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Identification of *Methanococcus jannaschii* Proteins in 2-D Gel Electrophoresis Patterns by Mass Spectrometry

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Introduction

The genome of *Methanococcus jannaschii* has been sequenced completely and has been found to contain approximately 1,770 predicted protein-coding regions. When these coding regions are expressed and how their expression is regulated, however, remain open questions. In this work, mass spectrometry was combined with two-dimensional gel electrophoresis to identify which proteins the genes produce under different growth conditions, and thus investigate the regulation of genes responsible for functions characteristic of this thermophilic representative of the methanogenic Archaea.

Methods

The proteins of *Methanococcus jannaschii* were separated by 2-D gel electrophoresis and detected using Coomassie blue or silver stain. A number of protein spots were chosen at random, excised from the gel, destained by 25 mM ammonium bicarbonate/50% acetonitrile, and digested *in situ* with trypsin. The resulting peptides were then recovered using 50% acetonitrile/5% TFA, and analyzed by Kratos Kompact mass spectrometry. α-Cyano-4-hydroxy-cinnamic acid was used as the matrix.

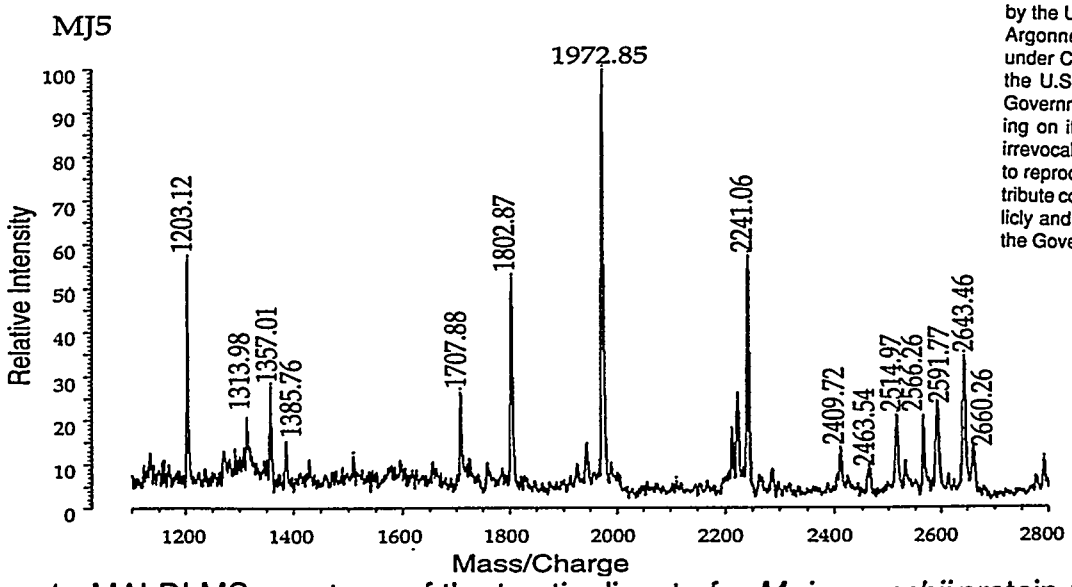

Figure 1. MALDI MS spectrum of the tryptic digest of a *M. jannaschii* protein (MJ5).

Results

Different staining methods were compared for their compatibility with mass analysis. A modified silver staining protocol turned out to be the method of choice due to its high protein detection

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sensitivity and low mass background. Figure 1 shows the MALDI MS spectrum of the tryptic digest of a protein labeled "Therm" in Figure 2. The resulting peptide masses were used to search public databases for matches with masses of predicted peptide fragments from known DNA and protein sequences. The search engine we used extensively is MS-Fit (http://prospector.ucsf.edu/ucsfhtml/msfit.htm). The molecular weight and pI obtained from the 2D gel were used to facilitate the search. Thirty spots were investigated and 20 of them have been identified so far. The 2D gel with identified gene products is shown in Figure 2. Four spots have been identified as MCRIγ (methyl coenzyme M reductase I, subunit gamma). Two genes encoding this protein have been identified in *M. Jannaschii*, suggesting two proteins should be produced. The additional two proteins could be post-translationally modified forms of the protein gene products.

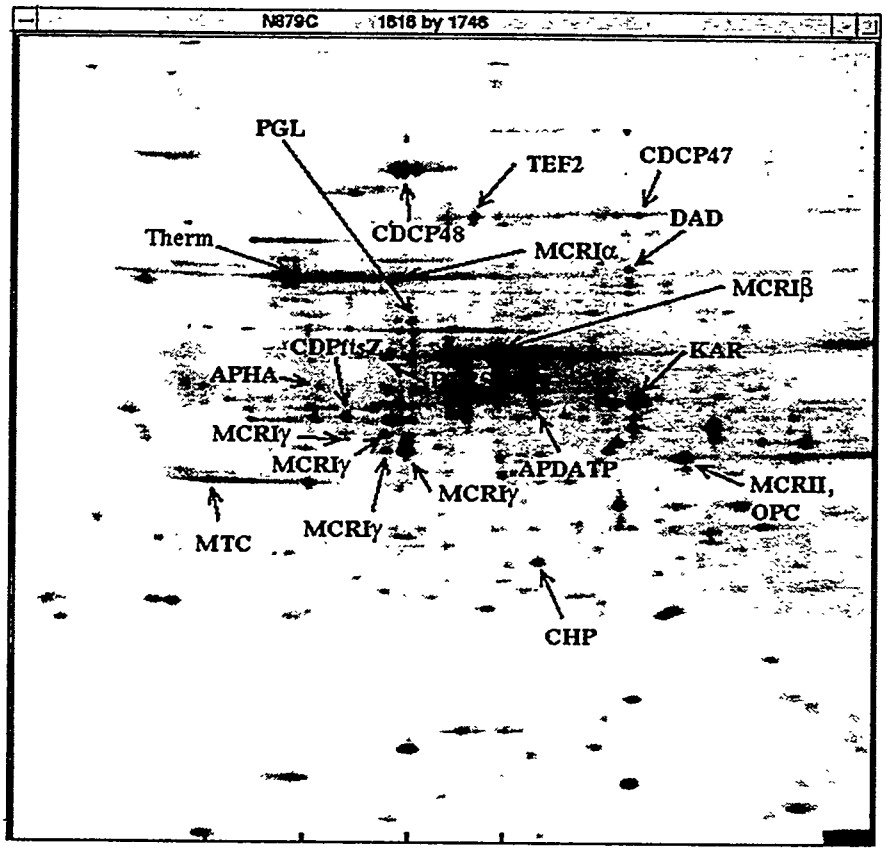

Figure 2. *Methanococcus jannaschii* proteins identified by MALDI MS.

Conclusions

This study demonstrated that fast and positive identification of proteins of *Methanococcus jannaschii* can be obtained by peptide mass mapping. A majority of the proteins analyzed thus far have been matched with a gene sequence. In subsequent studies, aspects of cell growth environment (e.g., temperature, ionic strength, gas pressure, growth phase) will be manipulated to trigger controlled adaptation responses that can be correlated with quantitative changes in specific proteins. Such changes will be monitored by 2-D gel electrophoresis coupled with mass spectrometry to reveal sets of proteins that are co-regulated and thus allow a description of regulation mechanisms in this organism.