Proteome Profiling of *Corynebacterium glutamicum* by 2-D PAGE and LC-MS/MS

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Overview

The proteomic profiles of *Corynebacterium glutamicum* are being studied under different growth phases by 2-D PAGE and LC-MS/MS. Characteristic MS/MS data from two types of tandem mass spectrometers are compared. The data presented represents one growth phase of *C. glutamicum*.

Introduction

Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) coupled with mass spectrometry are powerful tools for the characterization of complex proteomes. 2-D PAGE is used to separate the complex protein mixture: the first dimension resolves by pI, and the second dimension by molecular weight. Capillary HPLC and tandem mass spectrometry are employed to determine the identity of protein spots in the 2-D gels. Spots of interest are excised from the gel, subjected to proteolytic digestion, then analyzed by LC-MS/MS.

The resulting data are searched against protein databases. Peptide matches are found on the basis of MW and fragment ions; these peptide matches are collated to produce protein "hits". Typical MS/MS spectra from a quadrupole ion trap MS and hybrid quadrupole-time of flight MS are compared in terms of the characteristic ion types produced by each instrument.

Methods

2-D PAGE was conducted at the Research Institute of Innovative Technology for the Earth, using precast gels and apparatus from Amersham Biosciences (Uppsala, Sweden).[1,2] Briefly, collected cells were disrupted using a Sonicator (Misonix Inc., Farmingdale, NY). After ultracentrifugation, soluble proteins were acetone-precipitated and the pellet was resuspended in solubilization buffer (9 M Urea, 4% CHAPS, 1% w/v DTT, 1% Pharmalyte 3-10). For IEF, 300 µg of proteins were focused for 175 kVh in Immobiline Dry Strips (18 cm) in an IPGphor apparatus. ExcelGel Gradient XL 12-14 and Multiphor II were used for SDS-PAGE as recommended by the manufacturer. Gels were stained with colloidal Coomassie solution (Coomassie Blue G-250 0.1%, methanol 34%, acetic acid 0.1%, $(NH_4)_2SO_4$ 17%).[3]

Methods, continued

Spots of interest were excised from the gels and sent to Stanford University Mass Spectrometry for further analysis. Samples were reduced with dithiothreitol and alkylated with acrylamide, then digested with Promega modified trypsin. LC-MS/MS was run using peptide CapTraps (Michrom, Auburn, CA) for online desalting, followed by capillary HPLC and nanoelectrospray ionization of the column output directly into the mass spectrometer.

One of two HPLC-MS systems were employed for the LC-MS/MS analysis: 1) CapLC and hybrid quadrupole-time of flight MS (Q-Tof API US, Micromass, Manchester, UK) with 0.075 x 100 mm C18 column (LC Packings, Amsterdam, Netherlands); 2) Famos-Switchos-Ultimate capillary LC system (LC Packings, Amsterdam, Netherlands) and LCQ Deca XP Plus MS (ThermoFinnigan, San Jose, CA) with 0.150 x 100 mm C18 column (MicroTech Scientific, Vista, CA) .

The MS/MS data was searched against the NCBI NR database using the online Mascot MS/MS Ions Search program at www.matrixscience.com (Matrix Science, London, UK).

Results

FIGURE 1. 2-D gels produce visual profiles of *C. glutamicum* proteins. The reproducibility of the protein patterns can be seen in the overlapping pI ranges. Dotted red lines delineate the edges of the overlapping regions; yellow arrows indicate a few of the homologous protein spots.

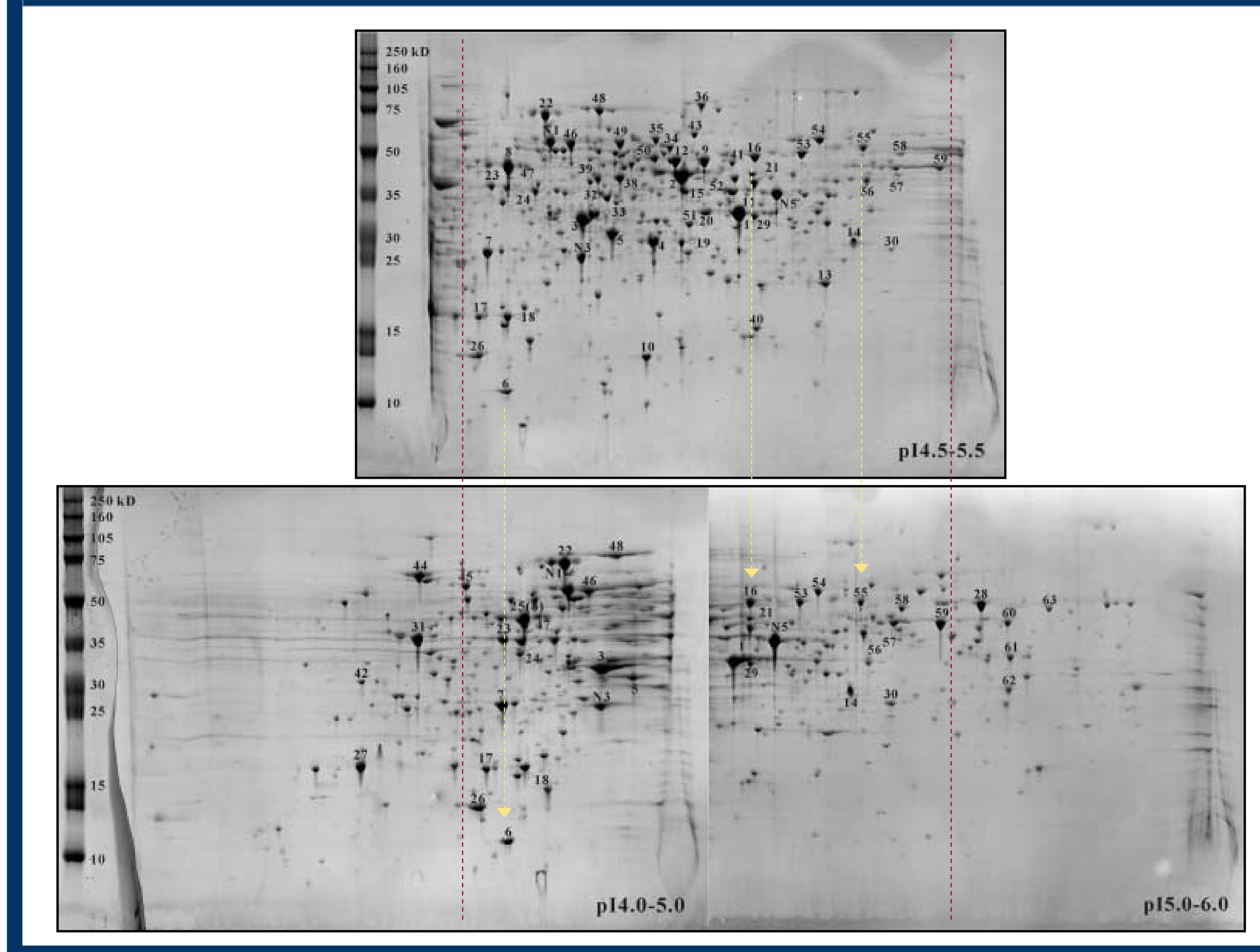


Table 1. Protein ID results. The top protein hit for each spot is listed along with the molecular weight of the protein, NCBI NR database accession number, and Mascot probability-based score.

Spot	MW	Accession	Score	Primary Protein Identified
1	37.4	21903368	>1500	Fructose-biphosphate aldolase
2	44.3	2625092	>1500	Argininosuccinate synthase
3	33.4	19553758	663	Cysteine synthase
4	27.3	19551647	860	Phosphoglycerate mutase 1
5	29.3	21324795	750	Tranlation elongation factor Ts
6	10.8	23813777	226	10 kDa Chaperonin
7	27.1	4062842	799	L-2,3-Butanediol dehydrogenase
8	45.0	23814062	940	Enolase (2-Phosphoglycerate dehydrogenase)
9	49.9	1955223	1937	Fumarase
10	44.9	21325141	128	Nucleoside diphosphate kinase
11	35.6	10039451	806	H+ ATPase gamma subunit
12	49.8	1955223	1670	Fumarase
13	22.1	19554112	334	Superoxide dismutase
14	24.6	19551984	461	Ribosome-associated protein Y
15	36.2	21324357	1051	Glyceraldehyde-3-phosphate dehydrogenase
16	54.5	19553765	1818	Acetyl Co-A hydrolase
17	17.9	19553894	306	Inorganic pyrophosphatase
18	20.1	19551284	134	Peptidyl-prolyl cis-trans isomerase
19	59.8	1324330	263	ABC-type transporter, ATPase component
20	37.4	98727	864	Fructose-biphosphate aldolase
21	35.6	19552240	850	Putative fructose-1,6-bisphosphatase
22	72.6	4579900	1079	Transketolase
23	40.2	19552055	953	Phosphoserine aminotransferase
24	39.4	19554190	1153	Myo-inositol-1-phosphate synthase
25	45.0	23814062	1005	Enolase (2-Phosphoglycerate dehydrogenase)
26	13.3	19551729	156	Ribosomal protein L7/L12
27	18.0	19552312	195	Peroxiredoxin
29	51.7	2497531	571	Pyruvate kinase
30	33.4	19553758	139	Cysteine synthase
31	42.7	19552799	932	3-Phosphoglycerate kinase
32	34.5	18073519	664	Malate dehydrogenase
33	31.9	19551923	212	Thioredoxin reductase
34	56.0	19551841	802	GMP synthase
35	65.7	23308896	664	1,4-Alpha-glucan branching enzyme
36	93.2	19553971	1686	ATPase with chaperone activity
38	38.7	12406798	404	Antigen 84
40	43.9	19553328	731	Methionine synthase II
41	51.1	19553865	654	NAD-dependent aldehyde dehydrogenase
42	32.1	19552452	319	Electron transfer flavoprotein alpha-subunit
43	63.4	19551930	1232	Acyl-CoA carboxylase
44	66.2	19553990	1661	70 kDa Heat shock chaperonin
45	59.6	19553137	1071	Phosphoenolpyruvate-protein kinase
46	56.7	19551832	>1500	Chaperonin GroEL
47	37.8	19553779	608	Phosphoribosylaminoimidazol (AIR) synthase
48	78.2	19551738	1232	Elongation factor G
49	58.9	9757619	817	H+ ATPase alpha subunit
50	55.3	23308979	650	NAD-dependent aldehyde dehydrogenase
51	33.7	19552014	546	Pyridoxine biosynthesis enzyme
52	46.8	19551884	1105	O-Acetylhomoserine sylfhydrylase
53	55.0	19553205	921	Predicted dehydrogenase
54	58.8	19551506	1064	Catalase
55	55.8	19552088	600	Phosphoribosylaminoimidazolecarboxamide formyltransferase
56	35.2	1620903	137	D-2-Hydroxyisocaproate dehydrogenase
57	46.6	19552219	883	Glycine hydroxymethyltransferase
58	53.3	19553476	775	Class II glycyl-tRNA synthetase
59	46.6	19552219	757	Glycine hydroxymethyltransferase
60	49.5	19553277	517	Glutamate dehydrogenase/Leucine dehydrogenase
61	46.6	19552219	106	Glycine hydroxymethyltransferase
63	50.9	20126695	583	Dihydrolipoamide dehydrogenase

Figure 2. Peptide fragment ion nomenclature.[4] The most commonly observed types are b and y ions, which are generated by collision-induced dissociation of the peptide bond.[5]

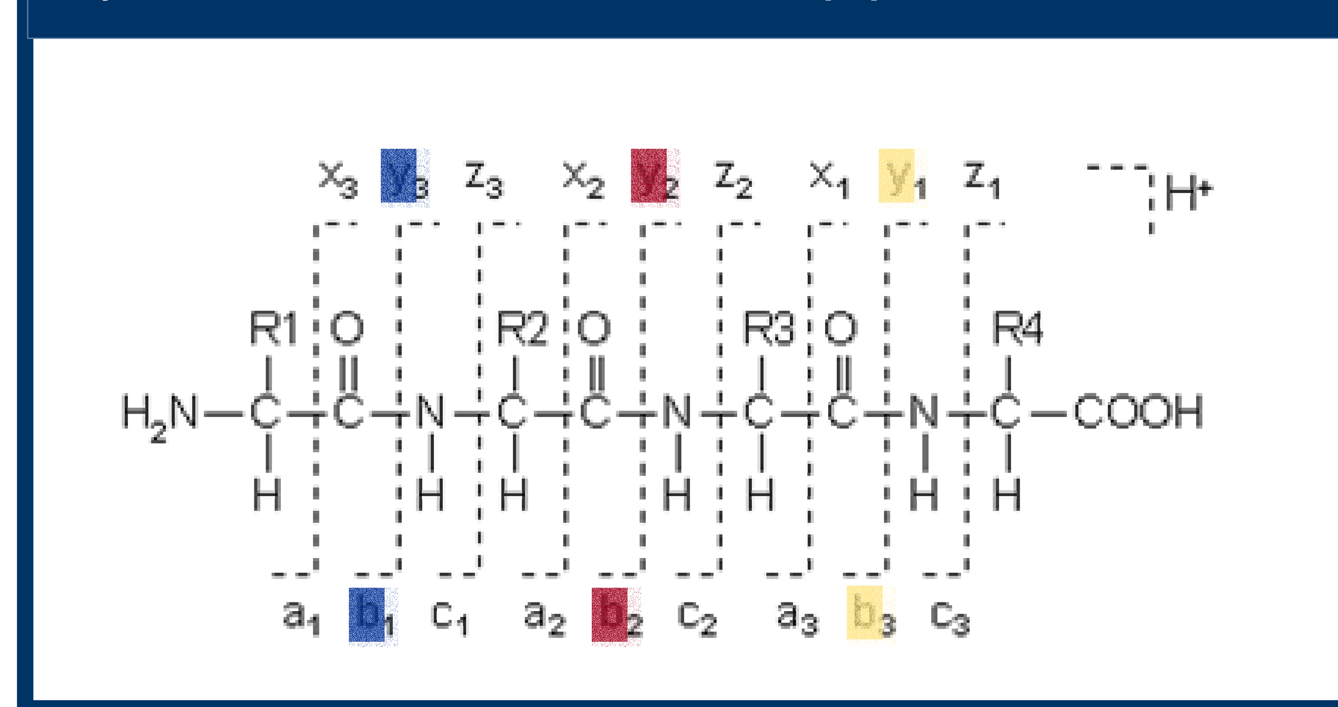
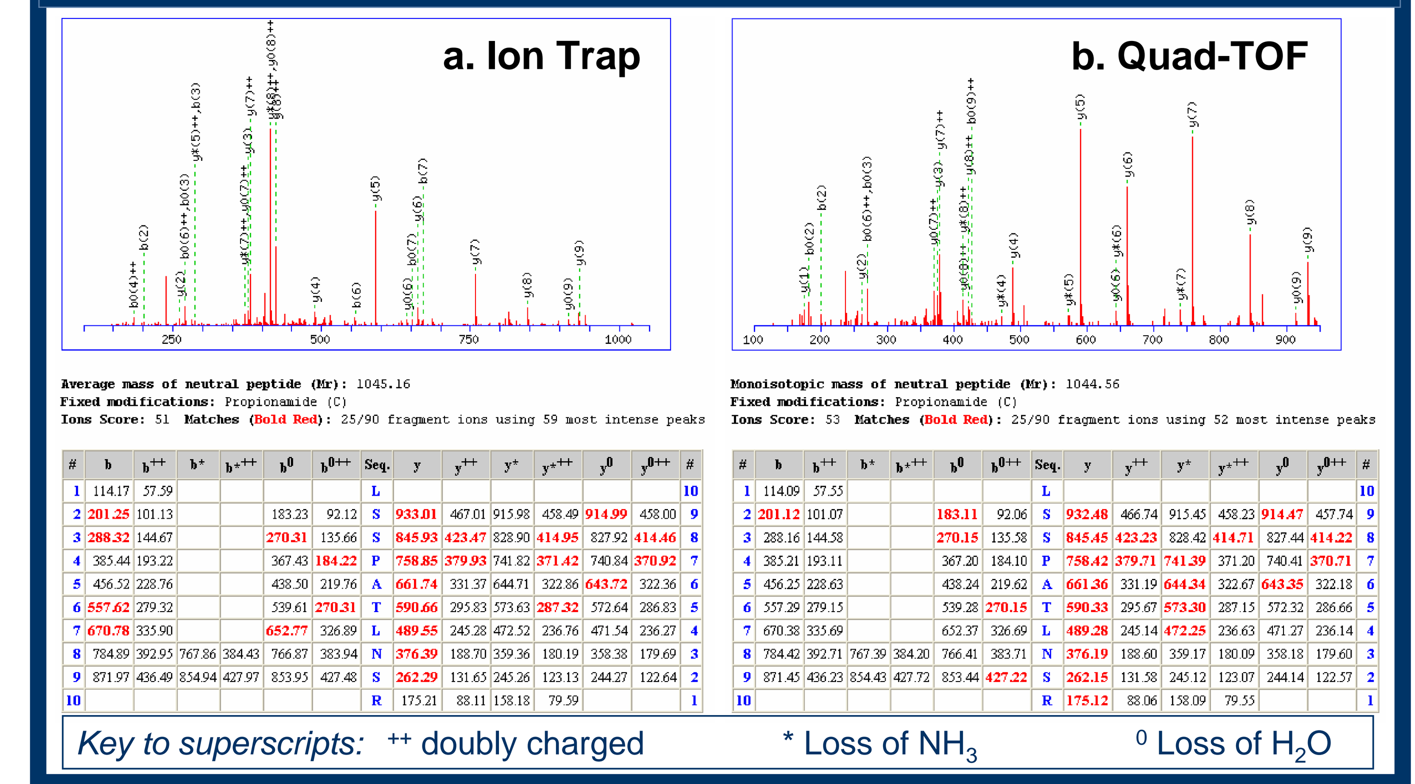


FIGURE 3. MS/MS spectra of peptide LSSPATLNSR produced by a.) Deca XP Plus ion trap MS and b.) Q-Tof hybrid quadrupole-time of flight MS. Low-energy collision-induced dissociation (CID) in the ion trap and high-energy CID in the quad-TOF produce different distributions of fragment ions. The high mass y-ion series seen in Fig. 3b is characteristic of Q-Tof MS/MS data. A large number of y* ions are also observed.



Conclusions

When investigating systemic changes in the proteome, large scale 2-D gel studies can be quite effective. With good gel-to-gel reproducibility, once the identity of each spot is determined by mass spectrometry, up- or down-regulation of the proteins can be monitored by simple visual imaging of the gels. Proteins which are differentially expressed under the various growth conditions will be focused on in further studies. Both quadrupole ion trap and quadrupole-time of flight mass spectrometers are effective in carrying out this type of investigation.

References

[1]Hermann, T., M. et al. *Electrophoresis* **2000**, *21*, p. 654-659.
[2]Schaffer, S. et al. *Electrophoresis* **2001**, *22*, p. 4404-4422.
[3]Matsui, N. M. et al. *Methods Mol. Biol.* **1999**, *112*, p. 307-311.
[4]Biemann, K. *Methods Enzymol.* **1990**, *193*, p. 886-887.
[5]Figure adapted from http://www.matrixscience.com/help/mis_help.html

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