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# Small Acid Soluble Proteins for Rapid Spore Identification

V. A. VanderNoot, S. Branda, A. Jokerst, and T.W. Lane

Prepared by
Sandia National Laboratories
Albuquerque, New Mexico 87185 and Livermore, California 94550

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# Small Acid Soluble Proteins for Rapid Spore Identification

V. A. VanderNoot, S. Branda, A. Jokerst and T.W. Lane
Biosystems Research Department
Sandia National Laboratories
P. O. Box 969
Livermore, CA 94551-0969

## Abstract

This one year LDRD addressed the problem of rapid characterization of bacterial spores such as those from the genus *Bacillus*, the group that contains pathogenic spores such as *B. anthracis*. In this effort we addressed the feasibility of using a proteomics based approach to spore characterization using a subset of conserved spore proteins known as the small acid soluble proteins or SASPs. We proposed developing techniques that built on our previous expertise in microseparations to rapidly characterize or identify spores.

An alternative SASP extraction method was developed that was amenable to both the subsequent fluorescent labeling required for laser-induced fluorescence detection and the low ionic strength requirements for isoelectric focusing. For the microseparations, both capillary isoelectric focusing and chip gel electrophoresis were employed. A variety of methods were evaluated to improve the molecular weight resolution for the SASPs, which are in a molecular weight range that is not well resolved by the current methods. Isoelectric focusing was optimized and employed to resolve the SASPs using UV absorbance detection. Proteomic signatures of native wild type *Bacillus* spores and clones genetically engineered to produce altered SASP patterns were assessed by slab gel electrophoresis, capillary isoelectric focusing with absorbance detection as well as microchip based gel electrophoresis employing sensitive laser-induced fluorescence detection.

## Acknowledgement

Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy. This work was sponsored by the U.S. DOE under contract DE-AC04-94AL85000.

The authors gratefully acknowledge the gift by Dr. Peter Setlow at the University of Connecticut of a series of *B. subtilis* clones that produce differing patterns of SASPs.

Thanks also go to Susan Jamison for her effort in evaluating polymer solutions and to Elizabeth Van Blarigan for her efforts at resolving SASPs by isoelectric focusing.

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## Abbreviations

| | |
|---|---|
| BW | biological warfare |
| SDS-PAGE | sodium dodecyl sulfate polyacrylamide gel electrophoresis |
| CGE | capillary gel electrophoresis or chip gel electrophoresis |
| CZE | capillary zone electrophoresis or chip zone electrophoresis |
| IEF | isoelectric focusing |
| LIF | laser-induced fluorescence |
| UV | ultraviolet |
| OD | optical density |
| MW | molecular weight |
| Da/kDa | Dalton, unit of molecular weight used when describing proteins (1 Da = 1atomic mass unit); 1kDa represents 1000 Da. |
| SASP | small acid soluble protein |

# 1. INTRODUCTION

The development of rapid methods for the characterization of pathogens is of vital importance to the DOE mission of detecting and responding to biological and chemical attacks. In the area of biological attack agents, the most well known techniques employ recognition of specific nucleic acid sequences in the organisms genetic material. These techniques can have very good sensitivity; however this sensitivity comes at the expense of response time and PCR based techniques are problematic for samples that contain polymerase inhibitors, or are "protein only" (*i.e.* biotoxins). In addition, PCR based techniques cannot detect things for which you have not developed and loaded expensive nucleic acid primers. These limitations are also characteristic of array based or "gene array" techniques.

A proteomic approach to agent identification is a strong alternative, one that is applicable to the full threat spectrum. By targeting the proteins themselves, developing methods that evaluate the proteins directly, we eliminate the need for specific recognition or capture elements and characterization can begin, even in the case of an unknown, by searching the public domain protein databases. The majority of the research related to proteomic profiling for bacterial identification involves mass spectrometry based approaches. These approaches have been successful in identifying bacteria at least to the species level, in some cases to the strain level and have the advantages of being very specific and sensitive. However, the methods can be time-consuming (eg. MS/MS analysis with enzymatic digestion), and more importantly, the instrument itself is a purely laboratory device, being fundamentally too bulky to be miniaturized to a man-portable/remote site deployable instrument in the foreseeable future.
This research will allow for the development of a proteomic approach to speciation that is complementary to nucleic acid based techniques, operating even in scenarios that are problematic for nucleic acid based assays. It promises to be rapid yet portable, making it suitable for a wide range of biodetection applications.

The proposed project is aimed at rapid identification of bacterial spores. A novel class of analytes that may prove advantageous for identifying spores is the Small Acid-soluble Proteins (SASPs). SASPs are highly promising as targets as they are found in all spore formers, including *Bacillus anthracis* and *Clostridium botulinum.* The SASPs constitute, depending on the bacterial species, 8-20% of total spore protein. They are degraded during spore germination and thus provide a pool of amino acids for protein synthesis during early phases of the germination process. Spores that lack SASPs do not have access to this amino acid pool and thus are slower in out growth after germination. Since spore germination is the first step in an anthrax infection one would expect that SASPs would play a large role in the virulence of *Bacillus anthracis*. In addition, the SASPs have been implicated in stability of the spore and resistance of to killing by DNA damage such as by UV light (1).

SASPs have been characterized from several species of gram positive spore forming bacteria such as members of the genus *Bacillus* and *Clostridia*. In *Bacillus* species two distinct types of major SASPs have been identified, named α and β as in *Bacillus subtilis*, where they were first identified. These α/β type SASPs are share peptide sequence similarity and are generally 5-7 kDa in size. Spores from each bacterial species of spore contains two major α/β type SASPs and several minor α/β typeSASPS. In addition, spores contain one γ type SASP. The peptide sequence of the γ type SASP is distinct from the α/β type SASPs and usually ranges from 8-

11kDa in size. Taken together, the major α/β type SASPs and the γ type SASP constitute 90% of the total SASP content of each spore (2).

Setlow and coworkers have utilized recombinant DNA methods to create knockout mutation in *B. subtilis* of the genes encoding the α, β and γ type SASPs, *sspA*, *sspB* and *sspE* respectively (3). In addition, they have created a strain that lacks *sspA* and *sspB* and instead expresses elevated levels of SspC, a minor α/β type SASP. Dr Setlow made samples of spores from each of these genetic variants along with wildtype available to us. These recombinant strains should allow us to unambiguously identify the major SASP types found in *B. subtilis* during our analysis. Moreover, these recombinant strains have been used to demonstrate the ability of using SASPs for spore identification purposes. This was successfully demonstrated using mass spectrometry based techniques (4).

The proposed work is a one-year LDRD study that was initiated in collaboration with Dr. Peter Setlow (University of Connecticut Health Center). Dr. Setlow is an expert in bacterial spore research and has published numerous papers on the characterization and function of SASPs in *Bacillus* species. The goal of the work was to develop rapid and sensitive detection methods for SASPs to identify bacterial spores. Building on our capabilities in sensitive detection and microfluidics, we proposed to devise small-scale extraction protocols that are amenable to microseparations and further develop high resolution *small-protein* separations. This approach, combined with isoelectric focusing and possibly zone electrophoresis, has great potential to allow rapid speciation of spores via their SASP patterns. Methods that separate proteins on the basis of molecular weight (chip gel electrophoresis or CGE), isoelectric point (isoelectric focusing or IEF) and/or charge-to-mass character (chip zone electrophoresis or CZE), in combination with the selective extraction protocols, is expected to yield species-specific patterns of SASPs.

## 2. EXPERIMENTAL APPROACH AND RESULTS

### 2.1 Spores, growth and purification.

*Wild type and Mutant strains*

Knockout mutant strains (listed below and described further in Table 2) of *B. subtilis* were supplied as a dry purified spore preparation by Peter Setlow and coworkers. These recombinant strains should allow us to unambiguously identify the major SASP types found in *B. subtilis* during our analysis.

PS 832 wild type;
PS 533 a wild type variant with $sspC^{WT}$ under control of the <u>*sspB*</u> promoter
PS 355 lacking *sspA* and *sspB* which encode the major α/β-type SASP
PS 260 lacking *sspA*
PS 1450 lacking *sspA* and *sspB* and also carrying a high-copy plasmid with *sspC* (minor α/β -type SASP), under control of the <u>*sspB*</u> promoter
PS 3019 Same as PS1450 with a modified *sspC*

For the bulk of the methods development experiments, spores were grown and purified in-house as previously described (5, 6). Briefly, *Bacillus subtilis* spores were introduced into 1L Growth Medium at an $OD_{600}$ of ~0.1. The cultures were grown with shaking (250 rpm) at 37°C for ~4 h. Once an $OD_{600}$ of ~0.8 was reached, corresponding to mid log growth, the cells were harvested by centrifugation (10,000 x *g* for 10 min), resuspended in 2L Sporulation Medium, and the new cultures shaken (200 rpm) at 37°C for ~48 h. Spores were harvested by centrifugation (10,000 x *g* for 10 min) and separated from cell debris through a series of salt and detergent washes followed by three water washes; all wash centrifugations were at 15,000 *x g* for 10 min. Washed spores were sonicated twice at 20kHz for 30 seconds, to increase spore yields. Finally, the spores were further purified using a Histodenz gradient. Purified spores were resuspended in water and stored at 4°C.

### 2.2 SASP Extraction

Initial SASP extraction protocols were obtained from the literature (4). Wild type *B. subtilis* spores (4mg of dry spores) were treated with 2N HCl at room temperature for 90 minutes with occasional vortexing. The samples were sedimented by centrifugation at 10 000 x g for 5 minutes and the supernatant was collected. Extraction protocols based on different acids including acetic acid (1-5M) and nitric acid (2N) were evaluated. Reactions times were varied between 10 minutes and 90 minutes. The reactions were also carried out at different temperatures ranging from 4°C to 60°C. Samples extracted using either nitric or hydrochloric acids were dialyzed against 25% (v/v) acetic acid at 4°C for 18-20 hours. Post extraction (and dialysis for methods 1 & 2), the SASPs were isolated using a 30,000 MWCO filter and dried using a Speed-Vac (approximately 2 hours).

Lyopholized SASPs samples were either run on a 16% Tricine gel, an IEF gel, or were used for separations on the uChemLab. For the 16% Tricine gel; samples were resuspended in 10 μL Tricine SDS sample buffer (2x), 2 μL NuPAGE reducing agent (10x), and 8 μL $dH_2O$ for a total volume of 20 μL (buffers from Invitrogen). The resuspended samples were then heated at 85° for 2 minutes. The samples were loaded on a 16% Tricine gel (Invitrogen) and run at 125V for

approximately 90 minutes. For the IEF gel; lyophilized SASPs were resuspended in 10 μL IEF sample buffer (pH 3-10; Invitrogen) plus 10 μL $dH_2O$ for a total volume of 20 μL. The resuspended samples were then loaded on a Novex pH 3-10 IEF gel (Invitrogen) and run at 100V for 1 hour, followed by 200V for 1 hour, and finally 500V for 30minutes. The IEF gel was then fixed in 12% Trichloroacetic acid (TCA) containing 3.5% sulfosalicylic acid for 30minutes. Both gel types were stained for SASPs with Coomassie blue, using standard procedures.

Optimized extraction conditions were used to extract SASPs from the 5 Setlow-supplied variants of *B. subtilis*, including PS260, PS355, PS533, PS1450 and PS 3019. The optimized extraction conditions for all were 5M acetic acid at room temperature for 10 minutes.

**2.3 Microscale Electrophoretic Separations**

*Capillary Isoelectric Focusing*

Reagents for IEF included IEF gel solution purchased from Beckman-Coulter and ampholyte purchased from Pharmacia (Pharmalyte 3-10). Capillary isoelectric focusing was carried out using an Agilent 1300 series CE system. Capillaries were obtained from Polymicro (uncoated fused silica), Agilent (poly vinyl alcohol coated bubble window) and Beckman (eCap neutral coated).

For all runs the anolyte solution was (91mM $H_3PO_4$ in IEF gel) and catholyte solution was (20mM NaOH in distilled water). Samples for focusing were prepared by mixing IEF gel (typically 200 uL) with ampholyte (typically 4 uL) and pI markers (1-3uL of each) The sample was pressure loaded onto the capillary at 2 bar for 0.3 minutes. Focusing was carried out at 20 kV and mobilization was initiated by pressure (30 mbar) after typically 5 minutes focusing. The electric field strength was typically ~750 V/cm. Absorbance was monitored at several wavelengths, including 214, 260 and 280 nm.

*Chip Gel Electrophoresis*

Chip gel electrophoresis (CGE) was carried out as previously described to serve as a baseline for attempts at improving resolution in the small molecular weight region (7). Protein and SASP samples were prepared in 5 mM SDS, 5 mM borate pH 9.0 buffer then labeled with 1 mM fluorescamine (Molecular Probes) dissolved in dry acetonitrile. The standard chip length was 10 cm. Extra

In order to try to improve molecular weight resolution, CGE resolution of small proteins was evaluated using a number of replaceable sieving gels. These included an alternative commercially available sieving matrix also from Beckman with a nominal fractionation range of 10-225 kDa. The SDS 14-200 kDa was also modified to incorporate ~0.1 – 0.4% (w/w) of high molecular weight polyethylene oxide (typically 900 kDa). long channels were also evaluated with a separation length of 20 cm. A suite of small molecular weight proteins were used to assess the resolution.

SASPs of the various strains were separated by both initial conditions as well as under the alternative conditions to demonstrate the ability to resolve.

# 3. RESULTS AND DISCUSSION

### 3.1 SASP Extraction:

A key aspect of the work was the development of methods for extraction that are at once relatively rapid and compatible with the subsequent fluorescent labeling and microseparations. The established extraction method is based on the exposure of spores to hydrochloric acid for 90 minutes. This is excessively long for a proposed "rapid" assay technology and is many times the length of time required for the separations themselves. In addition, the presence of a high concentration of a strong acid is problematic for subsequent reactions or separation steps including base catalyzed fluorescent labeling chemistries, the electrokinetic injection step (charge shielding) and the pH gradient formation in IEF. As a result, additional acid recipes were evaluated. An alternative acid, nitric acid was evaluated to determine if it was more rapid in extracting SASPs than hydrochloric acid. Volatile acids, including acetic and formic acid, were also evaluated to allow the facile replacement of the acid with a more suitable buffer after rapid evaporation. Alternatively, salt could be eliminated from the non-volatile acid containing samples using size exclusion columns. However, samples were effectively extracted using acetic acid (5 M) and both the amounts and overall patterns of proteins, as evidenced by side-by-side polyacrylamide gels, were comparable to that seen with the traditional non-volatile acid methods (see Figure 2).

Extraction temperatures were also compared (4°C, room temperature, 60°C) and found to be comparable, extracting similar amounts of total protein and achieving similar patterns of bands compared to the literature method. Extraction time was also varied between 10 minutes and 90 minutes. As can be seen in Figure 2, the bulk of the SASPs appear to be extracted in approximately 10 minutes, with little more change happening from between 10 and 90 minutes. Indeed, comparable patterns and band intensities were seen after only 10 minutes compared to 90 minutes with the HCl literature method. As a result, 10 minutes extraction time at room temperature was adopted for all further extractions.

The extraction protocol was also optimized for specificity with respect to other potentially interfering proteins. The vast majority of the proteins extracted from the intact spore were likely SASPs (correct molecular weight range) but some co-extraction of the high abundance larger proteins (in the 60+ kilodalton range) was seen. Size fractionation using ultrafiltration membranes (30 kDa molecular weight cut off) proved very successful for isolating the SASP containing fraction, in the size range below 15 kilodaltons.

Figure 3 shows the side by side comparisons of SASPs extracted from the variants of *B. subtilis* acquired from Dr. Setlow's group, including the wild type (WT) and the 5 variants PS260, PS355, PS533, PS1450 and PS3019. Figure 3 shows the SASPs for the variants at both 4°C and room temperature. There are differences in band patterns and relative amounts of bands of each variant though not as striking as one would have expected.

### 3.2 Microscale Separation Technologies

The bulk of the separations work in this year focused on capillary IEF and chip based gel electrophoresis.

*Capillary Isoelectric Focusing*

The isoelectric points of the various SASPs cover the pH range from 4 to 10, and as a result, broad pH range separation methods (pH 3-10) were developed based on commercially available IEF kits and reagents. A variety of capillaries were used including uncoated, PVA coated and eCAP neutral polymer coated. After optimization of the focusing parameters, resolution as shown by pI marker proteins appeared reasonably good, with a ΔpI of approximately 0.2 pH units possible (data not shown).

The extraction protocol and subsequent sample clean-up performed as hoped, with the introduction of SASP sample causing little if any noticeable effect on the pH gradient formation in terms of measured currents or degradation of the separation resolution. However, IEF with standard UV absorbance proved to be problematic for these species and it was extremely difficult to clearly identify the peaks associated with the addition of SASPs. The problem of poor sensitivity appears to stem from the lack of strong chromophores in the 280 nm region. As shown in Table 1, the amino acid sequences for any of the *B. subtilis* derived SASPs contain no tryptophans, the brightest of the three amino acid residues commonly known to generate protein absorbance in the UV region about 280 nm (8). Not only did the SASP sequences not contain tryptophans, but they also lacked tyrosines, the next brightest residue, containing only multiple phenylalanines. It was hoped that the multiple phenylalanines and monitoring at 260 nm (absorption maximum of phenylalanine is blue-shifted to that of the other two amino acids) sufficient to generate a measurable signal with a relatively large sample, sufficient for the proof of concept, but unfortunately that did not prove to be the case.

As a result, the remaining time was devoted to developing chip based IEF with laser induced fluorescence detection. Time and budget constraints prevented the full demonstration however it is expected that LIF detection would be successful for these samples. LIF is typically at least 2 orders of magnitude more sensitive that classical UV absorbance, but in this case, the enhancement in detectability over UV absorbance might be expected to be significantly higher.

*Chip Gel Electrophoresis*

The spores of *Bacillus* species typically display a larger proportion of small molecular weight proteins, corresponding to, among others, the high copy number spore coat proteins and this is comparable to what has been observed in the literature for these species (9, 10). Indeed the SASPs represent a small MW range…making the traditional separations difficult.

Initial separations were carried out with the standard Beckman gel to serve as a comparison. Traces for the 5 strains and wild type are shown in Figure 4. The ability to resolve the different SASP producing strains of *B. subtilis* was evident as each strain produced distinct patterns by optimized CGE. While it is true that each of the mutant strains did show distinctive CGE traces, it is not clear how these traces could correlate with the expected SASP populations as listed in Table 2. The situation is no doubt complicated by the relatively poor resolution of the gels but also by the possibility of additional and confounding proteins being present, coextracting with the expected SASPs.

Additional resolution was sought, possibly to better resolve the small molecular weight proteins that all were below the lower fractionation limit. Alternative sieving media, including an alternative of Beckman with a nominal 10-225 kDa MW range (cf 14-200 kDa) was unsuccessful (data not shown). Modified replaceable gels (0.1 to 0.4 % (w/w) PEO in Beckman SDS 14-200 gel), showed increased viscosity and slightly better resolution with small MW standards but not sufficient resolution to significantly enhance SASP traces (data not shown). Longer channels (20 cm vs the traditional 10 cm) were also explored but did not sufficiently improve the resolution of the samples to merit the 4x increase in separation time.

Given the observed difficulties with both UV absorbance and the relatively poor molecular weight resolution, it is likely that the best separation method to employ in concert with IEF coupled with LIF detection will prove to be zone electrophoresis. This will be pursued should follow on funding be made available.

## 4. CONCLUSIONS

This work represents the results of a one year LDRD project aimed at determining the feasibility of using small acid soluble proteins for spore characterization using rapid microscale separation techniques. Overall, the project had modest success. An alternative protocol for extracting the SASPs that was both relatively rapid and amenable to the microscale separations and protein labeling steps was developed. It appeared to have comparable effectiveness when compared to the slower literature method.

While the extraction method optimization was successful, the microseparations were less so. Poor sensitivity of the capillary IEF using UV absorbance made the measurement of SASP pIs impossible. This was due to the inherent lack of UV chromophores in the primary sequences of the SASPs themselves. To compensate, chip based IEF employing fluorescence detection was initiated but time and budget constraints limited the progress. Only modest success was achieved in the small molecular weight separations. Each *B. subtilis* variant did produce a distinct CGE protein pattern but it is not clear how the observed patterns might correlate with the expected SASPs. It is possible that co-extracted proteins may have masked the patterns.

Given the observed difficulties with both UV absorbance and the relatively poor molecular weight resolution, it is likely that the best separation method will prove to be zone electrophoresis with laser induced fluorescence detection. This will be pursued should follow on funding be made available. This work represents a necessary starting point and although not wholly successful, did serve to eliminate some strategies and point the direction towards more successful approaches.

**Table 1: Amino Acid Sequences for Selected SASPs**

| Name | AMINO ACID SEQUENCE | Calculated average mass (Da) | Calculated pI | Origin |
|---|---|---|---|---|
| SASP-α | ANNNSGNSNNLLVPGAAQAIDQMKLEIASEFGVNLGADTTSRANGSVGGEITKRLVSFAQQNMGGGQF | 6,939.6 | 4.94 | *B. subtilis* |
| SASP-ß | ANQNSSNDLLVPGAAQAIDQMKLEIASEFGVNLGADTTSRANGSVGGEITKRLVSFAQQQMGGRVQ | 6,848.6 | 5.00 | *B. subtilis* |
| SASP-γ | ANSNNFSKTNAQQVRKQNQQSAAGQGQFGTEFASETNAQQVRKQNQQSAGQQGQFGTEFASETDAQQVRQQNQSAEQNKQQNS | 9,136.5 | 8.47 | *B. subtilis* |
| SASP- γ | SKKQQGYNKATSGASIQSTNASYGTEFSTETDVQAVKQANAQSEAKKAQASGAQSANASYGTEFATETDVHSVKKQNAKSAAKQSQSSSSNQ | 9,540.1 | 9.26 | *B. cereus B. thuringiensis* |
| SASP- γ | ANSNNKTNAQQVRKQNQQSASGQGQFGTEFASETNVQQVRKQNQQSAAGQGQFGTEFASETDAQQVRQQNQSAEQNKQQNS | 8,889.3 | 8.47 | *B. globigii* |

**Table 2: Descriptors for Individual *B. subtilis* Strains**

| Strain Name | DESCRIPTOR | Expected masses (Da) | Expected pI |
|---|---|---|---|
| SP260 | No SASP-α | 6,850<br>9,135 | 5.00<br>8.47 |
| SP355 | No SASP-α, No SASP-ß | 9,135 | 8.47 |
| SP533 | Wild type like<br>+ $SspC^{WT}$ under sspB promoter control | 6,940<br>6,850<br>7,627<br>9,135 | <br><br><br>8.47 |
| SP1450 | No SASP-α, No SASP-ß<br>+ High copy # SspC | 7,627*<br>9,135 | <br>8.47 |
| SP3019 | No SASP-α, No SASP-ß<br>+ High copy # $SspC^{\Delta 11D13K}$ | 6,355*<br>9,135 | <br>8.47 |

Figure 1: SASP Patterns Projected for a Number of *Bacillus* Species Showing the Potential for Spore Identification

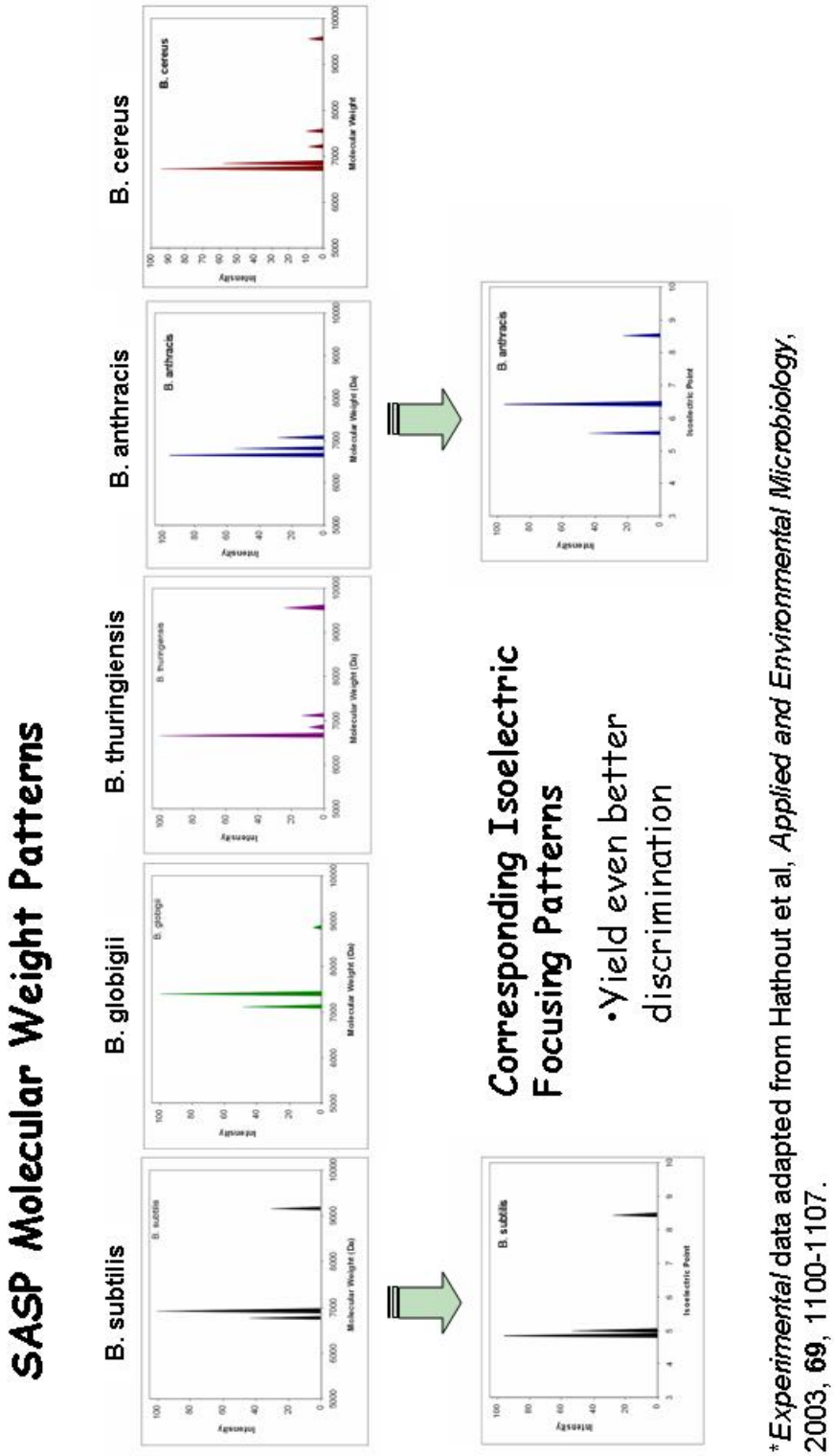


Figure 2: Modified Extraction Protocol of SASPs from *Bacillus subtilis*

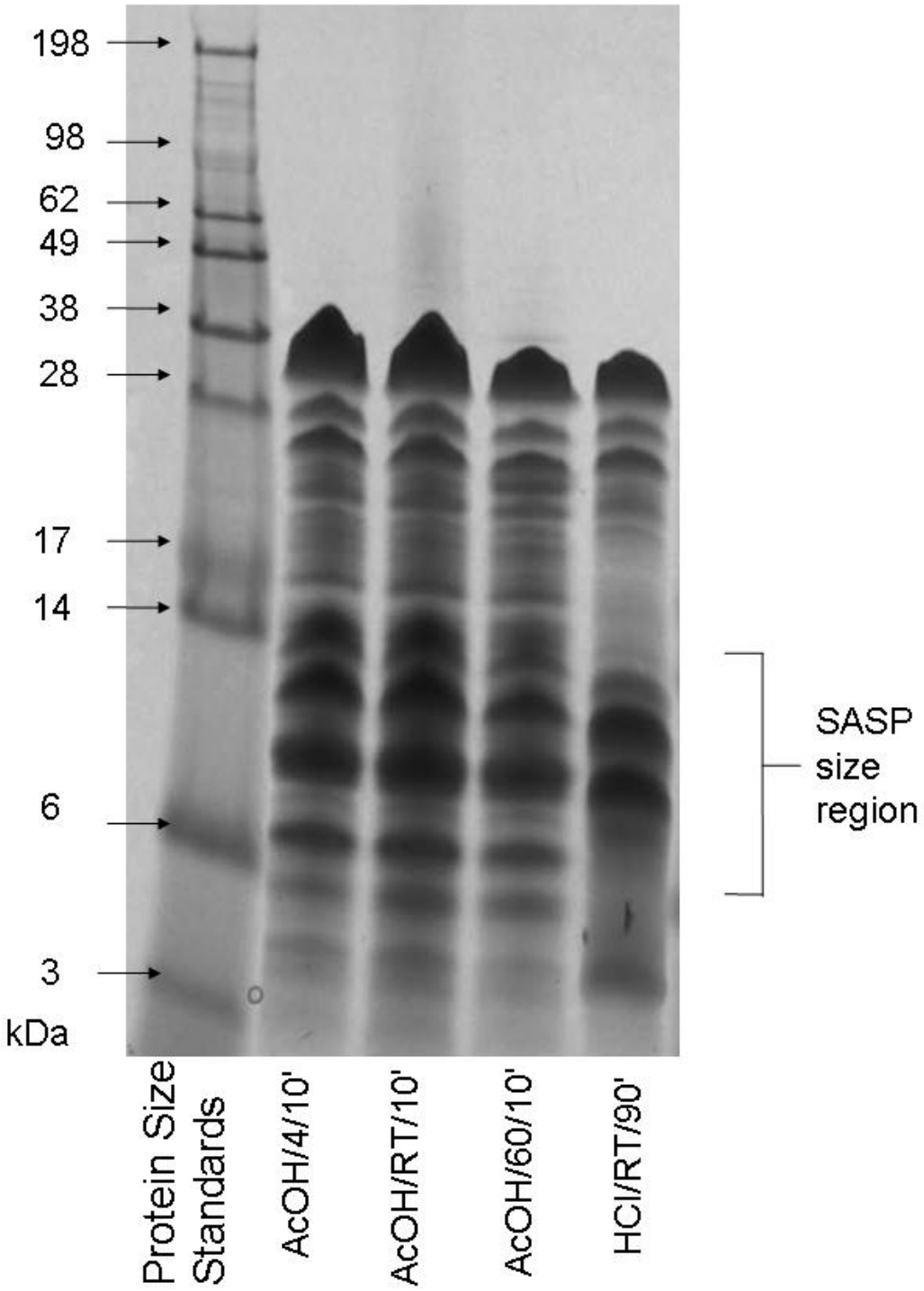


Figure 3: SASPs from *Bacillus subtilis* Variants Extracted at A) Room Temperature and B) 4$^{o}$C

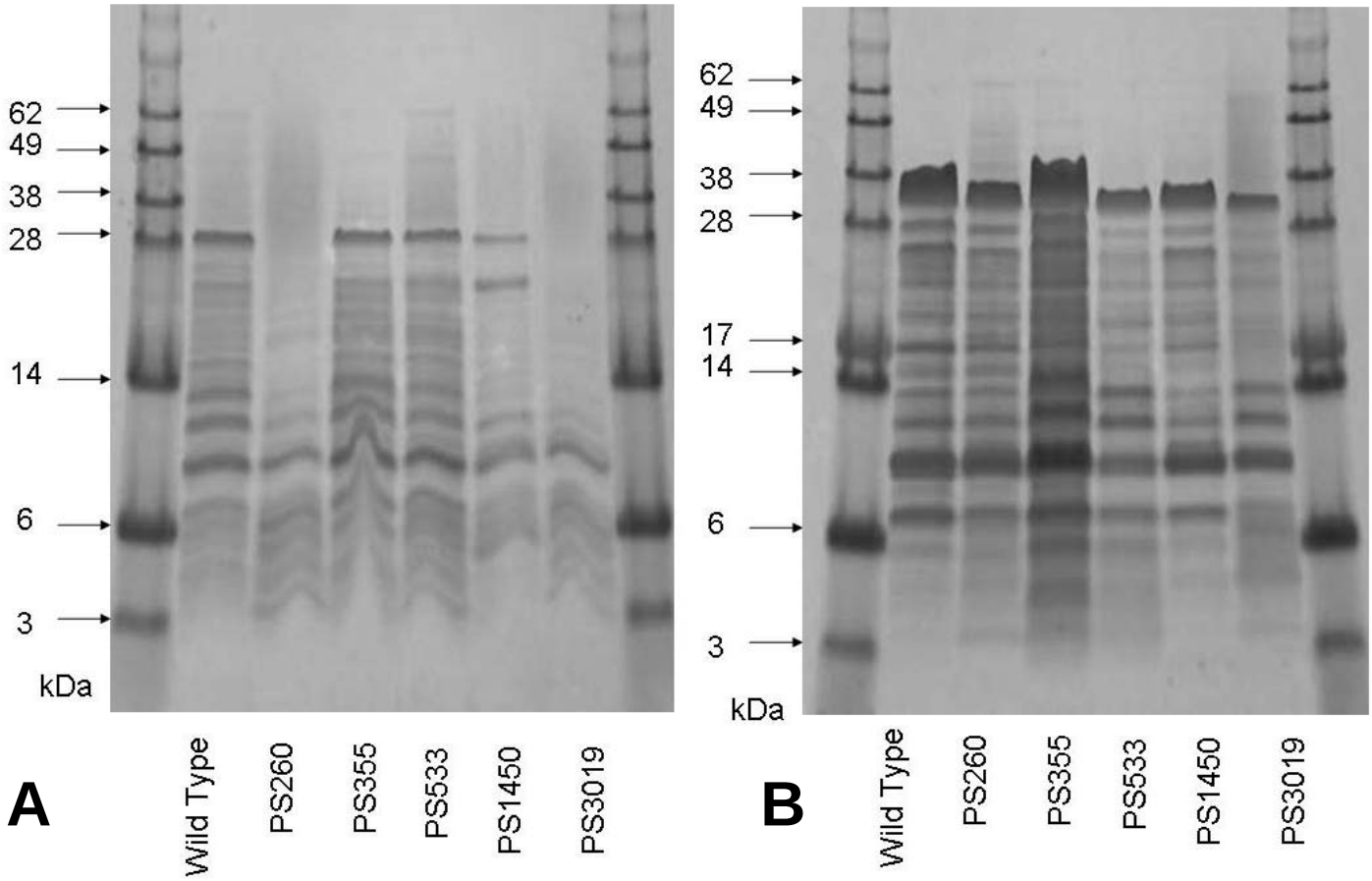
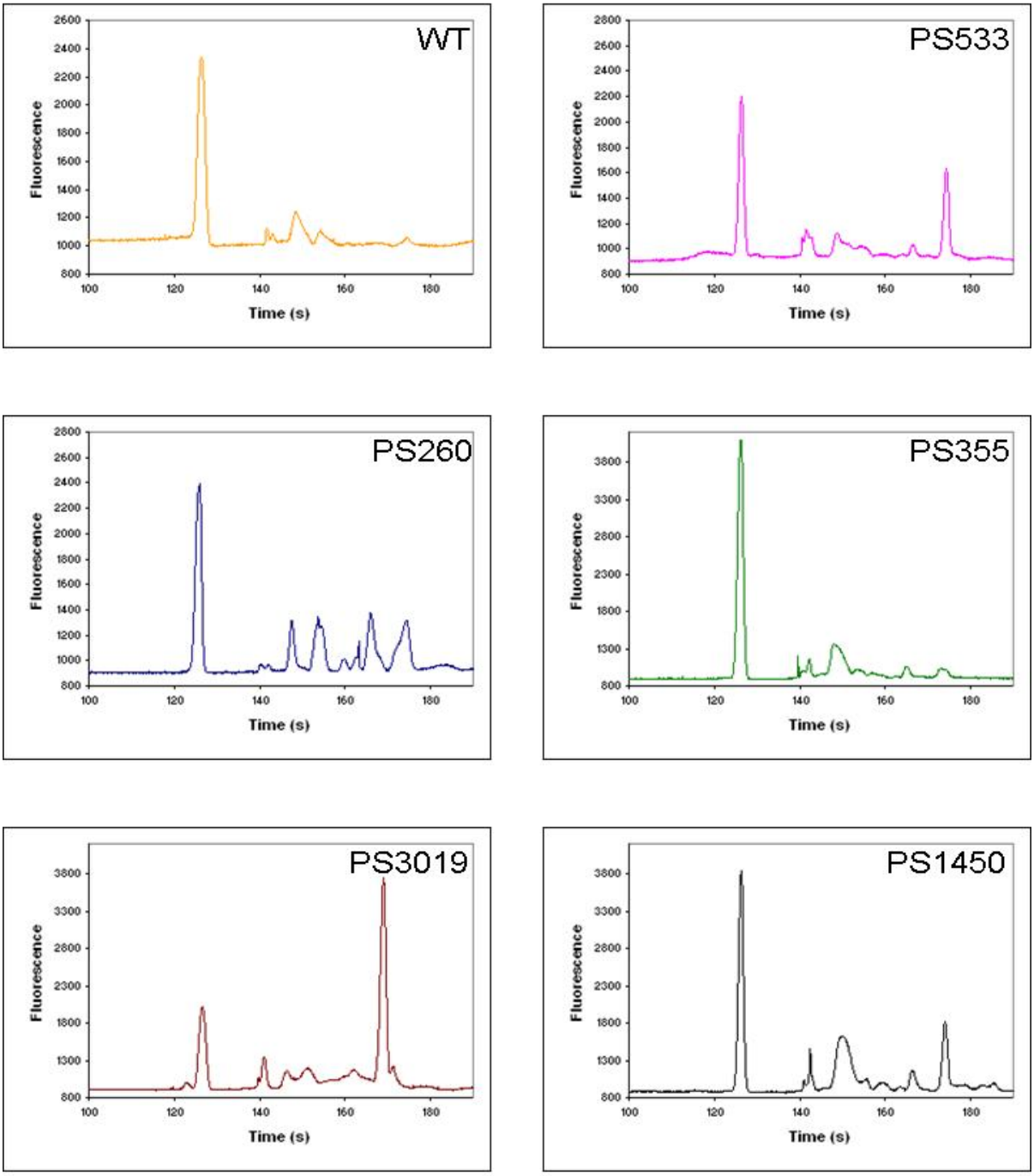


Figure 4: CGE Traces for SASPs extracted from Mutant *Bacillus subtilis* clones



## 5. REFERENCES


1. P. Setlow, Spores of *Bacillus subtilis*: their resistance to and killing by radiation, heat and chemicals, Journal of Applied Microbiology, 2006, 101, 514-525.
2. F. Tovar-Rojo and P. Setlow, Effects of Mutant Small, Acid-Soluble Spore Proteins from Bacillus subtilis on DNA In Vivo and In Vitro, Journal of Bacteriology, 1991, 173, 4827-4835.
3. D.L. Popham, S. Sengupta, P. Setlow, Heat, hydrogen peroxide, and UV resistance of Bacillus subtilis spores with increased core water content and with or without major DNA-binding proteins, (1995) *Applied and Environmental Microbiology*, **61**, 3633-3638.
4. Hathout, Y; Setlow, B; Cabrera-Martinez, RM; Fenselau, C; Setlow, P., Small, acid-soluble proteins as biomarkers in mass spectrometry analysis of Bacillus spores., *Applied and Environmental Microbiology*, 69, 1100-1107.
5. W. L. Nicholson, P. Setlow in C.R. Harwood, S. M. Cutting (Eds.) Molecular Biology Methods for *Bacillus*, John Wiley and Sons, Chichester, England, 1990, pp. 391-450.
6. V. A. VanderNoot, S. Branda, A. Jokerst, and T.W. Lane, Rapid Assessment of Spore Viability, Sandia Report, SAND #2005-7356, Printed December 2005.
7. Ronald F. Renzi, James Stamps, Brent A. Horn, Scott Ferko, Victoria A. VanderNoot, Jay A.A. West, Robert Crocker, Boyd Wiedenman, Daniel Yee and Julia A. Fruetel, Hand-Held Microanalytical Instrument for Chip-Based Electrophoretic Separations of Proteins, (2004) *Analytical Chemistry*, **77**, 435-441.
8. C. K. Mathews, K.E. Van Holde, *Biochemistry*, 2nd ed., The Benjamin/Cummings Publishing Company, Inc., 1996, pg. 136.
9. Lai, E., Phadke, N., Kachman, M., Giorno, R., Vazquez, S., Vazquez, J., Maddock, J., and Driks, A. (2003*) Journal of Bacteriology* 185, 1443-1454.
10. Chun-Ming Huang, K. Wade Foster, Tivanka S. DeSilva, Kent R. Van Kampen, Craig A. Elmets and De-chu C. Tang, Identification of *Bacillus anthracis* proteins associated with germination and early outgrowth by proteomic profiling of anthrax spores, *Proteomics* 2004, **4**, 2653–2661.

## 5. DISTRIBUTION

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