A 10 kb nucleotide sequence at the 5' flanking region (32°) of *srfAA* of the *Bacillus subtilis* chromosome

Yoshiyuki Fujishima and Kunio Yamane

Author for correspondence: Kunio Yamane. Tel./Fax: +81 298 53 6680.

Institute of Biological Sciences, University of Tsukuba, Tsukuba-shi, Ibaraki 305, Japan

The nucleotide sequence of approximately 10 kb at the 5' flanking region (32°) of *srfAA* of the *Bacillus subtilis* chromosome was determined. Eleven putative ORFs were identified. Three of them (*orf6*, *orf7* and *orf8*) coincided with known *B. subtilis* genes (*comJ*, *comI* and *tlpC*) encoding a competence-specific protein, a DNA-entry nuclease and a transducer-like protein, respectively. The products of two other ORFs showed similarity to GlnP of *Escherichia coli* (*orf1*) and β -glucosidase A of *B. polymyxa* (*orf5*).

Keywords: *Bacillus subtilis*, genome sequencing, 32° region, *srfAA*

INTRODUCTION

In a previous paper (Fuma *et al.*, 1993) we reported the isolation of four independent clones that hybridized to the *NotI* linking plasmids pNEXT13 and pNEXT61 (Itaya & Tanaka, 1991). Nucleotide sequence analysis revealed that they contained *srfAA* and *srfAB* (Cosmina *et al.*, 1993). One of these, clone 110, hybridized with pNEXT13 only and it contained an approximately 10 kb DNA segment at the 5' flanking region of *srfAA*. We have now sequenced and analysed this segment.

Clone 110 was obtained from a λ DASH II library of *Bacillus subtilis* chromosomal DNA partially digested with *Sau3AI* (provided by N. Ogasawara) using ^{32}P -labelled pNEXT13 as a probe. All sequences were determined using a 373A DNA sequencer from Applied Biosystems using the Taq Dye Primer Cycle Sequencing Kit and the Taq DyeDeoxy Terminator Cycle Sequencing Kit after preparing randomly overlapping libraries for the shotgun sequencing method as described by Ogawa *et al.* (1995). The compiled sequences were analysed for the location of possible ORFs using GENETYX-MAC from Software Development (Tokyo). The amino acid sequences of the products of each ORF were searched for similarity to sequences reported previously in non-redundant protein sequence data banks using FASTA and BLAST e-mail servers in the Human Genome Center, Institute of Medical Science, University of Tokyo.

Abbreviation: SD, Shine–Dalgarno.

The GSD/EMBL/DBJ/NCBI accession number of the nucleotide sequence reported in this paper is D30762.

Putative ORFs in the region sequenced were searched in all six possible translation frames. Based on the length of the ORF starting from ATG, GTG or TTG and preceded by a Shine–Dalgarno (SD) sequence, 11 putative ORFs were detected (Fig. 1 and Table 1). The initiation codon for 9 ORFs was ATG, but TTG for *orf6* and GTG for *orf11*. The direction of transcription and translation of *orf3*, 4, 5, 11 and *srfAA* was the same as that of DNA replication, whereas that of the other ORFs (*orf1*, 2, 6, 7, 8, 9 and 10) was in the opposite direction.

Comparison of the amino acid sequences of the putative products of each ORF with the non-redundant protein sequence data banks revealed that the nucleotide sequences of *orf6*, *orf7* and *orf8* and their predicted amino acid sequences have been reported and characterized genetically and biochemically (Vosman *et al.*, 1988;

Fig. 1. Organization of ORFs of the *B. subtilis* chromosome 32° area at the 5' flanking region of *srfAA*. The scale (in kb) starts at the G residue at the *BamHI/Sau3AI* junction site (Bm/S). *EcoRI* (E) and *BglII* (B) sites are indicated. ORFs are shown by thick black arrows and the arrowheads show the direction of their transcription and translation. Numbers on the arrows indicate each ORF.

Table 1. Putative ORFs in the 10 kb sequence at the 5' flanking region of *srfAA* (32°)

ORF	Endpoints (nucleotides)	Size of product (aa/kDa)	SD consensus sequence (upper case) and initiation codon (bold)
<i>orf1</i>	152 < 829	226/25.2	AGGAcGTtGAtttgtaatcgacgccatg
<i>orf2</i>	842 < 1408	189/21.0	AAgGAGaTGggcsattgacgggatg
<i>orf3</i>	2207 > 2533	109/12.2	AGGAaaaGAccaaacgacagcatg
<i>orf4</i>	2618 > 2947	110/12.8	GGAGGaataaaatggatg
<i>orf5</i>	3104 > 4534	477/55.1	AGGAGtgataaacactatg
<i>orf6</i>	4577 < 4972	132/15.0	AgAGGAGGaacagcacattg
<i>orf7</i>	5002 < 5310	103/11.5	AAAGaAtatgataaacaatg
<i>orf8</i>	5619 < 7337	573/61.8	agGGAGGaattatg
<i>orf9</i>	7700 < 8005	102/11.1	AAGGAtaactccgatg
<i>orf10</i>	8118 < 8642	175/19.0	AAGGgaGTGgatcaacaatg
<i>orf11</i>	8928 > 9233	102/12.0	AGaAGGaattaacgcttcagtg
<i>srfAA</i>	9810 >	—	GGAGGtatgacaatatg

Table 2. Similarity of predicted ORF products to known proteins

Product	Similar protein in database	Identity observed	BLAST score
Orf1	Glutamine permease operon GlnP protein in <i>E. coli</i>	39 % in the C-terminal half	209
	Histidine permease membrane M protein (HisM) of <i>E. coli</i>	33 % in the C-terminal region	190
	Arginine periplasmic transport system protein ArtQ in <i>E. coli</i>	36 % in the C-terminal half	177
Orf5	β -Glucosidase A of <i>B. polymyxa</i>	57 % in the N-terminal 135 residues	457
	β -Glucosidase A of <i>B. circulans</i>	61 % in the N-terminal 128 residues	457
Orf6	Competence specific protein of <i>B. subtilis</i>	100 % for the entire length	688
Orf7	DNA-entry nuclease of <i>B. subtilis</i>	73 % in 103 residues	406
Orf8	Transducer-like protein of <i>B. subtilis</i> homologous to methyl-accepting protein of <i>E. coli</i>	100 % for the entire length	—

Hanlon *et al.*, 1994); the functions of the products of two other ORFs (Orf1 and Orf5) were predicted on the basis of a considerable degree of similarity to known protein sequences from other bacteria (Table 2). The amino acid sequence of Orf1 showed considerable similarity (39 %, 33 % and 36 % identity, respectively) to the C-terminal regions of GlnP (219 residues) of the glutamine permease operon (Nohno *et al.*, 1986), histidine permease membrane M protein (HisM, 144 residues; Kraft & Leinwand, 1987) and arginine periplasmic transport system protein (ArtQ, 239 residues; Wissenbach & Uden, 1993) of *Escherichia coli*. However, the sequence of Orf1 did not show any similarity to those of *B. subtilis* GntP and lysine-specific permease of *E. coli*. Since *orf2* does not have a clear promoter sequence, and *glnH*, *glnP* and *glnQ* are included in an operon in *E. coli*, *orf1* and *orf2* seemed to comprise at least part of an operon. However, the amino acid sequence of Orf2 showed no similarity to GlnH or GlnQ, so this is probably not the case. Orf1 may be a member of a permease family. The *orf5* product shows 61 %, 57 %, 65 % and 58 % amino acid sequence identity with β -glucosidase A from *B. circulans* (450 residues; Paavilainen *et al.*, 1993), *B. polymyxa* (448 residues; Gonzalez-Candelas

et al., 1990), *Caldocellum saccharolyticum* (455 residues; Love & Bergquist, 1988) and *Clostridium thermocellum* (448 residues; Graebnitz *et al.*, 1991), respectively. This implies that Orf5 is the *B. subtilis* β -glucosidase.

The nucleotide sequences of *orf6* and *orf7* were the same as those reported by Vosman *et al.* (1988) except for one additional G residue in *orf7* at nucleotide position 5086. This addition resulted in a frameshift in *orf7* and consequently Orf7, corresponding to *comI* encoding a DNA-entry nuclease, was found to be only 103 amino acids (11.5 kDa) not 144 amino acids (17 kDa) as previously reported (Vosman *et al.*, 1988). *orf6* corresponds to *comJ* encoding a competence-specific protein and its amino acid sequence was completely identical to that in the previous report. The reading frame of *orf7* did not overlap with that of *orf6*. The nucleotide and amino acid sequences of Orf8 were the same as those reported by Hanlon *et al.* (1994). The functions of the other ORFs (Orf2, 3, 4, 9, 10 and 11) remain unknown.

The other two possible ORFs (5837–6292 and 9003–9462) would be transcribed in the opposite direction to, and are located within the *orf8* and *orf11* regions, respectively.

They did not exhibit sufficient consensus SD sequences and were not studied.

ACKNOWLEDGEMENTS

We thank Dr M. Itaya and Dr N. Ogasawara for pNEXT13, a *NotI* linking clone and the λ DASH II library of *B. subtilis* chromosomal DNA. This work was supported by a Grant-in-Aid for Creative Research (Human Genome Project) from the Ministry of Education, Science and Culture of Japan.

REFERENCES

- Cosmina, P., Rodriguez, F., de Ferra, F., Grandi, G., Perego, M., Venema, G. & van Sinderen, D. (1993). Sequence and analysis of the genetic locus responsible for surfactin synthesis in *Bacillus subtilis*. *Mol Microbiol* **8**, 821–831.
- Fuma, S., Fujishima, Y., Corbell, N., D'Souza, C., Nakano, M. M., Zuber, P. & Yamane, K. (1993). Nucleotide sequence of 5' portion of *srfA* that contains the region required for competence establishment in *Bacillus subtilis*. *Nucleic Acids Res* **21**, 93–97.
- Gonzalez-Candelas, L., Ramon, D. & Polaina, J. (1990). Sequence and homology analysis of two genes encoding β -glucosidases from *Bacillus polymyxa*. *Gene* **95**, 31–38.
- Graebnitz, F., Seiss, M., Ruecknagel, K. P. & Staudenbauer, W. L. (1991). Structure of the β -glucosidase A gene *bglA* of *Clostridium thermocellum*: sequence analysis reveals a superfamily of cellulases and β -glucosidases including human lactase/phlorizin hydrolase. *Eur J Biochem* **200**, 301–309.
- Hanlon, D. W., Rosardo, M. M. L., Ordal, G. W., Venema, G. & Van Sinderen, D. (1994). Identification of TlpC, a novel 62 kDa MCP-like protein from *Bacillus subtilis*. *Microbiology* **140**, 1847–1854.
- Itaya, M. & Tanaka, T. (1991). Complete physical map of the *Bacillus subtilis* 168 chromosome constructed by a gene-directed mutagenesis method. *J Mol Biol* **220**, 631–648.
- Kraft, R. & Leinwand, L. A. (1987). Sequence of the complete P protein gene and part of the M protein gene from the histidine transport operon of *E. coli*: compared to that of *S. typhimurium*. *Nucleic Acids Res* **15**, 8568.
- Love, D. R. & Bergquist, P. L. (1988). Sequence structure and expression of a cloned β -glucosidase gene from an extreme thermophile. *Mol & Gen Genet* **213**, 84–92.
- Nohno, T., Saito, T. & Hong, J.-S. (1986). Cloning and complete nucleotide sequence of the *Escherichia coli* glutamine permease operon (*glnHPQ*). *Mol & Gen Genet* **205**, 260–269.
- Ogawa, K., Akagawa, E., Nakamura, K. & Yamane, K. (1995). Determination of a 21 548 nucleotide sequence around the 24° region of the *Bacillus subtilis* chromosome. *Microbiology* **141**, 269–275.
- Paavilainen, S. K., Hellman, J. & Korpela, T. (1993). Purification, characterization, gene cloning and sequencing of a new β -glucosidase from *Bacillus circulans* subsp. *alkalophilus*. *Appl Environ Microbiol* **59**, 927–932.
- Vosman, B., Kuiken, G., Kooistra, J. & Venema, G. (1988). Transformation in *Bacillus subtilis*: involvement of the 17-kilodalton DNA-entry nuclease and the competence-specific 18-kilodalton protein. *J Bacteriol* **170**, 3703–3710.
- Wissenbach, U. & Uden, G. (1993). Physical map location of the new *artPIQMJ* genes of *Escherichia coli*, encoding a periplasmic arginine transport system. *J Bacteriol* **175**, 3687–3688.

Received 13 July 1994; revised 20 September 1994; accepted 13 October 1994.