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The signal peptidase II (*lsp*) gene of *Bacillus subtilis*

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The gene encoding the type II signal peptidase (SPase II) of *Bacillus subtilis* was isolated by screening a genomic DNA library of this bacterium for the ability to increase the levels of globomycin resistance in *Escherichia coli*, and to complement the growth deficiency at the non-permissive temperature of *E. coli* strain Y815 carrying a temperature-sensitive mutation in its *lsp* gene for SPase II. The deduced amino acid sequence of the *B. subtilis* SPase II showed significant similarity with those of other known SPase II enzymes. Activity of the *B. subtilis* SPase II was demonstrated by a pulse-labelling experiment in *E. coli*. In *B. subtilis*, the *lsp* gene is flanked by the isoleucyl-tRNA synthetase (*ileS*) gene and the pyrimidine biosynthetic (*pyr*) gene cluster, which is known to map at 139° of the chromosome. In the Gram-positive bacteria studied thus far, *lsp* appears to be the first gene in an operon. The promoter-distal gene (‘*orf4*’) of this operon specifies a hypothetical protein in bacteria and yeast.

Keywords: *Bacillus subtilis*, leader peptidase II, lipoprotein, Lsp, signal peptidase II

INTRODUCTION

Signal peptidases are integral membrane proteins which remove signal peptides from precursors of secretory proteins. In bacteria, three types of signal peptidases are known (for a review see von Heijne, 1994). The type II signal peptidases (SPase II; EC 3.4.23.36), or prolipoprotein signal peptidases (Lsp), specifically remove signal peptides from glyceride-modified prolipoproteins during their translocation across the membrane (Sankaran & Wu, 1994). Prior to the processing of lipoprotein precursors by SPase II, the Cys residue at the +1 position (relative to the processing site) of these proteins is modified by a diacylglyceryl moiety (Giam *et al*., 1984). The lipomodified precursor proteins are cleaved by SPase II on the amino-terminal side of the Cys residue, resulting in apolipoproteins and signal peptides. This step can be inhibited by the peptide antibiotic globomycin (Glm), which is a potent, reversible and noncompetitive inhibitor of SPase II (Inukai *et al*., 1978). Finally, the apolipoproteins are further modified by *N*-fatty acylation of the diacylglyceryl-cysteine amino group to yield mature lipoproteins (Tokunaga *et al*., 1982).

lsp genes encoding SPase II have been identified and sequenced from *Escherichia coli* (Yamagata *et al*., 1983; Tokunaga *et al*., 1983), *Pseudomonas fluorescens* (Isaki *et al*., 1990a), *Enterobacter aerogenes* (Isaki *et al*., 1990b), *Haemophilus influenzae* (Fleischmann *et al*., 1995), *Staphylococcus aureus* (Zhao & Wu, 1992), *Staphylococcus carnosus* (Witke & Götz, 1995), and *Mycoplasma genitalium* (Fraser *et al*., 1995). In Gram-negative bacteria, the *lsp* gene is part of the *ileS–lsp* operon, containing four other genes with unrelated functions, such as the isoleucyl-tRNA synthetase (*ileS*) gene (Miller *et al*., 1987). In *S. aureus*, *S. carnosus* and *M. genitalium*, the *lsp* and *ileS* genes are not organized in one operon (Zhao & Wu, 1992; Fraser *et al*., 1995; Witke & Götz, 1995).

In Gram-positive bacteria, lipomodified proteins play important roles in: (i) the uptake of nutrients; (ii) sporulation and germination; (iii) protein secretion; (iv) conferring resistance to certain antibiotics; and (v) targeting bacteria to different substrates, host tissues and bacteria via adhesins (Sutcliffe & Russell, 1995). To analyse the pathway for the export of lipoproteins in *B.*

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Abbreviations: Ap, ampicillin; Glm, globomycin; SD, Shine–Dalgarno; Tc, tetracycline.

The GenBank accession number for the sequence reported in this paper is U48870.

subtilis, the cloning and characterization of the *B. subtilis lsp* gene [*lsp*(Bsu)] is desirable. SPase II is also likely to be important for the secretion of non-lipoproteins, since the lipomodified protein PrsA is required for the correct folding of secreted proteins after their translocation across the membrane (Kontinen & Sarvas, 1993).

In this article, we report the isolation and characterization of the *lsp*(Bsu) gene of *B. subtilis* 168. The deduced amino acid sequence of the corresponding SPase II was compared with that of known type II SPases and the genomic location of *lsp*(Bsu) was determined.

METHODS

Bacterial strains, plasmids and media. *E. coli* DH5α [F⁻/*endA1 hsdR17*($r^- m^+$) *supE44 thi-1 recA1 gyrA* (Nal^r) *relA1* Δ(*lacIZYA–argF*)*U169 deoR* (*ϕ80dlac*Δ(*lacZ*)*M15*)] and *E. coli* JM110 [F′ *traD36 lacI*^{q} Δ(*lacZ*)*M15 proA*$^+$*B*$^+$/*rpsL* (Str^r) *thr leu thi lacY galK galT ara fhuA dam dcm supE44* Δ(*lac–proAB*)] were the hosts for cloning experiments. For the complementation analysis, *E. coli* strain Y815 was used, which has a temperature-sensitive SPase II, and which carries an IPTG-inducible *lpp* gene for the major outer-membrane lipoprotein, also known as Braun's lipoprotein, on the low-copy-number plasmid pHY001. Plasmid pHY001 also contains a tetracycline (Tc) resistance determinant (Yamagata *et al.*, 1982). *B. subtilis* strain 168 (*trpC2*) from our laboratory collection was used as a source of chromosomal DNA for the construction of a gene library in the phagemid pBluescript II KS(+) (Stratagene) vector. Plasmid pKSA11 and its deletion derivatives (pG1–pG5), constructed in pBluescript II KS(+), contain various inserts from the *B. subtilis lsp* region (Fig. 1a). Strains were grown in TY medium, M9 medium-1 or M9 medium-2 (van Dijl *et al.*, 1988). If required, 100 μg ampicillin (Ap) ml^{-1}, 100 μg Glm ml^{-1}, 10 μg Tc ml^{-1}, 0·6 mM IPTG and 40 μg X-Gal ml^{-1} were added.

DNA manipulations. Plasmid DNA preparation, restriction endonuclease digestion, ligation, filling-in of cohesive ends, agarose gel electrophoresis and transformation of electrocompetent *E. coli* cells were carried out as described by Sambrook *et al.* (1989). Total DNA of *B. subtilis* 168 was extracted according to Bron (1990). The genomic library of *B. subtilis* 168 was constructed by the method of partial filling-in of cohesive ends (Zabarovsky & Allikmets, 1986). Enzymes and deoxynucleotides were purchased from Boehringer and Pharmacia.

Complementation test for SPase II activity in *E. coli*. The growth of *E. coli* strain Y815 is sensitive to IPTG addition at high temperature due to the accumulation of lipid-modified prolipoprotein (Yamagata *et al.*, 1982). This strain was electrotransformed with plasmid DNAs. Dilutions were plated in two series on AB3 agar medium (Difco) supplemented with 100 μg Ap ml^{-1}, 10 μg Tc ml^{-1} and 0·6 mM IPTG, and the plates were incubated for 4 d at either the permissive (30 °C), or the non-permissive (42 °C) temperature for growth. Transformants carrying the *lsp*-containing plasmid DNA were able to grow at 42 °C in the presence of IPTG. Cloning vector pBluescript II KS(+) was the negative control.

Assay for SPase II activity in *E. coli*. *E. coli* Y815 was transformed with pBluescript II KS(+), pKSA11 or pG3, and transformants were grown at 30 °C in AB3 medium containing Ap and Tc. The overnight culture was diluted tenfold in M9 medium-1, and grown for 3 h at 30 °C. Next, the cells were washed and resuspended in M9 medium-2 (methionine- and cysteine-free medium) containing 0·6 mM IPTG, to induce the *E. coli lpp* gene on the resident plasmid pHY001, and incubated for 30 min at 42 °C to inactivate the SPase II of *E. coli* Y815. Pulse-chase labelling of proteins with [^{35}S]methionine (5 min, 42 °C), immunoprecipitation with serum against Braun's lipoprotein, tricine SDS-PAGE (Schagger & von Jagow, 1987) and fluorography were carried out as described by van Dijl *et al.* (1992).

Sequencing, data handling and sequence analysis. The DNA sequence was determined on both strands using the dideoxy chain-termination procedure (Sanger *et al.*, 1977). Clones were sequenced with universal, reverse and synthetic primers using [^{35}S]dATP-αS (Amersham) and the T7 polymerase sequencing kit (Pharmacia). For sequence assembly and analysis of the sequences, the programs COMPARE, BESTFIT, PILEUP, PRETTY and GAP of the University of Wisconsin Genetics Computer Group (GCG) software package (Devereux *et al.*, 1984) were used. Database searches were done using the BLAST program of the Kyoto-Centre, Japan (Altschul *et al.*, 1990).

RESULTS

Construction of a *B. subtilis* 168 gene library

B. subtilis 168 genomic DNA was partially digested with *Sau*3AI and after size fractionation by agarose gel electrophoresis, DNA fragments ranging from 1·5 to 4 kb were isolated. The 5′ sticky ends generated by *Sau*3AI treatment were partially filled-in using Klenow DNA polymerase and dATP plus dGTP. *Sal*I-digested pBluescript II KS(+) vector DNA was similarly partially filled-in with dTTP and dCTP. Chromosomal and vector DNA were ligated in a 1:1 ratio. To prevent the loss of plasmids containing inserts which are difficult to clone in *E. coli*, the library was not amplified in *E. coli*. For the initial analysis of the library, *E. coli* DH5α was electrotransformed with 1 μl of the ligation mixture, containing 0·04 μg vector and 0·04 μg genomic DNA. Transformants were selected on TY agar medium supplemented with Ap, IPTG and X-Gal. Of the $1{\cdot}7 \times 10^5$ Ap-resistant (Ap^r) colonies obtained, 90·5% were white, indicating that they carried potential recombinant plasmids. The average size of the insert DNAs was 2·5 kb, as determined by *Eco*RI and *Xho*I double digestion of the plasmids purified from 30 randomly chosen white colonies. Based on the average insert size and the known size of the *B. subtilis* genome (4·2 Mb), about 7700 white (or 8500 Ap^r) colonies would be necessary for a representative gene library (99% probability of the presence of the desired fragment).

Screening of the library for the presence of the *lsp*(Bsu) gene

Since the (over-)expression of cloned *lsp* genes from Gram-negative and Gram-positive bacteria caused Glm resistance (Glm^r) in *E. coli* (Isaki *et al.*, 1990a, b; Zhao & Wu, 1992; Witke & Götz, 1995), the *B. subtilis* 168

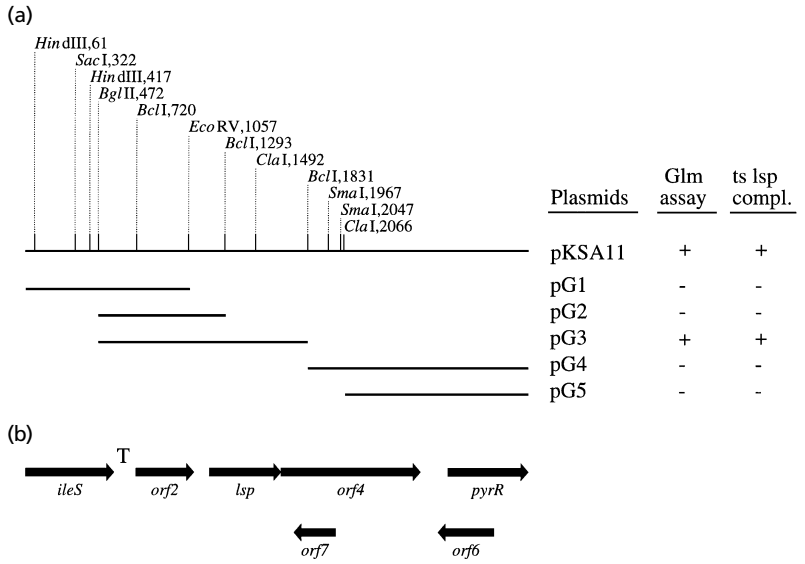

Fig. 1. (a) Restriction map and deletion analysis of the 3·3 kb insert in pKSA11. Deletion derivatives of pKSA11, designated pG1–pG5, were generated with restriction enzymes. The fragments still present in pG1–pG5 are shown below the map. For the localization of the *lsp* gene, the growth of *E. coli* DH5α transformants with the different deletion derivatives was tested on TY agar medium containing 100 μg Glm ml^{-1} (+, growth; −, no growth). The test for complementation of the temperature-sensitive (*ts*) mutation in the *lsp* gene of *E. coli* Y815 was carried out in the presence of IPTG at 42 °C after transformation with the various deletion derivatives of pKSA11 (+, complementation; −, no complementation). Vector pBluescript II KS(+) served as a negative control, and pKSA11 as a positive control. (b) ORFs (shown as heavy arrows) on the 3·3 kb insert in pKSA11. T, putative transcription terminator.

gene library was screened for this property. The minimal inhibitory concentration of Glm for *E. coli* DH5α is 30 μg ml^{-1}. For the screening of the library, 100 μg Glm ml^{-1} was used. *E. coli* DH5α was electrotransformed with an amount of DNA from the gene library, sufficient to obtain about 7×10^5 Ap^r transformants, and the transformation mixture was plated on TY agar medium containing Glm and Ap. Seventeen Glm^r transformants were obtained. Plasmids purified from these transformants were used for electrotransformation of *E. coli* strain Y815, which has a temperature-sensitive Spase II and an IPTG-inducible *lpp* gene. The resulting transformants were tested for complementation of the growth deficiency at 42 °C (non-permissive temperature) in the presence of IPTG for 4 d. Under these conditions, all *E. coli* Y815 transformants containing plasmids from the 17 Glm^r transformants were able to grow, indicating that each of these plasmids carried an *lsp* gene for a functional SPase II protein. As shown by restriction analysis and PCR, the 17 recombinant plasmids contained the same insert on overlapping *Sau*3AI fragments, the sizes of which ranged from 1·4 to 3·3 kb (data not shown). For further analysis, one of these plasmids, pKSA11, was chosen which carried the largest (3·3 kb) insert. Also, pKSA11 gave the largest colonies in the complementation tests suggesting that, compared to the other plasmids, pKSA11 resulted in the best expression of the cloned *lsp* gene.

Nucleotide sequence determination and analysis of the *B. subtilis lsp* gene

Various restriction endonucleases were used to construct a map of the 3·3 kb insert in plasmid pKSA11 (Fig. 1a). Based on this map, deletion derivatives (plasmids pG1–pG5) were constructed to delineate the part of the insert required for complementing the growth defect of *E. coli* Y815 at 42 °C in the presence of IPTG. Complementation was still observed with pG3 containing the 1·4 kb *Bgl*II–*Bcl*I fragment of pKSA11. This indicates that the *lsp*(Bsu) gene is located on this fragment. Direct evidence for this conclusion will be provided in the next section (see Fig. 4) in which an *in vivo* assay for SPase II activity is described.

The nucleotide sequence of the entire 3·3 kb insert was determined on both strands using subclones and synthetic primers. ORFs are shown in Fig. 1(b). The putative *lsp*(Bsu) gene was identified in the middle part of the insert. This gene comprises 462 nucleotides and can encode a protein of 154 amino acid residues with a molecular mass of 17395 Da. A Shine–Dalgarno (SD) sequence, AcuGGAGGaacg, was identified three nucleotides upstream of the putative GUG start codon. The deduced amino acid sequence of the *B. subtilis* SPase II was compared with that of the corresponding enzymes from several other bacteria. A multiple alignment is shown in Fig. 2. The levels of the amino acid identity (and similarity) were: *S. carnosus*, 50·0 % (73·3 %); *S. aureus*, 44·8 % (72·1 %); *P. fluorescens*, 36·8 % (63·2 %); *H. influenzae*, 36·2 % (66·5 %); *E. coli*, 34·2 % (64·4 %); *E. aerogenes*, 34·0 % (65·3 %); and *M. genitalium*, 22·3 % (58·1 %).

The hydropathy profile of the *B. subtilis* SPase II was determined as described by Kyte & Doolittle (1982) and four membrane-spanning segments (A–D) could be distinguished (Fig. 2). Following the positive-inside rule (von Heijne, 1992), a model for the membrane topology of the *B. subtilis* SPase II can be proposed which is shown in Fig. 3. Three conserved regions (I, II and III) were revealed by the amino acid sequence alignment shown in Fig. 2. According to the model for the membrane topology (Fig. 3), region I would be located in the carboxy-terminal part of the first extracytoplasmic loop. Region II would correspond to the membrane-spanning segment C and the amino-terminal part of the second extracytoplasmic loop, while region III would correspond to the carboxy-terminal portion of the

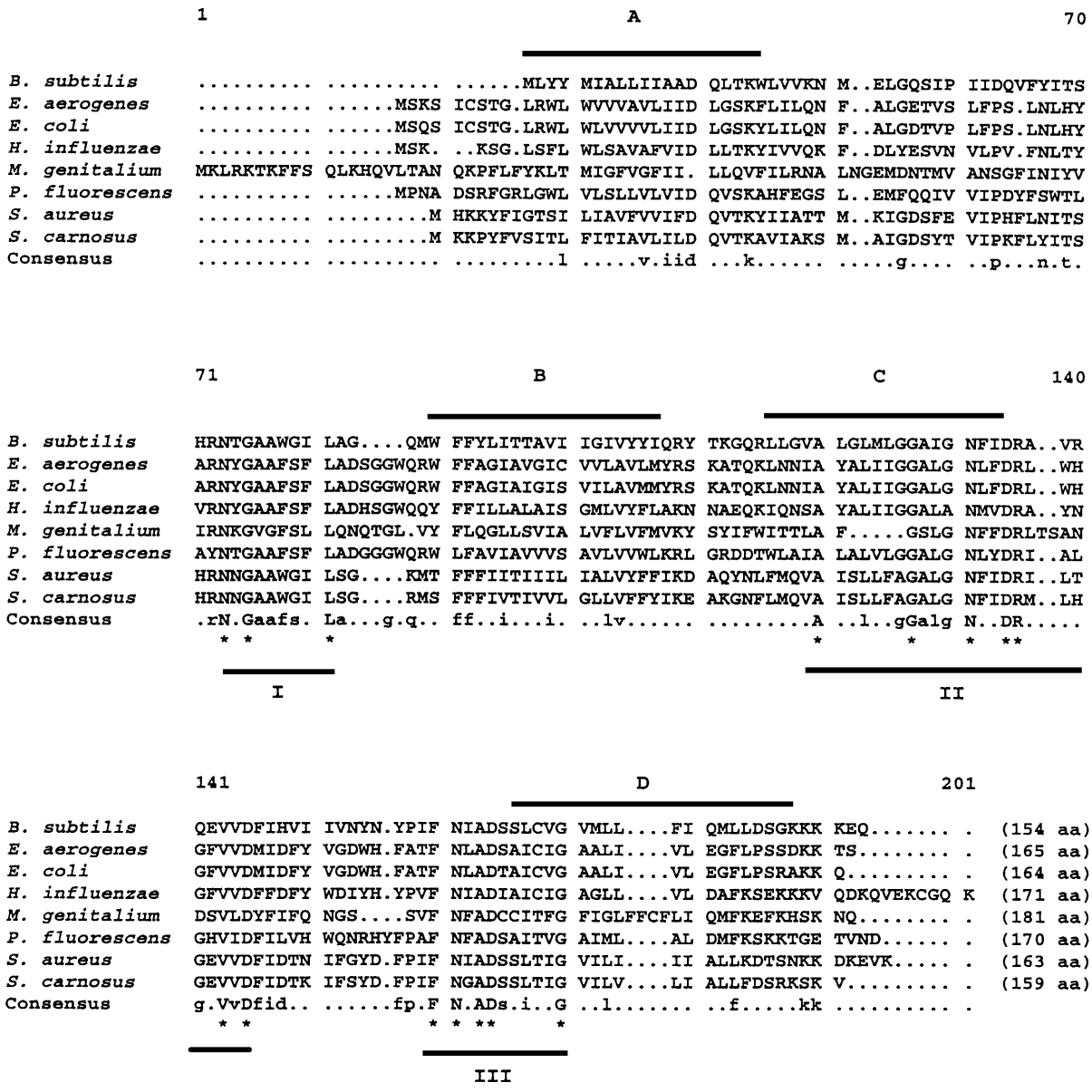

Fig. 2. Comparison of the deduced amino acid sequences of type II SPases. In the consensus sequence, shown below each block, points indicate lack of identity, lower-case letters show identity in at least five of the sequences, and upper-case letters and stars indicate identity in all sequences. The predicted membrane-spanning segments of *B. subtilis* SPase II (A–D) are overlined and the conserved regions (I–III) are underlined.

second extracytoplasmic loop and a part of the transmembrane segment D.

B. subtilis SPase II is active in *E. coli*

To verify the activity of the *B. subtilis* SPase II, processing of Braun's prolipoprotein was studied in *E. coli* Y815 transformed with pBluescript II KS(+), pKSA11 or pG3. The expression of the *lpp* gene was induced by adding IPTG and the temperature-sensitive SPase II of *E. coli* Y815 was inactivated by increasing the temperature to 42 °C. Processing of prolipoprotein was monitored by pulse-chase labelling, subsequent immunoprecipitation, and tricine SDS-PAGE (Fig. 4). Mature lipoprotein was only observed in *E. coli* Y815 carrying pKSA11 or its deletion derivative pG3, but not in *E. coli* Y815 carrying pBluescript II KS(+) (negative control). These results show that the *B. subtilis* SPase II is functional in *E. coli*.

Genes in the *lsp* region of the *B. subtilis* chromosome; map position of *lsp*

In the nucleotide sequence of the 3271 bp insert of plasmid pKSA11, five complete and two partial putative ORFs were identified (Fig. 1b). Six ORFs, starting with

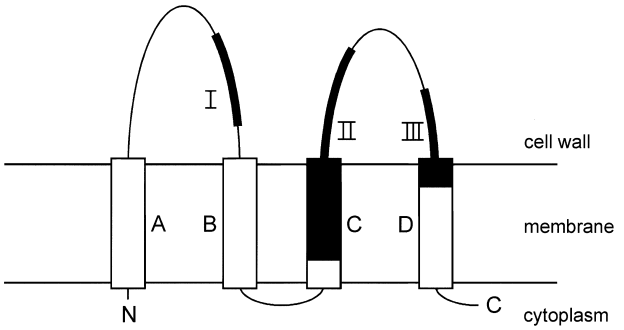

Fig. 3. Model for the membrane topology of the *B. subtilis* SPase II. The four predicted transmembrane domains (A–D) are indicated. The conserved regions (I–III) are indicated in bold, N, amino-terminus; C, carboxy-terminus.

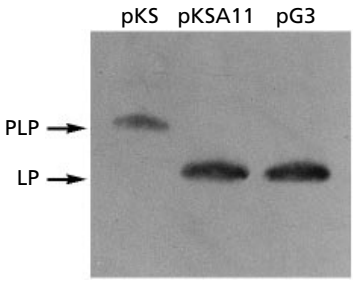

Fig. 4. Activity of the *B. subtilis* SPase II in *E. coli* Y815. Proteins were labelled by incubating cells with [^{35}S]methionine for 5 min at 42 °C. After a chase of 15 min with excess of non-labelled methionine, samples were precipitated with ice-cold trichloroacetic acid. Processing of prolipoprotein by SPase II in *E. coli* Y815 carrying pBluescript II KS(+) (pKS), pKSA11 or pG3 was analysed by immunoprecipitation and tricine SDS-PAGE (16·5 %, w/v, acrylamide). PLP, diacylglyceryl modified precursor form of Braun's lipoprotein; LP, mature form (probably fully lipomodified) of Braun's lipoprotein.

one of the three initiation codons AUG, UUG or GUG, were preceded by an appropriate SD sequence for the binding of mRNA to the ribosome (Table 1).

The deduced amino acid sequence (189 residues) of the partially sequenced gene on the left side of the insert showed 46·7 % (66·5 %) and 34·9 % (57·7 %) identity (and similarity) to the carboxy-terminal parts of the *S. aureus* and *E. coli* isoleucyl-tRNA synthetases (IleS), respectively. Nine bases downstream of the stop codon of *ileS*, an inverted repeat was identified which might function as a rho-independent transcriptional terminator. On the right side of this terminator, and in the same transcriptional orientation as *ileS*, the *orf2*, *lsp*, *orf4* and *pyrR* ORFs were identified, while on the complementary strand *orf6* and *orf7* were present (Fig. 1b). *orf2*, *orf4*, *orf6* and *orf7* could encode hypothetical proteins of unknown function; the deduced *orf2*, *orf6* and *orf7* products did not show significant similarity to any known protein in the databank, while the *orf4* product exhibited a high degree of similarity to 15 bacterial (both Gram-negative and Gram-positive) and three yeast hypothetical proteins.

In Gram-positive bacteria and *M. genitalium*, the *lsp* and *orf4* genes seem to be part of one operon (Table 2). The stop codon of the *lsp* gene overlaps with the initiation codon of *orf4* in *M. genitalium* and *S. aureus*, while in *B. subtilis* only one and in *S. carnosus* 15 nucleotides are present between the stop codon of *lsp* and the start codon of *orf4*. In *B. subtilis*, *M. genitalium* and *S. aureus* the putative SD sequence of *orf4* is located in the coding region of the *lsp* gene.

Downstream of *orf4*, the first gene (*pyrR*) of the pyrimidine biosynthetic gene cluster was identified, because 1209 bp of the sequenced fragment, starting at the *Cla*I site (position 2066; Fig. 1), were (nearly) identical with the published sequence of the *pyr* gene cluster of *B. subtilis* JH861 (1A610) (GenBank accession number M59757; Quinn *et al.*, 1991; Turner *et al.*, 1994); only ten bases were different in both sequences. Since the *pyr* gene cluster has been mapped at 139° of the *B. subtilis* chromosome (Quinn *et al.*, 1991), the *lsp* gene should also be located at this map position.

DISCUSSION

In this paper we describe the isolation of the gene (*lsp*) for a type II SPase of *B. subtilis*. The *B. subtilis lsp* gene encodes the smallest known SPase II protein, consisting of 154 amino acid residues (Fig. 2). In contrast to other type II SPases, the *B. subtilis* SPase II lacks an amino-terminal cytosolic extension of 6 to 26 residues, with positively charged amino acids (Isaki *et al.*, 1990a, b; Muñoa *et al.*, 1991; Zhao & Wu, 1992; Witke & Götz, 1995), suggesting that this region is not essential for catalysis.

The most recently published sequence alignment of type II SPases (Witke & Götz, 1995) revealed 30 conserved amino acid residues in the SPase II protein family. By including the sequences of type II SPases from *H. influenzae* (Fleischmann *et al.*, 1995), *M. genitalium* (Fraser *et al.*, 1995), and *B. subtilis* in the alignment, this number is now reduced to 15 (Fig. 2). It seems likely that some of these conserved residues form part of the catalytic site. In fact, as the type II SPases contain three conserved aspartic acid residues, the present data are in line with the hypothesis of Sankaran & Wu (1994), who suggested that these enzymes could belong to a novel class of aspartic proteases. Moreover, the known type II SPases lack conserved serine, cysteine and histidine residues, required for catalytic activity of serine, thiol and metalloproteases, respectively. Of the three conserved aspartic acid residues, two are located in the conserved region II and one in region III (Fig. 2). Accordingly, the active site could be located in the second extracytoplasmic loop of the type II SPases. In this respect, the type II SPases would resemble type I SPases, the active site of which is also located in an extracytoplasmic part of the enzyme (Dalbey & von Heijne, 1992; van Dijl *et al.*, 1992, 1995).

Table 1. Putative ORFs in the *lsp* region of the *B. subtilis* chromosome

ORF or gene	Coordinates*	Size of product (aa/kDa)	SD consensus sequence† and translation start codon (bold)	Stop codon
ileS‡	> 1..567	–	–	UAA
orf2	715..1086	124/14·0	gcAGGAGuUGugaaga**aug**	UAA
lsp	1192..1653	154/17·4	AcuGGAGGaacguuu**gug**	UAG
orf4	1658..2566	303/34·2	AAAGGAGcaauagc**aug**	UGA
pyrR‡	2752.. > 3271	–	cuguGAGGUGucacaca**uug**	–
orf6	3048..2689	120/13·6	cAAcGAGaaugaacuuu**uug**	UAG
orf7	2011..1745	89/10·1	cggaaAGGUcugugca**aug**	UGA

* Coordinates are according to the U48870 nucleotide sequence in GenBank.

† Nucleotides identical to the consensus Shine–Dalgarno sequence (5′ AAAGGAGGUGAU 3′) are shown in upper case.

‡ Partially sequenced ORF.

Table 2. Comparison of the intercistronic regions of the *lsp–orf4* operon in Gram-positive bacteria and *M. genitalium*

Species	Sequence* in the junction	Size of Orf4 (aa)
B. subtilis	AAAAGGAGCAAuagC**aug**AAU	303
M. genitalium	AUUCAAAAACCAGua**aug**GAA	308
S. aureus	UAAGGAGGUUAAGua**aug**GGA	94†
S. carnosus	uagUAAAGGAGCAUUUAC**aug**	74‡

* The stop codon (uaa or uag) of the *lsp* gene is indicated in lower case, while the SD sequence of the *orf4* gene is underlined and the translation start codon (**aug**) is indicated in bold lower case.

† The 3′ end of the available sequence is too short to conclude that the Orf4 protein of *S. aureus* consists of 94 amino acid residues only.

‡ Partially sequenced ORF.

In Gram-negative bacteria, the *lsp* gene is present in an operon consisting of the genes *x*, *ileS*, *lsp*, *orf149* and *orf315* (Miller *et al*., 1987; Isaki *et al*., 1990a, b). The organization of genes in the *lsp* regions of Gram-positive bacteria and *M. genitalium* seems to be different, as all known *lsp* genes from these organisms are likely to form an operon with *orf4*. Furthermore, none of the genes in the *lsp* regions of Gram-positive bacteria and *M. genitalium*, such as *orf2*, *orf4* and *pyrR* of *B. subtilis*, showed significant similarities with gene *x*, *orf149* and *orf315* of Gram-negative bacteria. Although *ileS* and *lsp* are located close together on the *B. subtilis* chromosome, it seems unlikely that these genes are transcriptionally linked, since a putative terminator sequence was identified between *ileS* and *orf2* (Fig. 1b). Transcriptional linkage of *orf2*, *lsp*, *orf4* and *pyrR* cannot, however, be excluded.

With the availability of the *lsp*(Bsu) gene and its nucleotide sequence, we can now analyse the function of the SPase II protein, and the effects of changing the levels of its production on the export of lipoproteins and non-lipoproteins by *B. subtilis*. In future work, the question will be addressed whether the expression of *lsp*(Bsu) is temporally regulated, and whether SPase II can be a limiting factor in the export of (lipo)proteins in *B. subtilis*.

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