). The aa sequence of YfS exhibits some similarity to those of two hypothetical proteins of *Synechocystis* sp. and *Mycobacterium smegmatis*, and the nickel resistance protein (NreB) of *Alcaligenes xylosoxidans*, and the multidrug resistance protein 2 (Bmr2) of *B. subtilis* (Table 2). The product of yfU, about 0.7 kb upstream of yfS, exhibits some aa sequence similarity to the hypothetical transport protein of *Streptomyces violaceoruber*, the tetracycline resistance protein homolog (Act) of *Streptomyces coelicolor*, and the tetracyclomycin C resistance protein (TcmA) of *Streptomyces glaucescens* (Table 2). Moreover the product of yfhI, which is located approximately 11 kb upstream of yfU, exhibits high aa sequence similarity to the chloramphenicol resistance proteins of *Streptomyces venezuelae* (CmiIV) and *Rhodococcus fascians* (Cmrt), and to the AraJ of *E. coli* which is induced by arabinose and may be involved in either the transport or processing of arabinose polymers (Table 2). Since YfS, YfU and Yfh have multiple transmembrane regions (data not shown), they may be drug resistance exporters and use transmembrane electrochemical gradients as an energy source. The aa sequence of YfB exhibits some similarity to those of the *phzC* and *phzF* gene products which are related to the phenazine biosynthesis of *P. aureofaciens* and *P. fluorescens*, respectively (Table 2). The aa sequence of YfhF exhibits high similarity to that of the cell division inhibitor homolog (SulA) of *Haemophilus influenzae*, but not SulA of *E. coli* (Table 2). YfhF may not be the cell division inhibitor, since SulA of *H. influenzae* has low similarity to one of *E. coli*. The aa sequence of YfhM exhibits very high similarity to those of the soluble epoxide hydrolases of *Arabidopsis thaliana* (AtsEH), *Solanum tuberosum*, and *Rattus norvegicus* (Seh) (Table 2). The aa sequence of YfhN exhibits high similarity to that of the *sspE* gene product.

Therefore, both genes cloned in *B. subtilis*, while at least four isozymes have been cloned in *B. megaterium*. Judging from the genome structure, there seems to be no ρ-independent terminator sequence among yfhR and *sspE*. Therefore, both genes may form an operon (Fig. 1). However, it has been reported that *sspE* gene was monocistronically transcribed by E-ρ. We are very interested in the finding that yfhR is located just upstream of *sspE*, and further work is needed to determine the role of the yfhR gene product.

Among the 29 ORFs in the 27.8-kb region, 15 did not exhibit any significant aa sequence similarity to ones in the non-redundant protein sequence data bank. Therefore, our research is now directed toward the isolation of gene-disrupted mutants for functional analysis in a systematic way under the international cooperative project.

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