Published in final edited form as:

Int J Mass Spectrom. 2019 January ; 435: 227–233. doi:10.1016/j.ijms.2018.10.033.

Microwave supported hydrolysis prepares *Bacillus* spores for proteomic analysis

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Abstract

Rapid identification of *Bacillus* spores in the environment has depended primarily on a family of small acid soluble proteins (SASPs) as biomarkers. However, SASP sequences and molecular masses are similar or identical in some critical cases. For example, some strains of *B. subtilis*, and *B. thuringiensis* cannot be distinguished from strains of *B. anthracis* based on SASPs. Consequently, additional or alternative biomarkers should be sought. In this study microwave-assisted hot acid hydrolysis was coupled with mass spectrometry as a potentially powerful approach to the rapid automatable characterization of *Bacillus* spores. Hot acid provides lysis of the spores, Asp-selective hydrolysis of proteins, and peptides compatible with automated analysis of either peptide fingerprints or tandem mass spectra. Peptide biomarkers are compared here for a selection of *Bacillus* spores, and peptides unique to each spore type are identified.

Keywords

Bacillus spores; Microwave heating; Hot acid digestion; MALDI-TOF; In-source activation; Bottom-up proteomics; LC-MS/MS

1. Introduction

Bacillus spores can cause serious infectious diseases, and methods that serve for the rapid detection of *Bacillus* spores are of great interest [1]. MALDI-TOF mass spectrometry provides several advantages, making it suitable to characterize molecular signals generated from microorganisms and the rapid detection of *Bacillus* spores [2].

Two MALDI-TOF mass spectrometry-based strategies have been developed previously for the characterization and identification of *Bacillus* spores: whole cell profiling and proteolytic peptide identification [3,4]. In the first strategy, mass signals generated from intact proteins and other molecules of microorganisms are recorded and characterized by MALDI-TOF mass spectrometry and the detection is accomplished by searching the spectrum of the unknown microorganism against a well-established spectral library [3]. One of the limitations of this strategy is that MALDI spectra from several intact *Bacillus* spores

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This paper is dedicated to Professor Shuying Liu on the occasion of her 75th birthday.

are very similar and only one or two mass signals are different, increasing the false positive rate [5]. In addition, proteins of higher molecular mass (>20,000 Da) are not readily detected from spores using MALDI-TOF mass spectrometry. In the bottom-up strategy, intact proteins released from spores are enzymatically or chemically hydrolyzed. Peptides are then identified using fragment ions produced by post-source decay (PSD) or collision-induced dissociation (CID) and identified by automated database searching [4]. Bottom-up strategies provide higher confidence and better specificity for the identification of *Bacillus* spores with high genetic similarity and have been used to distinguish *B. anthracis* Ames from *B. cereus* and *B. thuringiensis* spores [6].

A family of small acid soluble proteins (SASPs) has been shown to provide abundant biomarkers for *Bacillus* spores [7]. However, the similarity in SASP sequences and masses suggests that additional or alternative proteins should also be investigated as biomarkers [8]. In previous studies, we established a microwave-assisted hot acid method that provides extensive protein hydrolysis [9,10]. In this study, the method is applied to lyse four *Bacillus* spore types: *B. globigii*, *B. thuringiensis* (Hakam), *B. anthracis* (sterne), and *B. subtilis* (168) and to proteolyse a wider selection of released proteins. The sensitivity of this method is evaluated with MALDI-TOF mass spectrometry, and peptides unique to each spore type are identified in a parallel LC-MS/MS proteomic analysis.

2. Experimental

2.1. Safety

Bacillus spores can be dangerous to human health. The University of Maryland Institutional Biosafety Committee approved the protocols that were used in this study. All work with *Bacillus* spores were conducted in a biosafety cabinet (Biosafety Level II). NaOCl (5%) solution with 30 min contact time was used to inactivate *Bacillus* spores and all waste was autoclaved at 121 °C for 1 h.

2.2. Materials

B. globigii spores, *B. thuringiensis* (hakam) spores and *B. anthracis* (sterne) spores were >95% free of vegetative cells, based on phase contrast microscopy. *B. subtilis* (ATCC 168) spores were prepared at the University of Maryland using standard techniques [11,12] and shown to be 80% free of vegetative cells, using phase contrast microscopy. Established spores were dried, weighed, and stored in a -80 °C freezer. The MALDI matrix α -cyano-4-hydroxycinnamic acid (CHCA) was purchased from Sigma (St. Louis, MO). Glacial acetic acid, water, trifluoroacetic acid (TFA), formic acid (FA), and acetonitrile (ACN) were purchased from Fisher Chemical (Fair Lawn, NJ).

2.3. Microwave-assisted hot acid digestion

Microwave-assisted hot acid digestion was conducted in a laboratory microwave (CEM, Matthews, NC). *Bacillus* spores were suspended in 100 μ L of 12.5% acetic acid solution and the digestion was conducted at 140 °C for 15 min. The selection of pH, temperature and time for these experiments was based on previous optimization studies in this laboratory [9,13,14]. After hot acid hydrolysis, samples were centrifuged at 10,000 $\times$ g for 1 min. The

supernatant samples were carefully collected and concentrated using C18 ZipTips (Millipore, Billerica, MA).

2.4. MALDI-TOF mass spectrometry analysis

The CHCA matrix was prepared in 50% acetonitrile containing 0.1% TFA at a concentration of 10 mg/mL. Eluted peptides were mixed with 0.5 μ L CHCA solution and applied to the MALDI plate. The samples were evaluated on two MALDI-TOF mass spectrometers: Axima-CFR^{PLUS} (Kratos Analytical by Shimadzu Biotech, Manchester, U.K.) and Autoflex maX (Bruker, Billerica, MA). MALDI-TOF mass spectra were obtained in the positive linear mode and all spectra were collected as an average of 1000 profiles from 1000 to 10,000 Da. Mass accuracy is $\pm$ 0.5 Da for both MALDI-TOF mass spectrometers.

2.5. LC-MS/MS proteomic analyses

Protein hydrolysis patterns and peptide identities were confirmed using a bottom-up proteomics strategy with an Ultimate 3000 nano HPLC system (Dionex, Sunnyvale CA) interfaced by nano-electrospray to an orbitrap Fusion Lumos instrument (Thermo Fisher Scientific, San Jose CA). About 1 μ g of protein was injected. The column packing was C-18 (Dionex) and the gradient was developed from 5% to 55% solvent B (97.5% acetonitrile, 2.5% water and 0.1% formic acid) in 90 min. Solvent A was 97.5% water, 2.5% acetonitrile, and 0.1% formic acid. Tandem spectra were acquired using CID with 35% of total energy (orbitrap mass accuracy <2 ppm). Raw files were processed with Proteome Discovererv.2.2 (Thermo Fisher Scientific). A database of bacterial proteins (UniProt, 333,371 reviewed proteins) was searched.

3. Results and discussion

3.1. Characterization of peptides produced from *Bacillus* spores by microwave-assisted hot acid digestion

Peaks representing peptides formed with hot acid digestion of *Bacillus* spores were characterized on the Shimadzu MALDI-TOF mass spectrometer with a signal to noise ratio >5 in the mass range of 1000–10,000 Da: 68 peaks were characterized from *B.a.* Sterne spores, 56 peaks were characterized from *B.g.* spores, 55 peaks were characterized from *B.s.* spores and 65 peaks were characterized from *B.t.* spores (Fig. 1). Molecular mass peaks corresponding to intact SASPs were observed strongly in both TOF and orbitrap spectra. Some may be seen in the spectra of *B.a.* Sterne spores (Fig. 1), e.g., 6678.4 Da (small acid soluble protein 2), 6834.4 Da (small acid soluble protein A), and 9737.6 Da (small acid soluble protein gamma). This observation is consistent with reports that SASPs are abundant proteins and stable during acid extraction [10,15]. Limits of detection were determined in two MALDI-TOF mass spectrometers (Fig. 2) and the results showed that the lowest amount of spore sample whose peptides can be detected after hot acid treatment is ~0.005 μ g. Fig. 3 shows an amplified MALDI-TOF spectrum of 0.005 μ g in which multiple peptides are identified.

3.2. Identification of peptides unique to each spore type using an LC-MS/MS strategy

Peptides produced from *Bacillus* spores with hot acid treatment were identified using LC-tandem mass spectrometry and bioinformatics. A list of precursor proteins is shown in Table 1, which includes the most abundant 10 proteins from each spore type, based on peptide-spectrum matching numbers [16]. The LC-tandem mass spectrometry analysis showed that peptides characterized by MALDI-TOF MS are contributed by the most abundant proteins, including SASPs and spore wall proteins (S-layer proteins, Fig. 4). Cellular location analysis shows that proteins identified in the spore samples after hot acid digestion are mainly assigned to ribosome, membrane, external encapsulating structure, and cell wall. Of interest, proteins of high molecular masses, such as 60 kDa chaperonin (~60 kDa) and S-layer protein EA1 (~86 Da) were routinely identified from spore samples with hot acid, demonstrating a significant advantage of this proteolytic method.

Peptides that are uniquely observed from each spore type are listed in Table 2. Although SASPs were identified in all four spore types, peptides produced from certain SASPs are unique to a spore type, such as small acid-soluble protein gamma identified in *B.g.* spores [6,17,18]. Some proteins are found in only one *Bacillus* spore type such as Spore coating protein X from *B.t.* spores and S-layer protein EA1 from *B.a.* spores (Table 2), suggesting variable protein abundance in different spore types. As a result, peptides formed from those proteins with hot acid can be used as biomarkers for the identification. In addition, protein alignment analysis (UniProt.org) showed that, in some cases, peptides identified from nominally the same protein in different spore types have different sequences (Fig. 5). For example, 60 kDa chaperonin was identified in both *B.g.* and *B.t.* spores but the peptide KPEENAGGAGMPDMGGMGGMGGMM is unique to *B.g.* spores (Table 2, Fig. 5). Elongation factor Tu was identified in both *B.a.* spores and *B.s.* spores and the peptide GAPEERERGITISTAHVEYETETRHYAHV is only unique to *B.s.* spores (Table 2, Fig. 5). These genetically distinct proteins are potentially very definitive biomarkers. However, they still have to be evaluated in other variants of the spores of interest.

4. Conclusions

In this study, we have demonstrated a strategy using hot acid to lyse spores and hydrolyse their proteins for the rapid characterization of *Bacillus* spores with MALDI-TOF mass spectrometry. Unique peptide biomarkers were characterized for four *Bacillus* spore types. This strategy provides efficiency, sensitivity, specificity, and is readily susceptible to automation. The methods can be directly extended to the characterization of other variants of *Bacillus* spores and to analysis of other microorganisms.

Acknowledgments

This material is based upon work supported by, or in part by, the U. S. Army Research Office under contract number W911NF-15-C-0243. The content of the information does not necessarily reflect the position or the policy of the Government, and no official endorsement should be inferred. We acknowledge Dr. Yue Li and the Mass Spectrometry Facility of the Dept. of Chemistry and Biochemistry at the University of Maryland, and Dr. Yan Wang and the Proteomics Facility of the College of Math and Natural Sciences, University of Maryland for the use of some of their instruments.

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Fig. 1. MALDI-TOF (Top) and integrated LC-MS (Bottom) mass spectra of *B. anthracis* Sterne spores processed by hot acid digestion.

Fig. 2.
MALDI-TOF mass spectra of different amounts of hot acid samples detected by Shimadzu (Left) and Bruker (Right) mass spectrometers. The mass range is from 1000 to 10,000 Da.

Fig. 3. MALDI-TOF mass spectrum from 0.005 μg of *B. anthracis* spores (Shimadzu mass spectrometer). Sequence information is shown for selected peaks, provided by LC-MS/MS analysis.

Fig. 4. MS/MS spectra of two peptides assigned to S-layer protein EA1 identified in *B.a. sterne* spores: TPASAIKNVVFALD (Top) and TPASAIKNVVFALDADNDGVVNYGSKLSGKD (Bottom). The most intense peaks of *b* and *y* ions are labeled.

60 kDa chaperonin

P28598 ⁵²¹ KPEENG GAGMPDMGGMGGMGGMM ⁵⁴⁴
A8FAG3 KPEEGG SGGGMPDMGGMGGMGGMM

Elongation factor Tu

A7Z0N5 ⁵² GAPPEERERGITISTAHVEYET DTRHYAHVD ⁸²
P33166 GAPPEERERGITISTAHVEYET ETRHYAHVD

Fig. 5.

Protein sequence alignments of 60 kDa chaperonin (Top) and Elongation factor Tu (Bottom). Sequence difference is highlighted in dark ([UniProt.org](https://www.uniprot.org/sequence-alignments), “sequence alignments”) [18].

Table 1

Protein list generated from LC–MS/MS analysis. The most abundant 10 proteins are listed from each spore type, estimated by peptide-spectrum matching numbers.

Bacillus globigii	Bacillus thuringiensis
Small, acid-soluble spore protein gamma-type	Small, acid-soluble spore protein
Small, acid-soluble spore protein 2	Small, acid-soluble spore protein, alpha/beta type
60 kDa chaperonin	60 kDa chaperonin
Polyketide synthase PksN	Spore coat protein
Small, acid-soluble spore protein 1	Small acid-soluble spore protein C
Elongation factor Tu	Elongation factor Tu
Mycosubtilin synthase subunit B	Stage IV sporulation protein A
Spore coat protein X	Small acid-soluble protein gamma type
DNA-binding protein HU 1	Polyketide synthase PksL
Polyketide synthase PksL	Glyceraldehyde-3-phosphate dehydrogenase 1
Bacillus anthracis	Bacillus subtilis
Small, acid-soluble spore protein B	Small, acid-soluble spore protein C
Small, acid-soluble spore protein	Elongation factor Tu
S-layer protein EA1	Vegetative catalase
Bacitracin synthase 3	Small acid-soluble protein gamma type
Mycosubtilin synthase subunit B	DNA-binding protein HU 1
S-layer protein	Spore coat-associated protein N
Mycosubtilin synthase subunit A	Alkyl hydroperoxide reductase C
Bacitracin synthase 1	60 kDa chaperonin
Polyketide synthase PksN	Spore coat protein
Surfactin synthase subunit 2	Glyceraldehyde-3-phosphate dehydrogenase 1

Table 2

Peptides observed uniquely in MALDI spectra of each spore type and identified using LC-MS/MS.

orbitrap (MH ⁺ , Da)	TOF	Peptide sequences	Protein Accessions	Protein
<i>Bacillus globigii</i>				
2136.6	2136.7	PIYNSKLVSRINKMMID	A7Z0N3	30S ribosomal protein S7
1489.6	1489.3	LVAEVASKTNDVAGD	P28598	60 kDa chaperonin
4460.9	4459.9	EEVGSIAEAMERVGNDGVITIEESKGFTELEVVEGMQFD	P28598	60 kDa chaperonin
2287.6	2287.0	KPEENAGGAGMPDmGGMGGMGMM	P28598	60 kDa chaperonin ^a
1017.3	1017.3	MGMGGMGMM	P28598	60 kDa chaperonin
1900.2	1899.6	FPGDDVPVVKGSALKALEG	A7Z0N5	Elongation factor Tu
2015.3	2015.6	FPGDDVPVVKGSALKALEGD	A7Z0N5	Elongation factor Tu
1365.6	1365.1	AKVLYTIAHPHD	A8FA13	FMN-dependent NADH-azoreductase
2698.2	2698.7	LmLKERRFVPPLSGEKEMAHFD	P40806	Polyketide synthase PksJ
2212.5	2211.1	PSRVLAVVLDAGTNQESLLND	034389	Probable NAD- dependent malic enzyme 3
8890.3	8890.2	ANSNNKTNAQQVRKQNNQSSASGQGQFGTEFASETNVQQVRKQNNQSSAAGQGQFGTEFASETDAQQVRQNNQSSAEQNKQQNS	P84585	Small, acid-soluble spore protein gamma- type
6694.0	6693.2	ANSNNKTNAQQVRKQNNQSSASGQGQFGTEFASETNVQQVRKQNNQSSAAGQGQFGTEFASETD	P84585	Small, acid-soluble spore protein gamma- type
4910.1	4910.4	QVRKQNNQSSAAGQGQFGTEFASETDAQQVRQNNQSSAEQNKQQNS	P84585	Small, acid-soluble spore protein gamma- type
2045.2	2045.8	EEIAYTYPYSmASYSPR	P39620	Spore coat protein GerQ
1469.7	1469.0	mESRPYSWVALD	Q08313	Spore coat protein X
1355.6	1335.5	mESRPYSWVAL	Q08313	Spore coat protein X
1454.6	1454.8	MESRPYSWVALD	Q08313	Spore coat protein X
1208.3	1207.9	ESRMNESLEL	P37548	Sporulation-specific protease YabG
<i>Bacillus thuringiensis</i>				

orbitrap (MH ⁺ , Da)	TOF	Peptide sequences	Protein Accessions	Protein
1750.1	1750.8	KMEAVLENPYLITD	A8FAG3	60 kDa chaperonin
1019.2	1018.9	RRKAMLED	A8FAG3	60 kDa chaperonin
1347.5	1347.5	RGYASPYMVTDS	A8FAG3	60 kDa chaperonin
3074.5	3074.6	WLAVQFATAKIGAVLVTVNTNYQAHELD	031826	acyl-CoA synthetase YngI
1700.8	1701.1	KVTVLSSSAEEANHID	Q6HAY1	ATP synthase epsilon chain
1585.8	1586.3	KVTVLSSSAEEANH	Q6HAY1	ATP synthase epsilon chain
1212.4	1212.3	MYQLSKELSI	Q5WEF6	ATP-dependent6- phosphofructokinase
2776.9	2776.2	AHY YAGKTYDY YKATFNRSIND	P05806	Bacilloolysin
2263.5	2262.9	AAKHLEAGAKKVIISAPANE ED	P09124	Glyceraldehyde-3- phosphate dehydrogenase 1
1048.3	1048.5	LAAYIAKKGL	P09124	Glyceraldehyde-3- phosphate dehydrogenase 1
1240.3	1241.0	NESGYSNRVVD	P09124	Glyceraldehyde-3- phosphate dehydrogenase 1
1871.2	1871.2	LLAIPTPGHTPGSmSFLD	Q9R9J0	Mycosubtilin synthase subunit B
3104.5	3104.5	ATARANGSVGGEITKRLVSLAEQQLGGYQK	P0A4F4	Small, acid-soluble spore protein 2
1301.4	1302.1	LAQANLNKQVSD	0.08313	Spore coat protein X
2254.8	2254.1	IATLISTATNTLVAILVITL VL	Q08313	Spore coat protein X
1015.1	1014.7	GVIAELVAQD	Q08313	Spore coat protein X
1082.1	1082.3	FAHAKTEYD	P35149	Stage IV sporulation protein A
2913.2	2913.8	VESEFAPIGTEKQSEELVR YLMQD	P35149	Stage IV sporulation protein A
2798.2	2798.8	VESEFAPIGTEKQSEELVR YLMQ	P35149	Stage IV sporulation protein A
1919.3	1919.2	ALKMVKQTGYGIAAPALAD	P35149	Stage IV sporulation protein A

orbitrap (MH ⁺ , Da)	TOF	Peptide sequences	Protein Accessions	Protein
1804.2	1804.3	ALKMVKQTGYGIAAPALA	P35149	Stage IV sporulation protein A
<i>Bacillus anthracis</i> (Sterne)				
1460.6	1461.2	AILEAKQGEETVTA	B7HDV0	30S ribosomal protein S2
2174.5	2174.6	TNAAKMAVGLGADVTTIDLNAD	Q08352	Alanine dehydrogenase
3195.7	3195.7	LGRVSRYSVVRVNEFMAIEKYSHVmHI	P28820	Aminodeoxychorismate synthase component I
2833.3	2833.1	TVELLALAIENRFDILVPITEFDRD	P39579	D-alanyl carrier protein
3955.6	3955.2	QLEALSPLKVMmRGYGLVYSEKNQVLKSVKDVVSAGD	A7GSJ8	Exodeoxyribonuclease 7 large subunit
2062.3	2062.8	DAAQALAAIEETMKNEGLGE	Q9F166	Phosphocarrier protein HPi
4980.6	4981.1	NAGHRGAVTIATNMAGRGTDIKLGEGVRELGLHLVLTGERHESRRID	Q9K6W8	Protein translocase subunit SecA
2507.8	2507.8	NAQAYVTDVKVSEPTKLTLTGTGL	P94217	S-layer protein EA1
1542.7	1542.5	GVVNYGSKLSGKDFA	P94217	S-layer protein EA1
1863.1	1863.5	PENLEGYEVESKNLAVA	P94217	S-layer protein EA1
1693.9	1693.8	VVTDSGSIGTKTIGVTG	P94217	S-layer protein EA1
2856.3	2856.3	PFGANTAIAKEVLPKTGVVAEGGLDVVTT	P94217	S-layer protein EA1
1978.1	1978.8	PENLEGYEVESKNLAVAD	P94217	S-layer protein EA1
2731.2	2731.3	RASAAVIFTKILNLPVDENAPQPSFK	P94217	S-layer protein EA1
2219.5	2219.8	VITSEIGSQAVHVNVLNPNL	P94217	S-layer protein EA1
2569.8	2569.9	LVNSTTVEYQLSKYTSDRVYSD	P94217	S-layer protein EA1
1911.3	1911.3	AKIVGNKVVVTKTPGKVD	P94217	S-layer protein EA1
3026.3	3026.5	TKLDLNVSTTVEYQLSKYTSDRVYSD	P94217	S-layer protein EA1
2912.2	2912.5	TKLDLNVSTTVEYQLSKYTSDRVYS	P94217	S-layer protein EA1
3870.4	3869.3	PENLEGYEVESKNLAVADAKIVGNKVVVTKTPGKVD	P94217	S-layer protein EA1
4550.2	4550.1	GVVNYGSKLSGKDFALNSQNLVVGEKASLNKLVIATIGEDKVVVD	P94217	S-layer protein EA1
1748.9	1747.8	GSINYLVDKGAITGKPD	P94217	S-layer protein EA1
2971.4	2971.5	PFGANTAIAKEVLPKTGVVAEGGLDVVTTD	P94217	S-layer protein EA1
3796.4	3796.4	DIVKGINLTKETQHKVRVVKSGAEGQKLYLDRNG	P94217	S-layer protein EA1
2803.2	2803.1	FALNSQNLVVGEKASLNKLVIATIGED	P94217	S-layer protein EA1

orbitrap (MH ⁺ , Da)	TOF	Peptide sequences	Protein Accessions	Protein
2687.9	2688.1	EKGNADVEYLNLANHVDVKFVANNL	P94217	S-layer protein EA1
2291.6	2292.8	TKLDLNVSTTVEYQLSKYTS	P94217	S-layer protein EA1
3074.5	3074.5	STARANGSVGGGEITKRLVAMAEQSLGGFHK	W8YUHG	Small, acid-soluble spore protein
3088.5	3088.2	ATARANGSVGGGEITKRLVSLAEQQLGGFQK	Q81KU1	Small, acid-soluble spore protein B
6679.5	6680.3	ARSTNKLAVPGAESALDQMKYEIAQEFVQLGADATARANGSVGGGEITKRLVSLAEQQLGGFQK	Q81KU1	Small, acid-soluble spore protein B
3610.1	3611.0	ARSTNKLAVPGAESALDQMKYEIAQEFVQLGAD	Q81KU1	Small, acid-soluble spore protein B
3494.9	3494.3	ARSTNKLAVPGAESALDQMKYEIAQEFVQLGA	Q81KU1	Small, acid-soluble spore protein B
<i>Bacillus subtilis</i>				
1480.7	1480.9	DIIRHIVVKEEE	P21468	30S ribosomal protein S6
3115.7	3115.5	TILKAKPAAAKGVYVKNVAVTSTMGPVKVD	Q06797	50S ribosomal protein L1
3000.7	2999.9	TILKAKPAAAKGVYVKNVAV TSTMGPVKV	Q06797	50S ribosomal protein L1
1822.1	1822.1	VSEAVLVKKTNTAKFD	Q06797	50S ribosomal protein L1
1707.0	1707.5	VSEAVLVKKTNTAKF	Q06797	50S ribosomal protein L1
2208.3	2208.7	PSQTISRNFVDLDEETGLAD	P80239	Alkyl hydroperoxide reductase C
1643.9	1643.5	SSEKISKITYAMIGD	P80239	Alkyl hydroperoxide reductase C
2192.5	2192.5	MNKTELINAVAEASELSKKD	P08821	DNA-binding protein HU 1
2455.7	2455.2	GAPEERERGITISTAHVEYETETRHYAHVD	P33166	Elongation factor Tu ^a
3340.6	3340.2	GAPEERERGITISTAHVEYETETRHYAHV	P33166	Elongation factor Tu ^a
1442.7	1442.8	YVKNMITGAAQMD	P33166	Elongation factor Tu
1911.9	1911.2	TFNTSSTSNSTSSSSNSK	P24327	Foldase protein PrsA
2169.5	2169.9	YLKEQVKYELLTQKAAD	P24327	Foldase protein PrsA
2054.4	2054.5	YLKEQVKYELLTQKAAD	P24327	Foldase protein PrsA

orbitrap (MH ⁺ , Da)	TOF	Peptide sequences	Protein Accessions	Protein
3235.7	3235.3	PRKYLGPAREAIKETVIGKMREFGSSNQA	P13243	Probable fructose- biphosphate aldolase
1506.6	1506.5	GYVKENEKAHSED	P54507	Spore coat-associated protein N
1391.5	1391.8	GYVKENEKAHSE	P54507	Spore coat-associated protein N
1324.3	1324.5	FKANGGSNTSPED	P54507	Spore coat-associated protein N
1209.3	1209.3	FKANGGSNTSPE	P54507	Spore coat-associated protein N
2474.8	2474.8	FQFENNGSLAIKEVLMALNYGD	P54507	Spore coat-associated protein N
2359.7	2359.6	FQFENNGSLAIKEVLMALNYG	P54507	Spore coat-associated protein N
1971.3	1971.2	PEYGKRVAEGLGLPIKDD	P26901	Vegetative catalase
1523.6	1523.8	YHTEDLFNAIENG	P26901	Vegetative catalase

m: oxidation, + 16 Da.

^aPeptide is genetically unique to the spore type.