Published in final edited form as:

Mol Cell Probes. 2009 December ; 23(6): 291–297. doi:10.1016/j.mcp.2009.07.003.

THE SMALL ACID SOLUBLE PROTEINS (SASP α and SASP β) OF *BACILLUS WEIHENSTEPHANENSIS* AND *B. MYCOIDES* GROUP 2 ARE THE MOST DISTINCT AMONG THE *B. CEREUS* GROUP

Courtney Callahan, Karen Fox, and Alvin Fox *

Department of Pathology, Microbiology and Immunology, School of Medicine, University of South Carolina, Columbia, SC 29208

Abstract

The *Bacillus cereus* group includes *Bacillus anthracis*, *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus mycoides* and *Bacillus weihenstephanensis*. The small acid-soluble spore protein (SASP) β has been previously demonstrated to be among the biomarkers differentiating *B. anthracis* and *B. cereus*; SASP β of *B. cereus* most commonly exhibits one or two amino acid substitutions when compared to *B. anthracis*. SASP α is conserved in sequence among these two species. Neither SASP α nor β for *B. thuringiensis*, *B. mycoides* and *B. weihenstephanensis* have been previously characterized as taxonomic discriminators. In the current work molecular weight (MW) variation of these SASPs were determined by matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS) for representative strains of the 5 species within the *B. cereus* group. The measured MWs also correlate with calculated MWs of translated amino acid sequences generated from whole genome sequencing projects. SASP α and β demonstrated consistent MW among *B. cereus*, *B. thuringiensis*, and *B. mycoides* strains (group 1). However *B. mycoides* (group 2) and *B. weihenstephanensis* SASP α and β were quite distinct making them unique among the *B. cereus* group. Limited sequence changes were observed in SASP α (at most 3 substitutions and 2 deletions) indicating it is a more conserved protein than SASP β (up to 6 substitutions and a deletion). Another even more conserved SASP, SASP α - β type, was described here for the first time.

Introduction

The *Bacillus cereus* group consists of *Bacillus anthracis*, *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus mycoides* and *Bacillus weihenstephanensis*. As is well known, *B. anthracis* is the causative agent of anthrax. Some *B. cereus* strains induce severe pneumonia [1] while others are responsible for food-borne gastroenteritis and emesis [2] as well as opportunistic infections, particularly of the eye [3]. *B. thuringiensis* (an insect pathogen) and *B. mycoides* (a soil organism) do not generally cause human disease. Some strains of *B. weihenstephanensis* cause emesis like *B. cereus* [4]. Thus it is important that biomarkers be defined for distinguishing *B. weihenstephanensis* from other members of the *B. cereus* group. In the food industry contamination commonly occurs with *B. cereus*, *B. mycoides* and *B. weihenstephanensis* so it is also vital to discriminate among two species that have potential for causing human disease (*B. cereus* and *B. weihenstephanensis*) and an environmental

*Corresponding author: Alvin Fox Phone: 803 733 3288; Fax 803 733 3192; E-mail: Alvin.Fox@uscmed.sc.edu.

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contaminant (*B. mycoides*). The particular focus of the current work is the use of SASPs for characterization of *B. weihenstephanensis* and *B. mycoides*.

The most studied members of the *B. cereus* group are *B. anthracis*, *B. cereus* and *B. thuringiensis* which have recently been referred to as the ACT group. There is high genetic similarity among certain members of the *B. cereus* group (*B. anthracis*, *B. cereus* and *B. thuringiensis*) with *B. anthracis* being a distinct lineage [5–7]. All share a significant degree of genetic similarity as demonstrated by DNA-DNA hybridization [8] and 16S and 23S rRNA sequence [9,10]. *B. anthracis* is generally characterized by the presence of the virulence plasmids, pXO1 and pXO2 which code for the tri-partite toxins and the poly-D-glutamic acid capsule respectively. The extra-chromosomal plasmid markers can be lost during culture; and naturally occurring strains without plasmids have been isolated from the environment. Furthermore other species occasionally contain these plasmids [1,11–13]. Chromosomal markers include the presence of Ba813 [14] or locus tag BA_5345 [15] and single nucleotide substitutions in *rpoB* [16]. Locus tag BA_5345 appears to be the most unique. Polymorphisms in 16S rRNA have been used in discrimination of *B. anthracis* from other members of the *B. cereus* group [17]. Tandem repeats such as are found in the *vrroA* gene [18] and in the *bclA* gene [19] are used for strain not species discrimination. A distinguishing characteristic of *B. thuringiensis* is its ability to produce insecticidal protein crystals (parasporal bodies) but loss of this trait makes *B. thuringiensis* difficult to distinguish from *B. cereus* by other physiological or morphological traits.

Discrimination of *B. mycoides* requires further examination. *B. mycoides* on initial isolation displays rhizoid colony morphology unlike other members of the *B. cereus* group. Unfortunately loss of the ability to form mycoid colonies during laboratory culture is often observed, thus this is not a reliable phenotypic feature [20]. Furthermore even newly isolated strains of *B. mycoides* may lack this property [21]. Multi-locus enzyme electrophoresis and restriction fragment length polymorphism analyses of *B. mycoides* show two genetically distinct groups of strains [20]. This was confirmed by others using DNA-DNA hybridization and fatty acid profiling [22]. Subsequently Nakamura, 1998 named these two groups *B. mycoides* (equivalent to group 1) and *B. pseudomycoides* (equivalent to group 2). The same fatty acids are present in the ACT group and *B. mycoides* with only differences in relative levels providing discrimination [22,23]. In addition, 16S rRNA sequencing suggested that one group of *B. mycoides* is essentially indistinguishable from the ACT group although the other contains significant sequence variation and appears quite unique. Nine of 10 strains of *B. mycoides* studied (including the type strain DSM 2048) contain 4 substitutions within positions 175–193 (*E. coli* numbering) of the 16S rRNA versus the ACT group. The remaining strain of *B. mycoides* exhibited only one substitution versus the ACT group [22]. Others also using 16S rRNA sequencing referred to groups A and B where *B. mycoides* and *B. cereus* strains are intermixed. *B. pseudomycoides* was lumped with some *B. mycoides* strains by 16S rRNA sequence (group A) [24]. Thus in the present report, to avoid confusion, we have chosen to retain the terms *B. mycoides* groups 1 and 2.

B. weihenstephanensis is the species most recently described and its relatedness to other members of the *B. cereus* group also requires further examination [2]. Like *B. cereus*, *B. weihenstephanensis* strains are known to cause food poisoning and emesis involving enterotoxin production [4]. Unlike other *B. cereus* group organisms, *B. weihenstephanensis* are psychrotolerant having optimal growth at approximately 25–35°C. They can be distinguished by their ability to grow at 7°C and below [2]. This is proving importance in food microbiology since there is an increasing demand for ready-to-eat foods and these strains grow at refrigeration temperatures [4]. Sequence differences are found in 16S rRNA, the 23S rRNA, the 16S–23S rRNA spacer region and the cold-shock protein gene *cspA* [4].

SASPs were originally discovered by Peter Setlow's group in *Bacillus subtilis* and their functions include protecting the spore DNA from damage by ultra-violet light [25]. However, the function of the analogous proteins in *B. anthracis*, have not been extensively studied. SASPs were first characterized by determination of MW using matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry by Catherine Fenselau and co-workers [26] are located in the core region of *Bacillus* spores and have been previously demonstrated to be biomarkers for differentiating *B. anthracis* and *B. cereus*. *B. cereus* strains have been divided into two clades [27,28] one more closely related to *B. anthracis* and one more distantly related. Clade 1 can't be readily distinguished from some *B. thuringiensis* strains. Most *B. thuringiensis* strains are grouped with *B. cereus* clade 2 [7]. SASP β of *B. cereus* exhibits one or two amino acid substitutions (correlating with clade 1 and 2) being distinct from *B. anthracis* [29,30]. SASP α does not vary in sequence among these organisms. Hoffmaster et al. [1] phylogenetically divided *B. cereus* strains into 10 branches by amplified fragment length polymorphism with Branch F including all *B. anthracis* strains and pneumonia-causing strains of *B. cereus*. There are four sub-branches within Branch F, with *B. anthracis* strains being found within sub-branch F1-B. SASPs of *B. cereus* strains found in F1-B showed a single amino acid substitution, while strains in the other three sub-branches were more variable generally showing one or two amino acid substitutions [31].

The SASPs of *B. thuringiensis*, *B. mycoides* and *B. weihenstephanensis* have not been previously characterized. In the current study the MWs of SASP α and β were compared for the 5 *B. cereus* species. Available genomic sequences were also downloaded for comparison. The primary aim of the study was to determine the utility of SASPs as possible markers for discrimination of *B. weihenstephanensis* and *B. mycoides* from other members of the *B. cereus* group.

Methods

Organisms and growth conditions

All strains used are listed in Table 1: *Bacillus anthracis* strains were a gift from Stephen Leppla, NIH and previously characterized by carbohydrate profiles using gas chromatography-mass spectrometry [32,33]; this distinguishes *B. anthracis* from *B. cereus* and *B. thuringiensis*. The *B. cereus* and *B. thuringiensis* strains were a gift from Paul Jackson and were characterized using amplified fragment length polymorphism [1]. *Bacillus weihenstephanensis* strains were a gift from Markus Antwerpen and originated from the Weihenstephan Culture Collection of *Bacillus cereus* group species, Munich Technical University, Freising-Weihenstephan, Germany (WSBC); 5 of the 6 strains are described in [2]. *B. mycoides* strains were obtained from the Bacillus Genetic Stock Culture Collection (BSGC). Three of the strains are described in [34] and were deposited in the BSGC by Leonard Peluski (6A11 [98/1883] 6A12 [96/3308] and 6A14 [Gibson 71] and originated from the Food Safety and Microbiology Laboratory, Central Public Health Laboratory, London, United Kingdom, and Niall Logan, Department of Biological Sciences, Glasgow Caledonian University, Glasgow, United Kingdom); 6A13 (NRS-306, ATCC 6463) was characterized by fatty acid profiling and DNA:DNA homology and previously shown to be a member of group 1 [22]. *B. mycoides* 6A19 and 6A20 are ATCC strains (31101 and 11986 respectively). Strains were kept as stocks at -70°C in glycerol. Bacteria were plated on sheep blood agar and incubated for 24 hr at 37°C to allow primary growth and to test hemolysis and then plated on nutrient agar and grown at room temperature to allow sporulation.

Spore preparation

Spores were prepared as previously [31]. Briefly, bacteria were grown on nutrient agar plates, at room temp until sporulation was essentially complete ($>95\%$). Cultures were harvested and

spores were centrifuge-washed three times in distilled water. Samples were freeze-thawed to activate autolysis (by freezing overnight then thawing) and incubating at room temp for 1–2 hr generating preparations >99% spores. The degree of sporulation was assessed, by phase contrast microscopy. The spores were harvested by centrifugation, washed three times in distilled water, autoclaved, lyophilized and stored at -70°C until analysis.

MALDI TOF mass spectrometry analysis

1–3 mg of spores was suspended in 1 ml of water, immediately before mass spectrometry analysis. SASPs were selectively extracted using a strong acidic solution of 10% trifluoroacetic acid (TFA) (Sigma Chemical Co., St. Louis, MO) in water. Extraction of SASPs was performed by mixing 1 μl of the spore suspension in water and 1 μl of 10% TFA in water and dried on the MALDI plate. The matrix consisted of 1 μl of α -cyano-4-hydroxy-cinnamic acid (α -CHCA, 10 mg/ml) (Sigma Chemical Co., St. Louis, MO), which was added to the MALDI plate, and allowed to dry. Samples were analyzed in positive ion, linear mode by MALDI TOF MS for the determination of the MW of native intact SASPs using a Bruker Ultraflex 2 (Bruker Daltonics, Billerica, MA).

Results and Discussion

The measured MWs of SASP α and β , determined using mass spectrometry, are reported here for representative strains of the 5 species within the *B. cereus* group. Both these proteins have been previously studied for a limited number of strains for *B. anthracis* and *B. cereus* [29–31]). The other SASP α - β type described here has not been previously characterized. Three types of α - β SASPs were identified in Genbank searches. The α SASPs, β SASPs and α - β type SASPs were not clearly defined by the annotations. All of the identified SASPs were listed as either β or α - β type. Sequence searches using either α or β SASP sequences would identify the α - β SASPs types since the sequences QMKYEIAQEF, RANGSVGGEI, and TKRLVAMAEQ are conserved within all three types of SASPs. The majority of the sequence differences were isolated within the N and C-termini. Once the α - β type was delineated, utilizing the newly identified alpha-beta type sequence, this type of SASP was identified for all strains examined.

For all 6 strains of *B. weihenstephanensis*, SASP α had the same MW (range 6869.87 to 6874.18) which differed from strains representing the other 5 species (generally range 6832.39 to 6838.71); see Fig 1 and Table 1. SASP β was also unique for 5/6 strains of *B. weihenstephanensis* (MW range 6523.62 to 6524.73). Although for one strain (WSBC 10201) the measured MW for SASP β differed from the other 5 strains of *B. weihenstephanensis* and all other strains for the other 4 species studied (see Table 1). It is unclear whether this strain represents a distinct clade within *B. weihenstephanensis* or is an example of a different *B. cereus* group species as yet undefined.

One of the 6 *B. mycoides* strains characterized in the current report (6A13) has been previously defined as a member of group 1 [22]. The SASPs of 5/6 strains (including 6A13) are reported here to be indistinguishable (based on MW of both SASP α and SASP β) from one another and those of the ACT group (see Table 1) suggesting that all 5 strains are members of group 1. The 6th strain (6A19) has distinct measured MWs from the other 5 strains and contains a unique SASP α and β (MW: 6636.92 and 6536.10 respectively) which presumably makes it a member of group 2.

A search of Genbank for members of the *B. cereus* group (as of June 1, 2009) provided a total of 19 *B. anthracis*, 44 *B. cereus* and 17 *B. thuringiensis* genomes. Only 1 genome was available each for *B. weihenstephanensis*, and *B. pseudomycoides*, and 3 for *B. mycoides*.

A BLAST search using the SASP α sequence (MANQNSSNQL VVPGATAAID QMKYEIAQEF GVQLGADSTA RANGSVGGEI TKRLVAMAEQ SLGGFHK) to search the above genomes determined that all 19 *B. anthracis*, 31 of 44 *B. cereus* and 14 of 17 *B. thuringiensis* contain an identical sequence. Of the remaining strains, 5 *B. cereus* and the remaining 3 *B. thuringiensis* strains have a single amino acid substitution at the carboxyl terminus (glutamine to histidine switch). Two *B. cereus* strains (sub-species cytotoxis and Rock 3–44) contain sequences almost identical to the other 31 *B. cereus* strains with one or two amino acid substitutions respectively unique to the strains. The remaining 6 *B. cereus* strains have sequences identical to those found in *B. mycoides* strain DSM 2048 and *B. weihenstephanensis* KBAB4. The other 2 *B. mycoides* strains and *B. pseudomycoides* contain sequences unique for this group of 3 strains. Table 2 shows the single deposited *B. weihenstephanensis* sequence (and for other strains sharing this sequence) and the unique *B. mycoides* and *B. pseudomycoides* sequences. Only examples are given of the common *B. anthracis*, *B. cereus* and *B. thuringiensis* sequences. The other unusual sequences (*B. cereus* sub-species cytotoxis and Rock 3–44) are also provided.

A majority of the strains within the ACT group were found to contain a SASP α - β type which is identical: MARNRNSNQL ASHGAQAALD QMKYEIAQEF GVQLGADTSS RANGSVGGEI TKRLVAMAEQ QLGGGYTR. However, 5 *B. cereus* strains (AH1273, AH603, AH621, and BDRD-ST196) contain a single amino acid substitution (serine to alanine) at position 7 which is identical to the sequence found in *B. weihenstephanensis* KBAB4 and *B. mycoides* DSM 2048. The other *B. mycoides*/*B. pseudomycoides* sequences have a single amino acid substitution at position 11 (alanine to valine); see Table 3. This SASP α - β type has not been previously detected using MALDI mass spectrometry since it is generally expressed at lower abundance than SASP α , being almost undetectable in many strains. For the ACT group this protein (see Table 3) would have a calculated mass of 7064.77; for *B. weihenstephanensis* 7064.78 and for *B. mycoides* and *B. pseudomycoides* 7085.80. Fig. 2 shows an example of a mass spectrum where the SASP α - β type is present at similar levels to SASP α and one where it is present at lower levels. SASP β is expressed at even higher levels than SASP α in both cases which is typical.

SASP β was found to be more variable in sequence (see Table 4). Using the *B. anthracis* sequence (MARSTNKLAV PGAESALDQM KYEIAQEFGV QLGADATARA NGSVGGEITK RLVSLAEQQL GGFQK) 8 distinct sequence variations were seen. The 19 *B. anthracis* strains all contain identical sequences, but only 2 *B. cereus* and 1 *B. thuringiensis* share this sequence. Like many other markers SASP β indicates *B. anthracis* is a unique lineage but does not serve as a totally unique discriminator from *B. cereus* and *B. thuringiensis*. Fourteen strains of *B. cereus* and 6 strains of *B. thuringiensis* contain a 1 amino acid substitution (phenylalanine to tyrosine) at the carboxyl end of the protein, while 2 *B. cereus* and 1 *B. thuringiensis* contain a single amino acid substitution (alanine to serine) at the amino end of the protein in agreement with previous mass spectrometry analysis [29–31]. Fourteen *B. cereus* and 4 *B. thuringiensis* strains contain a double substitution which reflects both single amino acid substitutions (also in agreement with previous studies [29–31]).

B. weihenstephanensis KBAB4 and *B. mycoides* DSM 2048 share identical SASP β sequences with 5 strains of *B. cereus*. These *B. cereus* strains (AH1273, AH603, AH621, BDRD-ST196 and AH1272) contain the *B. weihenstephanensis* SASP α sequence as well. Whether these 5 *B. cereus* strains are psychrotolerant and share other features with *B. weihenstephanensis* remains to be determined. Again *B. weihenstephanensis* KBAB4 and *Bacillus mycoides* DSM 2048 contain identical sequences. The calculated MW, from these deposited sequences, are also indistinguishable from the measured MWs for *B. weihenstephanensis* strains characterized at USC (calculated MW α : 6874.65, calculated MW β : 6527.27). While the other 3 strains (two *B. mycoides* and one *B. pseudomycoides*) share a different sequence. The calculated MWs of

both SASP α and β for *B. pseudomyoides* DSM 12442, *B. mycoides* Rock 1–4 and *B. mycoides* Rock 3–17 (6636.41 and 6536.28 respectively) are also consistent with measured MWs (see above). *Bacillus cereus* Rock 3–44 shares the *B. mycoides* and *B. pseudomyoides* sequence for SASP β , but has a unique SASP α sequence

B. cereus subsp. cytotoxis NVH 391–98 is unique among members of the ACT group. Each SASP examined contained single amino acid substitutions which are distinct for this organism. Unique single amino acid substitutions were also seen in *B. cereus* Rock 3–44 (SASP α), *B. thuringiensis* IBL 200 (SASP α - β type), and *B. cereus* AH1271 (SASP β).

In summary, based on MW determined experimentally and the genomic sequences present in Genbank, *B. weihenstephanensis* can generally be distinguished from the 4 other species of the *B. cereus* group. Members of the *B. mycoides* species contain 2 distinct deposited sequences, one of which is identical to *B. weihenstephanensis* and another which is distinct for the *B. mycoides* group. The measured MWs also show 2 types; one consistent with the ACT group and the other also distinct for *B. mycoides*. Further studies on *B. mycoides* are clearly warranted.

Conclusions

In previous work it was demonstrated that SASP β is useful in discriminating *B. anthracis* from *B. cereus* but SASP α is highly conserved [29–31]. In the current work it was confirmed that *B. thuringiensis* has similar characteristics to *B. cereus*. However, *B. mycoides* group 1 is also lumped with the ACT group as regards both SASP α and β . This is consistent with previous reports of the 16S rRNA sequence of *B. mycoides* group 1 [21]. *B. mycoides* group 2 (including *B. pseudomyoides*) and *B. weihenstephanensis* are quite distinct from the ACT group for both SASP α and β . This is also in agreement with 16S rRNA analysis [21]. The sequences available from genomic analysis by others are in general agreement with the results from mass spectrometry analysis. However, it appears that some strains of *B. mycoides* are still difficult to distinguish from *B. cereus* and other strains of *B. mycoides* can be confused with *B. weihenstephanensis*. A previously un-described SASP α - β type is described here for the first time that may have utility in aiding discrimination of *B. mycoides* and *B. weihenstephanensis*.

In conclusion, food contamination commonly occurs with *B. cereus*, *B. mycoides* and *B. weihenstephanensis* so it is essential to discriminate among two species that have potential for causing human disease (*B. cereus* and *B. weihenstephanensis*) and an environmental contaminant (*B. mycoides*). *B. weihenstephanensis* displays a unique feature (psychrotolerance) usually distinguishing it from other members of the *B. cereus* group. However, the rhizoidal nature of *B. mycoides* is not apparent for many strains and regardless is a feature readily lost on culture. Thus there is a particular need for further markers to be discovered for better characterization of both *B. mycoides* and *B. pseudomyoides*.

Acknowledgments

The authors are grateful to Markus Antwerpen for his help in supplying strains of *B. weihenstephanensis* which were previously not available from any culture collection in the US. Support for this study was provided by the Sloan Foundation Indoor Air Program. Courtney Callahan received fellowships from the Sloan Foundation, NIH (INBRE Program) and the NSF SEAGEP Program. Automated Methods LLC (A. and K. Fox) received a Phase Zero SBIR through the South Carolina Research Authority (SCRA).

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Fig 1. MALDI MS spectra of SASP α and β : A: *B. mycoides* strain 6A13 (group 1) B: *B. mycoides* strain 6A19 (group 2) and C: *B. weihenstephanensis* 10415. Note α - β type is expressed below detection limits for these strains.

Fig 2. MALDI MS spectra of SASP α , β , and $\alpha\text{-}\beta$ type A: *B. cereus* 03BB87 and B: *B. weihenstephanensis* 10204. Note that for strain 03BB87 $\alpha\text{-}\beta$ type is present at similar levels to α . However for strain 10204, $\alpha\text{-}\beta$ type is present at much lower levels.

Table 1

Measured MWs of SASP α and SASP β of the *B. cereus* group using MALDI mass spectrometry

Species and strain	MW*	SASP α		Amino acid deletions	MW*	SASP β		Amino acid deletions
		Amino acid substitutions				Amino acid substitutions		
<i>B. welshstephanensis</i> WSBC 10204	6874.18	3		0	6524.73	5		1
<i>B. welshstephanensis</i> WSBC 10206	6870.42	3		0	6523.87	5		1
<i>B. welshstephanensis</i> WSBC 10210	6869.87	3		0	6523.62	5		1
<i>B. welshstephanensis</i> WSBC 10211	6872.46	3		0	6525.06	5		1
<i>B. welshstephanensis</i> WSBC 10415	6872.33	3		0	6524.27	5		1
<i>B. welshstephanensis</i> WSBC 10201	6872.89	3		0	6739.27	ND		ND
<i>B. mycoides</i> 6A19	6636.92	3		2	6536.10	6		1
<i>B. mycoides</i> 6A11	6837.74	0		0	6696.59	1		0
<i>B. mycoides</i> 6A12	6835.93	0		0	6695.84	1		0
<i>B. mycoides</i> 6A13	6837.32	0		0	6695.89	1		0
<i>B. mycoides</i> 6A14	6837.15	0		0	6696.50	1		0
<i>B. mycoides</i> 6A20	6834.76	0		0	6694.36	1		0
<i>B. anthracis</i> ANR	6834.55	0		0	6678.08	0		0
<i>B. anthracis</i> Δ Sterne-1	6833.36	0		0	6677.03	0		0
<i>B. anthracis</i> Δ Texas-1	6838.71	0		0	6676.80	0		0
<i>B. anthracis</i> VNR-1- Δ -1	6832.39	0		0	6678.95	0		0
<i>B. cereus</i> 03BB87	6833.84	0		0	6710.21	2		0
<i>B. cereus</i> 03BB102	6834.72	0		0	6694.01	1		0
<i>B. cereus</i> 03BB108	6834.37	0		0	6694.36	1		0
<i>B. cereus</i> ATCC 14579	6833.43	0		0	6709.68	2		0
<i>B. cereus</i> E33L	6835.74	0		0	6694.88	1		0
<i>B. cereus</i> G9241	6834.71	0		0	6709.96	2		0
<i>B. thuringiensis</i> 974	6835.64	0		0	6694.71	1		0
<i>B. thuringiensis</i> AH516	6835.16	0		0	6695.43	1		0
<i>B. thuringiensis</i> HD30	6834.91	0		0	6694.29	1		0
<i>B. thuringiensis</i> 538	6837.65	0		0	6709.95	2		0
<i>B. thuringiensis</i> 4D2	6833.45	0		0	6710.20	2		0
<i>B. thuringiensis</i> 4F4	6837.60	0		0	6710.17	2		0

* MW was measured by MALDI-TOF mass spectrometry. The amino acid substitutions and deletions were inferred from genomic sequences and corresponding calculated MW (see Table 2 and 3).

ND – not determined

Sequence and calculated molecular weight of small acid soluble spore protein α for different members of the *B. cereus* group

Table 2

Accession Number	Species and Strain	Sequence					MW		
EEM06729	<i>B. mycoides</i> Rock 1–4	MANQN–NPL	VVPGATAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGYQK	6636.41
EEM12504.1	<i>B. mycoides</i> Rock 3–17	MANQN–NPL	VVPGATAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGYQK	6636.41
YP_001643650	<i>B. pseudomycoides</i> DSM 12442	MANQN–NPL	VVPGATAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGYQK	6636.41
ZP_04167490	<i>B. mycoides</i> DSM 2048	MANQSSYNQL	VVPGATAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGFQK	6874.65
YP_001643650.1	<i>B. weihenstephanensis</i> KBAB4	MANQSSYNQL	VVPGATAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGFQK	6874.65
EEL95191.1	<i>B. cereus</i> AH1273	MANQSSYNQL	VVPGATAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGFQK	6874.65
ZP_04196028	<i>Bacillus cereus</i> AH603	MANQSSYNQL	VVPGATAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGFQK	6874.65
ZP_04293561	<i>Bacillus cereus</i> AH621	MANQSSYNQL	VVPGATAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGFQK	6874.65
ZP_04260665	<i>Bacillus cereus</i> BDRD–ST196	MANQSSYNQL	VVPGATAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGFQK	6874.65
ZP_04243863	<i>Bacillus cereus</i> Rock 1–3	MANQSSYNQL	VVPGATAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGFQK	6874.65
ZP_04226484	<i>Bacillus cereus</i> Rock 3–29	MANQSSYNQL	VVPGATAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGFQK	6874.65
ZP_02935943.1	<i>Bacillus anthracis</i> A0174	MANQSSNQL	VVPGATAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGFHK	6834.59
YP_002748204.1	<i>Bacillus cereus</i> 03BB102	MANQSSNQL	VVPGATAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGFHK	6834.59
ZP_04221172	<i>Bacillus cereus</i> Rock 3–42	MANQSSNQL	VVPGATAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGFHK	6834.59
ZP_04070479	<i>Bacillus thuringiensis</i> IBL 200	MANQSSNQL	VVPGATAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGFHK	6834.59
ZP_04077171.1	<i>Bacillus thuringiensis</i> serovar pulsiensis	MANQSSNQL	VVPGATAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGFHK	6834.59
YP_893660.1	<i>Bacillus thuringiensis</i> Al Hakam	MANQSSNQL	VVPGATAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGFHK	6834.59
ZP_04299209	<i>Bacillus cereus</i> MM3	MANQSSNQL	VVPGATAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGFQK	6825.58
ZP_04232340	<i>Bacillus cereus</i> Rock 3–28	MANQSSNQL	VVPGATAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGFQK	6825.58
ZP_04178961.1	<i>Bacillus cereus</i> AH1272	MANQSSNQL	VVPGATAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGFQK	6825.58
EEM67610.1	<i>Bacillus thuringiensis</i> serovar berliner ATCC 10792	MANQSSNQL	VVPGATAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGFQK	6825.58
YP_001374031	<i>Bacillus cereus</i> subsp. cytotoxis NVH 391–98	MANQSSNQL	VVPGATAAID	QMKYEIAQEF	GVQLGADTTA	RANGSVGGEI	TKRLVAMAEQ	SLGGFQK	6839.61
ZP_04216357	<i>Bacillus cereus</i> Rock 3–44	MANQSSNPL	VVPGASAAID	QMKYEIAQEF	GVQLGADSTA	RANGSVGGEI	TKRLVAMAEQ	SLGGFQK	6780.54

Sequence and calculated molecular weight of small acid soluble spore protein α - β type for different members of the *B. cereus* group

Table 3

Accession Number	Species and Strain	Sequence					MW		
ZP_04162490	<i>B. mycoides</i> Roc k 1–4	MARNRNSNQL	VSNQAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7085.80
EEM11640.1	<i>B. mycoides</i> Rock 3–17	MARNRNSNQL	VSNQAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7085.80
ZP_04150977	<i>B. pseudomycoides</i> DSM 12442	MARNRNSNQL	VSNQAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7085.80
ZP_04168511	<i>B. mycoides</i> DSM 2048	MARNRNSNQL	ASHGAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7064.78
EEK73596.1	<i>B. welihenstephanensis</i> KBAB4	MARNRNSNQL	ASHGAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7064.78
EEL93985.1	<i>B. cereus</i> AH1273	MARNRNSNQL	ASHGAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7064.78
ZP_04197062	<i>B. cereus</i> AH603	MARNRNSNQL	ASHGAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7064.78
ZP_04294637	<i>B. cereus</i> AH621	MARNRNSNQL	ASHGAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7064.78
ZP_04261703	<i>B. cereus</i> BDRD–ST196	MARNRNSNQL	ASHGAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7064.78
ZP_04244908	<i>B. cereus</i> Rock 1–3	MARNRNSNQL	ASHGAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7080.78
ZP_04227499	<i>B. cereus</i> Rock 3–29	MARNRNSNQL	ASHGAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7080.78
ZP_02933616.1	<i>B. anthracis</i> A0174	MARNRNSNQL	ASHGAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7080.78
YP_002749323.1	<i>B. cereus</i> 03BB102	MARNRNSNQL	ASHGAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7080.78
ZP_04222223	<i>B. cereus</i> Rock3–42	MARNRNSNQL	ASHGAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7080.78
EEM96704.1	<i>B. huringiensis</i> IBL 200	MARNRNSNQL	ASHGAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7080.78
EEM89968.1	<i>B. thuringiensis</i> serovar pulsiensis	MARNRNSNQL	ASHGAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7080.78
YP_894590.1	<i>B. thuringiensis</i> AI Hakam	MARNRNSNQL	ASHGAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7080.78
EEK68013	<i>B. cereus</i> MM3	MARNRNSNQL	ASHGAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7080.78
ZP_04233325	<i>B. cereus</i> Rock 3–28	MARNRNSNQL	ASHGAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7080.78
EEL88267.1	<i>B. cereus</i> AH1272	MARNRNSNQL	ASHGAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7064.78
ZP_04101745	<i>B. thuringiensis</i> serovar berliner	MARNRNSNQL	ASHGAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7080.78
YP_001374814	<i>B. cereus</i> subsp. cytotoxis NVH 391–98	MARNRNSNQF	ASNGAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYNR	7104.76
EEL51039.1	<i>B. cereus</i> Rock 3–44	MARNRNSNQL	ASHGAQAALD	QMKYEIAQEF	GVQLGADTSS	RANGSVGGEI	TKRLVAMAEQ	QLGGGYTR	7080.78

Sequence and calculated molecular weight of small acid soluble spore protein β for different members of the *B. cereus* group

Table 4

Accession Number	Species and Strain	Sequence				MW
ZP_04164615.1	<i>Bacillus mycoides</i> Rock 1–4	MA–STNKL	AVSGAQAAALD	QMKYEIAQEF	GVQLGADSTS	TKRLVSLAEQ
ZP_04159010.1	<i>Bacillus mycoides</i> Rock 3–17	MA–STNKL	AVSGAQAAALD	QMKYEIAQEF	GVQLGADSTS	TKRLVSLAEQ
ZP_04153294.1	<i>Bacillus pseudomycoides</i> DSM 12442	MA–STNKL	AVSGAQAAALD	QMKYEIAQEF	GVQLGADSTS	TKRLVSLAEQ
ZP_04219355.1	<i>Bacillus cereus</i> Rock 3–44	MA–STNKL	AVSGAQAAALD	QMKYEIAQEF	GVQLGADSTS	TKRLVSLAEQ
ZP_04170979.1	<i>Bacillus mycoides</i> DSM 2048	MA–STNKL	AVSGAQAAALD	QMKYEIAQEF	GVQLGADATS	TKRLVSLAEQ
YP_001647263.1	<i>Bacillus weihenstephanensis</i> KBAB4	MA–STNKL	AVSGAQAAALD	QMKYEIAQEF	GVQLCADATS	TKRLVSLAEQ
ZP_04182480.1	<i>Bacillus cereus</i> AH1273	MA–STNKL	AVSGAQAAALD	QMKYEIAQEF	GVQLGADATS	TKRLVSLAEQ
ZP_04199651.1	<i>Bacillus cereus</i> AH603	MA–STNKL	AVSGAQAAALD	QMKYEIAQEF	GVQLGADATS	TKRLVSLAEQ
ZP_04297083.1	<i>Bacillus cereus</i> AH621	MA–STNKL	AVSGAQAAALD	QMKYEIAQEF	GVQLCADATS	TKRLVSLAEQ
ZP_04264270	<i>Bacillus cereus</i> BDRD–ST196	MA–STNKL	AVSGAQAAALD	QMKYEIAQEF	GVQLGADATS	TKRLVSLAEQ
ZP_04182480.1	<i>Bacillus cereus</i> AH1272	MA–STNKL	AVSGAQAAALD	QMKYEIAQEF	GVQLGADATS	TKRLVSLAEQ
ZP_02933923.1	<i>Bacillus anthracis</i> A0174	MARSTNKL	AVPGAESALD	QMKYEIAQEF	GVQLGADATA	TKRLVSLAEQ
EEL43435.1	<i>Bacillus cereus</i> Rock 3–42	MARSTNKL	AVPGAESALD	QMKYEIAQEF	GVQLGADATA	TKRLVSLAEQ
EEM87426.1	<i>Bacillus thuringiensis</i> serovar pulsiensis	MARSTNKL	AVPGAESALD	QMKYEIAQEF	GVQLGADATA	TKRLVSLAEQ
YP_002752005.1	<i>Bacillus cereus</i> 03BB102	MARSTNKL	AVPGAESALD	QMKYEIAQEF	GVQLGADATA	TKRLVSLAEQ
ZP_04302872	<i>Bacillus cereus</i> MM3	MARSTNKL	AVPGAESALD	QMKYEIAQEF	GVQLGADATA	TKRLVSLAEQ
YP_896938.1	<i>Bacillus thuringiensis</i> Al Hakam	MARSTNKL	AVPGAESALD	QMKYEIAQEF	GVQLGADATA	TKRLVSLAEQ
ZP_04230040.1	<i>Bacillus cereus</i> Rock 3–29	MARSTNKL	AVPGAESALD	QMKYEIAQEF	GVQLCADATS	TKRLVSLAEQ
ZP_04247495.1	<i>Bacillus cereus</i> Rock 1–3	MARSTNKL	AVPGAESALD	QMKYEIAQEF	GVQLGADATS	TKRLVSLAEQ
ZP_04104374.1	<i>Bacillus thuringiensis</i> serovar berliner	MARSTNKL	AVPGAESALD	QMKYEIAQEF	GVQLGADATS	TKRLVSLAEQ
YP_001376530.1	<i>Bacillus cereus</i> subsp. cytotoxis NVH 391–98	MARNTNKL	AVPGAESALD	QMKYEIAQEF	GVQLCADATS	TKRLVSLAEQ
EEM94077.1	<i>Bacillus thuringiensis</i> IBL200	MARSTNKL	AVPGAESALD	QMKYEIAQEF	GVQLGADATS	TKRLVSLAEQ
EEL32428.1	<i>Bacillus cereus</i> Rock 3–28	MA–STNKL	AVSGAQAAALD	QMKYEIAQEF	GVQLGADATS	TKRLVSLAEQ