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Following the Biochemical and Morphological Changes of Bacillus atrophaeus during Sporulation using Bioaerosol Mass Spectrometry

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Disclaimer

Following the Biochemical and Morphological Changes of *Bacillus atrophaeus* during Sporulation using Bioaerosol Mass Spectrometry

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54th ASMS Seattle WA: Mon 05-29-2006

Talk Outline:

(1) Introduction to BAMS

(2) Sporulation time-series experiment

Bio-Aerosol Mass Spectrometry Team: Today and (Yesterday)

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EE & CMS, LLNL

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Technical Support Working Group, Department of Defense (TSWG)
LLNL Lab Directed Research and Development Program (02-ERD-002)
LLNL CMS & PAT Postdoctoral Program
Defense Advanced Research Projects Agency (DARPA)

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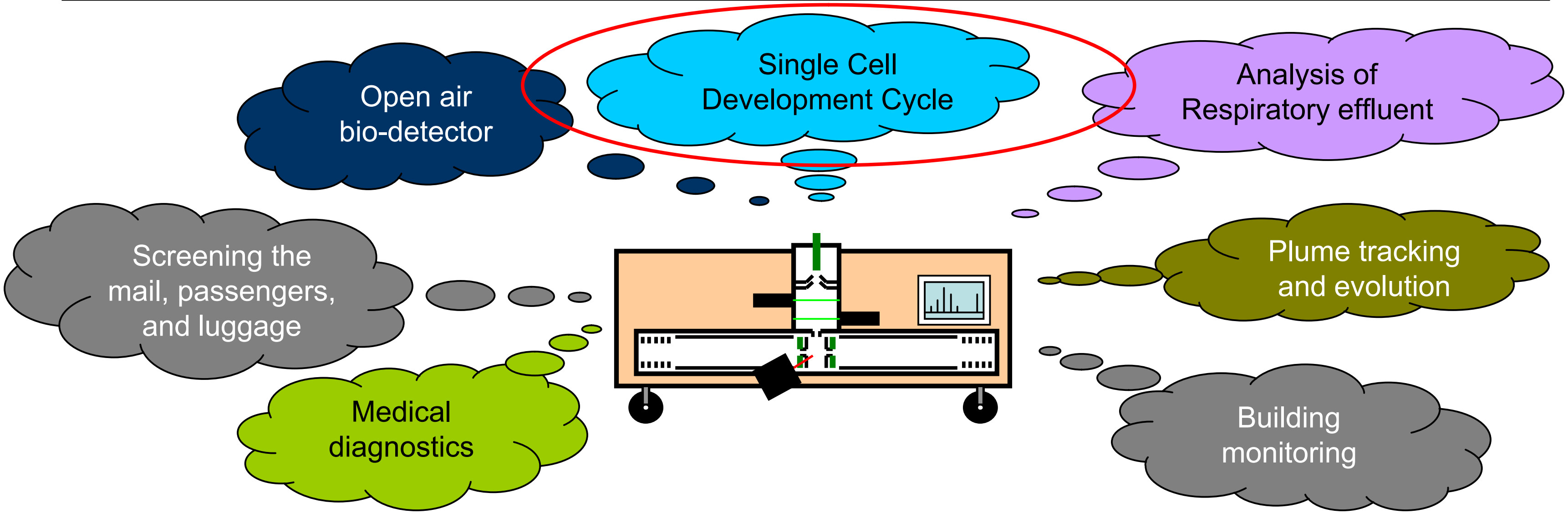
Research Objectives

Overall Group Objective:

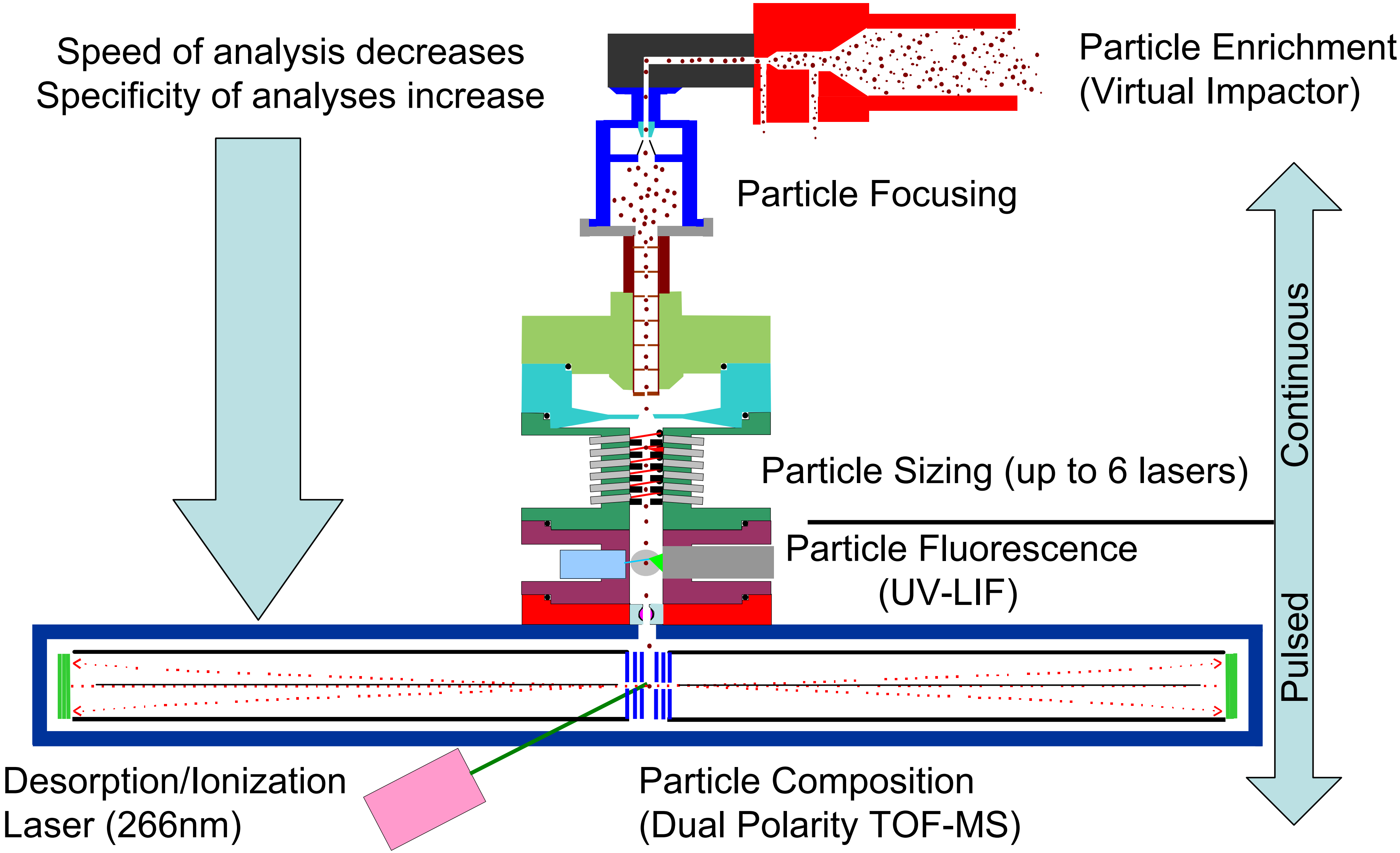
Develop a real-time single-particle mass spectrometry technique called Bio-Aerosol Mass Spectrometry (BAMS) in order to efficiently screen and identify bioaerosols and single cells of national security and public health concern.

Individual spore, bacterial cell, virus, and toxin

identification → species level



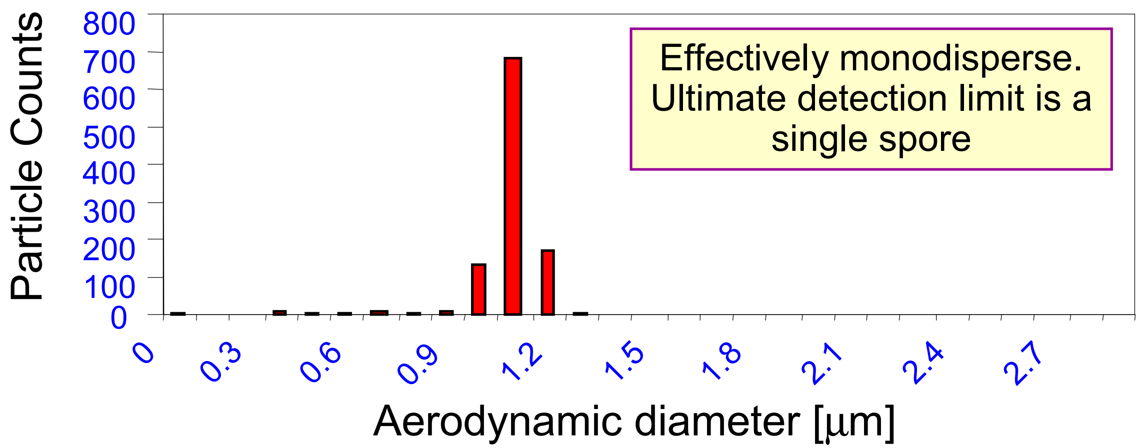
Bioaerosol Mass Spectrometry Today, BAMS 1.4 (LDRD, TSWG, DARPA)



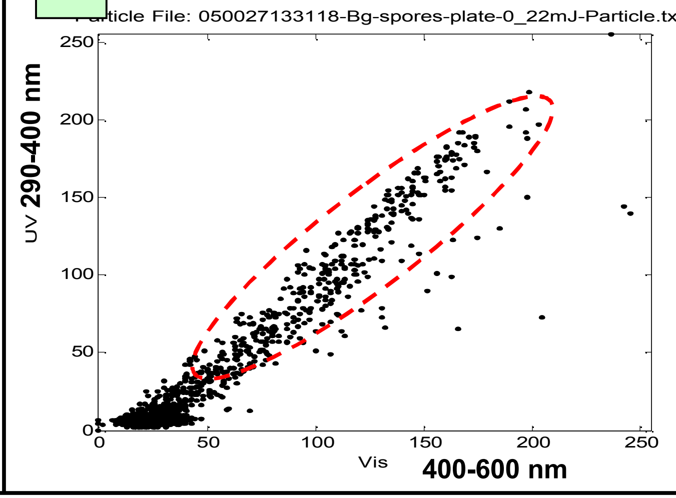
Modular design of instrument stages and associated electronics and analysis

Example BAMS 1.4 Signatures for Bacterial Spores

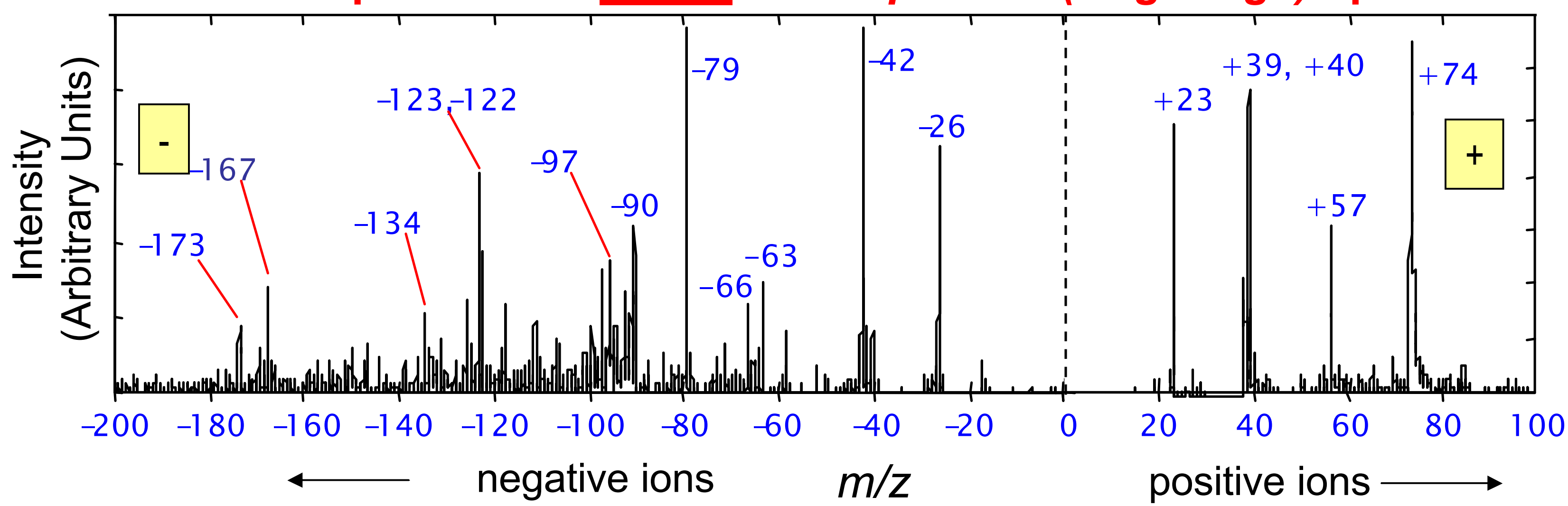
1 ***B. atrophaeus* spore size distribution**



2 **UV-LIF *B. atrophaeus***



3 **Mass spectrum of <u>single</u> *B. atrophaeus (B. globigii)* spore**



Basic Research Application: Model of Bacterial Sporulation

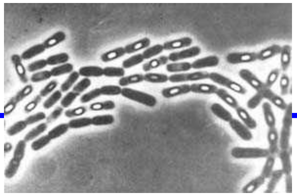
Gram positive bacteria in the genera *Bacillus* and *Clostridium* are able to undergo physical and biochemical changes during periods of starvation and stress in order to form dormant and robust endospores for survival.

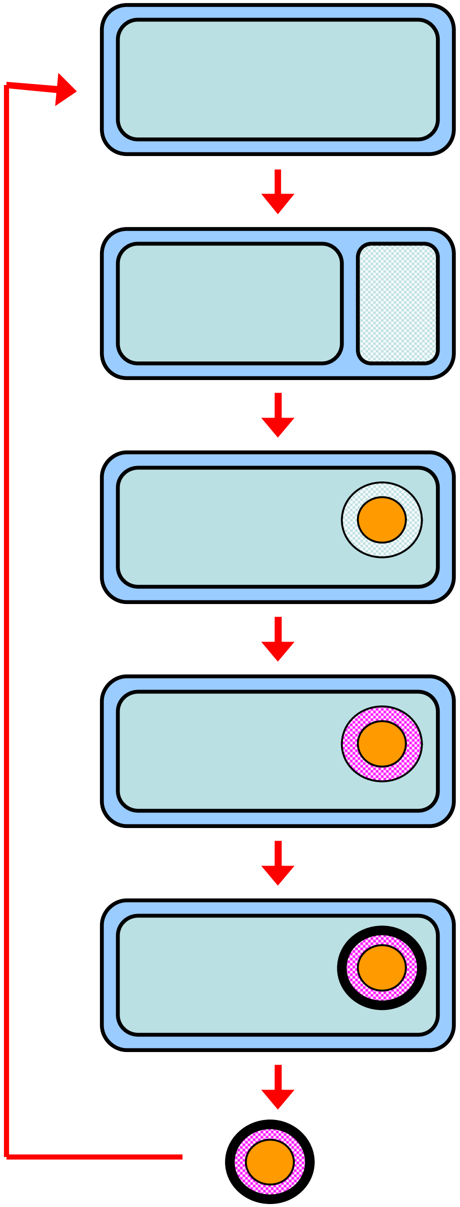
The process of sporulation well studied over the years.

Commonly used model for the study of microbial development and cell differentiation in the cell development cycle.

A simple set of experiments is presented in order to introduce and demonstrate: potential utility of Bioaerosol Mass Spectrometry (BAMS) for the study of cell development processes at the *single cell* level.

Stages of sporulation





(A) Starvation promotes sporulation gene expression.

(B) Asymmetrically positioned septum divides cell into the forespore and mother cell comparments.

(C) Edge of septum migrates toward forespore pole pinching off a protoplast with double layer membrane in mother cell cytoplasm.

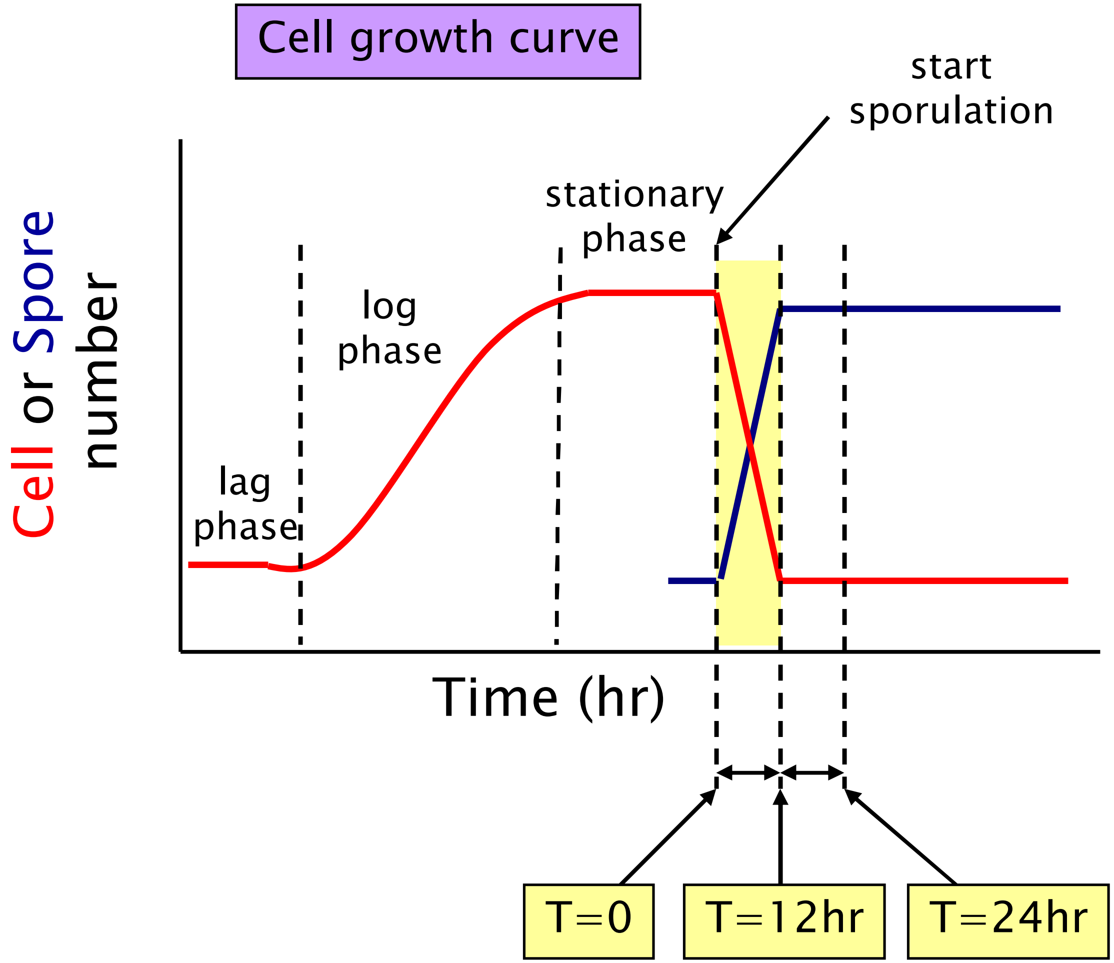
(D) Cell wall like material is deposited between membrane layers. Two layers, inner= germ cell wall , outer= cortex.

(E) Formation of coat of proteins assemble around forespore.

(F) Lysis of mother cell and release of fully formed spore.

Time series samples: sporulation of *B. atrophaeus*

Prepare series of samples at different time points from the same batch of sporulating cells.
Aliquot of cells from mother broth spun down, washed 3 times, and resuspended into d.i. Water.



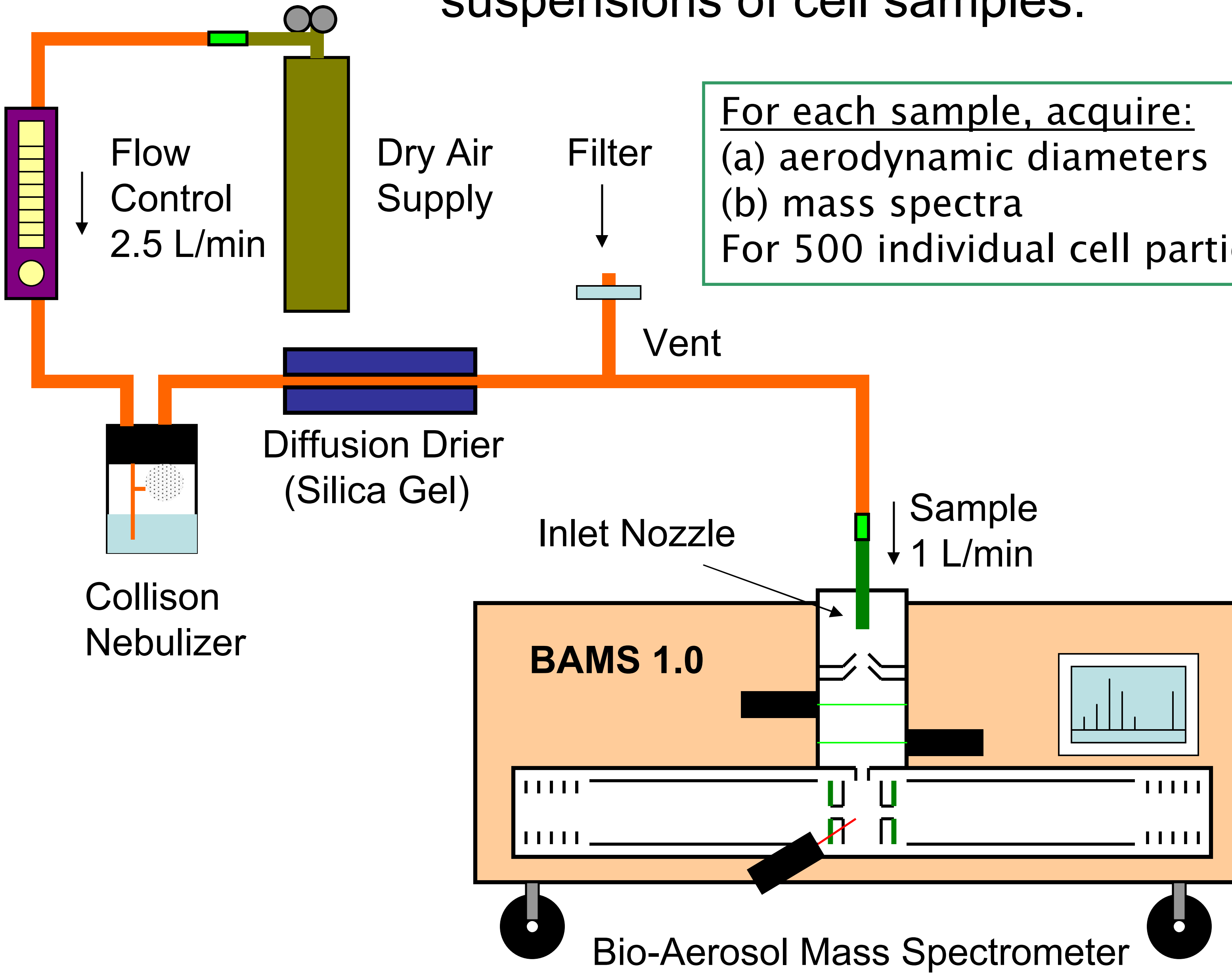
B. atrophaeus cells at:

T = 0 min
T = 2 hr 15 min
T = 5 hr
T = 7 hr 45 min
T = 10 hr 15 min
T = 12 hr
T = 24 hr

B. at "DPA mutant" at:
(spo VF)

T = 24 hr

Experimental Setup: Generation of aerosols from liquid suspensions of cell samples.



<u>For each sample, acquire:</u>
(a) aerodynamic diameters
(b) mass spectra
For 500 individual cell particles.

BAMS Mass Spectra Analysis: Pattern Matching

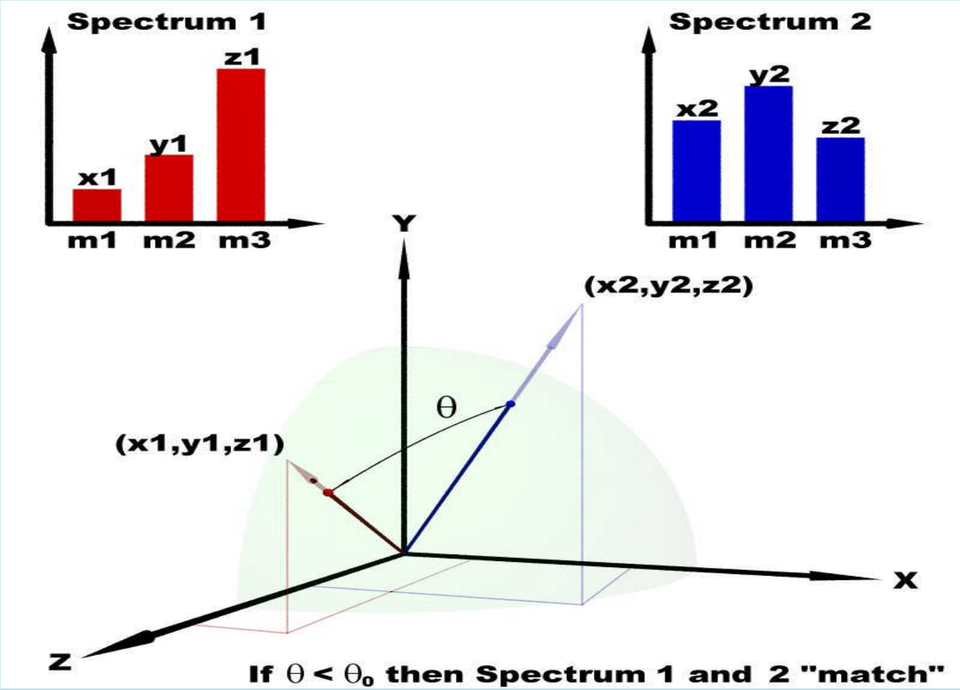
The entire data set of mass spectra was pooled and analyzed together.

For each individual cell particle:

(a) Feature extraction: Compute numeric information from observation (mass spectra). The positive and negative mass spectrum treated as a 350-element vector (i.e. *m/z* range).

(b) Classification: This vector is compared to all other vectors in data set in multidimensional space. If vectors are within certain angle they are clustered together using a neural network algorithm.

Visual example for comparison of 3-element vectors in 3-dimensional space:

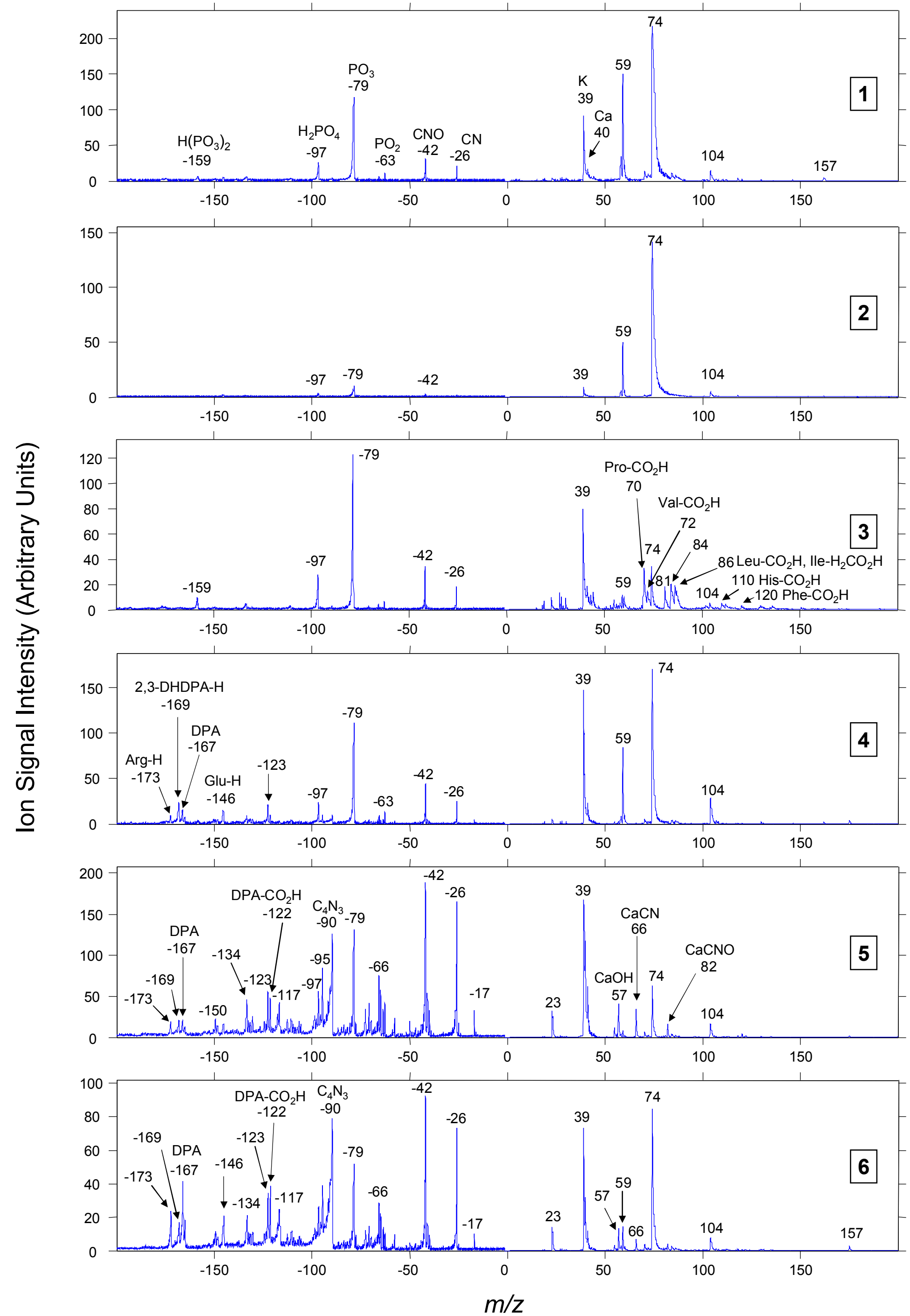


Presented according to a numerical arrangement that reflects their rough order of appearance in the time series experiment.

Shot-to-shot mass spectral variations.

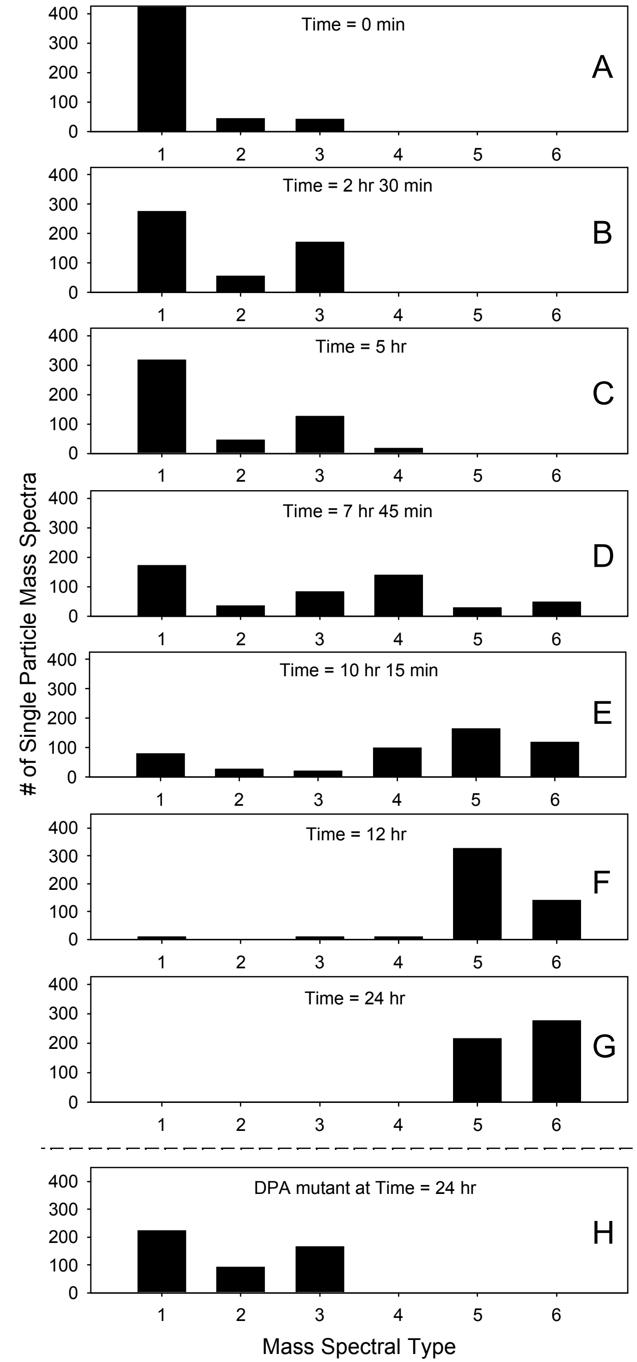
Averages presented.

~4000 spectra (culled ~1%)



Six General Mass Spectral Types

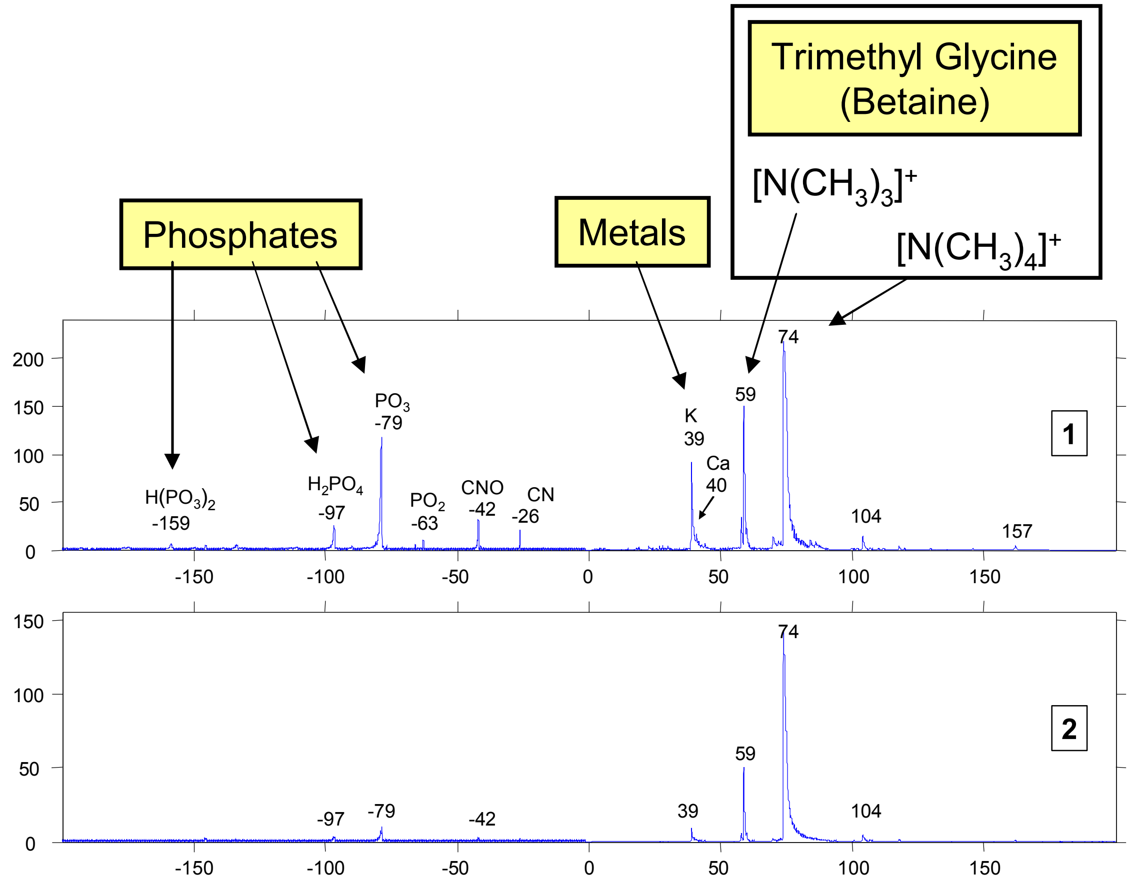
Number of BAMS single cell particle mass spectra that are clustered into each of the six general mass spectral types for the time series samples and the "DPA mutant" sample.

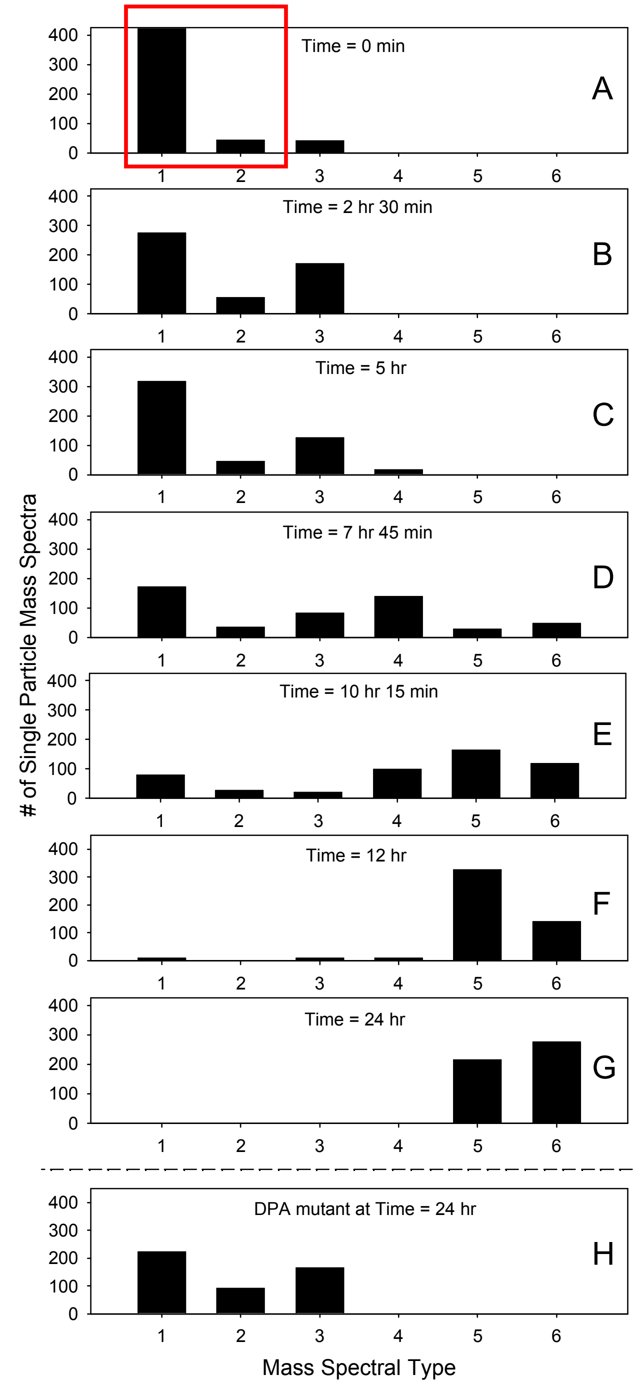


Time = 0 min

Mass spectral types 1 and 2 were most prevalent.

Represented the earliest vegetative state of *B. atrophaeus* cells.



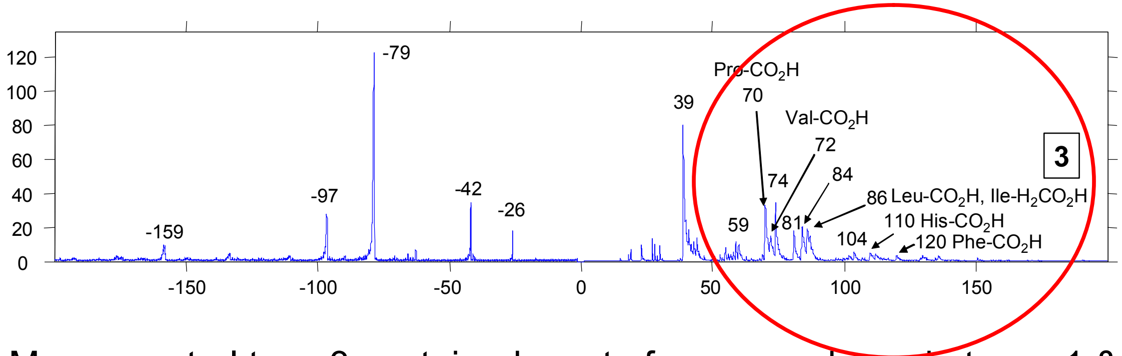


Time = 2 hr 30 min to 7 hr 45 min

Intermediate stages of *B. atrophaeus* sporulation.

Mass spectral types 1 and 2 were still prevalent.

Mass spectral type 3 began to grow more prevalant.



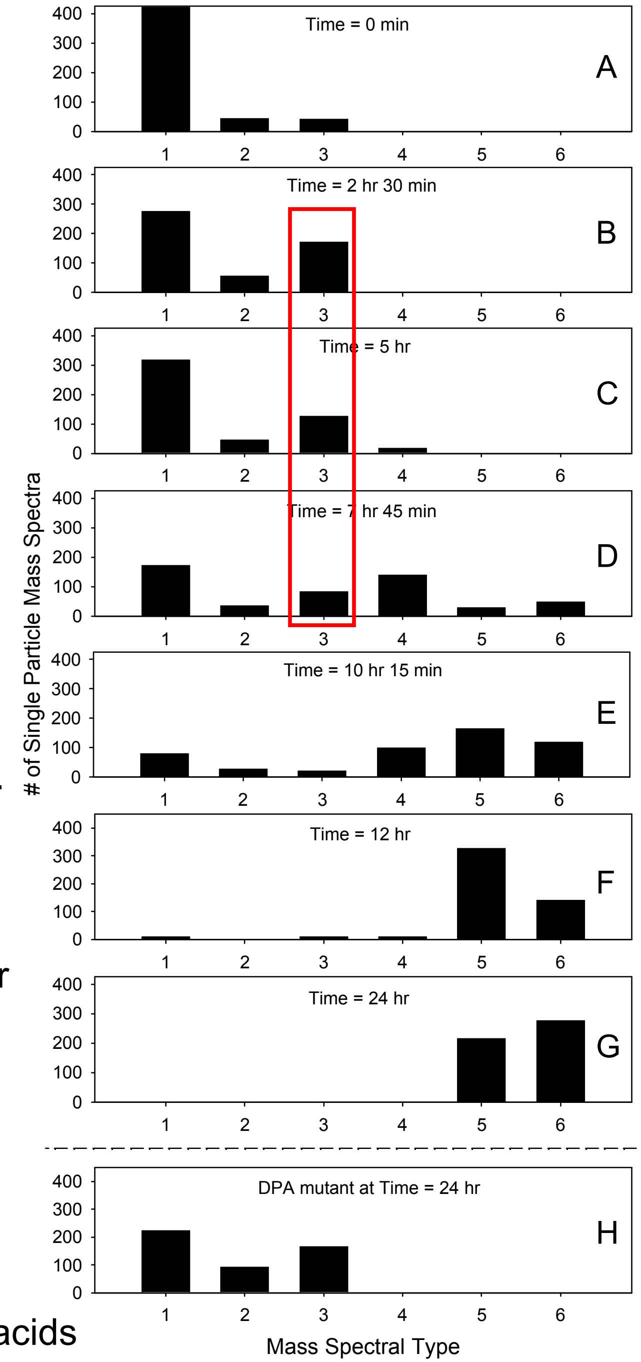
Mass spectral type 3 contained most of same peaks as in types 1 & 2.
Also contained additional peaks due to amino acid residues.

Source: increase in the availability of amino acids during intermediate stages where the cell, in its increased level of activity, required greater resources for endospore formation processes.

Examples of some of these processes may include:

-Production of proteinaceous coat around forespore by mother cell,

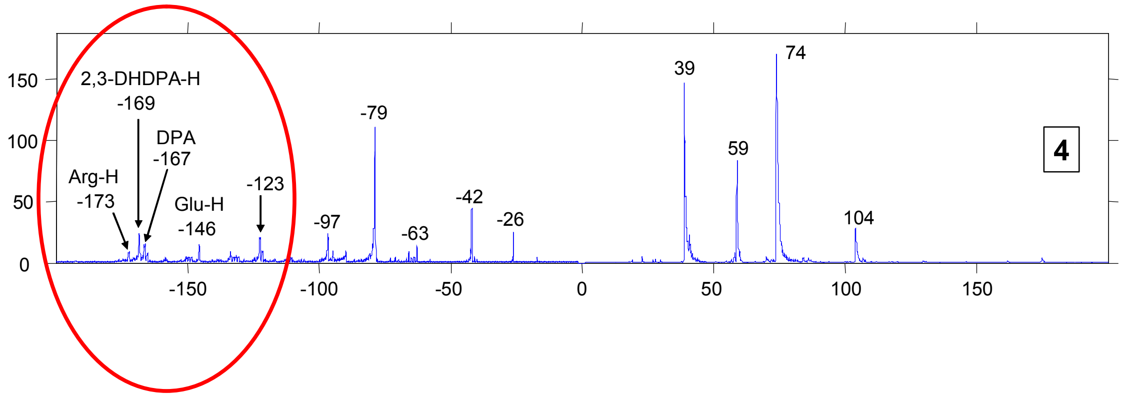
-Production of large amounts of small acid soluble proteins (SASPs) by the developing forespore, which provide a ready source of amino acids



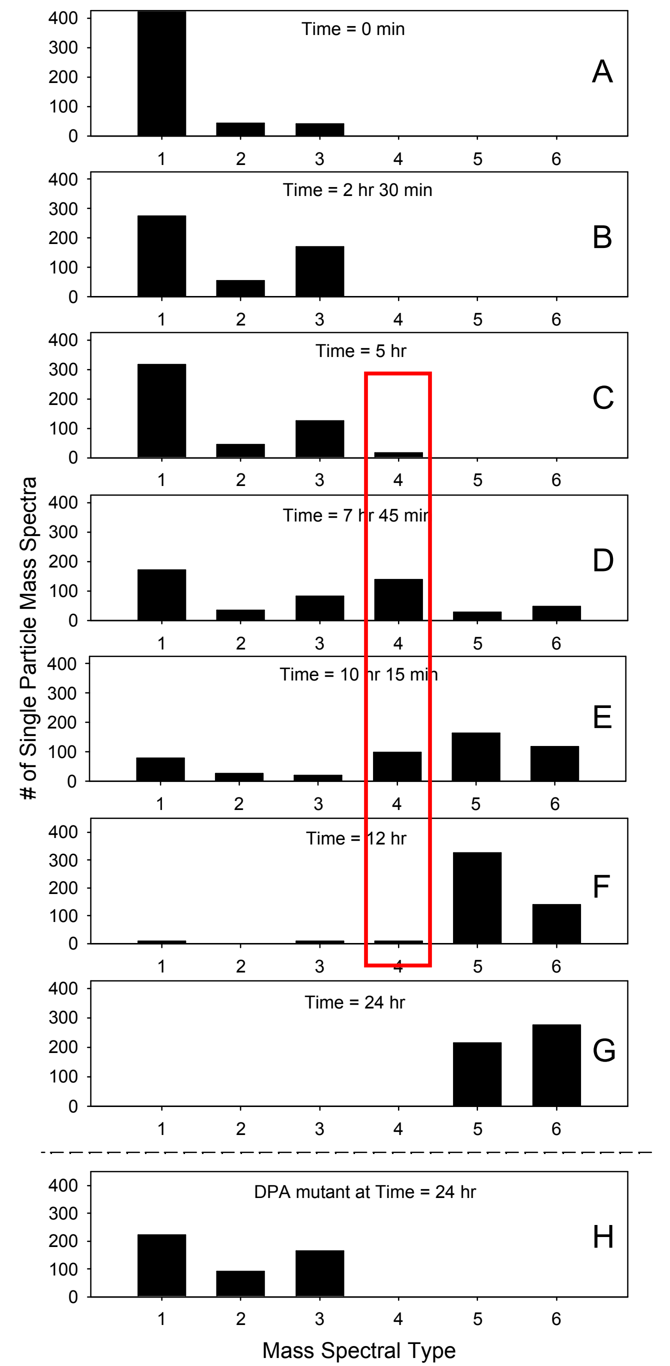
Time = 5hr to 12 hr

Mass spectral type 4 began to grow in, peaked at 7 hr 45 min.

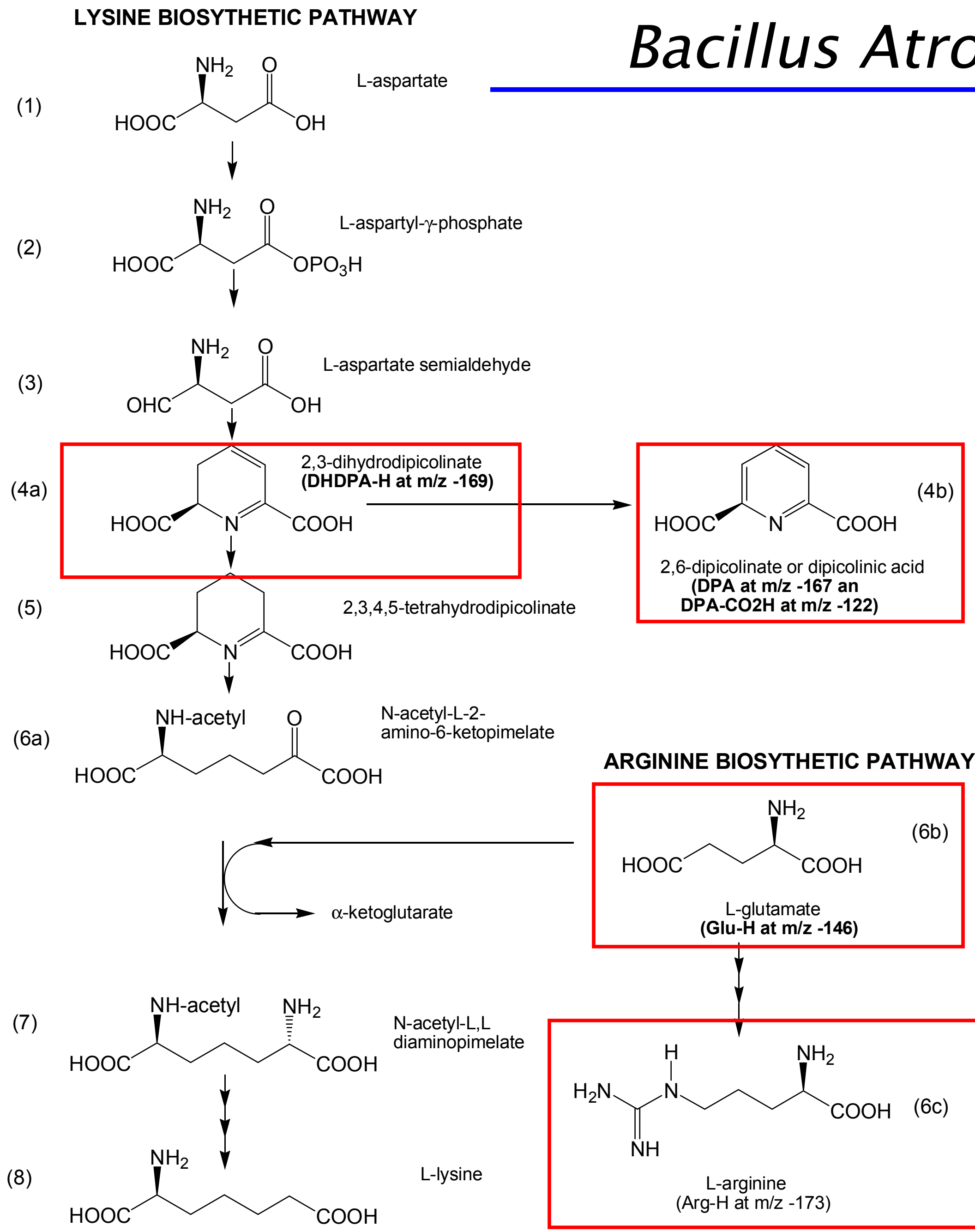
Negative ion portion of mass spectral contain many additional peaks.



Representative of the biochemical makeup, as seen by BAMS, of developing spores undergoing parts of the lysine and arginine biosynthetic pathways.



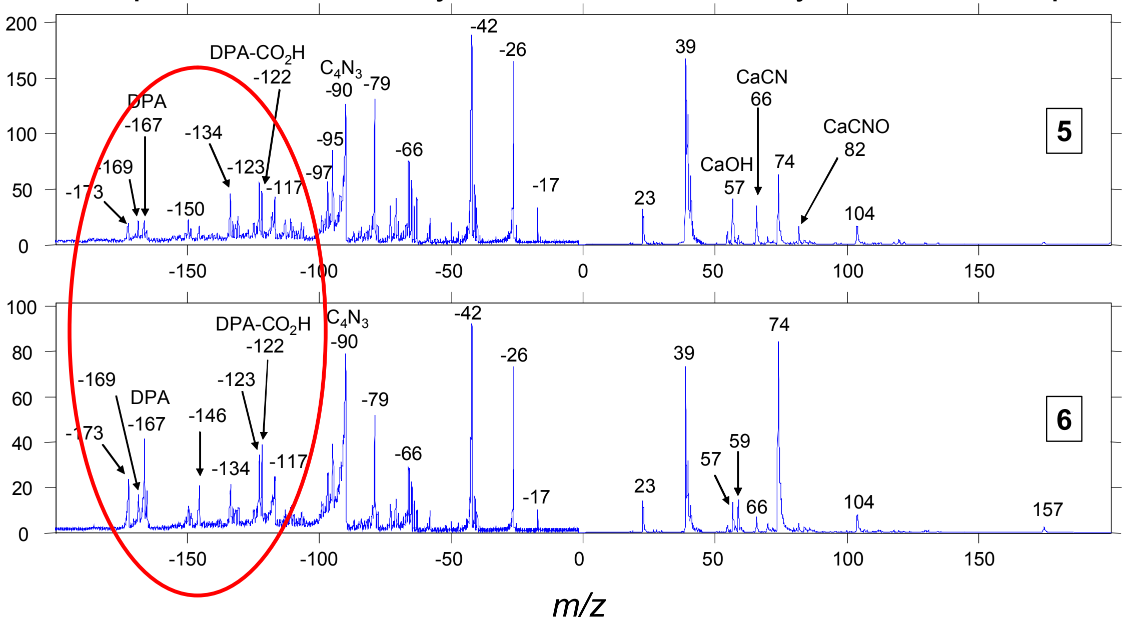
Bacillus Atropheaus

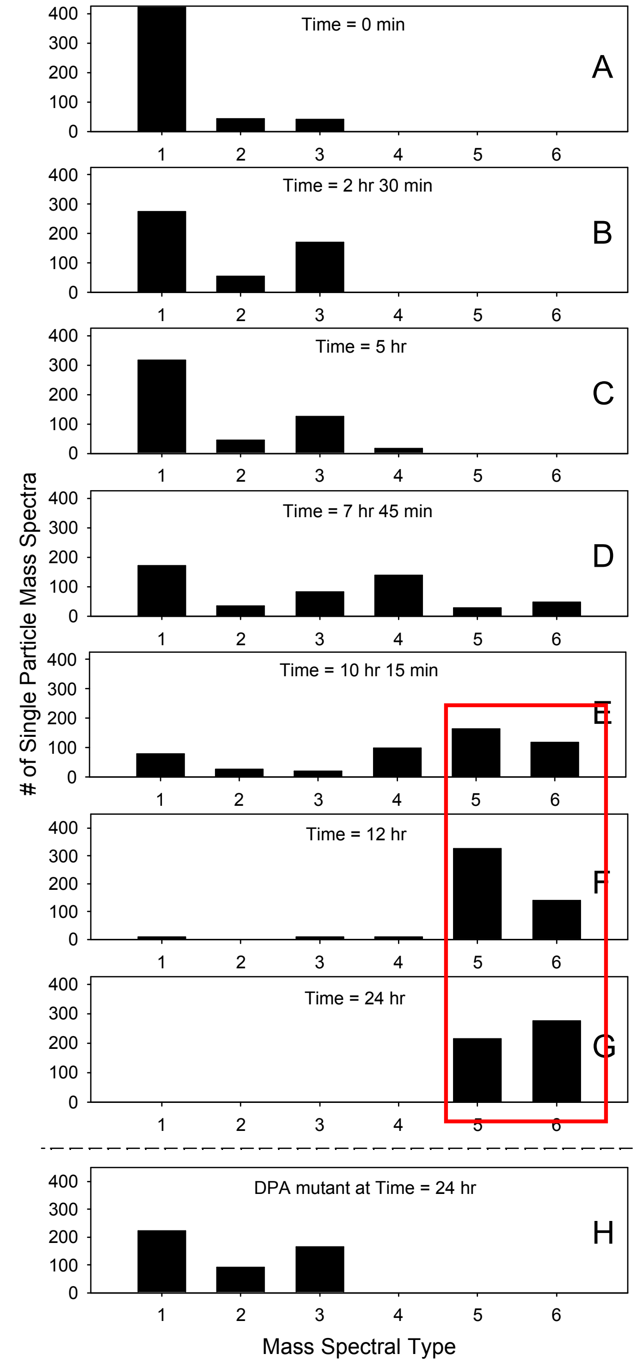


Time = 10hr 15min to 24 hr

Mass spectral type 5 and 6 began to grow in and dominate.

Most representative of fully formed/and near fully formed endospores.





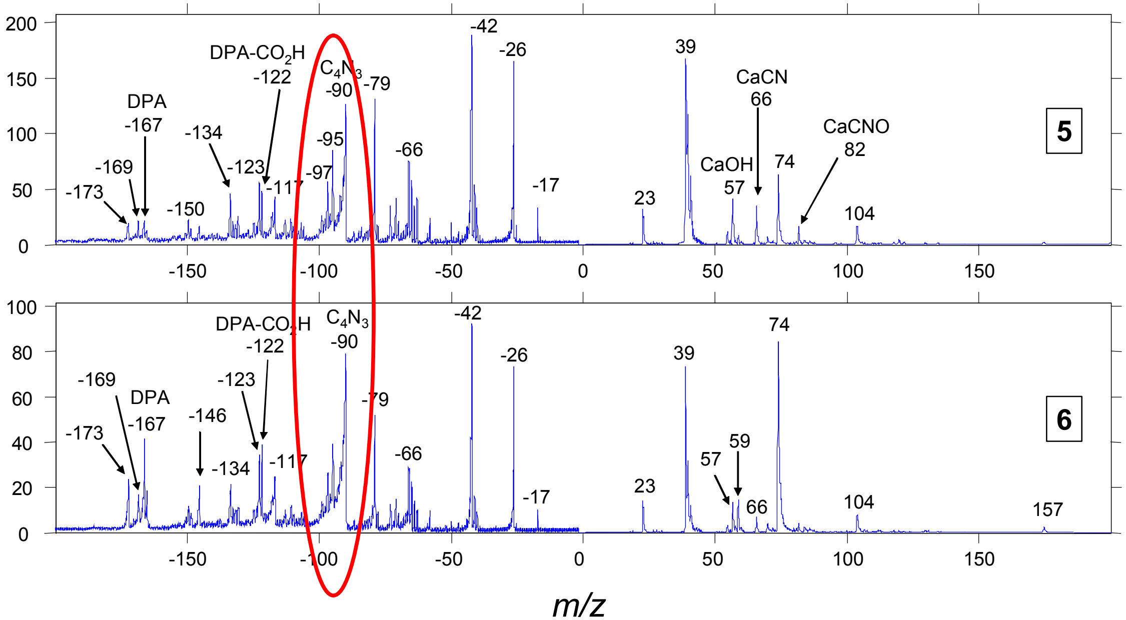
DPA (*m/z* -167), DPA - CO_2H (*m/z* -122), and arginine – H (*m/z* -173) grew in successively from mass spectral type 4 to 5 to 6.

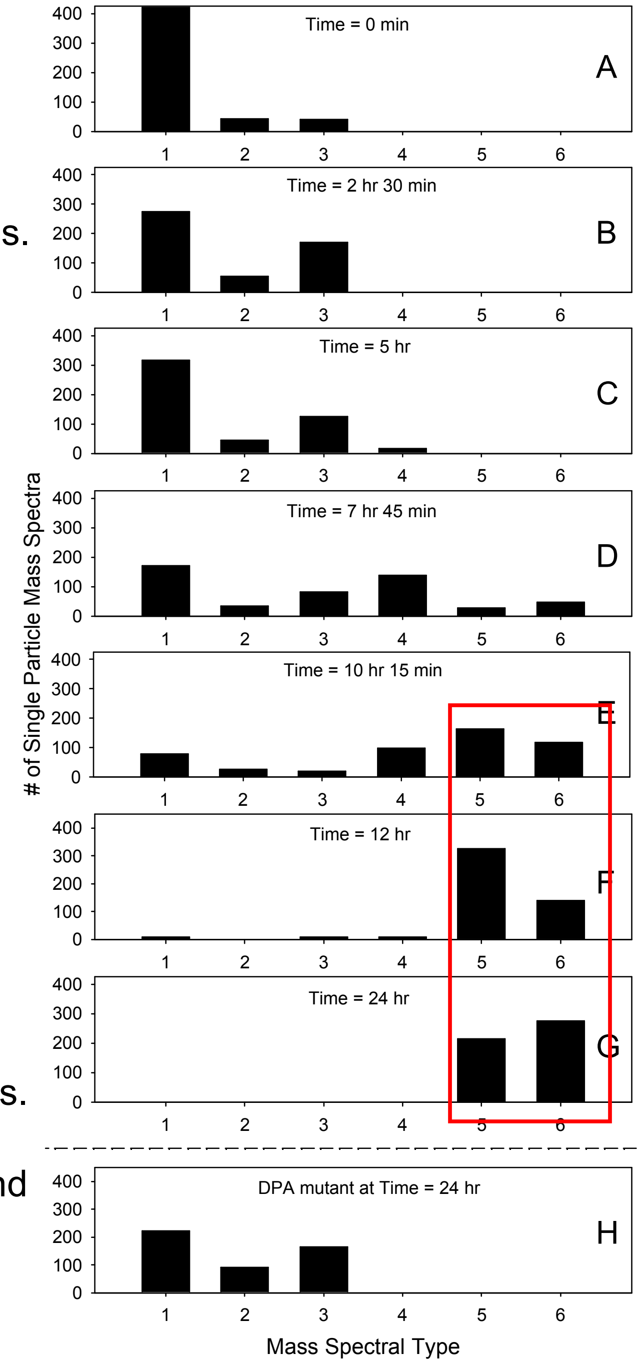
2,3-dihydrodipicolinate-H (2,3-DHDPA-H, *m/z* -169) decreased in relative abundance to others, probably indicating DPA production was complete and/or nearly complete in these cells.

Time = 10hr 15min to 24 hr

Mass spectral type 5 and 6 began to grow in and dominate.

Most representative of fully formed/and near fully formed endospores.





Increase in relative intensity of a *m/z* -90 peak, previously shown to be due to contributions from adenine and guanine nucleotides through a common fragment $[C_4N_3]^-$

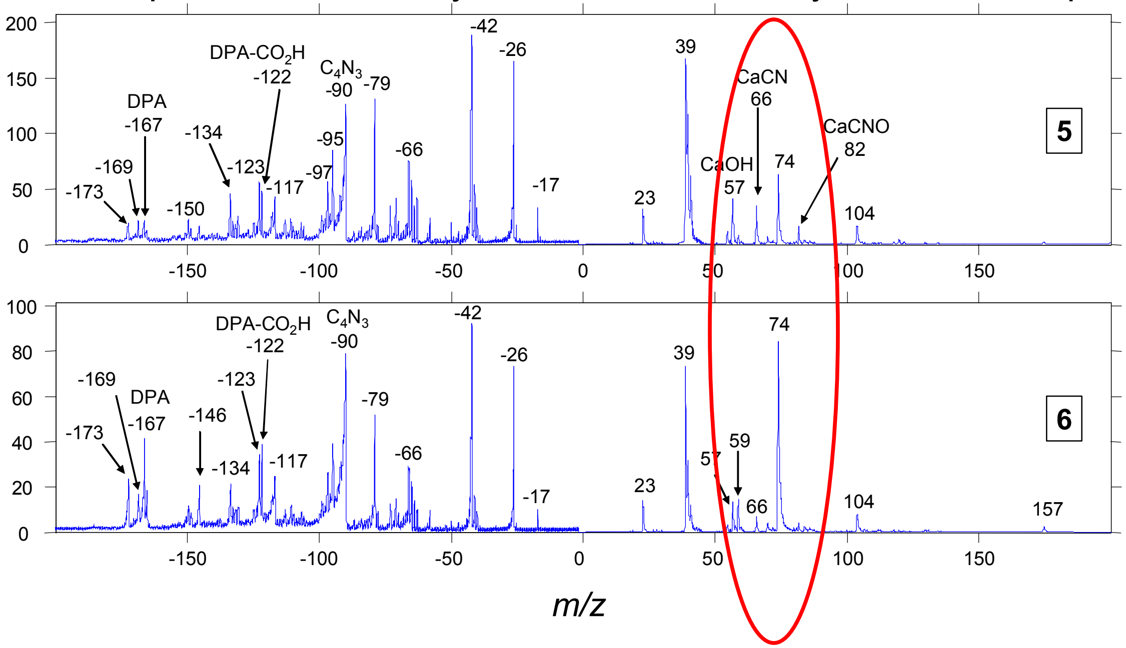
m/z -90 in part represents the higher levels of AMP present in spores.

BAMS ionization efficiency for AMP is much greater than for ATP and ratio of AMP/ATP is much greater in spores than vegetative cells.

Time = 10hr 15min to 24 hr

Mass spectral type 5 and 6 began to grow in and dominate.

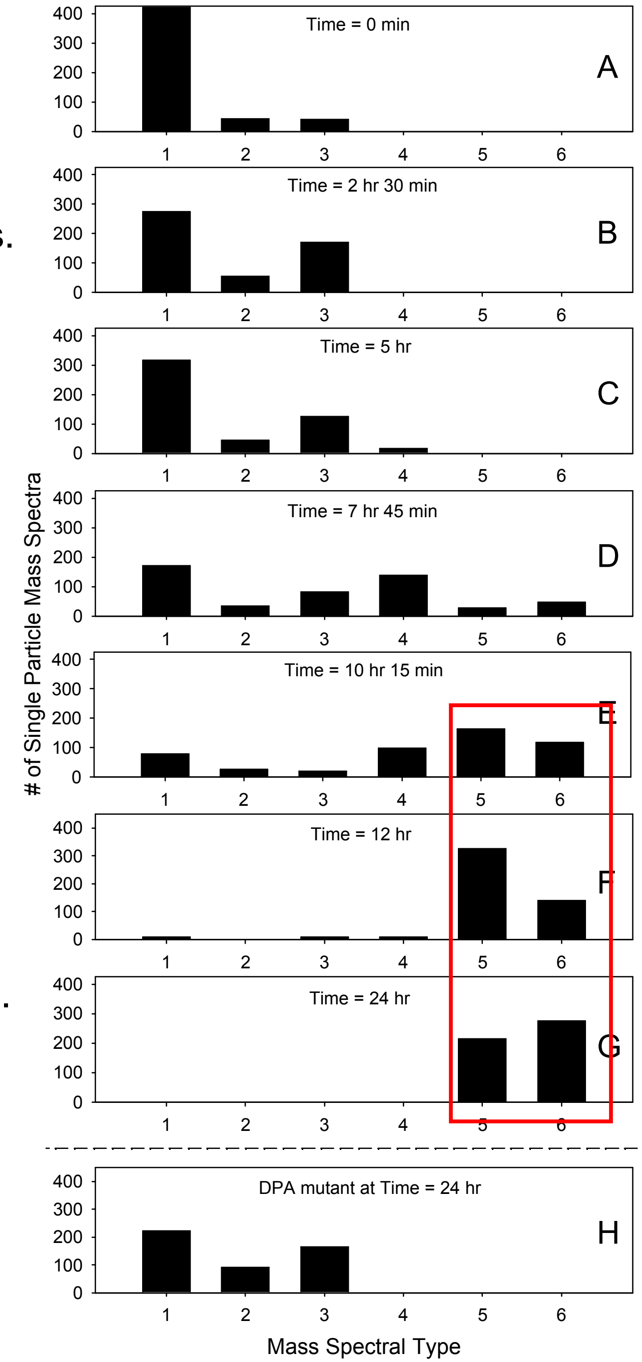
Most representative of fully formed/and near fully formed endospores.



Increased abundance of calcium from positive ion peaks such as [CaOH]+ at *m/z* +57, [CaCN]+ at *m/z* +66, and [CaCNO]+ at *m/z* +82.

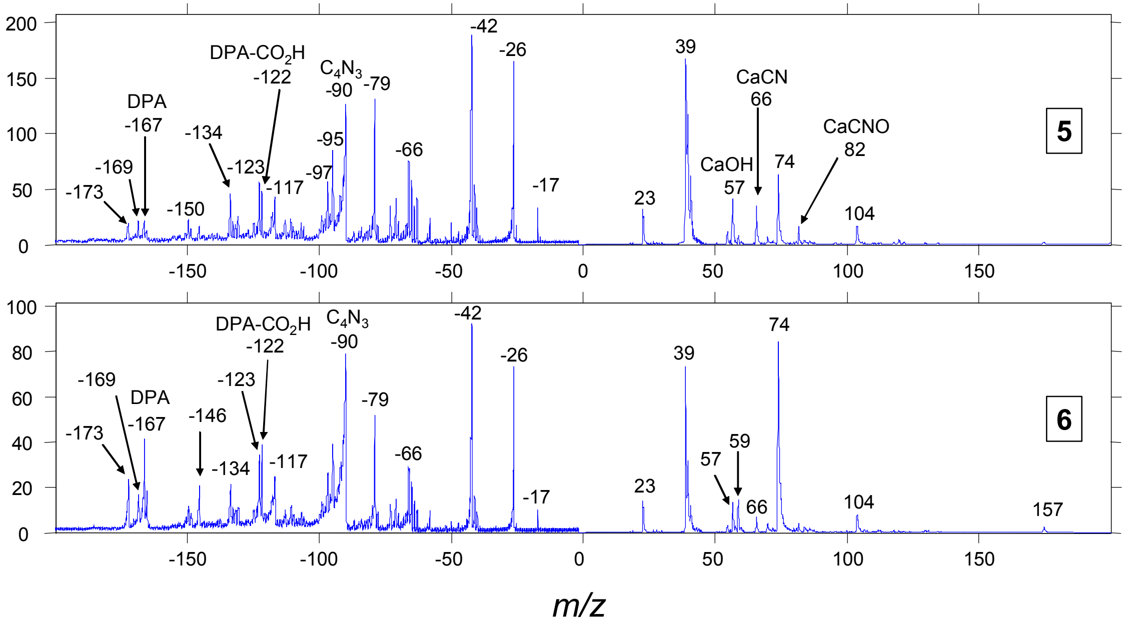
Characteristic of positive ion BAMS mass spectra of *B. atrophaeus* spores.

Calcium is well known to be abundant in endospore cores where it is found complexed with DPA.

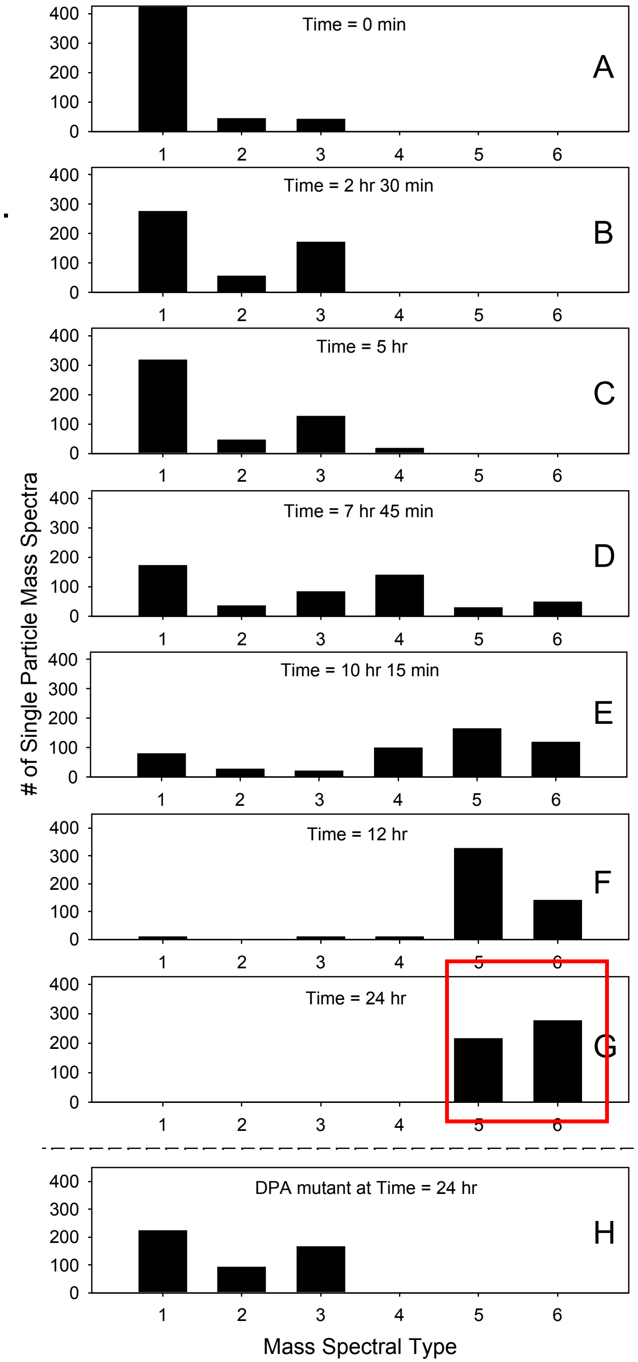


Time = 24 hr

Most representative of fully formed/and near fully formed endospores.



All BAMS mass spectra of *B. atrophaeus* cells are of mass spectral type 5 or 6.

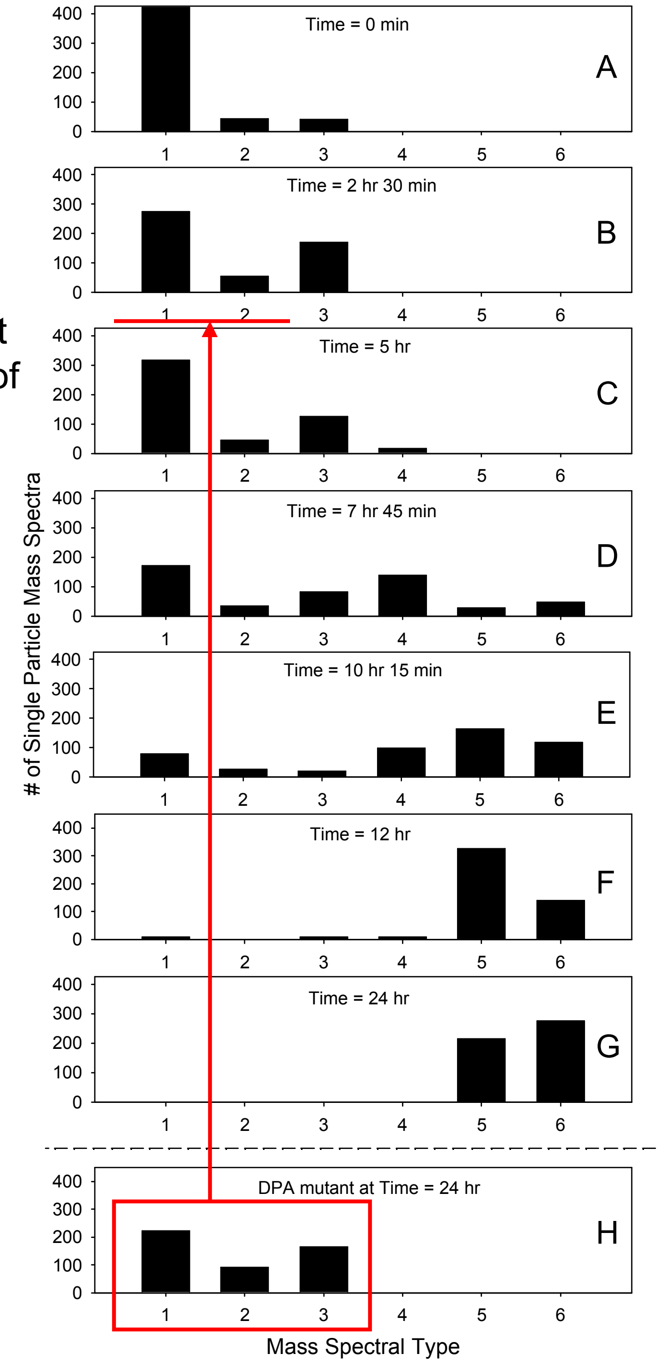


T = 24 hr: DPA Mutant

No evidence of DPA production.

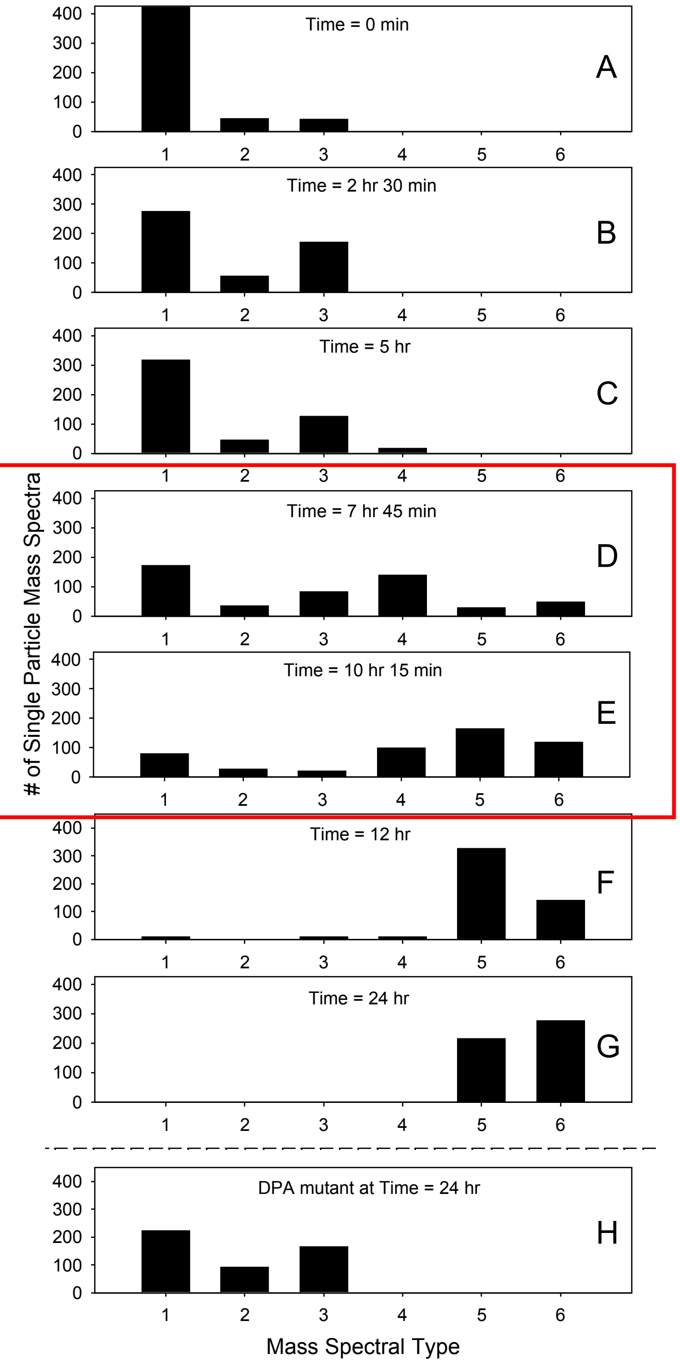
Distribution of mass spectral type abundances suggested DPA mutant cells may fit between the time = 2 hr 30 min and time = 5 hr samples of the normal *B. atrophaeus* sporulation time series experiment.

On a biochemical level, BAMS indicated that the DPA mutant cells were at an early stage in the sporulation process.



Asynchronous Sporulation

Large subpopulations of cells were at different points in the sporulation process at any given time.



BAMS analysis of spore preparations in our laboratory often quickly revealed if those preparations were non-sporulated, partially sporulated, or fully sporulated

BAMS valuable as a rapid diagnostic of spore samples.

Morphological Structure of *B. atrophaeus* Cells

Characterization techniques:

Phase contrast microscopy (staining)
Electron microscopy (staining and dehydration)
Automated scanning microscopy
High resolution Atomic force microscopy

Vegetative Cells:

Rod-like
Diam ~0.8 μm and length ~2-3 μm (Madigan et al., 2003)

Endospores:

B. thuringiensis (Westphal et al., 2003); *B. atrophaeus* (Plomp et al., 2005)
Oval-like
Diam ~0.7 μm and length ~1.8 μm (dried state)
Approximately 12% larger (wet state)

Aerodynamic diameters: that would be expected to be measured in BAMS…

Diam of spherical particle w/ d=1g/cm3 that has = settling velocity as particle.

Vegetative cells	~1.2-1.4 μm
Endospores	~1.1-1.2 μm

Aerodynamic size distributions measured by BAMS generally fit these estimated sizes

BAMS Aerodynamic Diameters of *B. atrophaeus* Cells

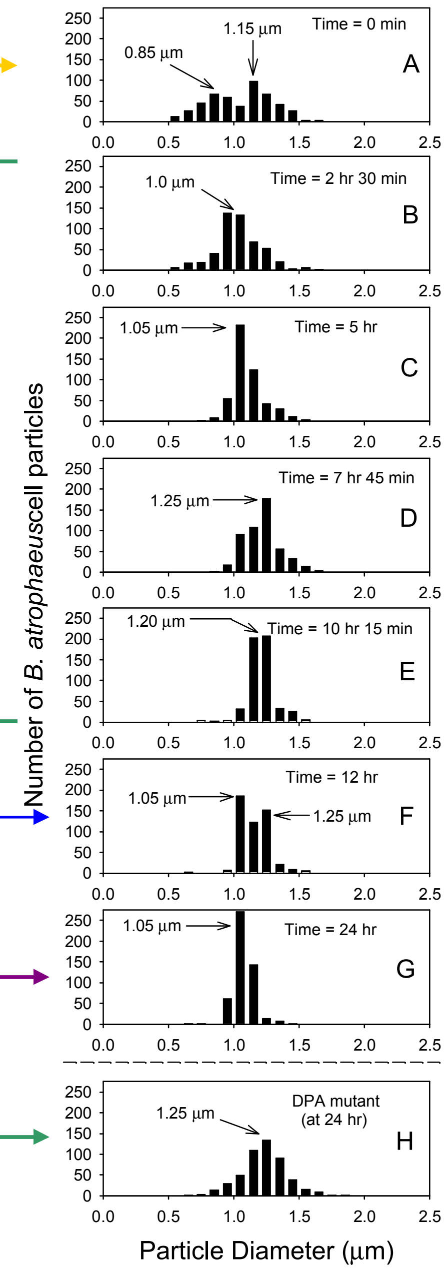
Initial vegetative cell size distribution is broad

Trend for cell transformation in the first ~10 hours: progression from broad distribution of sizes (~1µm) toward tighter size distribution of sizes (~1.25µm).

Almost fully formed spores began to reduce in size

Endospores continued to reduce in size and attained the tightest size distribution.

DPA Mutant cell size distribution is broad and similar to early transforming cells



Possible Sources of Aerodynamic Size Distribution Differences

All samples:

1) Natural biological variability in individual cell sizes in each sample.

Broad size distribution of vegetative-like cells earlier in time series:

2) Smaller cell particle sizes due to cell fragments.

3) Larger cell particle sizes due to the clumping of numerous cells.

4) tumbling of rod-like shaped vegetative-like cells into BAMS.

Tighter distribution sizes of later time series samples:

5) Greater homogeneity of cell shapes.
(i.e. spores: shorter rods more resembling spheres).

Increase in cell size towards middle time series:

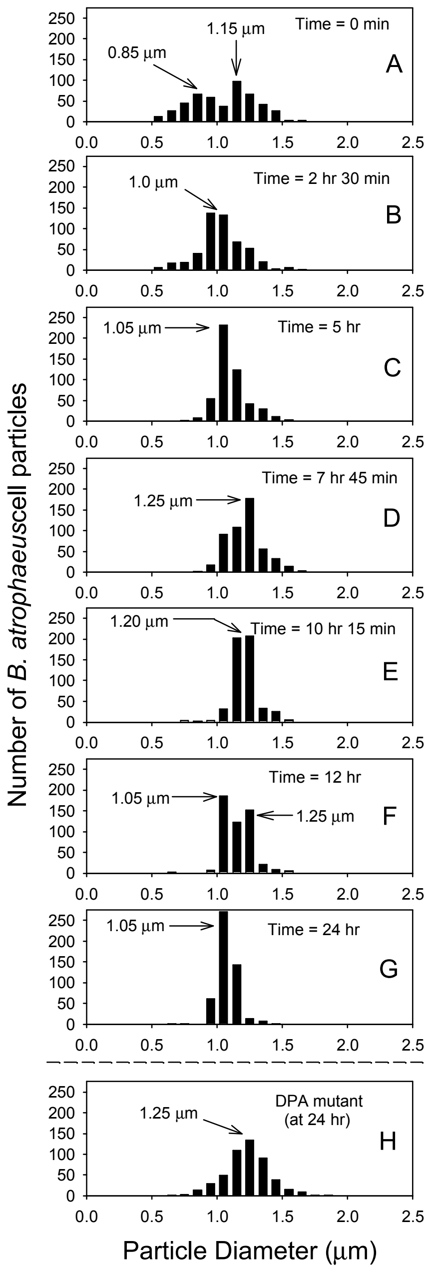
6) Physically larger when forespore develop in mother cell.

Reduction in cell size towards end of time series:

7) Fully formed endospores more likely smaller than developing ones.
Mother cell lysed to release forespore.
DPA helps to dehydrate spore making it smaller.

DPA mutant cells more like early stage vegetative-like cells:

1) DPA is important in reducing a spore's core water content.
2) DPA is required for spore dormancy.
Known to lyse and/or germinate during sporulation/purification.

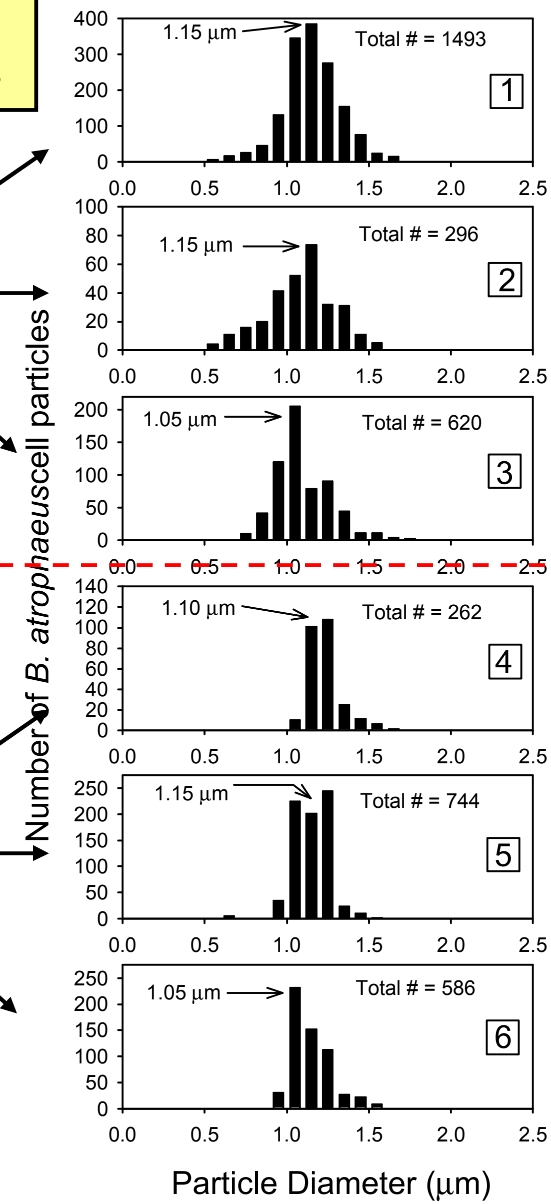


BAMS Aerodynamic Diameters of *B. atrophaeus* Cells

Distribution of aerodynamic diameters measured for cells matching each of the six mass spectral types

Mass spectral types (1-3): Broad size distributions.
Represent the vegetative cell states and the early to intermediate sporulation stage states

Mass spectral types (4-6): Tighter size distributions.
Represent the towards fully formed and fully formed endospores.



Final Points

(1) BAMS could be used to rapidly follow gross morphological and metabolic changes in large populations of *B. atrophaeus* cells during the process of endospore formation, at the *single cell* level.

(2) More work will be needed to improve and fully understand the data acquired by BAMS.

(3) With some work and improvement, BAMS may prove to be a powerful complementary technique to those commonly used and established in the field.

Thank you