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On-Probe Digestion of Bacterial Proteins for MALDI-MS

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On-probe digestion combined with MALDI mass spectrometry is studied as a rapid method for the analysis and identification of bacterial proteins. The use of trypsin adsorbed to the probe surface reduces the digestion time from hours to minutes. A high amount of trypsin must be applied to the probe for the successful digestion of bacterial proteins. Mass spectra of the digest contain a number of low-mass digest fragments. Several components of a *B. subtilis* bacterial digest can be identified through postsource decay and database searching.

Much research in analytical chemistry has been directed toward the mass spectral analysis of microorganisms.[1−6] The speed of the method is one of its most attractive features. With quick analysis times and the ability to handle mixtures, mass spectrometry is naturally amendable to the analysis of complex biological samples, such as bacteria.[7,8]

Both matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI) have been successfully applied in studies of bacteria.[7−10] Each ionization method has its own particular advantages. On-line chromatography is easily adapted to ESI.[11,12] However, multiple charge states yield more complicated spectra for samples with many components. Since it produces primarily singly charged ions, MALDI spectra are simpler and therefore more easily interpretable. Although ions of up to 500 kDa have been detected in MALDI experiments on bacteria, most observed masses are less than ∼50 kDa.[13,14] While time-of-flight (TOF) mass spectrometry is amenable to both MALDI and ESI, only MALDI takes particular advantage of the extremely large mass/charge range that time of flight provides. In summary, the complexity of bacteria samples and the quickness, simplicity, and greater tolerance toward impurities of MALDI-TOF mass spectrometry have led to its being the most popular mass spectrometric method for analyzing bacteria.

The initial work on bacterial identification by MALDI-MS primarily focused on finding peaks that are characteristic of different species.[15−18] These peaks are often referred to as biomarkers. Analysis of different bacterial species and strains has demonstrated that MALDI can produce unique mass spectral fingerprints.[19] These can be informative, independent of whether the biomolecules corresponding to the peaks have been identified. Although mass spectral fingerprints and biomarkers have been successfully used in the identification of bacteria, results can be complicated by experimental conditions.[20−23] Bacterial mass spectra are affected by cell growth and extraction conditions and even by the different instruments and operators that recorded the data.[20,23]

Lately, some mass spectrometric studies have focused on identifying the proteins in bacterial lysates.[24−30] This typically involves cellular fractionation, digestion, mass spectrometric

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analysis, and peptide mass mapping. Although the separation of proteins in bacterial extracts requires time, it usually results in more informative experimental data. For example, Arnold and Reilly extracted ribosomes from *Escherichia coli* cells and identified 55 of the 56 possible ribosomal proteins by MALDI-MS.[25] Holland et al. isolated proteins from various bacterial extracts by HPLC and identified proteins based on their precursor ion masses and partial amino acid sequences obtained by Edman analysis.[26] Wall et al. separated proteins in a whole-cell lysate in 15 min with RP-HPLC.[27] After an 18-h digestion of the HPLC fractions, 33 components were identified from the molecular weights and tryptic peptide maps. Dai et al. performed HPLC on an *E. coli* extract, detecting over 300 components in the various fractions.[28] They performed 2-h digestions of several of the HPLC fractions and analyzed the digests by MALDI. Database searches identified two cold shock proteins and a DNA-binding protein. Several intense MALDI digestion peaks could not be identified. Another approach that has been tried involves separating the bacterial proteins by gel electrophoresis before digestion and analysis by MALDI-TOF.[29,31−36] Proteins were identified by comparing the digest peptide masses to genomic data. Larsson et al. used biotinylation to isolate low-abundance proteins.[30] They selectively tagged the proteins of interest before isolating them with streptavidin beads. Proteins extracted from the beads were separated by electrophoresis before digestion. Mass spectra of digests were interpreted through database searches.

A rapid enzyme digestion of bacterial proteins may provide additional fingerprinting information. Typically digestions are performed for at least several hours.[37,38] However recent research has been devoted to minimizing protein digestion times.[39−45] Good digestion has been obtained with enzymes adsorbed or covalently bound to surfaces.[46−49] Immobilized enzyme has the advantage of reducing autolysis product formation, allowing the use of higher trypsin concentrations that yield faster digestion times. Gobom et al. demonstrated rapid tryptic digestion of bovine serum albumin (BSA) in a Poros microcolumn.[39] Identification was confirmed through further digestion by adding V8 to the tryptic digest already deposited on the MALDI probe. Dogruel et al. used trypsin immobilized on MALDI probes to digest picomoles of protein in times under 30 min.[40,41] Goa et al. employed a miniaturized membrane reactor with trypsin adsorbed to a porous membrane.[42] With reaction times as low as 3 min, complete coverage was obtained for cytochrome *c* by ESI. Marie et al. demonstrated that myoglobin could be digested in 5 min with pepsin nonpermanently attached to PEEK tubes yielding 83% sequence coverage by ESI.[43] Wang et al. interfaced trypsin-immobilized beads on a microchip for 3−6-min digests of melittin and BSA.[44] Recently, Ericsson et. al demonstrated rapid digestion on a nanovial MALDI target with adsorbed trypsin.[45] While digestion was seen in the low-femtomole and even high-attomole range, this technique required deposition of the substrate by a piezoelectric flow-through microdispenser whereby samples were continuously deposited in 100-pL droplets. Evaporation during the sample dispensing allowed the protein concentration to increase.

In this paper, we discuss the rapid digestion of *Bacillus subtilis* proteins by trypsin adsorbed to a MALDI probe and the subsequent identification of the proteolytic fragments. Though not as fast as analysis of whole bacterial cells, it is almost as fast as direct MALDI analysis of bacterial lysates and it adds the fingerprinting selectivity of protein identification. PSD was performed on some of the digest fragments to obtain sequence information. Peptide ions and PSD fragments were used in database searches to identify bacterial proteins.

EXPERIMENTAL SECTION

Materials and Bacteria. α-Cyano-4-hydroxycinnamic acid was purchased from Aldrich (Milwaukee, WI). Trypsin was purchased from Sigma (St. Louis, MO). Samples of *B. subtilis* and *Bacillus thuringiensis* were obtained from colleagues at Indiana University. The bacteria were lysed with a BioNeb Nebulizer (Glas-Col, Terra Haute, IN). The bacterial lysates were then concentrated and frozen until analysis.

Bacteria Growth. Bacteria samples were grown overnight and harvested by centrifugation. The optical density of *B. subtilis* at 600 nm was measured to be 0.626 ± 0.004, corresponding to 0.626×10^9 cells/mL based on there being 10^9 cells/mL at an optical density of 1 and a path length of 1 cm.[50] The 30-mL solution of cells was then divided into six samples, washed with 10 mM NH_4HCO_3, and centrifuged (10 min at 5000 rpm) to form a pellet. The bacteria were lysed with a BioNeb Nebulizer with 200 psi N_2 as

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the carrier gas.[51] Samples were run through the nebulizer three times to ensure all cells were lysed. The lysates were then centrifuged for 25 min at 13 000 rpm. The supernatant was separated and vacuum-dried. It was stored in the freezer before further analysis. The lysates were vacuum-dried and stored at −20 C. Before analysis, they were diluted in 140 μL of 25 mM NH_4HCO_3, resulting in a concentration of 22 × 10^6 cells/μL.

Digestion. On-probe digestions were performed on a Bruker 26 spot stainless steel MALDI probe. Trypsin was initially adsorbed to the probe by depositing 1 μL of trypsin in water and allowing the spot to dry in air. Bacteria samples were suspended in 25 mM NH_4HCO_3, and 1 μL of the bacteria solution was added to the probe, which was then placed in a humidified enclosure at room temperature. After a desired length of time, the probe was removed from the enclosure and 0.5 μL of 1% TFA was added to stop the digestion process. As an alternative to a timed humidified digest, the reaction was allowed to proceed until the solution evaporated in air (7−8 min). After sample drying, 0.65 μL of 10 mg/mL α-cyano-4-hydroxycinnamic acid (in 2:1 ACN/0.1% TFA) was added on top of the digest and this was also air-dried. The probe was then inserted into the mass spectrometer for analysis. When smaller spot sizes were employed, the volume of reactants added to the probe was reduced by a factor of 5. In-solution digestions were performed with the same quantities of trypsin (0.5 μg) and bacteria (22 × 10^6 cells) as with on-probe digestion but were performed in 4 μL of 25 mM NH_4HCO_3.

MALDI Mass Spectrometry. All MALDI spectra were obtained using a Bruker Reflex III time-of-flight mass spectrometer (Billerica, MA) with delayed extraction in positive ion mode. Spectra were obtained by irradiating the spot with a high laser power for 10 laser shots before reducing the power and acquiring 100 shots. Trypsin autolysis peaks were used as internal calibrants. Some ion fragmentation data were recorded through postsource decay (PSD) experiments, where parent ions were isolated to a 10-Da window with an ion gate.

Database Search. Database searches were performed based on peptide and PSD ion fragment masses. Digest searches were conducted in the SwissProt database using PROWL Pepfrag and limiting the organism to *B. subtilis*.[52] PSD peaks were selected visually based on peak intensities being twice the noise level of higher. In spectra where many PSD fragments were observed, the most well-resolved and intense peaks were used for database searching. Precursor ion and PSD fragment mass errors were set at 0.05 and 1 Da, respectively. One missed cleavage was allowed. Fragment matches were limited to a-, b-, and y-ion types.

Safety. TFA is corrosive and causes burns. Suitable caution should be excercised when highly concentrated TFA is used. Appropriate care should also be exercised when working with biological organisms.

RESULTS AND DISCUSSION

Most solution-phase digestions are performed over a period of hours, since autolysis limits the amount of enzyme that may be used and still result in efficient analyte digestion.[53] When the enzyme is immobilized, a larger ratio of enzyme to analyte can be used since the enzyme lacks mobility and autolysis is prevented.[54] However, immobilizing an enzyme requires time or the purchase of a commercially available immobilized product. In this work, trypsin is first applied to a stainless steel probe and allowed to dry. Later the analyte is added. Although some of the trypsin may redissolve when the analyte is added, that which remains adsorbed at the probe surface has limited mobility. Although autolysis is not eliminated, when 1 μg of trypsin was used, it could be reduced by up to half when compared to digestions where trypsin is in solution (for experiments both on-probe and in an Eppendorf tube). This allowed a greater amount of enzyme to be used with on-probe digestion and still observe the same amount of autolysis that could be seen with solution experiments. However, the major advantage of on-probe digestion is that is allows the use of 1-μL volumes, whereas evaporation/condensation of a solution that small would present problems in an Eppendorf tube. To demonstrate this, experiments were performed to compare on-probe digestion with liquid-phase digestions in 600-μL Eppendorf tubes. Digestion time was maintained at 10 min for both media. A reaction of 1 μg of trypsin and 22 × 10^6 lysed cells took place in 4-μL solution in the 600-μL tube. A 1-μL sample of in-solution digest was added to the probe, acidified, and allowed to dry. Matrix was then added to the spot. For the on-probe digestion, 1 μg of trypsin was applied to the probe and allowed to dry. Then 1 μL of the bacteria lysate in buffer was added. Following the reaction period, the solution was acidified and allowed to dry. Matrix was then added to the spot. Figure 1 compares the digestion of *B. subtilis* lysate in the two cases. The

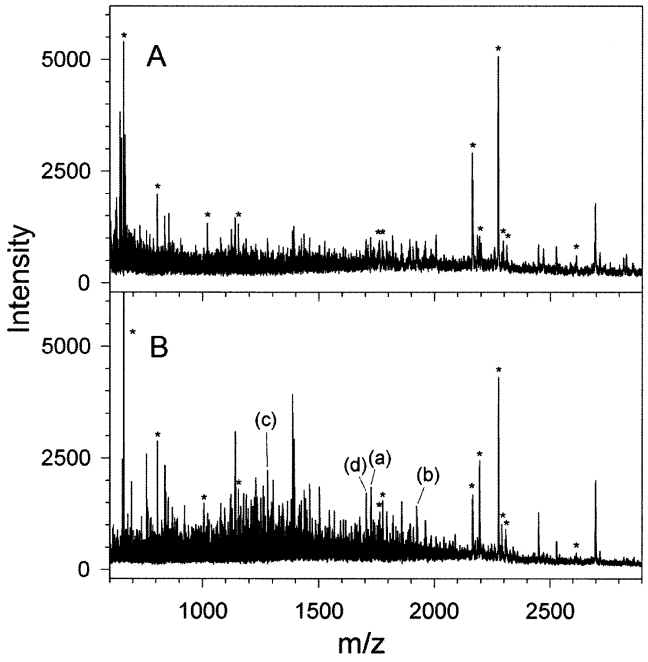

Figure 1. MALDI mass spectra obtained following 10-min digestions of 22 × 10^6 *B. subtilis* cells using (A) 1 μg of trypsin in a 4-μL solution reaction volume and (B) 1 μg of trypsin dried on the probe before deposition of the bacteria. Trypsin autolysis peaks are marked with an *. Peaks labeled a−d were selected for PSD fragmentation experiments.

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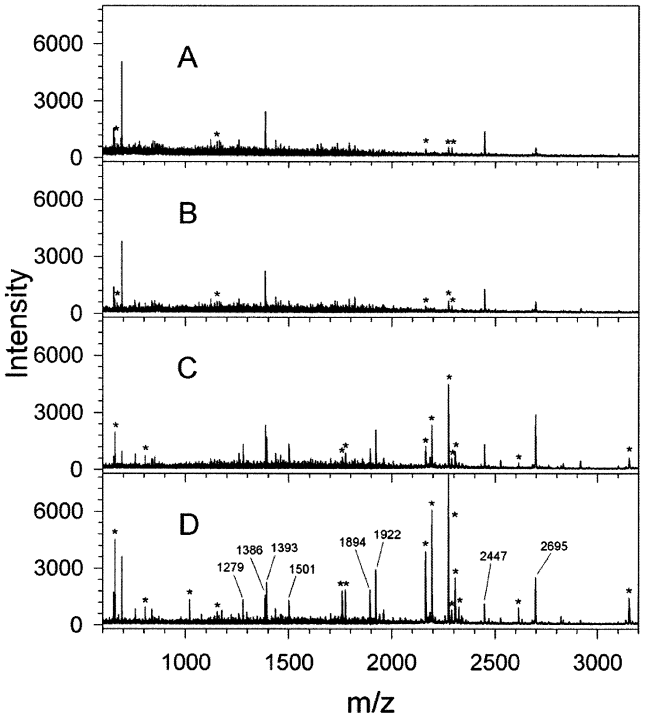

Figure 2. MALDI spectra of 10-min on-probe digestions of *B. subtilis* with (A) 0.05, (B) 0.1, (C) 0.5, and (D) 1 μg of trypsin applied to the probe. Trypsin autolysis peaks are marked with an *.

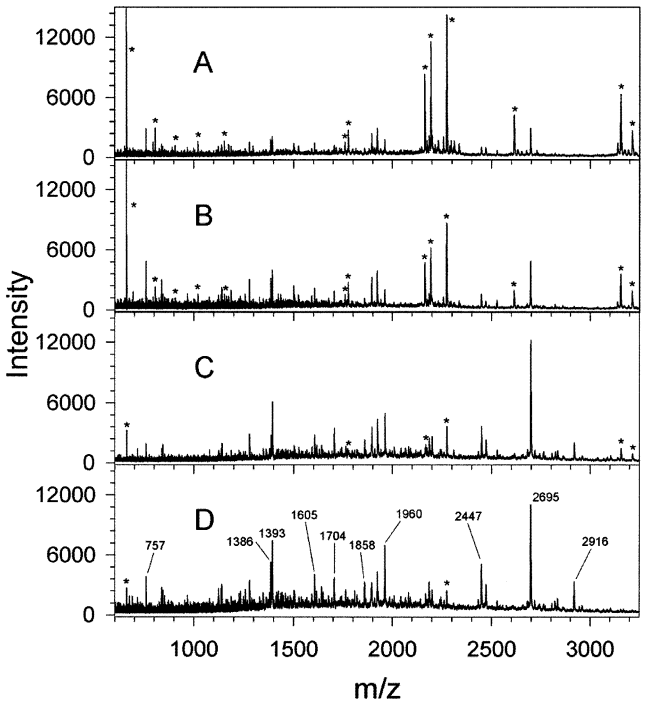

Figure 3. MALDI spectra of 10-min on-probe digestions of *B. subtilis* using 1 μg of trypsin applied to the probe: (A) 3×10^6, (B) 7×10^6, (C) 22×10^6, and (D) 44×10^6 cells in the bacterial lysate. Trypsin autolysis peaks are marked with an *.

on-probe digestion occurred quickly, with many mass spectral peaks visible after 10 min of reaction. The solution-phase digestion also occurred quickly although less signal was visible than with on-probe digestion because the solution-phase sample was diluted in the Eppendorf tube. It was desired to perform the solution-phase digestion in as small a volume as possible. Nevertheless, because evaporation reduced the volume somewhat, digestions were initiated in 4 μL of solution. Masses of common tryptic digestion products of *B. subtilis* are displayed in Supporting Information, Table 1.

Experimental conditions were optimized to maximize the efficiency of on-probe digestion. First, the amount of trypsin necessary for obtaining digestion products was determined. The sample size was set as 22×10^6 cells in the lysate. Figure 2 demonstrates the effect of varying the amount of trypsin deposited on the surface from 0.05 to 1 μg. High amounts of trypsin resulted in intense autolysis products dominating the spectra, while low amounts of trypsin resulted in insufficient digestion of bacterial proteins. Although relatively high amounts of trypsin can be used in on-probe digestion, as the amount applied to the surface is increased beyond monolayer coverage, it is likely that some trypsin may become mobile and induce autolysis. Although a high amount of autolysis is undesirable, the occurrence of some is acceptable since the peaks can be used as internal mass calibrants. Assuming a 4-nm diameter for a trypsin molecule,[55] 0.01 μg of trypsin amounts to about one monolayer of coverage on a 2-mm-diameter MALDI spot. However, in our experiments, the best spectra of cell digests were obtained using substantially more than this amount of trypsin. It is possible that some trypsin must enter the solution for optimal digestion. Alternatively, upper layers of adsorbed trypsin may be more enzymatically active. In some conditions, substantial autolysis is seen. One method of reducing autolysis is through the use of trypsin whose lysines have been methylated in order to reduce autolysis.[56] Experiments involving modified trypsin for on-probe digestion are currently underway.

The amount of bacteria on the probe was varied to determine the number of cells necessary to obtain intense digestion peaks. In Figure 3, the amount of *B. subtilis* applied to the probe was varied from 3×10^6 to 44×10^6 cells. The intensity of the digestion peaks increased as the number of cells applied to the probe increased. The digestion of the bacteria occurs quickly enough that, even with a higher cell concentration, the same digestion peaks are seen. These peaks are not visible when *B. subtilis* is applied to a spot without trypsin.

On-probe digestions times were also varied. Figure 4 displays spectra from 1- to 10-min digestions of *B. subtilis*. Although some peaks were seen in 1 min, they grew in number and intensity in the 5-min digest. After 10 min, spectral changes were relatively modest. No major increase in signal was seen with digestion times as long as 30 min, as spectra quality was similar to the 5- and 10-min digests.

Another parameter that can affect the degree of cellular protein digestion is temperature. The standard temperature for tryptic digestions is 37 °C. These bacterial digestions were performed at room temperature for simplicity. While on-probe digest experiments at higher temperatures demonstrated an increase in digestion, some difficulties arise when rapid digestions are performed. Higher temperatures increase the evaporation rate, resulting in changes in the reaction volume when the probe is

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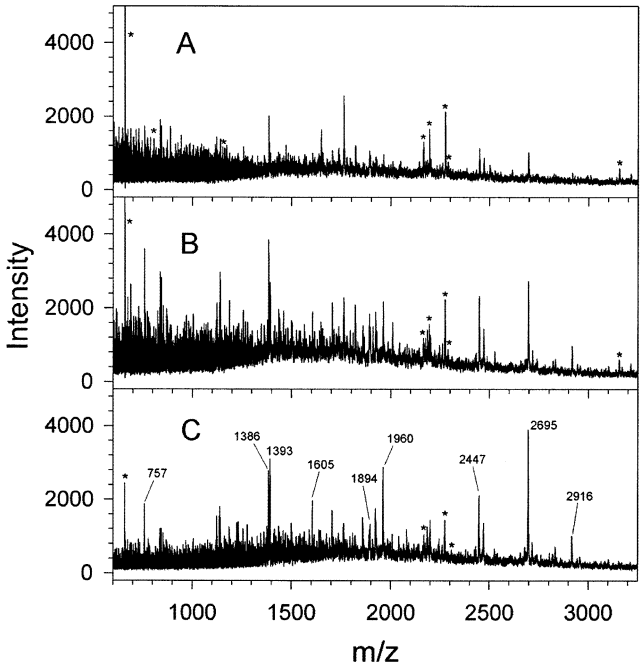

Figure 4. MALDI spectra of an on-probe digestion of 22×10^6 cells of *B. subtilis* with 1 μg of trypsin applied to the probe: (A) 1-, (B) 5-, and (C) 10-min digestion time. Trypsin autolysis peaks are marked with an *.

not humidified during sample deposition and when the process is stopped. Significant evaporation can occur while depositing samples and stopping the reaction, particularly when a number of sample spots are being studied. Since the increase in digestion at higher temperatures was small, reactions were run at room temperature. Digestion at room temperature is also more compatible with automation since heating or humidification control is not available in many automatic sample preparation systems.

Standard conditions for tryptic digestion of *B. subtilis* were chosen to be 0.5 μg of trypsin on the probe, 22×10^6 cells in the lysate, and a 10-min reaction time. The latter was chosen over a 5-min digest to improve reaction completeness. The cell number and trypsin amount were chosen to yield high digest peak intensities. Fewer cells could be used if necessary, although the signal-to-noise ratio falls. The 0.5 μg of trypsin needed for on-probe digestion of our bacteria proteins is greater than the 0.01 μg of trypsin needed for on-probe digestion of 0.5 μg of a standard biomolecule like hemoglobin. This is presumably due to the complexity of the bacteria sample, only a fraction of which corresponds to each bacterial protein. Assuming 155 fg of protein/cell, 22×10^6 cells corresponds to 3.4 μg of total protein.[57] For 0.5 μg of a bacterial protein to be present in the sample, the bacteria must contain 15% of that protein by weight, an unreasonably high amount.[58] Even if a bacterial protein was present in the lysate at 2 wt %, that would correspond to the very low picomoles to high femtomoles. Assuming the bacterial proteins have an average molecular mass of 10 000 Da, the 3.4 μg applied to the probe would be equivalent to 340 pmol of proteins. With the 0.5 μg of trypsin (21 pmol) on the probe, the resulting substrate-to-enzyme molecular ratio is 16:1. The amount of trypsin necessary to obtain a rather complete digestion has been well documented.[59,60] While solution-phase digestions are often performed at substrate-to-enzyme ratios of 50:1−100:1, enzyme kinetics are slower at low substrate concentrations. Therefore, a relatively large amount of trypsin is needed to ensure the maximum amount of digestion will occur in a short amount of time.

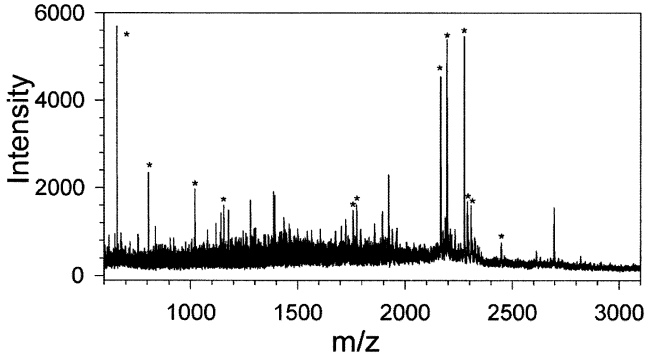

Figure 5. MALDI spectra of a 10-min on-probe digestion of *B. subtilis* in a reduced volume. The amount of trypsin on the probe was reduced to 0.2 μL of 1 μg/μL of solution and the bacterial lysate volume was reduced to 0.2 μL of 22×10^6 cells/μL of solution. Trypsin autolysis peaks are marked with an *.

In an attempt to reduce the amount of sample needed, an on-probe digestion was performed in a smaller lysate volume. Only 0.2 μg (at 1 μg/μL) of trypsin was applied to the probe along with the lysate from 4×10^6 cells. As seen in Figure 5, the bacteria digested although less intense peaks were obtained in comparison to typical on-probe digestion volumes as seen in Figure 1b. Although a normal probe spot is 2 mm in diameter, only a fraction of this is used for analysis. Reducing the sample volume from 1 to 0.2 μL provided reasonably good digestion although the reduced intensity was believed to result from spreading of the sample spot on the probe. Another way to improve sensitivity could be through the use of anchor chips.[61]

With an established protocol for rapid on-probe digestion, efforts were directed toward identifying the proteins that contributed to our mass spectra. Searching the *B. subtilis* database with just the mass of one digestion fragment at a mass accuracy of 0.05 Da resulted in up to 50 possible protein matches. To narrow down this number, more information is required. Some sequence information was obtained through PSD experiments. De novo sequencing is not typically achieved by PSD without chemical derivatization since too many ion types are produced.[62] In our experiments, candidate ions for PSD analysis were selected based on their intensities and separations from nearby peaks. With our ion gate, peaks to be isolated needed to be at least 5 Da apart. PSD fragment ion spectra were acquired by scanning the reflector over 14 stages and pasting the segments together. For database searches, the mass error of the fragment ions was set at 1 Da, while that of the precursor was 0.05 Da. Four examples of tryptic peptide PSD spectra that resulted in good database matches to

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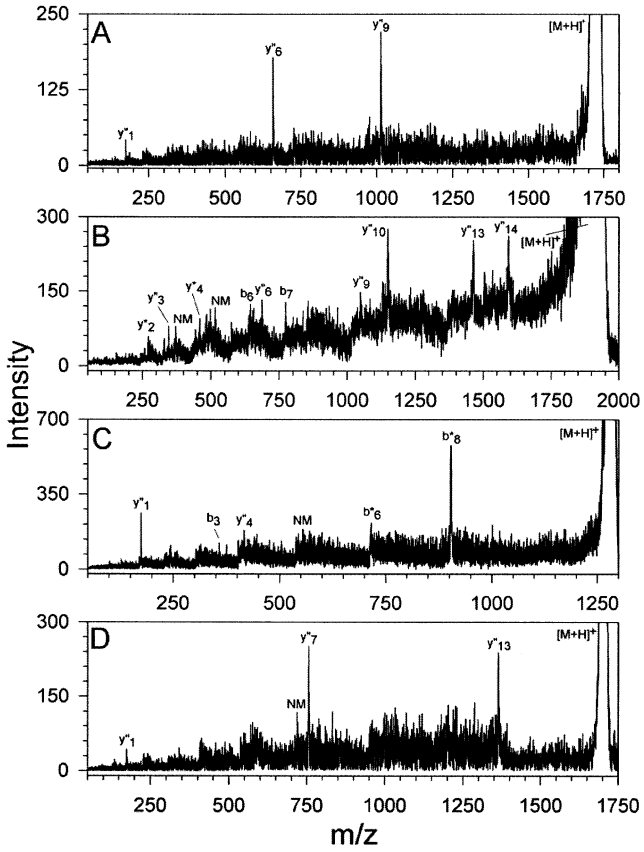

Figure 6. MALDI postsource decay spectra for (A) 1723.75, (B) 1921.91, (C) 1278.63, and (D) 1703.89 *m*/*z* on-probe digestion products of *B. subtilis*. Assigned peaks were identified through database searches. NM, no match.

B. subtilis proteins are displayed in Figure 6 and labeled in Figure 1b.

The quality of PSD spectra varied considerably from one tryptic digest fragment to another. For example, the peptide ion at 1723.75 *m*/*z*, labeled a in Figure 1b, yielded only three interpretable fragments. The postsource decay mass spectrum of this peak is seen in Figure 6a. Without PSD data, 13 possible proteins matched this tryptic peptide mass. Database searching identified only one protein matching the mass of the peptide and at least two of its fragments. The digestion fragment (GTAMAYDQIDGAPEER) from this particular protein, elongation factor tu (EF-TU), matched all three of the fragments, y''_1, y''_6, and y''_9. (The $''$ signifies the addition of two hydrogens to the C-terminal side of the peptide after cleavage of the peptide amide bond.[63]) The production of y-ion fragments is consistent with the C-terminal residue of this peptide being arginine. Masses of PSD fragments used in database searching are listed in Supporting Information, Table 2.

Other tryptic peptides provided more PSD fragments. As seen in Figure 6b, peak b (1921.91 *m*/*z*) in Figure 1b resulted in 12 apparent fragments. Although 23 possible proteins matched the peptide mass to within 0.05 Da, only one protein contained a peptide that matched at least 5 of its PSD fragments. In fact, this tryptic digestion fragment (SLEEGQEVSFEIVEGNR) from cold shock protein CSPD matched 10 of the 12 fragments. Of the 10 matching fragments, 8 corresponded to y-ions.

PSD of peak c (1278.63 *m*/*z*) in Figure 1b yielded six fragments, as shown in Figure 6c. Database searching yielded only one protein matching more than two of the fragments. This protein, spore coat polysaccharide biosynthesis protein SPSJ, matched five of the six fragments. The sequence of the digestion fragment that resulted from database searching was QEVTFEEGIAR. As shown in Figure 6d, PSD of peak d (1703.89 *m*/*z*) in Figure 1b resulted in four major fragments. Database searching resulted in one protein matching three of the four fragments. This protein, EF-TU, was the same protein identified from the Figure 6a spectrum. The corresponding sequence of the digestion fragment was LLDYAEAGDNIGALLR.

PSD was performed on 12 different tryptic peptides and 8 resulted in good assignments from database matching. At higher precursor masses (>2500 *m*/*z*), lower mass accuracy resulted in less definitive search results and in some cases no high-quality matches, even after the number of missed cleavages was increased to allow for incomplete digestion. However, fragments were usually seen in the PSD spectra and the lack of matches could arise from errors in the *B. subtilis* proteome, posttranslational modifications that were not accounted for in searching, or sample impurities. Since the bacterial proteins were not reduced and alkylated before their tryptic digestion, the existence of disulfide bonds could also lead to unpredicted peptide masses.

To help confirm the identities of bacterial proteins, an overnight digestion of the bacterial lysate was derivatized by guanidination. This is a simple 5-min reaction that efficiently modifies lysines to homoarginines resulting in mass shifts of 42 Da for each lysine modified.[64−66] The four proteolytic fragments that were analyzed by postsource decay as discussed above did not shift in mass, confirming that the peptides were arginine-terminated. A preference for the appearance of arginine-containing peptides in a MALDI spectrum of tryptic digest is expected since arginine residues are more basic than lysine and this enhances their rate of protonation.[67] In fact, most of the bacterial digest peaks did not shift in the guanidination experiment. However, the reaction was observed to be complete in these experiments since all lysine-containing autolysis fragments were fully modified. Under reaction conditions used in these experiments, guanidination has previously been found to be complete.[66]

B. subtilis was chosen as the principal bacterial subject for this work since its genome has been sequenced. To demonstrate that this method is applicable to fingerprinting, *B. thuringiensis* was also digested. Figure 7 displays the results of a 10-min on-probe digestion. Different tryptic digestion peaks are seen with *B. thuringiensis* than with *B. subtilis*. However, database searching on *B. thuringiensis* was not possible since its genome has not been sequenced.

These experiments were performed by lysing the bacterial cells, which took about 20 min. On-probe digestion was also performed on whole bacterial cells. While digestion products were still obtained, peak intensities were generally much smaller than

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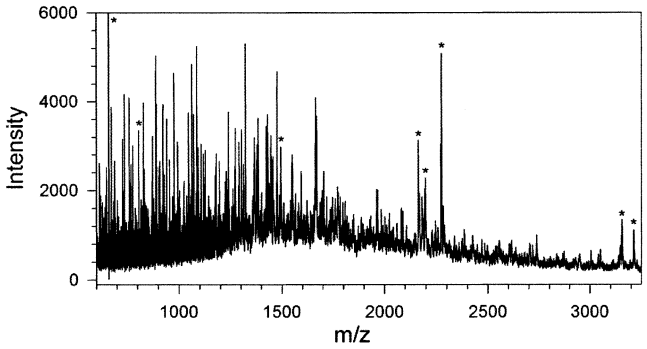

Figure 7. MALDI spectra of a 10-min on-probe digestion of 22 × 10^6 cells of *B. Thuringiensis* with 1 μg of trypsin. Trypsin autolysis peaks are marked with an *.

with lysed cells and database searching with MS/MS results did not yield any definite protein matches.

An important advantage of using tryptic peptides rather than bacterial proteins for cellular fingerprinting is the better accuracy with which their masses can be measured. The mass error on many of the larger masses in bacterial fingerprints is typically 1 Da or greater. However, digestion products below 3 kDa can easily be measured to 0.05 Da. Autolysis products also provide internal calibrants that improve mass accuracy without having to add proteins to a sample. Multiple commercial instruments are currently available that can provide better mass accuracy. For example, a Q-TOF mass spectrometer can provide mass accuracies of 5 ppm or better.[68,69] A TOF/TOF mass spectrometer could also improve fragmentation data with excellent mass accuracy and high throughput.[70] This would enable rapid acquisition of fragmentation data on digest peaks. These new developments could enhance automatic acquisition and identification of bacteria.

In summary, PSD of several of the bacterial digestion fragments produced successful identifications. All identified proteins are known to exist in bacteria in high abundance.[58] For example, elongation factor tu has been found to be one of the most abundant proteins in *E. coli*, accounting for up to 6% of the bacterial cell's proteins.[58] While rapid digestion of unfractionated bacterial lysates will probably not be able to provide identification about low copy number proteins, useful fingerprint and sequence information may be obtained for more abundant proteins.

CONCLUSION

Rapid on-probe digestion provides a quick and simple way to obtain information about bacteria. Database searches of PSD data yield information that enable high-abundance bacterial proteins to be identified.

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SUPPORTING INFORMATION AVAILABLE

Tables containing the masses of common *B. subtilis* tryptic digestion peak and the PSD fragment peak matches. This information is available free of charge via the Internet at http://pubs.acs.org.

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