Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Immediate and Sensitive Detection of Sporulated *Bacillus subtilis* by Microwave Release and Tandem Mass Spectrometry of Dipicolinic Acid

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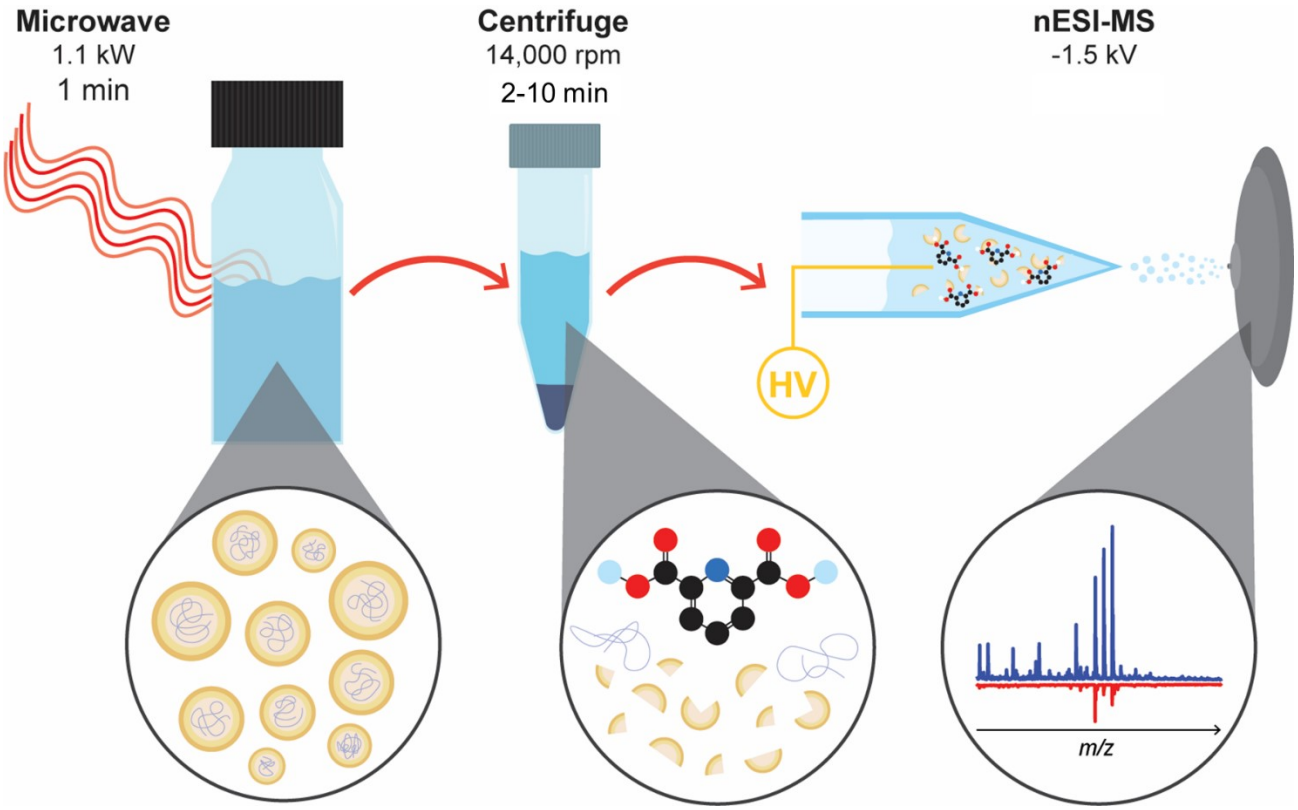

Figure S1. Scheme describing the experiment in which spores are lysed for 1 min with 1.1 kW of microwave irradiation and subsequently analyzed by nESI-MS in the negative ion mode.

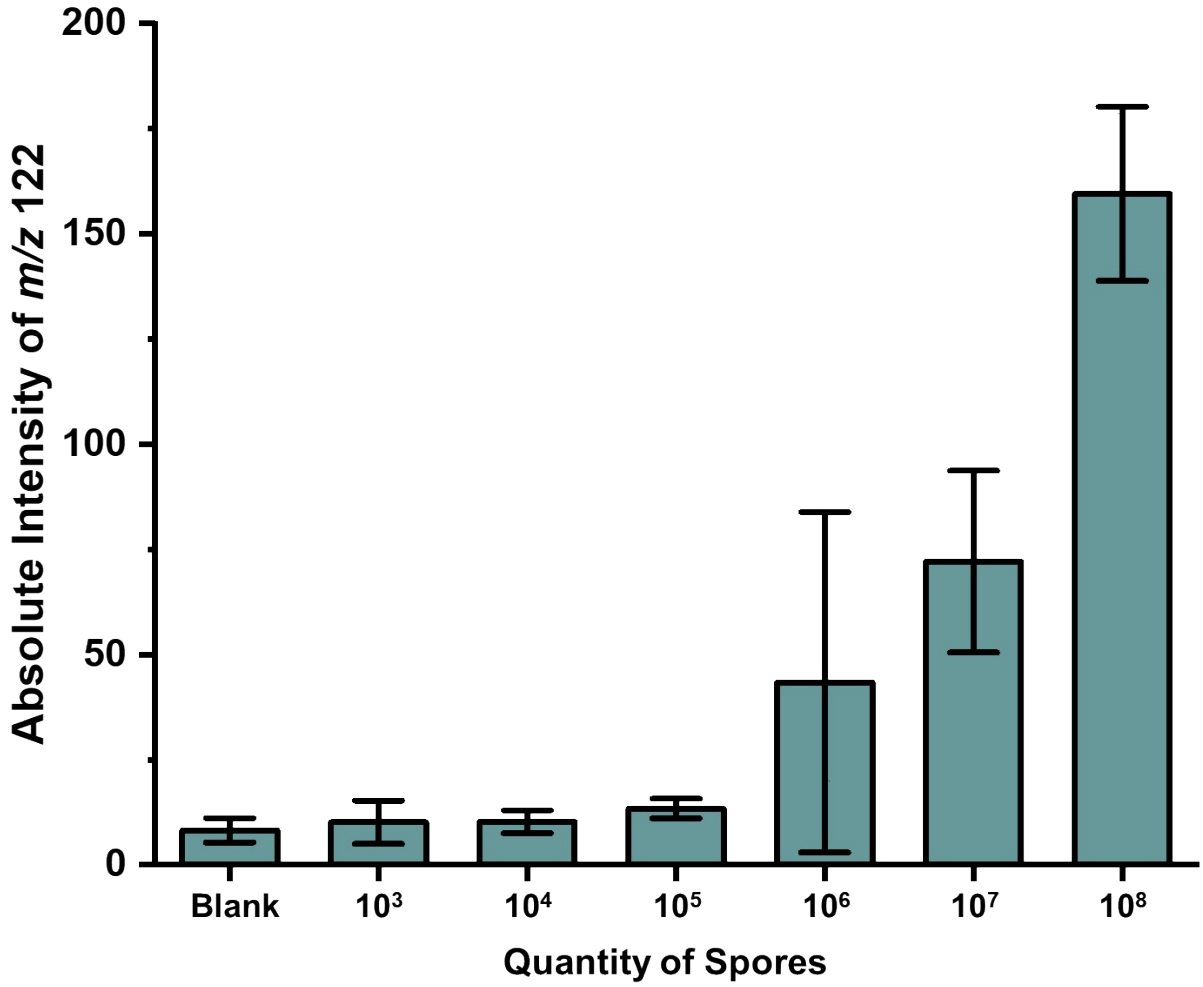

Figure S2. Bar graph of absolute intensity (arbitrary units) of the product ion *m/z* 122 generated by MS/MS of *m/z* 166 from the supernatant of irradiated *Bacillus subtilis* spores.

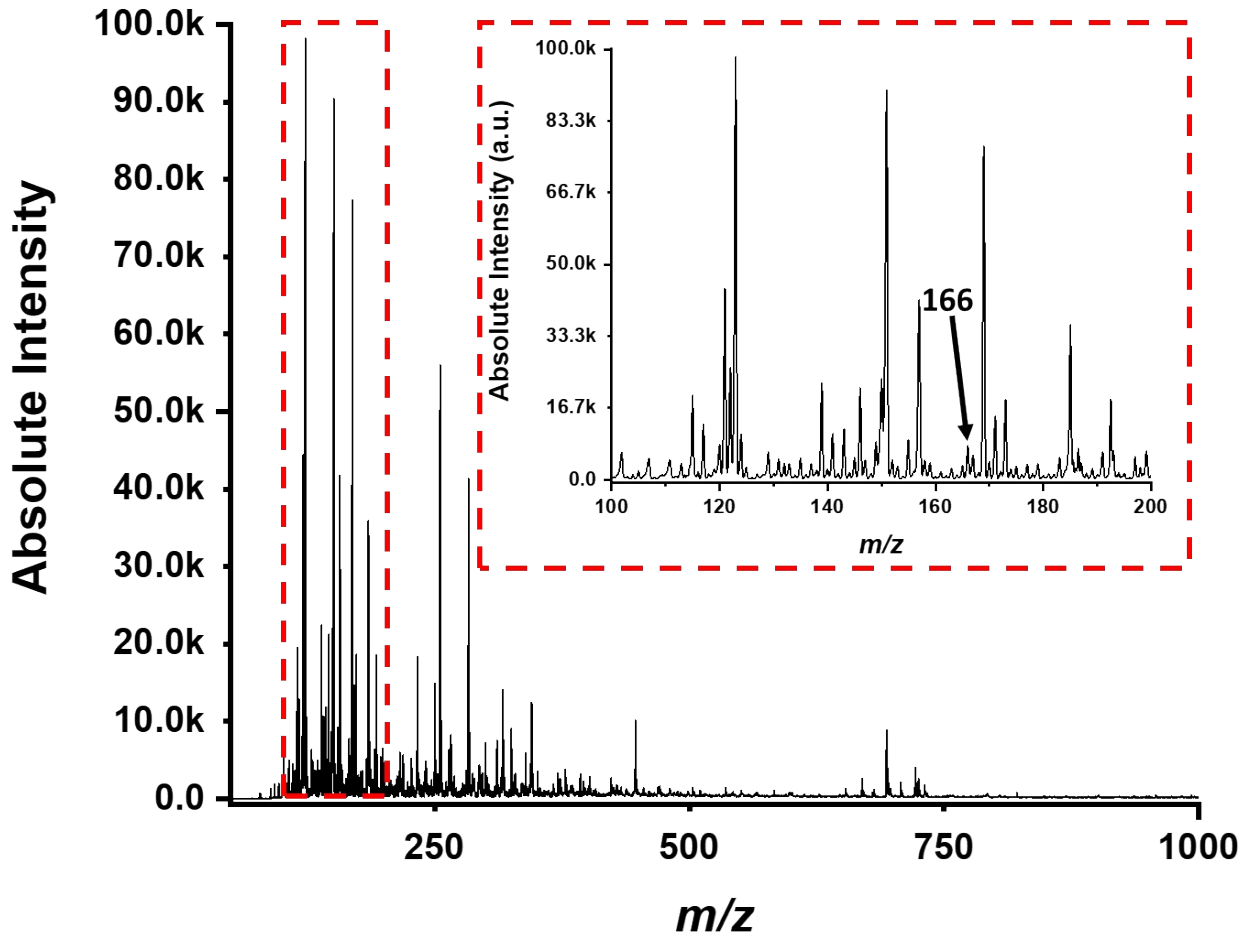

Figure S3. Mass spectrum of supernatant after irradiation of 2.2×10^8 *Bacillus subtilis* spores, recorded in the negative ion mode. The inset shows mass range *m/z* 100 - 200.

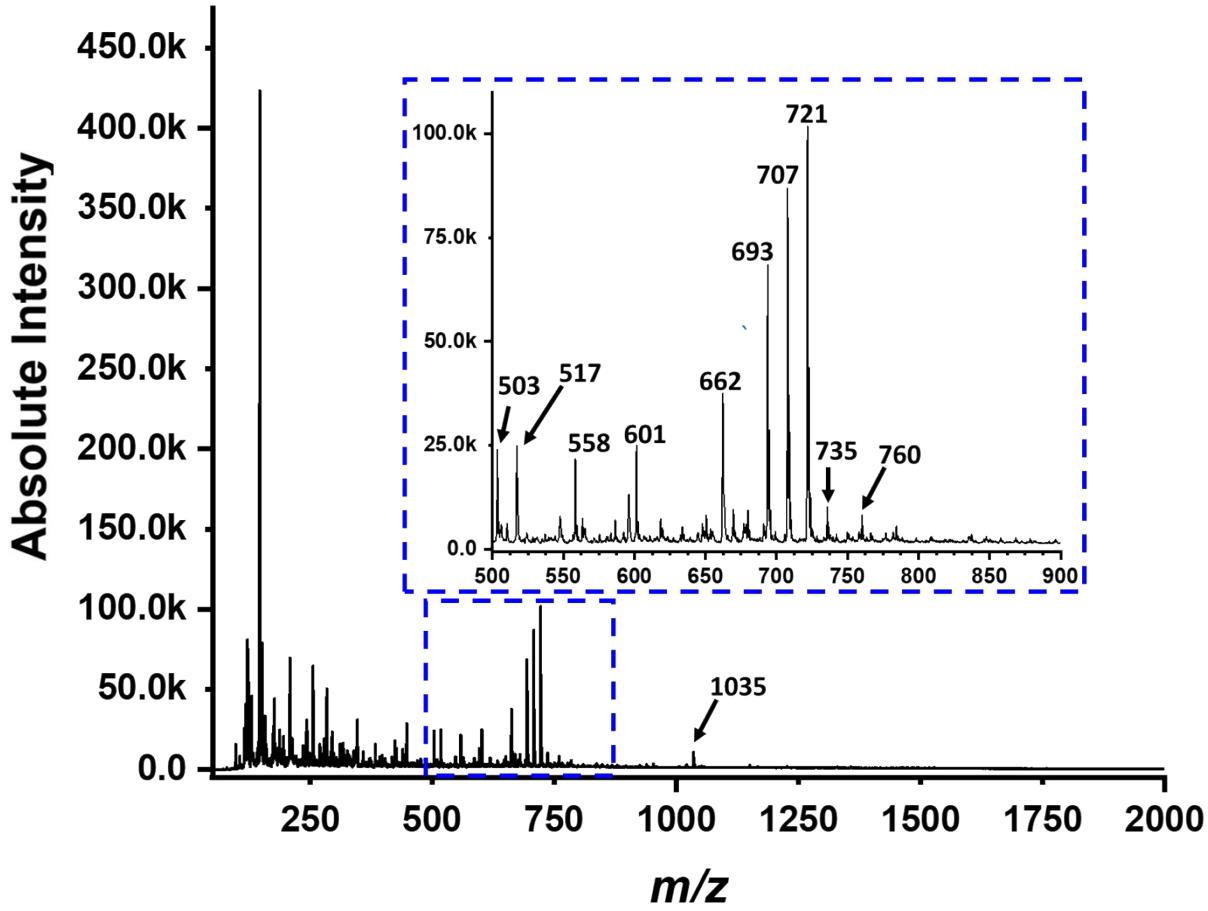

Figure S4. Mass spectrum of microwaved supernatant of *Bacillus subtilis* (5.0×10^8 spores) in the negative ion mode acquired using nESI-MS.

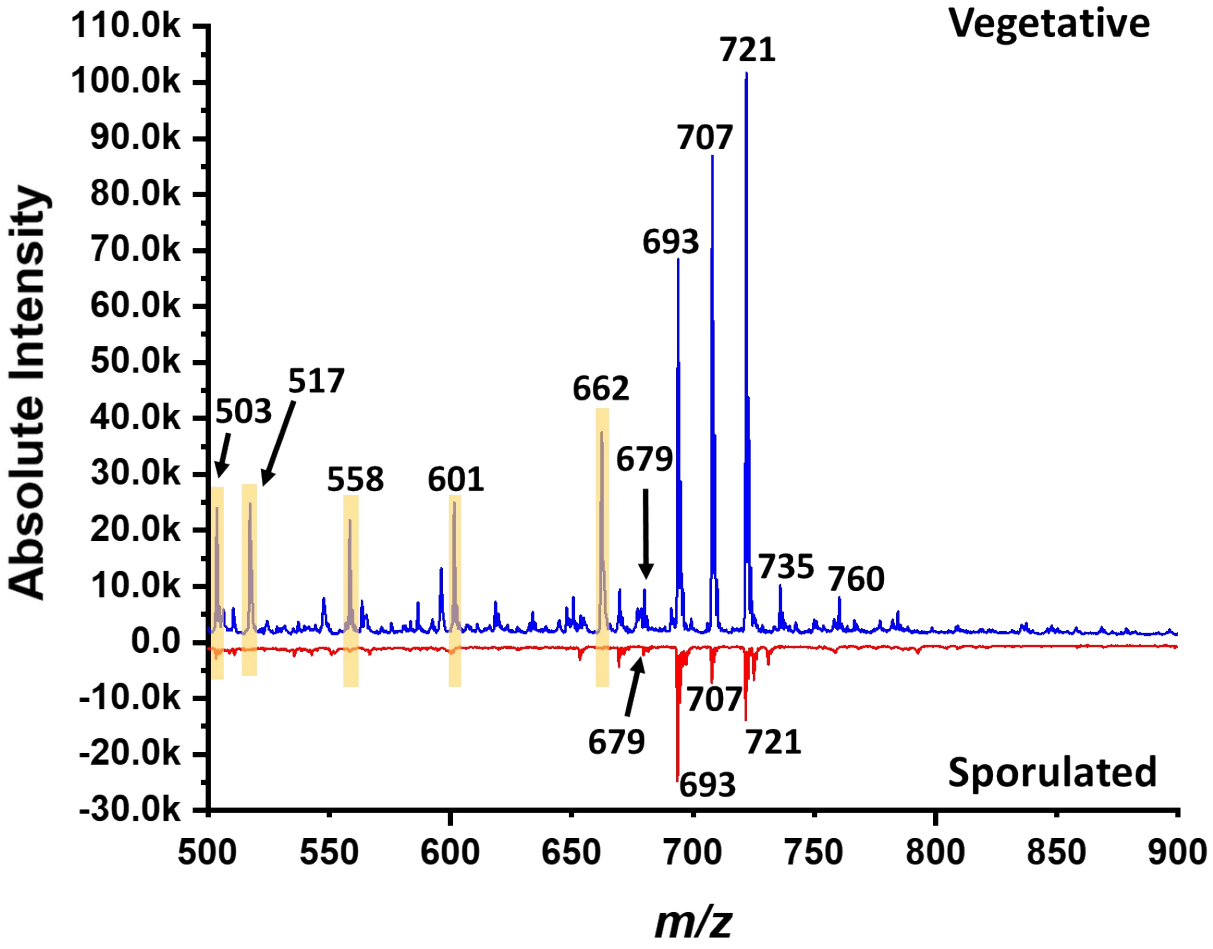

Figure S5. Mass spectrum of the lipid region of microwaved (Blue line) vegetative ($5.0x10^8$ bacteria) and (Red line) sporulated ($2.2x10^8$ spores) *Bacillus subtilis* in the negative ion mode. The mass range shown includes the lipid region between *m/z* 650-*m/z* 750. The yellow boxed peaks denote lipids that are unique to vegetative cells.

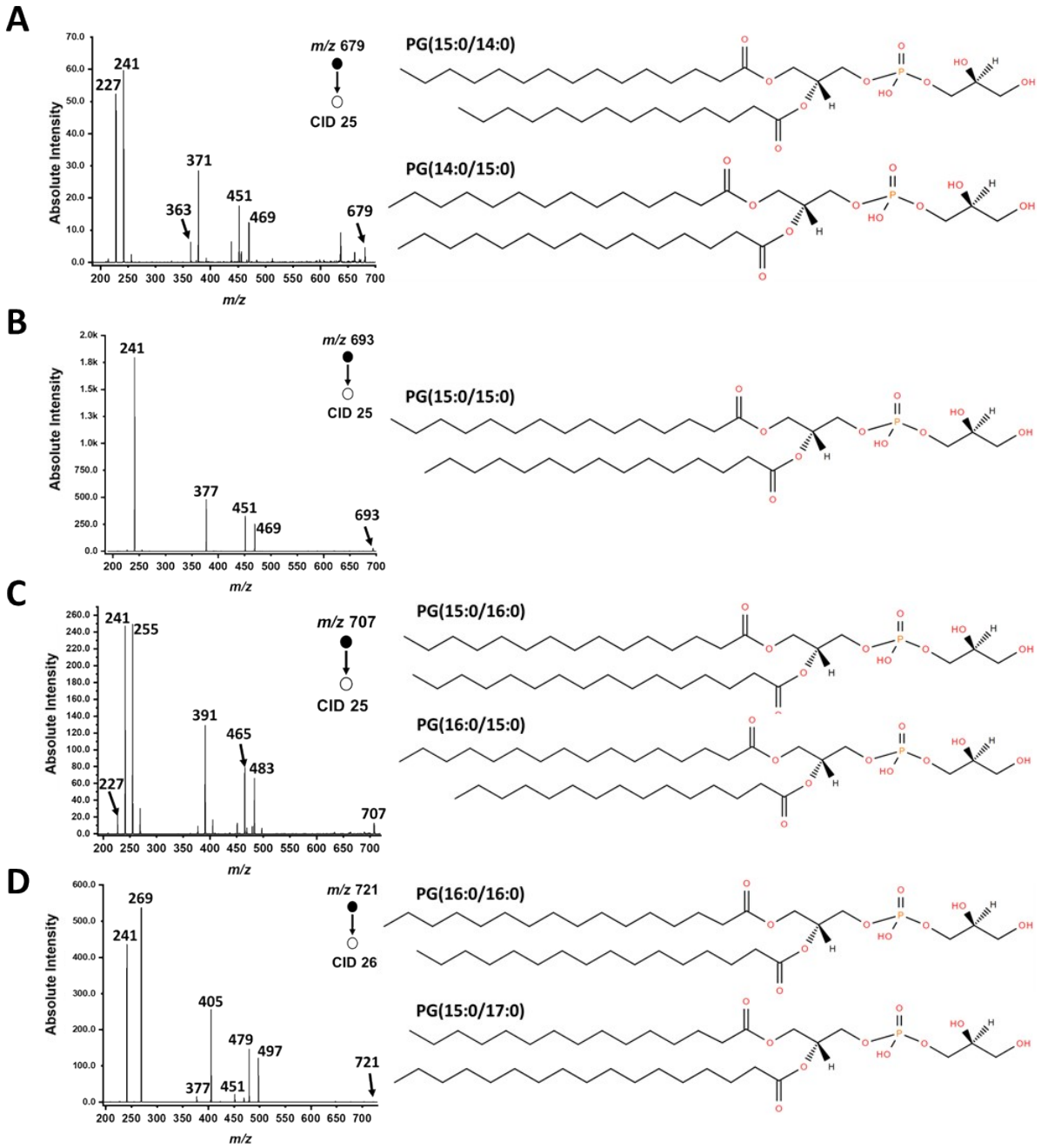

Figure S6. MS/MS spectra of A) PG(31:0), B) PG(30:0), C) PG(29:0), and D) PG(32:0) with collision energies of 25, 25, 25, and 26, respectively. Insets next to each spectrum show plausible isobaric lipid species, however in the case of PG(30:0)-PG(15:0/15:0), an alternative isobaric lipid species is not plausible since both the lipid tails for PG(15:0/15:0) are saturated. All MS/MS spectra of the lipids of interest were acquired using 2.2 x 10^8 spores with nESI-MS in the negative ion mode.

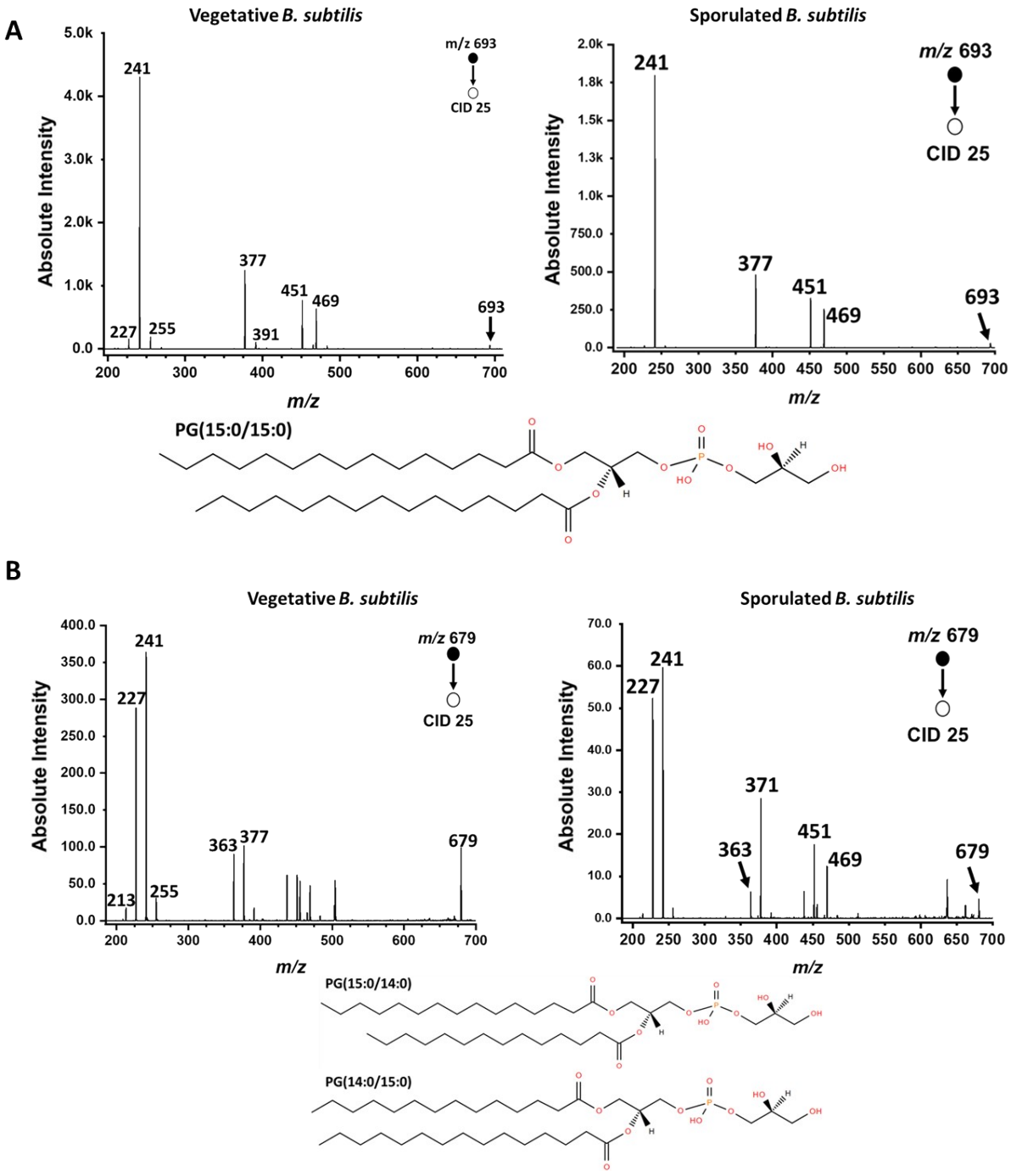

Figure S7. MS/MS spectra of A) PG (30:0) and B) PG (29:0) in the negative ion mode. Spectra on the left side are from microwaved vegetative bacteria ($5.0x10^8$ cells) and spectra of spores ($2.2x10^8$ spores) are on the right. Proposed structures of the lipids are shown underneath each set of spectra.

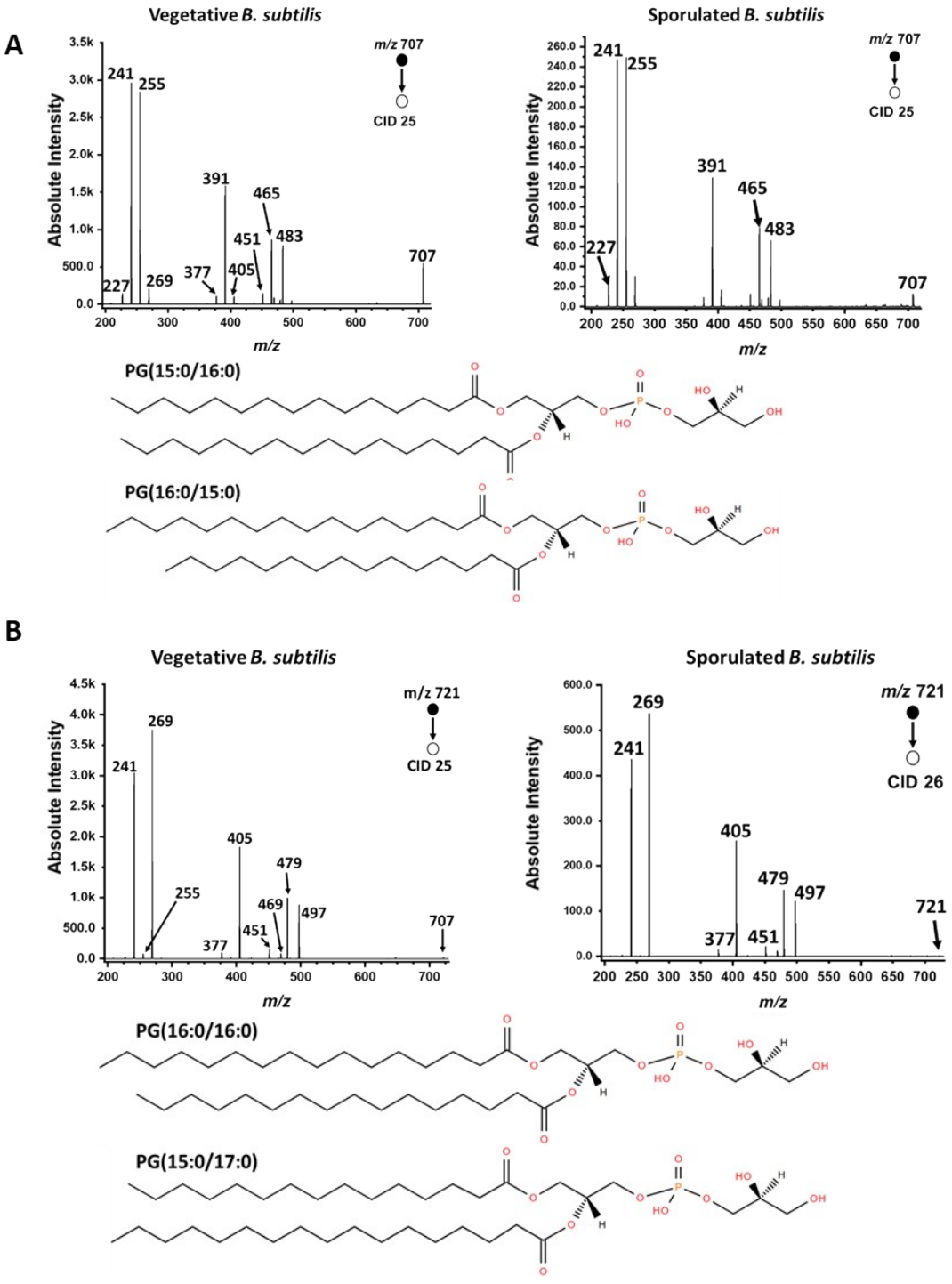

Figure S8. MS/MS spectra of A) PG (31:0) and B) PG (32:0) in the negative ion mode. Spectra on the left side are from microwaved vegetative bacteria ($5.0x10^8$ cells) and spectra of spores ($2.2x10^8$ spores) are on the right. Proposed structures of the lipids are shown underneath each set of spectra. Spectra were acquired in triplicate and averaged.

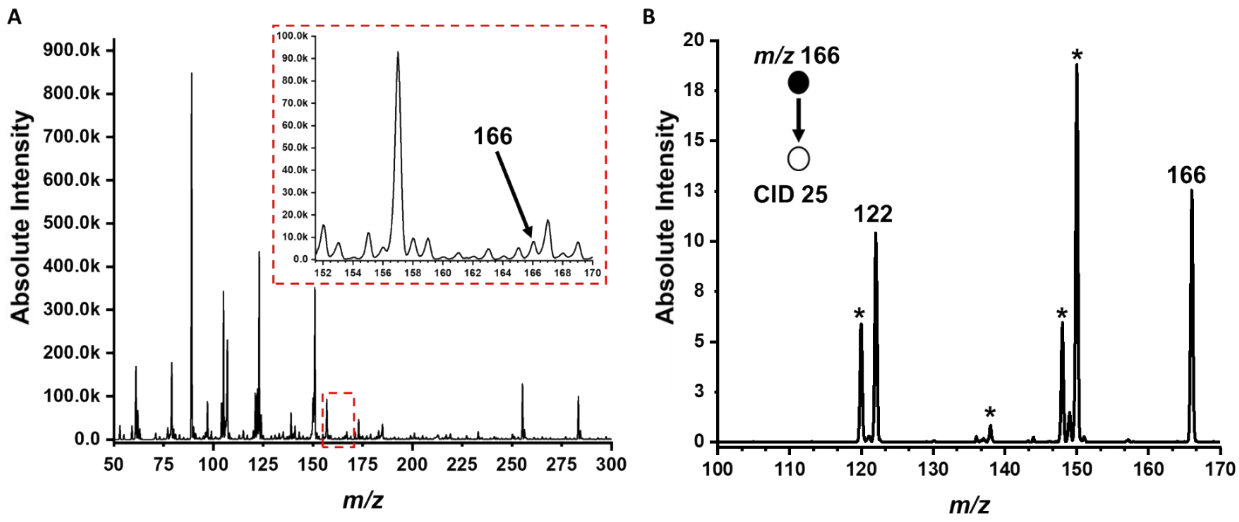

Figure S9. A) Mass spectrum of microwaved *B subtilis* spores ($2.2x10^6$ spores) and B) MS/MS spectrum of *m/z* 166, the molecular ion for DPA, in the negative ion mode using nESI-MS. Inset in figure A shows the *m/z* range of *m/z* 151.5 – 170. All replicate samples were microwaved in for 1 min. Background peaks are labeled with asterisk.

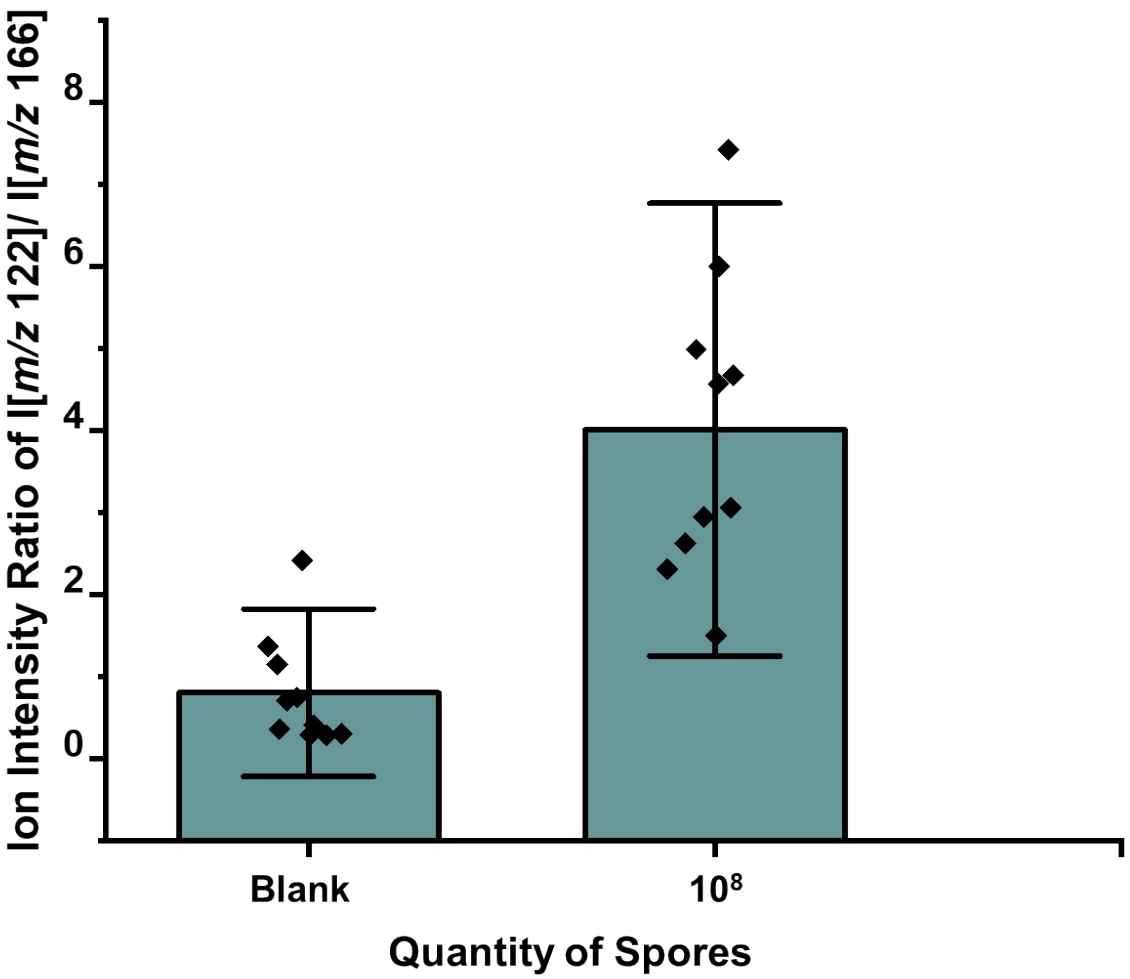

Figure S10. Ratio of ion intensity of *m/z* 122 generated by fragmentation of *m/z* 166 comparing the blank to the signal for 10^8 spores. The data for this experiment was acquired using paper spray ionization in the negative ion mode.