

# On an Archaeal Proteome: Quantitative Proteomics of *M. acetivorans* by $^{14}\text{N}/^{15}\text{N}$ Metabolic Labeling and Nano-LC LTQ-FT MS

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## Introduction

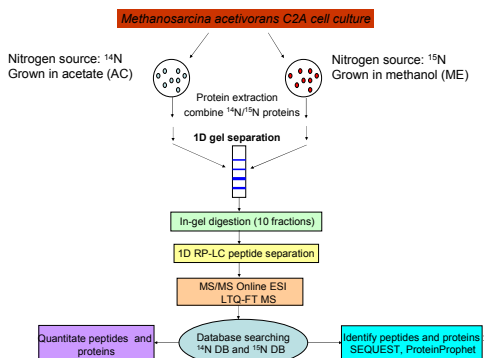
All of life is classified into three domains (Archaea, Bacteria and Eukarya) of which the Archaea is the least understood despite their importance to the biosphere. Methane-producing microbes, the largest known group representing the Archaea, are essential to the global carbon cycle as well as to the alternative energy source<sup>1,2</sup>. *Methanosarcina acetivorans* is ideally suited for characterization of the proteome of an acetate-utilizing methanogenic species as it has a tractable genetic exchange system, and the genome sequence is available<sup>3</sup>. Furthermore, the genome of *M. acetivorans* at 5,751,492 base pairs is the largest reported for any of the Archaea, reflecting the expanse of metabolic diversity yet to be discovered.

Mass spectrometry (MS) is playing an ever-increasing role in biological research, especially in the proteomics area. Quantitative proteomics based on isotopic labeling and MS detection enables high throughput investigation of relative protein abundances<sup>4,5</sup>. Achieving quantitative accuracy, especially for low abundant proteins, requires high resolution and high mass accuracy mass spectrometry, such as time of flight (TOF) or Fourier transform ion cyclotron resonance (FTICR) MS. FTICR-MS is playing an increasingly important role in the characterization of complex biosamples because of its capability to provide higher confidence of identification, unmatched resolution and increased dynamic range. In this study, an  $^{14}\text{N}/^{15}\text{N}$  metabolic labeling strategy was applied to an anaerobic archaea, *Methanosarcina acetivorans*, grown on different substrates (acetate and methanol). Using ESI LTQ-FTICR MS combined with 1D SDS PAGE and nano-LC separation techniques, we performed protein identification and quantitative analysis based on the LC-MS data.

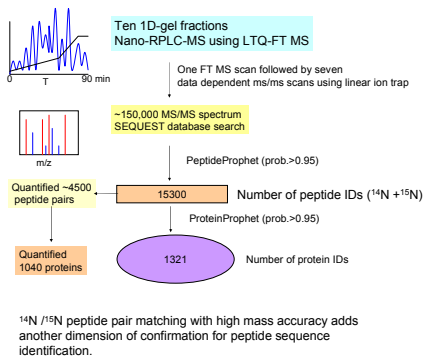
## Methods

*M. acetivorans* C2A was grown in acetate or methanol media, enriched with  $^{14}\text{NH}_4\text{Cl}$  or  $^{15}\text{NH}_4\text{Cl}$  (>98%), respectively, as the sole nitrogen sources. Whole cell lysates of acetate- and methanol-grown cells were combined to generate 1:1 (w/w) mixture of  $^{14}\text{N}$ - and  $^{15}\text{N}$ -labeled proteins. Following 1D gel pre-fractionation, band excision and in-gel digestion, nano-RPLC coupled with the online ESI LTQ-FT MS were used to analyze the peptide mixtures. After database searching with Sequest and protein identification using Protein Prophet, relative abundance ratios of identified peptide pairs were calculated using an in-house program, which compares the chromatography peak areas of the  $^{14}\text{N}/^{15}\text{N}$  peptide pairs.

## 1. Experimental steps involved in the qualitative and quantitative approach



## 2. Data acquisition and processing



## 3. Distribution of identified proteins on the gel fractions

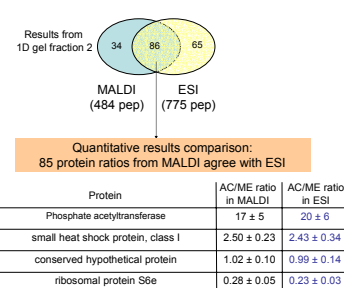
kDa	Fractions	Protein ID Number		Unique Number	
		AC/ME	AC/ME	AC/ME	AC/ME
265	10	61/56	17/30		
116	9	81/96	51/56		
	8	76/86	52/57		
55	7	102/121	64/88		
	6	103/122	81/93		
36	5	125/160	101/131		
	4	78/98	59/75		
29	3	124/151	110/132		
	2	130/149	105/119		
20	1	109/101	109/101		
<b>Total</b>		<b>989/1040</b>	<b>749/882</b>		

\* Unique means the proteins are only identified in that fraction.

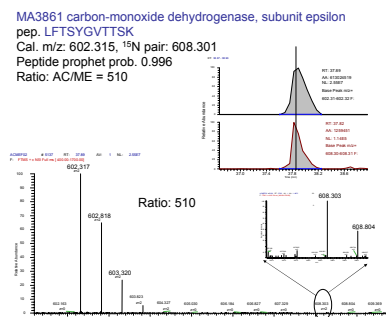
## 4. 2D gel image vs. LC-MS approach for quantitation

- Less than 100 proteins have been quantified using a 2D gel image analysis approach; LC-MS combined with isotopic labeling was enabled the quantification of more than 1000 proteins;
- LC-MS provide higher dynamic range and more accurate ratios than gel image analysis;
- LC-MS is a relatively high-throughput approach compared with the 2D gel image;
- 2D gels can provide protein MW. and pI directly, while bottom-up LC-MS approach can not readily provide this.

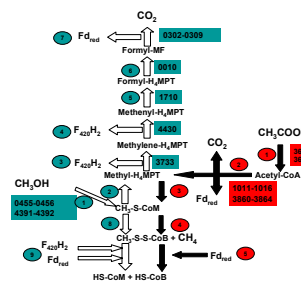
## 5. LC-MALDI TOF/TOF MS vs. LTQ-FT MS



## 6. LTQ-FT MS quantitation example



## 7. Some regulated proteins in the methanogenesis pathway



## Conclusions

- With the nano-LC MS approach, ~25% of *Methanosarcina acetivorans* proteome, which is the largest in the archaea domain, has been quantitatively detected using  $^{14}\text{N}/^{15}\text{N}$  metabolic labeling in different growth media (methanol and acetate).
- Compared to 2D gel image analysis, quantitative proteomics analysis based on high resolution FTICR MS data increased the accuracy and throughput for protein abundance measurement, especially for low abundant proteins and proteins which have similar pI and MW.
- The combination of accurate mass from FTICR MS, Sequest Xcorr value, ProteinProphet prob. score and pair matching of co-eluting  $^{14}\text{N}/^{15}\text{N}$  isotopic labeled peptides provided highly confident protein identification and quantitation.
- A comparison of the relative abundance of proteins between the two growth conditions (acetate and methanol) provided a more detailed understanding of the acetate pathway in this species (to be published).

## References

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Fig 7. Pathways of methanogenesis from methanol or acetate in *Methanosarcina* species showing differential synthesis of proteins of *Methanosarcina acetivorans*.

- Open arrows and circled numbers in green show the steps (1-9) for dismutation of methanol to methane. The solid arrows and circled numbers in red show the steps (1-5) for the conversion of acetate to methane.
- Substrates and products are shown in large bold font.
- The four-digit numbers in green box correspond to the loci numbering of genes for which the products were found to be elevated in methanol- versus acetate-grown *M. acetivorans*. The products of genes in red box were found to be elevated in acetate- versus methanol-grown cells.
- Abbreviations: Fd<sub>red</sub>, ferredoxin (reduced); MF, methanofuran cofactor; H<sub>4</sub>MPT, tetrahydromethanopterin cofactor; CoA, coenzyme A; CoM, coenzyme M; CoB, coenzyme B; F<sub>420H2</sub>, coenzyme F<sub>420</sub> (reduced).