Suping Zheng[1]
Monica A. Ponder[2, 3]
Janice YangJen Shih[4]
James M. Tiedje[2, 3]
Michael F. Thomashow[2, 3]
David M. Lubman[1, 5]

[1]Department of Chemistry, University of Michigan, Ann Arbor, MI, USA
[2]Center for Genomic and Evolutionary Studies on Microbial Life at Low Temperature, Michigan State University, East Lansing, MI, USA
[3]Center for Microbial Ecology, Michigan State University, East Lansing, MI, USA
[4]Department of Biological Chemistry, University of Michigan, Ann Arbor, MI, USA
[5]Department of Surgery, University of Michigan Medical Center, Ann Arbor, MI, USA

Received March 24, 2006
Revised June 29, 2006
Accepted July 10, 2006

Research Article

A proteomic analysis of *Psychrobacter articus 273-4* adaptation to low temperature and salinity using a 2-D liquid mapping approach

Psychrobacter 273-4 was isolated from a 20 000–40 000–year-old Siberian permafrost core, which is characterized by low temperature, low water activity, and high salinity. To explore how *273-4* survives in the permafrost environment, proteins in four *273-4* samples cultured at 4 and 22°C in media with and without 5% sodium chloride were profiled and comparatively studied using 2-D HPLC and MS. The method used herein involved fractionation *via* a pH gradient using chromatofocusing followed by nonporous silica (NPS) RP-HPLC and on-line electrospray mass mapping. It was observed that 33 proteins were involved in the adaptation to low temperature in the cells grown in the nonsaline media while there were only 14 proteins involved in the saline media. There were 45 proteins observed differentially expressed in response to salt at 22°C while there were 22 proteins at 4°C. In addition, 5% NaCl and 4°C showed a combination effect on protein expression. A total of 56 proteins involved in the adaptation to low temperature and salt were identified using MS and database searching. The differentially expressed proteins were classified into different functional categories where the response of the regulation system to stress appears to be very elaborate. The evidence shows that the adaptation of *273-4* is based primarily on the control of translation and transcription, the synthesis of proteins (chaperones) to facilitate RNA and protein folding, and the regulation of metabolic pathways.

Keywords: Cold adaptation / Combination effect / Salinity adaptation / 2-D HPLC and MS
DOI 10.1002/elps.200600173

1 Introduction

Psychrobacter is a "cold-loving" bacterium, which has been isolated from various sources, including sea water [1, 2], sediments [3, 4], coast [5], soil [6], permafrost [7], ice [8–10], refrigerators [11], animals [12, 13], and food [13–17]. *Psychrobacter 273-4* examined in this work was isolated from a 20 000–40 000–year-old Siberian permafrost core [18], which is characterized by low temperature (−10°C), low water activity (aw = 0.85–0.9), poor nutrition, and high salinity. *Psychrobacter 273-4* is able to survive at a temperature of −10°C with optimal growth rate at 22°C. Furthermore, it grows rapidly at low temperatures. In addition, it is able to tolerate up to 15% salt. The permafrost habitat of *Psychrobacter 273-4* has very similar characteristics to that of certain known astral bodies. Therefore, *Psychrobacter 273-4* was selected as a model to study cold and salinity adaptation for understanding further the adaptations of life on other astral bodies since it is well adapted to the permafrost environment.

Temperature and salinity are two crucial factors that influence the distribution of bacteria in nature. Low temperature and high salinity are usually treated as environmental stress factors for the survival of bacteria. In order to survive in an extreme environment, bacteria must develop special biochemistry and its regulation to maintain the integrity of the cell and sustain its proper function. As one of the fundamental components of a cell, proteins play an important role in cell function. Hence, the investigation on the proteome is essential to understand the impact of low temperature and high salinity.

Correspondence: Professor David M. Lubman, Department of Surgery, The University of Michigan, 1150 West Medical Center Drive, Building MSRB1, Rm A510B, Ann Arbor, MI 48109, USA
E-mail: dmlubman@umich.edu
Fax: +1-734-615-2088

Abbreviations: CF, chromatofocusing; **CHCA**, α-cyano-4-hydroxycinnamic acid; **EB**, elution buffer; **NPS**, nonporous silica; **SB**, start buffer; **TCEP**, tri(2-carboxyethyl) phosphine hydrochloride; **TSB**, tryptic soy broth

Salt effects have been extensively studied, especially in halophiles. The response of bacteria to low temperature has been extensively studied in certain bacteria and various regulation systems have been proposed to elucidate the mechanism [19, 20]. However, most of these studies focused on cold shock. Research on long-term low-temperature adaptation has been initiated recently as a result of growing interest in astrobiology due to exceptional features of psychrophiles. In particular, low-temperature adaptation of an archae *Methanococcoides burtonii* and a Gram-positive bacterium *Bacillus sychrosaccharolyticus* were investigated using proteomic tools [21–24], and proteins related to low temperature were identified.

Research on salt adaptation has focused primarily on plants in high-salinity environments and halophilic bacteria, with recent interest directed to other bacteria [25–30]. Most of the studies of bacteria were conducted on a Gram-positive soil bacterium *Bacillus subtilis*. No proteomic studies of salt adaptation of any psychrophile have been reported to date. It is the first time that the salt adaptation of a Gram-negative bacterium is studied.

Progress on low-temperature and salinity adaptation research has been achieved mainly through genomic or physiological studies. Proteomic analysis provides the dynamic information of cells, which reflects the actual live status of cells. The genome of *273-4* with 2147 ORFs is relatively small which could make it simpler to understand its cold adaptation mechanisms. How it survives in a harsh environment with such a small genome is of great interest. Identification of all the proteins including those differentially expressed under different conditions will facilitate the understanding of the adaptation process.

As a traditional proteomic separation method, 2-DE provides high resolution and sensitivity for the separation of proteins [31, 32]. However, it also has several inherent limitations, including poor reproducibility, low recovery, and it is labor intensive. To overcome these drawbacks, a column-based 2-D liquid phase separation method has been developed as an alternative [33, 34]. This method fractionates proteins by p*I* in the first dimension by chromatofocusing (CF), and subsequently separates proteins by hydrophobicity using nonporous RP-HPLC (NPS-RP-HPLC). The final eluent is collected for PMF, and/or is introduced into an ESI mass spectrometer for molecular weight determination.

In an effort to understand the biological process involved in cold and salt adaptation, four *273-4* samples cultured at 22 and 4°C in 1/2 tryptic soy broth (TSB) with or without 5% NaCl were selected to study the impact of low temperature and salt on adaptation using 2-D HPLC and MS. All the proteins including those differentially expressed were further identified using MALDI-TOF-MS or MALDI-TOF/TOF-MS/MS and ESI-TOF-MS.

2 Materials and methods

2.1 Chemicals

N-Octyl-β-D-glucoside (OG), urea, thiourea, iminodiacetic acid, bis-tris propane, Trizma base (Tris), PMSF, ammonium bicarbonate, TFA, formic acid, sodium iodide, NaCl, hydrogen chloride, α-cyano-4-hydroxycinnamic acid (CHCA), isopropanol, and ACN were purchased from Sigma (St. Louis, MO, USA). Tri(2-carboxyethyl) phosphine hydrochloride (TCEP) was purchased from Pierce (Rockford, IL, USA). Sequencing grade TPCK-modified porcine trypsin was purchased from Promega (Madison, WI, USA). Standard buffer solutions of pH 4, 7, and 10, were obtained from Fisher Scientific (Burr Ridge, IL, USA). Protein assay and BSA were purchased from BioRad Laboratories (Richmond, CA, USA). Start and elution buffers were obtained from Beckman Coulter (Fullerton, CA, USA), and the pH was adjusted when necessary. Water was purified by a Milli-Q water filtration system (Millipore, Bedford, MA, USA). The reagents were used without further purification.

2.2 Cell culture

Psychrobacter articus 273-4 was acclimatized to low water activity and low temperature by culturing at least four times in 1:2 dilution of 1/2 TSB or 1/2 TSB + 5% NaCl at 4 or 22°C. The permafrost isolate was grown to an absorbance (OD600) of 0.3 in 1/2 TSB + 5% NaCl or 1/2 TSB at 4 and 22°C in 325 mL of media in four 500 mL Erlenmeyer flasks. A 300 mL culture of each sample was spun down for proteomics. Samples were prepared in duplicates.

2.3 Sample lysis

The cells were rinsed using 50 mM Tris-HCl solution (pH 7.6) three times. The cell pellet was resuspended in 4 mL of 50 mM Tris-HCl solution (pH 7.6), and sonicated on ice for 10 s with 30 s intervals until the solution became clear. PMSF was added at 2 mM concentration once the cells were disrupted. Urea, thiourea, *n*-octylglucoside, and TCEP were added into the solution at the end so that the final lysate contains 6 M urea, 2 M thiourea, 50 mM Tris-HCl, 2% *n*-octylglucoside, and 5 mM TCEP. The lysate was ultracentrifuged at 125 000 × *g* for 60 min. The supernatant was collected and stored at −80°C for further use.

2.4 Protein quantification

The lysates were quantified using the BioRad protein assay based on the Bradford method using different dilutions of BSA as the standard according to the procedure described in the manual. The protein concentration of four samples varied from 2 to 12 mg/mL.

2.5 CF and RP separation

Protein mapping (CF and RP separation) was performed on the PF2D ProteomeLab (Beckman Coulter). In the CF, equilibration/start buffer (SB) and elution buffer (EB) were used to generate the internal pH gradient in the column. The pH range was set by the pH of the SB (pH 8.6) and the EB (pH 3.9), respectively. The sample was buffer exchanged using a PD-10 G-25 column purchased from Amersham Pharmacia Biotech (Piscataway, NJ, USA) before loading onto the CF column. The CF column was first equilibrated with SB at 0.2 mL/min, and then the exchanged sample (5 mg proteins) was loaded onto the column. EB was applied to elute proteins by their p*I* when a stable baseline was reached 30 min later. Following pH elution the column was washed with 1 M NaCl to remove the proteins binding on the column by electrostatic interaction at 105 min, then deionized water was applied to remove the NaCl from the column. Finally, isopropanol was employed to clean the column. The CF fractions were collected at 2.5 min intervals using a fraction collector. At the end of the collection certain fractions were further combined based on the assumption that intensity of the peak on the chromatogram is proportional to the abundance of the proteins; therefore, two adjacent CF fractions having very low UV absorbance were combined to shorten the run time of the second dimension. Each CF fraction (250 μL) was subsequently loaded onto the NPS-RP column for the second dimension separation by an autosampler. The separation was performed using water with 0.1% TFA (solvent A) and ACN with 0.1% TFA (solvent B) at a flow rate of 0.75 mL/min. The gradient profile was as follows: 5% B for 3 min, 5–25% B in 1 min, 25–31% B in 1 min, 31–37% B in 8 min, 37–41% B in 8 min, 41–67% B in 2 min, 67–100% B in 2 min, 100% B for 1 min, and 100–5% B in 1 min. The column was maintained at 45°C. The fractions were collected every 0.93 min intervals from 5 to 35 min using a Gilson FC204 fraction collector and stored at −80°C for further analysis.

2.6 HPLC-ESI-TOF-MS

HPLC-ESI-TOF-MS was conducted on a Beckman Coulter Gold model 126 binary pump (Beckman Coulter) coupled with an ESI-TOF-MS (Micromass, Manchester, UK) using the CF fractions for protein molecular weight analysis. The separation was performed using the same NPS-RP column and the same gradient at a flow rate of 0.5 mL/min but with the addition of formic acid to the solvents A and B to a final concentration of 0.3% to increase the ionization efficiency for ESI-MS. The eluate was split 2:3 after column to be introduced into the ESI-TOF-MS and UV detector, respectively; the eluent from the UV detector was manually collected by peak for further analysis. The LCT was operated under positive ion mode, and the parameters were set as follows: capillary voltage 3200 V, sample cone 45 V, RF lens 750.0 V, extraction cone 2.0 V, desolvation temperature 350°C, source temperature 110°C, desolvation gas flow 650 L/h, with the maximum nebulizer gas flow. The external calibration was performed using direct infusion of NaI-CsI solution prior to the experiment, where spectra between *m/z* 500 and 4000 were acquired, and deconvoluted into mass spectra to obtain experimental molecular weights of proteins using the MaxEnt 1 deconvolution algorithm provided by Masslynx.

2.7 Tryptic digestion of the NPS-RP-HPLC fractions

The selected fractions collected from PF2D were digested using trypsin. Each fraction was concentrated to 80 μL using a SpeedVac, followed by the addition of 10 μL of 1 M ammonium bicarbonate to adjust the pH, and finally 0.25 μg of TPCK-modified trypsin was added. The solution was incubated at 37°C for 12 h on a shaker. The digestion was terminated by the addition of 50 μL of fresh 0.1% TFA into the solution. The fractions collected from the HPLC detector coupled with ESI-MS were digested using the same protocol, but 40 μL of 1 M ammonium bicarbonate was added to each fraction instead to neutralize the TFA and formic acid.

2.8 MALDI sample preparation and data acquisition

The tryptic digested peptides were desalted using 2 μm C18 ZipTips (Millipore) and eluted with 5 μL of 60% ACN with 0.1% TFA. A saturated CHCA solution in 60% ACN with 0.1% TFA was diluted in 1:4 with 60% ACN with 0.1% TFA. Angiotensin I, ACTH 1-17, and ACTH 18-39 were added to the solution as internal standards. About 0.5 μL of the matrix solution and 0.5 μL of sample were spotted on the MALDI plate using a two-layer method [35, 36]. MALDI-MS analysis was performed on a TOF-SPEC 2E (Waters-Micromass) in the delayed-extraction reflectron mode with positive polarity. A 337 nm Nd:YAG laser was used at 50% of coarse laser energy and 20–90% of fine laser energy. The source voltage was 20 kV

and extraction voltage was set at 1:1 to the source voltage. The pulse voltage was set at 2300 V, and reflectron voltage was 24 500 V. The delay time was 520 ns. The laser frequency was 5 Hz and 15–20 shots were collected over the *m/z* 0–4000 range for each spectrum.

2.9 MALDI data analysis and database search

Each spectrum was calibrated using internal standards and the monoisotopic peptide mass list was generated using Masslynx and submitted to the *273-4* unpublished database provided by Joint Genome Institute through the MS-FIT search engine for protein identification. The search was carried out allowing one missed cleavage, including peptide *N*-terminal Gln to pyroGlu, oxidation of M and protein *N*-terminal acetylated as possible modifications, and allowing 100 ppm mass tolerance. No limitation was set for the molecular weight and p*I*. The criteria for protein identification by MS was established based upon previous work [37]. In order to obtain a confident result, the spectrum was subjected to manual inspection if the sequence coverage was greater than 20% when the molecular weight was less than 60 K, or the sequence coverage was greater than 15% when proteins were greater than 60 K and the MOWSE score was greater than 10^3. In addition, a 300 Da mass discrepancy between theoretical and experimental molecular weight was acceptable considering possible post-translational modifications when several components coeluted during the separation. When the mass discrepancy was greater than 300 Da, the molecular weight was accepted as the deconvoluted value when there was only one component; under coeluting conditions, if the sequence coverage was greater than 20% and the MOWSE score was greater than 10^4, or the sequence coverage was greater than 25% and the MOWSE score was greater than 10^3, the match was considered an acceptable match even if there was no molecular weight available.

2.10 MALDI-MS/MS

MALDI-MS/MS was conducted using an Applied Biosystems 4700 MALDI-TOF-TOF (Applied Biosystems, Foster City, CA, USA) when the protein could not be identified using MALDI-MS peptide mapping. The sample was spotted onto a plate using the same protocol as MALDI analysis without internal standards. A 337 nm Nd:YAG laser was used. The parent ions were fragmented in the TOF collision cell using collision energy of 1 kV. The mass window was set at ± 4 Da for the TOF/TOF timed ion selector. The parent ions and the daughter ions were analyzed in a reflectron MS/MS mode. The MS/MS spectra were acquired and processed using the 4700 Explorer. The parent ion and daughter ions monoisotopic peak list were submitted to the *273-4* database using MS-tag for protein identification. The search was performed as described above. The mass tolerance of the parent ion was 100 ppm and the daughter ion was 0.5 Da. The identification of proteins based on MS/MS analysis of one peptide was considered sufficient for such a relatively simple genome. The MS/MS analysis on multiple peptides was performed to increase confidence in protein identification.

2.11 Software

Virtual 2-D UV maps of the samples were generated by ProteoVue software (Beckman Coulter) with the p*I* along the *X*-axis and the hydrophobicity along the *Y*-axis. Each peak in the RP chromatograms was visualized into a band, where the darkness of the band was proportional to the peak intensity. Each map was normalized with the highest peak intensity. The differential expression of proteins was displayed using DeltaVue software (Beckman Coulter). The differential 2-D map was achieved by point-to-point substraction of two 2-D UV maps.

The theoretical molecular weight and p*I* of proteins were calculated using Compute p*I*/MW provided by expasy (http://us.expasy.org/tools/#primary). PSORTb version 2.0 (http://www.psort.org/psortb/) was used to predict subcellular localization of proteins. The integration of peaks in each chromatogram was performed by a software written in-house.

3 Results and discussion

3.1 2-D HPLC separation

Four duplicate samples of *Psychrobacter 273-4* cultured at 22 and 4°C in 1/2 TSB medium with or without 5% NaCl were selected to study the impact of temperature and salt. The protein extractions were examined using 2-D HPLC and MS. A pH range of 4.0–8.6 was chosen for the first dimension CF separation, as 80% of ORFs are known to have a p*I* below 8.6 (Fig. 1). The pH was usually monitored by a pH electrode with a flow cell placed before the detector; however, the extra dead volume resulting from the flow cell causes band-broadening for the separation (data not shown). NPS-RP-HPLC was performed by loading an identical volume of sample from each CF fraction using a PF2D system, where obtaining the identical volume of CF fractions from different samples was very important for subsequent comparison between samples. When CF fractions were collected by pH intervals, certain fractions with an identical pH range collected from different samples may have different volumes. This

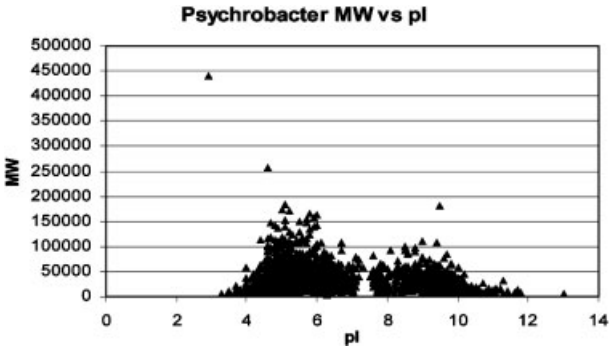

Figure 1. MW and p*I* distribution of protein predicted from *273-4* database.

result can make the comparison between samples difficult since the CF fractions were loaded onto the RP column by volume and not by proportion in the current software used by PF2D. In order to eliminate the extra band-broadening, the separation of the samples was carried out without monitoring the pH, and the fractions were collected at time intervals. However, the pH was monitored when running the blank prior to the protein separation using the same solvents. Owing to the reproducibility of the column-based separation, the pH intervals of the CF fractions of samples were obtained using the pH titration curve acquired from the blank as a reference. The second-dimensional separation was conducted on NPS-RP column based on hydrophobicity. The chromatograms of the NPS-RP-HPLC from one sample were converted into a virtual 2-D map based upon UV absorption detection using ProteoVue software.

The virtual 2-D maps are shown in Fig. 2 and the p*I* ranges of lanes in Fig. 2 are summarized in Table 1. These four samples display similar protein profiles where more than 500 bands are observed on each map and most of the bands observed fit the p*I* range between pH 4.1 and 7.1.

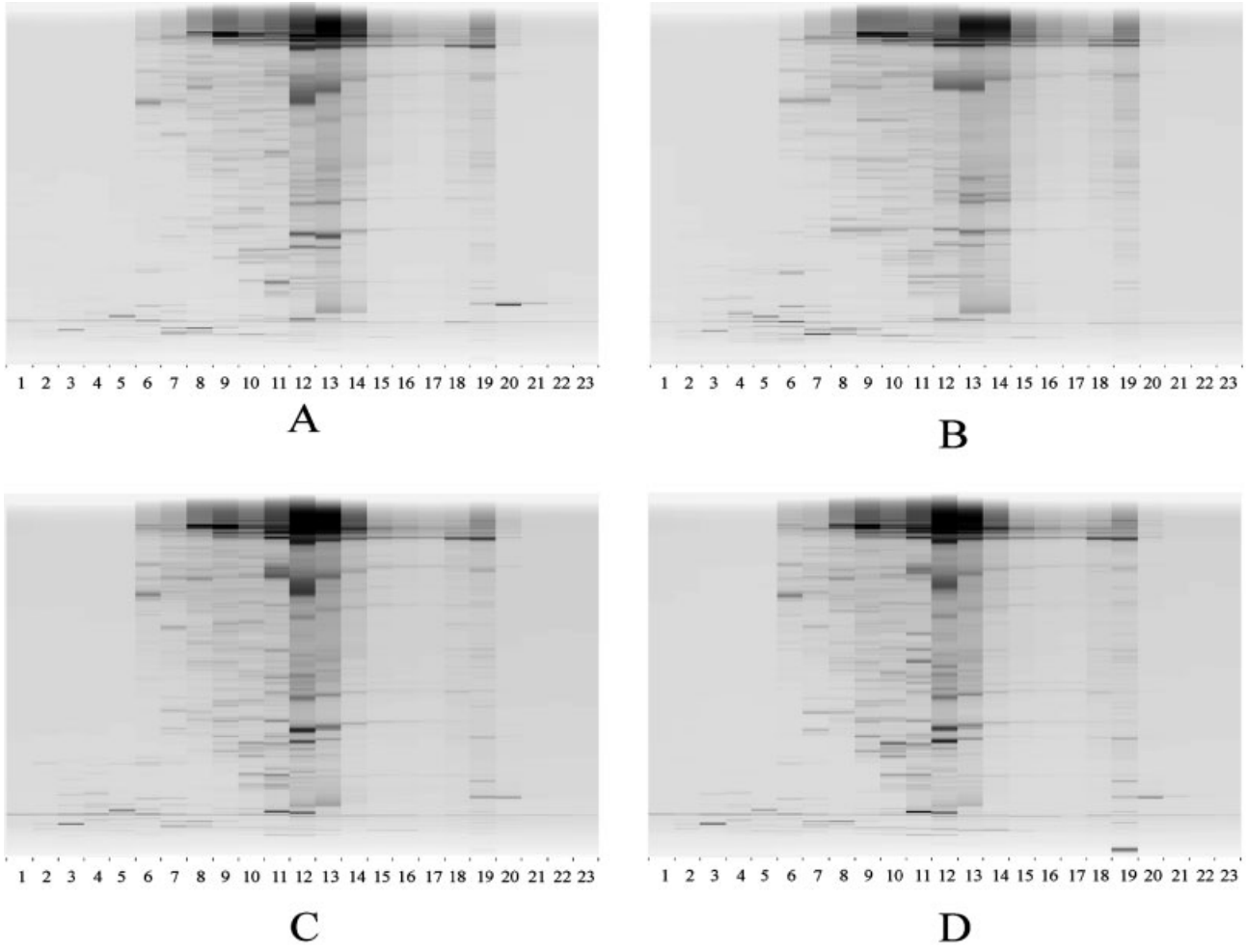

Figure 2. Virtual 2-D maps of four samples after PF2D separation generated by ProteoVue. The *X*-axis of the map is the p*I* and the *Y*-axis is the hydrophobicity. (A) 4°C, (B) 22°C, (C) 4°C with 5% NaCl, and (D) 22°C with 5% NaCl.

Table 1. p*I* range of lanes in Fig. 2

Lane	pH start	pH end
1	8.2	8.6
2	7.9	8.2
3	7.5	7.9
4	7.0	7.5
5	6.6	7.0
6	6.3	6.6
7	5.9	6.3
8	5.6	5.9
9	5.3	5.6
10	5.0	5.3
11	4.9	5.0
12	4.7	4.9
13	4.4	4.7
14	4.1	4.4
15	3.9	4.1
16	3.8	3.9
17	3.8	3.8

3.2 Identification of differentially expressed proteins

In order to study the influence of low temperature and salinity, the maps of cells grown in the same media but different temperature or the maps of cells grown at the same temperature but different media were compared using DeltaVue software. The proteins differentially expressed due to temperature are shown in Fig. 3, and the proteins differentially expressed due to salinity are displayed in Fig. 4. MALDI mass spectra of interesting fractions collected from the PF2D were acquired for the identification of these proteins. More than one protein was observed in certain fractions collected using the PF2D by time intervals, which causes difficulties in distinguishing these proteins. Therefore, an RP-HPLC was coupled with an ESI-TOF-MS and a UV detector to obtain the molecular weight as well as the peptide mass fingerprint in order to provide more evidence for the identification based on fractions collected from the PF2D.

The identification of differentially expressed proteins is summarized in Table 2. All identifications were performed using the unpublished *Psychrobacter 273-4* database with MS-FIT or MS-TAG search engine. It is not surprising that only one protein was identified in the majority of peaks representing differentially expressed proteins on the UV chromatogram since the genome is so small. Therefore, it is reasonable to quantify protein expression by integration of the peak area on the UV chromatogram. The quantification comparison in Table 2 was obtained from the average of two replicates. However, two proteins encoded from gene 2003 and gene 2751 were identified

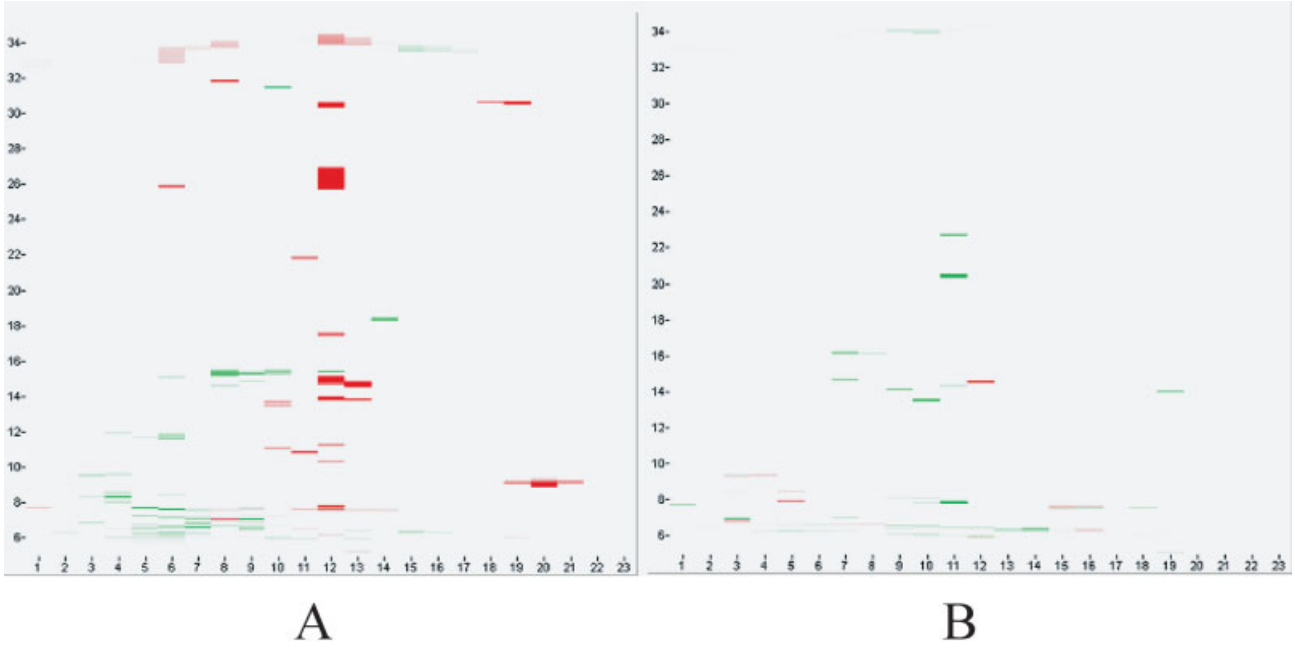

Figure 3. Influence of temperature shown by differential maps generated by DeltaVue. The *X*-axis of the map is the p*I* and the *Y*-axis is the hydrophobicity. (A) Differential map of cells grown in 1/2 TSB and (B) the differential map of cells grown in 1/2 TSB with 5% NaCl. On both maps, red color represents cells cultured at 4°C and green color represents cells cultured at 22°C.

Table 2. Identification of differentially expressed proteins

Gene Name	Protein Name	Function	Protein MW (Da)/p/	Exp. MW (Da)	Exp. p/	Presence				Quantitation comparison					Lane #	Retention time (min)
						22	4	22S	4S	4/22	4S/22S	22S/22	4S/4	4S/22		
psyc_30jun03_gene1759	Ribosomal protein S15	Translation	**10186/10.4**	10143	7.0–7.5	X	X	X	X	0.2	3.2	0.1	1.4	0.3	4	11.91
psyc_30jun03_gene2443	Hypothetical protein	Hypothetical	**14112/6.2**	14208	7.0–7.5	X	X	X	X	0.2	2.3	0.5	6.2	1.1	4	8.43
psyc_30jun03_gene2003	D-3 phosphoglycerate dehydrogenase	Amino acid metabolism	**44837/5.4**	44820	4.9–5.0	X	X	X	X	1.6	2.1	1.9	2.5	3.9	11	27.25(*)
psyc_30jun03_gene2751	Aminotransferase	Amino acid metabolism	**44236/4.8**	44325	4.9–5.0	X	X	X	X	1.6	2.1	1.9	2.5	3.9	11	27.25(*)
psyc_30jun03_gene2103	30S ribosomal protein S4	Translation	**24252/10.0**	24126	6.3–7.0	X	X	X	X	0.0	1.9	0.0	0.7	0.0	5, 6	11.59
psyc_30jun03_gene2089	Ribosomal protein L14	Translation	**13395/10.5**	13396	7.0–7.5	X	X	X	X	1.6	1.7	7.7	7.7	12.7	4	9.33
psyc_30jun03_gene2085	Ribosomal protein S3	Translation	**27241/10.2**	27115	6.3–6.6	X	X	X	X	0.5	1.6	0.4	1.2	0.6	6	12.4
psyc_30jun03_gene1391	Fumarate hydratase, class II (fumarase)	Energy metabolism	**50295/5.2**	50210	5.6–5.9	X	X	X	X	4.5	1.5	6.3	2.2	9.7	8	31.62
psyc_30jun03_gene14	tRNA synthetases, class-II (G, H, P and S):seryl-tRNA synthetase, class IIa:aminoacyl-transfer RNA synthetase, class II	Translation	**47740/5.2**	47787	5.0–5.3	X	X	X	X	2.0	1.5	0.8	0.6	1.2	10	23.63
psyc_30jun03_gene935	Conserved hypothetical protein	Conserved hypothetical	**47210/6.1**	47890	5.6–5.9	X	X	X	X	4.9	1.4	4.9	1.4	6.7	8	31.83
psyc_30jun03_gene166	Putative outer membrane protein	Unassigned	**43506/4.7**	24480	4.7–4.9	X	X	X	X	2.4	1.3	1.5	0.8	1.9	12	26.5
psyc_30jun03_gene2698	Ribosomal protein S6	Translation	**15560/5.2**	15564	4.9–5.0	X	X	X	X	0.5	1.3	1.8	4.2	2.2	11	15.49
psyc_30jun03_gene2268	Bacterial stress protein, probable tellurium resistance TerD	Unassigned & other	**20381/4.4**	20250	4.4–4.7	X	X	X	X	1.9	1.2	0.8	0.5	0.9	13	14.41
psyc_30jun03_gene1277	Alkyl alkyl hydroperoxide reductase, subunit c	Unassigned	**20603/4.7**	20516	4.9–5.0	X	X	X	X	1.8	1.2	3.9	2.6	4.6	11	19.26
psyc_30jun03_gene2633	Putative adenylosuccinate lyase	Nucleotide metabolism	**51743/5.0**	44272	5.6–5.9	X	X	X	X	0.3	1.2	0.3	1.1	0.3	8	21.28
psyc_30jun03_gene1511	Putative two-component response regulator	Transcription	**27361/5.0**	27275	4.9–5.0	X	X	X	X	1.1	1.1	7.5	7.7	8.3	11	17.91
psyc_30jun03_gene62	Putative adenylate kinase	Nucleotide metabolism	**23843/5.0**	23846	4.9–5.0	X	X	X	X	1.5	1.1	8.9	6.6	9.7	11	14.01
psyc_30jun03_gene148	Possible acetone carboxylase gamma subunit	Unassigned & other	**18097/5.0**	18097	4.9–5.3	X	X	X	X	3.1	1.1	2.2	0.8	2.4	10, 11	10.82
psyc_30jun03_gene1407	Enolase	Carbohydrate metabolism	**47200/4.9**	47200	4.9–5.0	X	X	X	X	0.6	1.0	1.8	3.2	1.9	11	31.6
psyc_30jun03_gene2104	DNA-directed RNA polymerase, 30–40 kDa subunit:RNA polymerase, alpha chain, bacterial and organelle	Transcription	**37373/4.7**	37374	4.7–4.9	X	X	X	X	2.6	1.0	3.4	1.3	3.5	12	31.59
psyc_30jun03_gene2392	Conserved hypothetical protein	Conserved hypothetical	**23012/5.5**	23222	5.0–5.3	X	X	X	X	2.5	1.0	2.0	0.8	2.0	10	25.22
psyc_30jun03_gene1195	Translation elongation factor Tu (EF-Tu)	Translation	**43099/5.1**	43066	4.7–4.9	X	X	X	X	3.8	0.9	4.4	1.1	4.0	12	30.34
psyc_30jun03_gene1039	Heat shock protein Hsp70	Unassigned	**69551/4.5**	69419	<3.9	X	X	X	X	1.1	0.9	0.2	0.2	0.2	19	27.79
psyc_30jun03_gene295	Putative exodeoxyribonuclease III	Replication and repair	**31730/5.3**	31604	5.6–5.9	X	X	X	X	0.1	0.9	0.2	1.1	0.1	8	14.59
psyc_30jun03_gene2098	Ribosomal protein L15	Translation	**15516/10.9**	15388	6.3–6.6	X	X	X	X	0.2	0.9	0.2	0.8	0.2	6	11.85
psyc_30jun03_gene2082	Ribosomal protein L2	Translation	**30399/11.3**	30360	5.3–5.9	X	X	X	X	0.4	0.9	0.4	1.0	0.3	8, 9	15.51
psyc_30jun03_gene1762	Conserved hypothetical protein	Conserved hypothetical	**41365/5.3**	41367	4.9–5.0	X	X	X	X	1.8	0.8	2.6	1.2	2.0	11	21.38
psyc_30jun03_gene1964	Acyl-CoA dehydrogenase	Amino acid metabolism	**43183/5.3**	43098	5.0–5.6	X	X	X	X	0.9	0.7	0.5	0.4	0.4	9, 10,11	30.7
psyc_30jun03_gene3	Argininosuccinate synthase	Amino acid metabolism	**46198/5.0**	46074	4.9–5.0	X	X	X	X	1.0	0.7	5.1	3.7	3,7	11	18.52
psyc_30jun03_gene2859	Delta-aminolevulinic acid dehydratase	Metabolism of cofactors	**37105/5.2**	37312	4.9–5.0	X	X	X	X	3.4	0.7	0.6	0.1	0.4	11	21.93
psyc_30jun03_gene1640	Putative DnaK suppressor protein	Signal transduction	**16906/4.9**	16772	<3.9	X	X	X	X	0.7	0.7	0.5	0.5	0.4	19	10.85
psyc_30jun03_gene1202	Ribosomal protein L7/L12	Translation	**12657/4.6**	12526	4.7–4.9	X	X	X	X	0.3	0.7	0.2	0.4	0.1	12	30.79
psyc_30jun03_gene1053	Putative phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase	Amino acid metabolism	**26466/5.0**	7440	<3.9	X	X	X	X	1.2	0.7	3.0	1.8	2.1	19	10.38
psyc_30jun03_gene855	Conserved hypothetical protein	Conserved hypothetical	**32493/4.6**	32363	4.4–4.7	X	X	X	X	0.1	0.7	0.1	0.8	0.1	13	18.26
psyc_30jun03_gene2825	Putative NADH:flavin oxidoreductase/NADH oxidase: FMN/related compound-binding core	Energy metabolism	**39617/5.3**	39491	5.9–6.3	X	X	X	X	0.4	0.6	0.6	1.0	0.4	8	16.25
psyc_30jun03_gene1793	Probable ketopantoate hydroxymethyltransferase	Metabolism of cofactors	**28498/5.4**	28412	5.9–6.3	X	X	X	X	0.7	0.6	0.8	0.7	0.5	7	31.54
psyc_30jun03_gene518	Nucleoside diphosphate kinase	Nucleotide metabolism	**15610/4.9**	15480	5.0–5.3	X	X	X	X	2.3	0.6	4.4	1.2	2.7	10	12.55
psyc_30jun03_gene1164	Putative superoxide dismutase (Mn/Fe binding)	Cellular processes	**23475/5.0**	23345	4.4–4.7	X	X	X	X	1.9	0.6	3.0	0.9	1.7	13, 14	13.94
psyc_30jun03_gene2399	Probable ABC transport permease	Cellular processes	**21186/4.6**	20918	5.0–5.3	X	X	X	X	1.3	0.6	2.0	0.9	1.1	10	13.01
psyc_30jun03_gene1766	Probable fructose-bisphosphate aldolase	Carbohydrate metabolism	**33294/5.1**	33340	5.0–5.3	X	X	X	X	0.5	0.6	0.3	0.4	0.2	10	21.07

Table 2. Continued

Gene Name	Protein Name	Function	Protein MW (Da)/p*I*	Exp. MW (Da)	Exp. p*I*	Presence				Quantitation comparison					Lane #	Retention time (min)
						22	4	22S	4S	4/22	4S/22S	22S/22	4S/4	4S/22		
psyc_30jun03_gene536	Alkyl hydroperoxide reductase/ thiol-specific antioxidant	Unassigned & other	**23573/4.9**	23486	4.9–5.3	X	X	X	X	1.7	0.6	3.1	1.0	1.8	10, 11	13.57
psyc_30jun03_gene1201	Ribosomal protein L10	Translation	**18811/9.1**	18741	5.6–5.9	X	X	X	X	1.8	0.5	3.9	1.0	1.9	8	16.01
psyc_30jun03_gene831	Putative peptidylprolyl isomerase, FKBP-type	Translation	**17426/4.6**	17297	4.9–5.0	X	X	X	X	0.9	0.5	4.9	2.4	2.2	11	14.33
psyc_30jun03_gene2137	Putative chaperonin HSP60 family	Cellular processes	**57708/4.8**	57590	4.7–4.9	X	X	X	X	2.8	0.4	6.2	1.0	2.8	12	31.17
psyc_30jun03_gene705	Putative phage terminase, large subunit	Replication and repair	**65480/5.3**	65638	4.7–4.9	X	X	X	X	1.7	0.4	4.0	1.0	1.7	11	26.61
psyc_30jun03_gene889	Conserved hypothetical protein	Conserved hypothetical	**15149/5.0**	15150	5.0–5.3	X	X	X	X	0.2	0.4	0.3	0.4	0.1	10	24.52
psyc_30jun03_gene1906	Conserved hypothetical protein	Conserved hypothetical	**32642/6.0**	11276	7.5–7.9	X	X	X	X	1.4	0.4	1.5	0.4	0.6	3	6.96
psyc_30jun03_gene645	Putative PhoH-like protein, predicted ATPase	Signal transduction	**40824/5.4**	41130	4.9–5.0	X	X	X	X	3.0	0.3	6.7	0.8	2.3	11	27.71
psyc_30jun03_gene2871	Aldehyde dehydrogenase family protein	Energy metabolism	**55714/5.0**	55760	4.7–4.9	X	X	X	X	2.7	0.3	6.0	1.0	2.6	12	31.43
psyc_30jun03_gene2321	Peptidyl-prolyl *cis-trans* isomerase, cyclophilin type	Unassigned & other	**18693/5.1**	18696	5.9–6.3	X	X	X	X	2.2	0.3	3.9	0.6	1.3	7	14.74
psyc_30jun03_gene381	Putative 3'-phosphoadenosine 5'-phosphosulfate sulfotransferase	Metabolism of cofactors	**24335/4.8**	24210	4.9–5.0	X	X	X	X	1.1	0.0	66.4	0.7	0.7	11	20.42
psyc_30jun03_gene393	Protein of unknown function DUF6	Conserved hypothetical	**27670/8.9**	9571	<3.9	ND	X	X	X	N/A	0.7	N/A	0.3	N/A	20	9.12
psyc_30jun03_gene414	Protein of unknown function DUF541	Conserved hypothetical	**26040/9.2**	9456	<3.9	ND	X	X	X	N/A	1.5	N/A	1.2	N/A	19	9.12
psyc_30jun03_gene2136	Putative chaperonin HSP10	Cellular processes	**10358/4.9**	10770	<3.9	ND	X	X	X	N/A	1.0	N/A	1.0	N/A	19	29.8
psyc_30jun03_gene2415	Putative cold-shock protein	Unsigned	**8111/4.9**	8770	<3.9	ND	X	X	X	N/A	1.0	N/A	1.0	N/A	19	30.23
psyc_30jun03_gene1450	Ribosomal protein L28	Translation	**9111/11.8**	8990	6.6–7.0	X	ND	ND	ND	N/A	N/A	N/A	N/A	N/A	5	15.15

Function is derived from the database. Presence indicates culture conditions at which a protein was detected. 22: at 22°C in 1/2 TSB media; 4: at 4°C in 1/2 TSB media; 22S: at 22°C in 1/2 TSB with 5% NaCl media; 4S: at 22°C in 1/2 TSB with 5% NaCl media. Quantitation comparison is depicted in ratio of expression level of proteins between different culture conditions. Proteins differentially expressed more than two-fold at culture conditions compared are marked as gray. 4/22: comparison between 4 and 22°C in 1/2 TSB media; 4S/22S: comparison between 4 and 22°C in 1/2 TSB with 5% NaCl media, 22S/22: comparison between media with and without with 5% NaCl at 22°C; 4S/22: comparison between media with and without with 5% NaCl at 4°C.
ND: not detected, N/A: not applicable, (*): proteins coeluted.

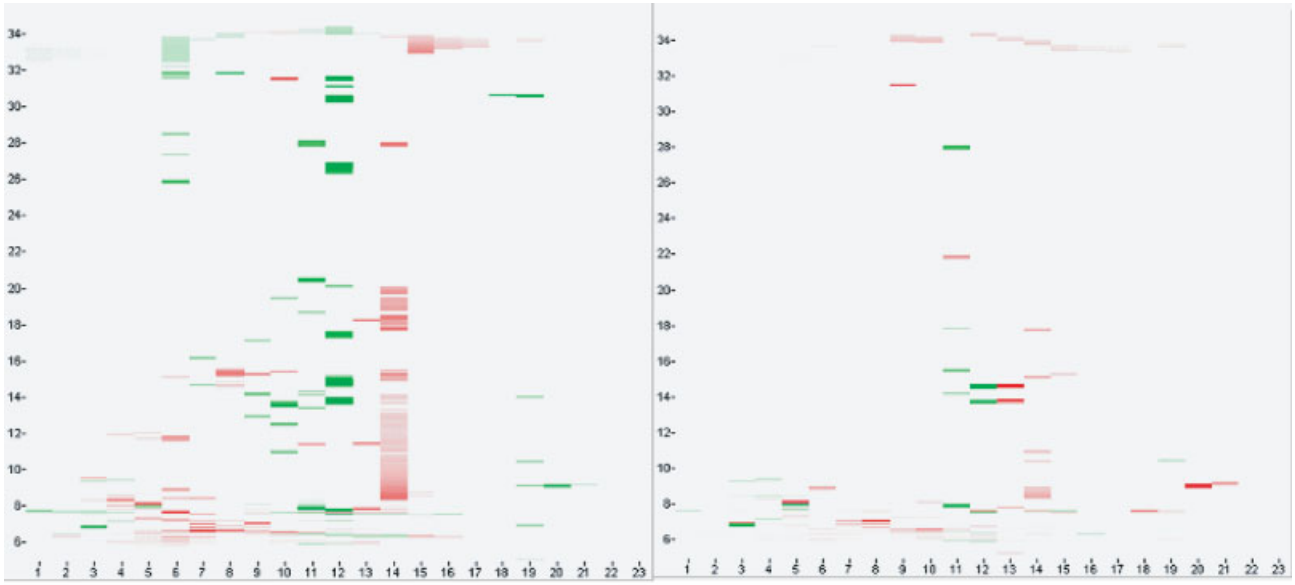

Figure 4. Impact of salt shown by differential maps generated by DeltaVue. The *X*-axis of the map is the p*I* and the *Y*-axis is the hydrophobicity. (A) Differential map of cells grown at 22°C and (B) the differential map of cells grown at 4°C. On both maps, red color represents cells grown in 1/2 TSB and green color for 1/2 TSB with 5% NaCl.

in one peak. It is still not clear whether one or both of them were differentially expressed under the given conditions using the current methods. The shot-gun proteomic method may be employed in the future to solve this problem. It is worth noting that only proteins differentially expressed more than two-fold were considered as significant in differential expression and included in Table 2.

Most of the proteins in Table 2 were observed to be differentially expressed in four samples. However, a number of proteins were only detected under certain culture conditions, which provides a definite connection between their function and the culture condition. For instance, one protein encoded from gene 1450 was only detected in cells cultured at 22°C in 1/2 TSB media; the other four proteins encoded from gene 393, 414, 2136, and 2415 were only observed in cells grown at low temperature (4°C) and/or in saline media. The expression of protein encoded from gene 393 is shown in Fig. 5 as an example. In addition, the function of the proteins encoded from gene 393 and 414 was unknown in the database. Response to temperature and/or salt may be part of their functions. Proteins encoded from gene 2136 and 2415 are annotated as putative HSP10 and putative cold-shock protein. HSP10 contains a homolog of the bacteria cochaperonin GroES, which is a functional regulator of the chaperonin. HSP10 was identified as an essential component of the mitochondrial protein folding apparatus, participating in various aspects of HSP60 function [38, 39]. The cold-shock protein is usually induced by cold shock. This study reports for the first time that HSP10 and cold-shock protein were also overexpressed in the saline media.

The experimental molecular weights of the majority of proteins identified are in accordance with the molecular weights predicted from the protein sequences. However, the observed molecular masses of a number of proteins in Table 2 differed by up to 300 Da from predicted molecular weights. Molecular weight is a basic property of a protein sequence, so any change of molecular weight indicates a change in the protein sequence, which reflects the possible occurrence of post-translational modifications on the protein.

3.3 Temperature effect

3.3.1 Temperature effect in nonsaline media

Virtual 2-D maps of cells cultured in 1/2 TSB media at 22 and 4°C were compared to illustrate the effect of temperature. The differential map is shown in Fig. 3A, and 18 up-regulated proteins and 15 down-regulated proteins at 4°C in 1/2 TSB are summarized in Table 2.

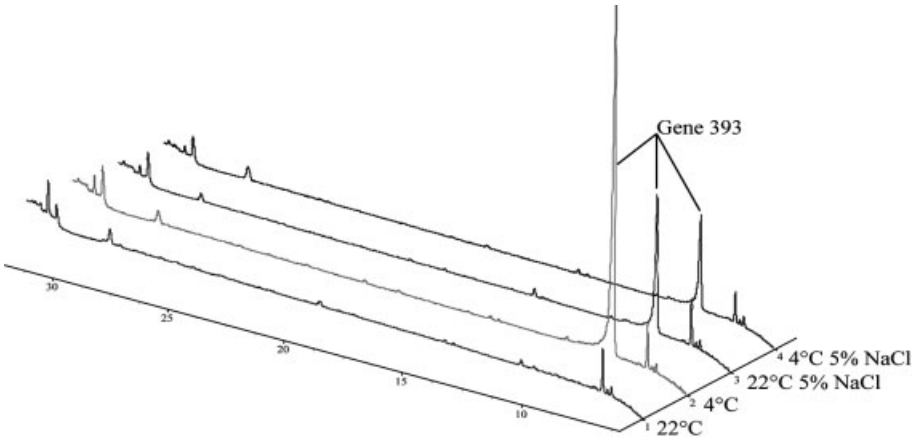

Figure 5. 3-D view of comparison of RP chromatograms of one fraction of four samples from salt elution, the proteins indicated by arrows were only observed at low temperature (4°C) and/or salinity media.

The majority of proteins down-regulated at 4°C are ribosomal proteins. For example, ribosomal proteins S3, S4, S6, S15, L2, L7/L12, and L15 were observed down-regulated more than two-fold at 4°C. In particular, ribosomal protein L28 was only detected at 22°C. Ribosomal proteins are directly related to protein synthesis. The decrease of ribosomal proteins at low temperature suggests that protein synthesis is decreased at low temperature to conserve energy, prevent the synthesis of unwanted proteins, and selectively synthesize more proteins that can help the cell survive under stress conditions [40].

The decrease in activity of cells at 4°C reflects not only the decrease of certain ribosomal proteins but also of some other proteins. One protein annotated as putative NADH was down-regulated at 4°C. NADH is responsible for electron transfer in cells. The decrease in NADH suggests a decrease of oxidoreductase activity at 4°C. Two enzymes related to metabolism, probable fructose-bisphosphate aldolase, and putative adenylosuccinate lyase were also down-regulated. Certain enzymes purified from psychrophilic organisms are cold-active enzymes, their enzymatic activity increases with the decrease of temperature [41]. However, little is known about correlation of catalytic activity of these two enzymes and temperature. It is still not clear if the change in their expression level is due to an increase of catalytic activity or low metabolic rate at 4°C.

While proteins down-regulated at 4°C help cells grow at 22°C, proteins up-regulated at 4°C increase the resistance of cells to the cold environment. In addition to induction of four proteins encoded from gene 393, 414, 2136, and 2415 at 4°C discussed in the previous section, overexpression of other general stress-related proteins is another strategy used by *273-4* to adapt to cold environment. HSP60 and peptidyl-prolyl *cis–trans* isomerases, cyclophilin type were observed overexpressed at 4°C. HSP60 was reported to prevent misfolding and promote the refolding and proper assembly of unfolded polypeptides generated under stress conditions [42–49]. Peptidyl-prolyl *cis–trans* isomerases, cyclophilin-type could accelerate protein folding [50]. The overexpression of HSP60 and peptidyl-prolyl *cis–trans* isomerases, cyclophilin type at 4°C facilitates protein folding, which is an effective way to overcome the stress caused by low temperature. In addition, the overexpression of HSP60 is consistent with the increase of HSP10 at 4°C. Moreover, overexpression of peptidyl-prolyl *cis–trans* isomerases, cyclophilin-type at 4°C in this study is in accordance with the finding of cold adaptation of *M. burtonii* [21].

In contrast to the down-regulation of certain ribosomal proteins, some other translation-related proteins, such as tRNA synthetase encoded from gene 14 and EF-Tu encoded from gene 1195, were also up-regulated. Overexpression of tRNA synthetase promotes tRNA synthesis, and subsequently increases the amino acid transportation and protein synthesis at 4°C. EF-Tu promotes the GTP-dependent binding of aminoacyl-tRNA to the A-site of ribosomes during protein biosynthesis [51]; its overexpression also improves protein synthesis at low temperature. These results show that the modulation of protein expression at the translational level appears to be an important mechanism involved in cellular response to low temperature.

3.3.2 Temperature effect in saline media

The maps of cells grown in saline media at 4 and 22°C were compared using DeltaVue software; the differential map is shown in Fig. 3B. Fewer proteins were observed to respond to the temperature change in saline media than in 1/2 TSB. Fourteen proteins were identified as contributing significantly to the temperature change in the saline media in Table 2. Four proteins were up-regulated while ten proteins were down-regulated at 4°C.

It is worthwhile to notice that proteins significantly regulated due to temperature effects in saline media differ from those in nonsaline media by either the amount or the direction of regulation. A conserved hypothetical protein encoded from gene 889 was significantly down-regulated in both media at 4°C; however, it is two times less significant in saline media than nonsaline media, indicating both salinity and temperature regulate its expression. Three proteins encoded from gene 831 (putative peptidylprolyl isomerase, FKBP-type), gene 2003 (D-3 phosphoglycerate dehydrogenase), and gene 2751 (aminotransferase) were also regulated in the same direction in both media; however, they were regulated in saline media but not in nonsaline media at 4°C. Ten other proteins were observed to be regulated in the opposite direction from the nonsaline media. For example, HSP60 and peptidyl-prolyl *cis–trans* isomerase, cyclophilin type were down-regulated in contrast to their up-regulation in nonsaline media. Down-regulation of these two proteins and putative peptidylprolyl isomerase, FKBP-type suggests that protein folding is easier in saline media at 4°C than 22°C. Down-regulation of these general stress-related proteins at 4°C in saline media suggests that 4°C is favored over 22°C in saline media by *273-4*. This phenomenon is related to the chemical properties of salt in solution. It is well known that the ionic strength and the ion activity of salt in the solution decrease with the decline of the temperature, which results in lower external osmotic pressure exposed on cells in saline media at 4°C than 22°C. Therefore, 5% NaCl exposes less stress on cells at 4°C than 22°C.

Only ribosomal protein S15 was differentially expressed resulting from temperature change in saline media while eight ribosomal proteins were differentially expressed in nonsaline media due to temperature change. The decrease of the number of ribosomal proteins involved in temperature change in saline media illustrates that regulation on the translation level plays a less significant role in temperature change in saline media than it does in nonsaline media. This may be due to less active biological processes of cells in saline media, as shown in Fig. 2 where less ribosomal proteins were expressed in cells cultured at low temperature or in saline media than at optimal culture conditions.

The expression level of proteins in saline media was determined by salinity and temperature. Low temperature is favored by cells in saline media. The adaptation to temperature change in saline media relies mainly on the translation level overexpression of chaperons and peptidylprolyl isomerase to aid in protein folding and refolding, regulation of metabolism pathways and proteins with unknown functions.

3.4 Salt effect

The salt effect is depicted in Fig. 4 by comparing virtual 2-D maps of cells grown at the same temperature but in different media. Forty-five proteins were differentially expressed at 22°C while only 22 proteins were differentially expressed at 4°C as summarized in Table 2.

3.4.1 Proteins regulated by salt at both 22°C and 4°C

A number of proteins were regulated toward the same direction at 22°C and 4°C in the saline media, and certain proteins were even changed by the same levels. For example, ribosomal protein L14 encoded from gene 2089 was up-regulated seven-fold at both 22 and 4°C in saline media, indicating the influence of salt on its expression dominates over the influence of temperature, as is seen from the quantification data shown in Table 2. Proteins encoded from gene 1511, 1964, 1640, and 1039 also fall into this category. Seven proteins encoded from gene 62, 2089, 1511, 1391, 3, 831, and 127, were up-regulated while six proteins encoded from gene 1964, 1640, 1766, 889, 1039, and 1201 were down-regulated at 22 and 4°C in the saline media. The protein encoded from gene 1640 was annotated as putative DnaK suppressor protein. This protein has been reported to suppress the response to the temperature change, where down-regulation of the protein would promote the synthesis of DnaK protein which belongs to the HSP70 family and helps the proteins maintain their structure [52, 53]. However, it was down-regulated more significantly in saline media than at low temperature in our study. Protein encoded from gene 1511 is a putative two-component response regulator. The two-component response system first senses the temperature change, where the regulation system is triggered to adapt to the temperature change due to the cold shock in *Escherichia coli* [19]. Its overexpression in saline media may contribute to sensing the salt and triggering the cascade regulation system. Protein encoded from gene 831 is a putative peptidylprolyl isomerase, FKBP-type. Its increase greatly helps denatured protein refold in saline media [51].

3.4.2 Proteins regulated by salt only at one temperature

Aside from the proteins' response to salinity at both temperatures, a large number of proteins responded significantly to salinity at only one temperature. Three proteins encoded from gene 414, 2136, and 2415 were significantly up-regulated by salt at 22°C while they remained unchanged at 4°C in saline media. Another protein en-

coded from gene 393 induced by salt and low temperature was regulated toward different directions by salt at 4°C and 22°C. These facts indicate that gene 393 and three other induced proteins are not in the same reaction chain during the response. Putative 3'-phosphoadenosine 5'-phosphosulfate sulfotransferase encoded from gene 381, which acts as a metabolism cofactor to catalyze thioredoxin (Trx)-dependent reduction of 3'-phosphoadenosine-5'-phosphosulfate (PAPS) [54], was highly expressed at 22°C in the saline media. Two alkyl hydroperoxide reductases, alkyl hydroperoxide reductase subunit c encoded from gene 1277 and alkyl hydroperoxide reductase/thiol specific antioxidant encoded from gene 536 were also up-regulated at 22°C in the saline media. NaCl may stimulate cells to generate alkyl hydroperoxide at 22°C. These reductases were induced by the salt at 22°C as they were induced by other oxidative stress [55]. Similar to the response to temperature in the cells cultured in the 1/2 TSB, HSP10, HSP60, the cold-shock protein, EF-Tu, DNA-directed RNA polymerase, peptidyl-prolyl *cis–trans* isomerase, cyclophilin-type, ribosomal protein S3, ribosomal protein L2, two conserved hypothetical proteins encoded from genes 2392 and 935, and a number of metabolism-related proteins were up-regulated in response to salinity at 22°C while some other ribosomal proteins were down-regulated. It suggests that salt at 22°C in some instances causes similar damage to the cell as does low temperature, so that cells in the saline media at 22°C use a similar strategy to survive as do those at 4°C in 1/2 TSB including aiding in protein folding and modulation on the translation level.

In contrast to the response to salinity at 22°C, the response to salinity at 4°C involves much smaller number of proteins as demonstrated in Fig. 4, which is due to lower external osmotic pressure at 4°C in saline media as discussed in Section 3.4.1.

The two-component regulation system, DnaK suppressor, HSP70, peptidylprolyl isomerase, FKBP-type, and other translation and metabolism-related proteins play important roles in the adaptation to salinity at 4 and 22°C. Alkyl hydroperoxide reductases were highly expressed in response to salinity at 22°C while they were not observed to have changed significantly at 4°C. In addition, low temperature and salinity showed a combination effect on a number of proteins.

3.5 Combination effect

More proteins were differentially expressed under the influence of temperature in cells grown in nonsaline media than in saline media, as shown in Fig. 3. Furthermore, the proteins differentially expressed in both media were regulated in opposite directions, as shown in Table 2. The presence of 5% NaCl in the media has apparently increased the cells' resistance to temperature change and changed the mechanism of the response. For instance, in the 1/2 TSB cells grown at 22°C experienced less stress than at 4°C; however, in the saline media, 4°C is favored over 22°C.

In order to study the influence of salt, cells cultured at the same temperature but in different media were compared. The virtual differential maps are shown in Fig. 4. Fewer proteins were observed on the differential maps of cells cultured at 4°C than at 22°C, which indicates that low temperature (4°C) may have blocked the sensitivity of cells to salt (5% NaCl). In addition, most of the proteins differentially expressed at 4°C were regulated in the same direction except for a number of proteins. The effect of salt dominates over the effect of low temperature. However, the expression level change of the same protein at both temperatures suggests that temperature also influences the response to the salt.

Low temperature and 5% NaCl shows a combination effect on the expression of certain proteins. The combination effect of low temperature and salt is very complicated. As seen in the quantification column of Table 2, the comparison between cells grown at 4°C in saline media and cells grown at 22°C in nonsaline media (1/2 TSB) clearly shows the complexity of the combination effect. For a certain protein expressed in all four samples, if the ratio generated from the comparison between cells grown at 4°C in saline media and cells grown at 22°C in nonsaline media is greater or smaller than those generated from comparison of salt effect and temperature effect, it suggests that low temperature and salt regulate the protein in the same direction; if the ratio is in between those generated from comparison of salt effect and temperature effect, it indicates that low temperature and salt regulate the protein in opposite ways. For a protein expressed only under certain conditions, the combination effect may be observed by comparison of the quantification information of low temperature or salt if available. It is observed from quantification information in Table 2 that 30 proteins were regulated in opposite directions by low temperature and salt while 23 proteins were regulated in the same direction, including 15 proteins down-regulated and eight proteins up-regulated. Protein encoded from gene 1793 is a good example of proteins regulated in the same direction by salt and low temperature. It was down-regulated less significantly either at low temperature or in saline media. However, it was down-regulated more than two-fold in cells grown at 4°C in saline media than in cells at 22°C in 1/2 TSB. Three proteins, ribosomal protein L28, HSP10, and the cold-shock protein were only affected by temperature or salt.

3.6 Classification of proteins differentially expressed

In order to explore the mechanism of cold and salt adaptation, the differentially expressed proteins were classified on the basis of their functional categories and location. The results are shown in Figs. 6 and 7. The differentially expressed proteins can be classified into 15 functional categories. Translational proteins constituted the majority of proteins identified, and the second category was conserved hypothetical proteins. Aside from proteins associated with transport and lipid metabolism, proteins in each category were involved in regulation. The location of the differentially expressed proteins varied from inside the cell to the outer membrane, which suggests that the regulation of the cell is not limited in any subunit; instead, it is a cell wide procedure. How these proteins associate with each other in the biological process is still not clear. Future metabolic pathways analysis may help to explain the origin of the regulation.

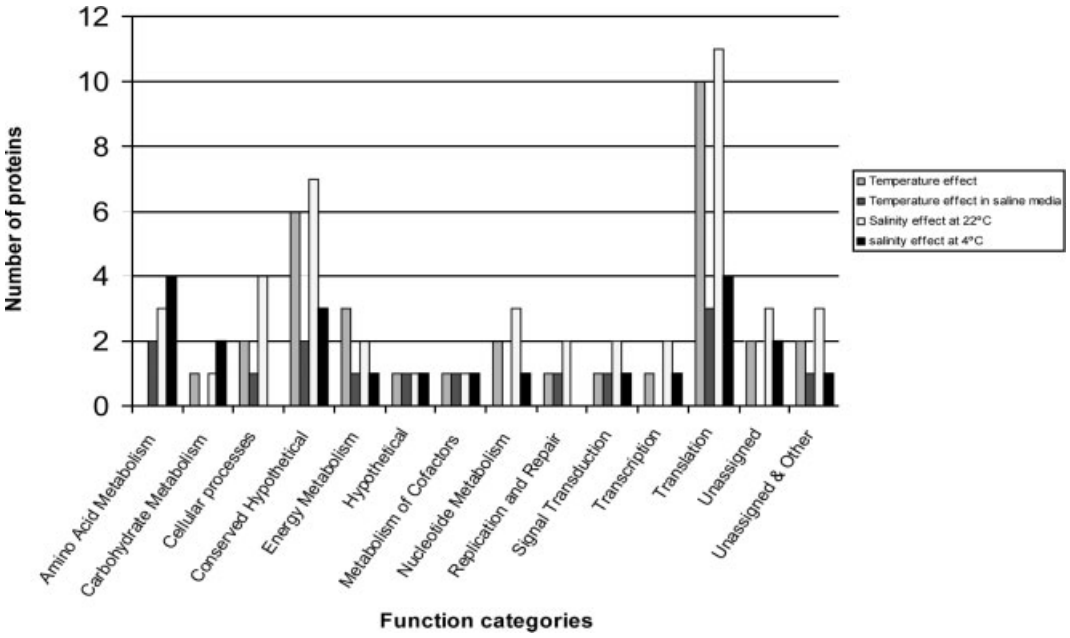

Figure 6. Function categories of differentially expressed proteins.

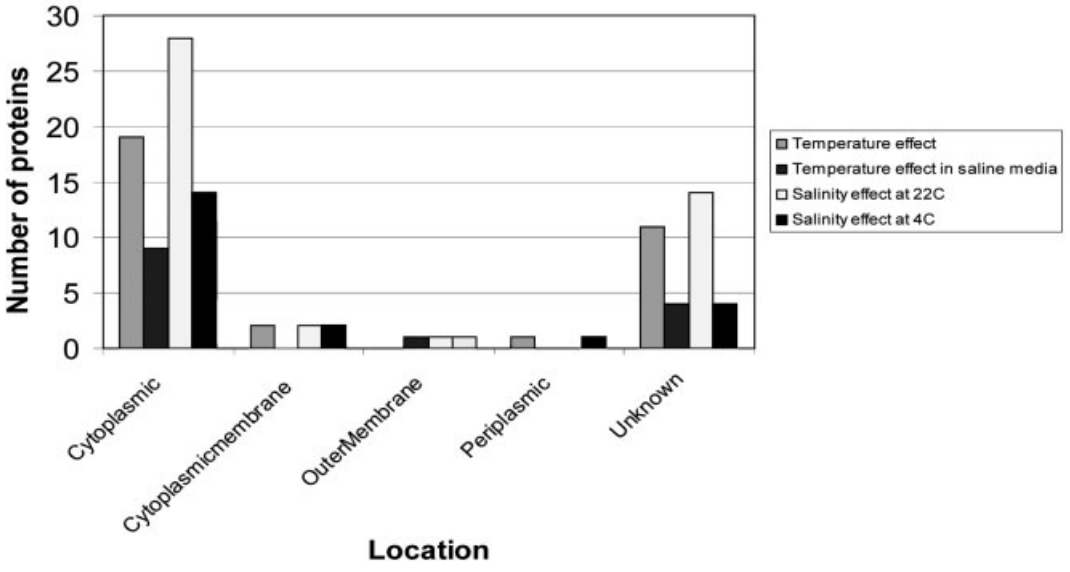

Figure 7. Location analysis of differentially expressed proteins.

3.7 Global protein profiling

The mechanisms of salt adaptation are similar to those of cold adaptation. Salt adaptation also relates to changing membrane fluidity [56, 57] and cell wall biosynthesis [25, 58], the induction of general and stress-specific proteins [59], and overexpression of chaperons [60].

In spite of such extensive research on bacterial stress adaptation, no proteins related to membrane fluidity or cell wall biosynthesis have been observed in a comparative proteomic study. They may not be detected using this method or they could be detected, but not differentially expressed. In order to clarify this finding and provide a more comprehensive and accurate understanding of the response to the adaptation of low temperature and salinity, further global protein profiling was performed using the method described above. More than 250 proteins were identified as shown in Table 3 (see Adddendum). A cell wall biosynthesis-related protein (UDP-glucose pyrophosphorylase) was detected during global protein profiling. However, there was no evidence that it was differentially regulated in cells cultured under different conditions in this study though it was expressed in other bacteria under stress [61]. Among the proteins identified, none associates with membrane fluidity. Specific enrichment may be required to analyze these proteins. Therefore, it is still too early to conclude if there is a connection between membrane fluidity and low temperature or saline media in *273-4*.

It was surprising that several chaperones (HSP70, HSP90, HSP60, and HSP10) related to protein folding were identified along with the PPIase and stress-related proteins such as TerD, TerZ, even under optimal growth conditions, which suggests that *273-4* experienced the stress even under optimal conditions. Besides two of the differentially expressed peptidyl-prolyl *cis–trans* isomerases, cyclophilin- and FKBP-type, putative ppiC-type peptidyl-prolyl *cis–trans* isomerase (gene 135), probable peptidyl-prolyl *cis–trans* isomerase (gene 2185), and probable ppiC-type peptidyl-prolyl *cis–trans* isomerase (gene 2824) were also observed, which indicates peptidyl-prolyl *cis–trans* isomerases are actively involved in the adaptation process. Clearly, *273-4* adapted to the stress in a manner different from some other species.

The proteins identified during global protein profiling were then further classified into different functional groups, shown in Table 3 (Addendum) and Fig. 8. Proteins in 18 functional categories were identified, where translational proteins represented the largest category, followed by conserved hypothetical proteins. Among the entire metabolism group, more proteins were found in amino acid metabolism, and a small number of proteins were found in carbohydrate metabolism, which suggests that amino acids may be a part of its carbon source. Pseudogenes are inactive sequences of genomic DNA. However, a pseudogene (gene 2036) predicted from the database was translated into the protein, which enhances our knowledge about *273-4*.

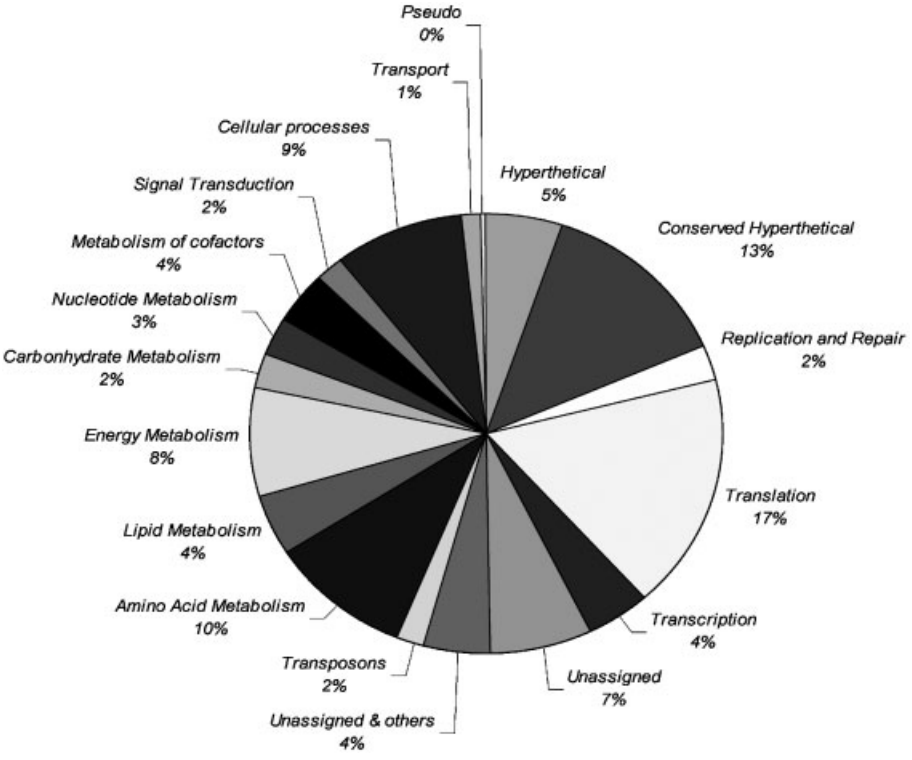

Figure 8. Functional categories of proteins identified from global protein profiling.

4 Concluding remarks

Global protein profiling and a comparative proteomic study of *273-4* cultured at 22°C and 4°C in the media with or without 5% NaCl have been performed using 2-D HPLC and MS. More proteins were involved in adaptation to low temperature in the cells grown in the nonsaline media than in saline media; more proteins were observed differentially expressed in response to the salt at 22°C than 4°C. In addition, 5% NaCl and 4°C showed a combination effect on the protein expression. Fifty-six proteins involved in temperature and salt regulation were identified using MS and database searching. Cold adaptation in nonsaline media relies on modulation of translation level, regulation of metabolism pathways, overexpression of proteins facilating protein folding, and certain proteins with unknown functions. In saline media 4°C is favored over 22°C by cells. The involvement of ribosomal proteins in low temperature adaptation and salinity adaptation at 22°C suggests that manipulation of the expression at the translational level was an important mechanism for cellular stress response. Overexpression of certain chaperones and stress-related proteins such as HSP60, HSP10, a cold-shock protein, and peptidyl-prolyl *cis–trans* isomerases facilitate protein biosynthesis at low temperature in the saline media. The differentially expressed proteins were found in different functional categories and locations, suggesting that the regulation system for adaptation to low temperature and salinity is cell-wide and very elaborate. There were no data from the study showing a connection between changing of membrane fluidity and/or cell wall adaptation. Peptidyl-prolyl *cis–trans* isomerase was actively involved in cold and salt adaptation process. The optimal growing condition of 22°C and nonsaline media still exposes cells to some stress. The evidence supports that the adaptation of *273-4* is based on control of translation, the synthesis of proteins (chaperones) to facilitate RNA and protein folding, and regulation of metabolism pathways.

This work is supported by the National Aeronautics and Space Administration (NASA) through the NASA Astrobiology Institute under Cooperative Agreement no. CAN-00-OSS-01 issued through the Office of Space Science.

5 References

[1] Shivaji, S., Reddy, G. S., Suresh, K., Gupta, P. *et al.*, *Int. J. Syst. Evol. Microbiol.* 2005, *55*, 757–762.

[2] Bozal, N., Montes, M. J., Tudela, E., Guinea, J., *Int. J. Syst. Evol. Microbiol.* 2003, *53*, 1093–1100.

[3] Rabus, R., Ruepp, A., Frickey, T., Rattei, T. *et al.*, *Environ. Microbiol.* 2004, *6*, 887–902.

[4] Heuchert, A., Glockner, F. O., Amann, R., Fischer, U., *Syst. Appl. Microbiol.* 2004, *27*, 399–406.

[5] Yumoto, I., Hirota, K., Sogabe, Y., Nodasaka, Y. *et al.*, *Int. J. Syst. Evol. Microbiol.* 2003, *53*, 1985–1989.

[6] Cavanagh, J., Austin, J. J., Sanderson, K., *Int. J. Syst. Bacteriol.* 1996, *46*, 841–848.

[7] Rivkina, E., Laurinavichius, K., McGrath, J., Tiedje, J. *et al.*, *Adv. Space Res.* 2004, *33*, 1215–1221.

[8] Shivaji, S., Reddy, G. S., Raghavan, P. U., Sarita, N. B. *et al.*, *Syst. Appl. Microbiol.* 2004, *27*, 628–635.

[9] Romanenko, L. A., Lysenko, A. M., Rohde, M., Mikhailov, V. V. *et al.*, *Int. J. Syst. Evol. Microbiol.* 2004, *54*, 1741–1745.

[10] Bowman, J. P., McCammon, S. A., Brown, M. V., Nichols, D. S. *et al.*, *Appl. Environ. Microbiol.* 1997, *63*, 3068–3078.

[11] Garcia-Lopez, I., Otero, A., Garcia-Lopez, M. L., Santos, J. A., *J. Appl. Microbiol.* 2004, *96*, 878–886.

[12] Kampfer, P., Albrecht, A., Buczolits, S., Busse, H. J., *Syst. Appl. Microbiol.* 2002, *25*, 31–36.

[13] Denner, E. B., Mark, B., Busse, H. J., Turkiewicz, M. *et al.*, *Syst. Appl. Microbiol.* 2001, *24*, 44–53.

[14] Yoon, J. H., Yeo, S. H., Oh, T. K., Park, Y. H., *Int. J. Syst. Evol. Microbiol.* 2005, *55*, 171–176.

[15] Rodríguez-Calleja, J. M., Patterson, M. F., García-Lopez, I., Santos, J. A. *et al.*, *J. Food Prot.* 2005, *68*, 538–543.

[16] Jung, S. Y., Lee, M. H., Oh, T. K., Park, Y. H. *et al.*, *Int. J. Syst. Evol. Microbiol.* 2005, *55*, 577–582.

[17] Shaw, B. G., Latty, J. B., *J. Appl. Bacteriol.* 1988, *65*, 7–21.

[18] Vishnivetskaya, T., Kathariou, S., McGrath, J., Gilichinsky, D. *et al.*, *Extremophiles* 2000, *4*, 165–173.

[19] Wick, L. M., Egli, T., *Adv. Biochem. Eng. Biotechnol.* 2004, *89*, 1–45.

[20] Inouye, M., Phadtare, S., *Sci. STKE* 2004, *2004*, pe26.

[21] Goodchild, A., Saunders, N. F. W., Ertan, H., Raftery, M. *et al.*, *Mol. Microbiol.* 2004, *53*, 309–321.

[22] Goodchild, A., Raftery, M., Saunders, N. F., Guilhaus, M. *et al.*, *J. Proteome Res.* 2005, *4*, 473–480.

[23] Nichols, D. S., Miller, M. R., Davies, N. W., Goodchild, A. *et al.*, *J. Bacteriol.* 2004, *186*, 8508–8515.

[24] Seo, J. B., Kim, H. S., Jung, G. Y., Nam, M. H. *et al.*, *Proteomics* 2004, *4*, 3654–3659.

[25] Piuri, M., Sanchez-Rivas, C., Ruzal, S. M., *J. Appl. Microbiol.* 2005, *98*, 84–95.

[26] Mutnuri, S., Vasudevan, N., Kastner, M., Heipieper, H. J., *Curr. Microbiol.* 2005, *50*, 151–154.

[27] Liu, Y., Gao, W., Wang, Y., Wu, L. *et al.*, *J. Bacteriol.* 2005, *187*, 2501–2507.

[28] Steil, L., Hoffmann, T., Budde, I., Volker, U. *et al.*, *J. Bacteriol.* 2003, *185*, 6358–6370.

[29] Hoffmann, T., Schutz, A., Brosius, M., Volker, A. *et al.*, *J. Bacteriol.* 2002, *184*, 718–727.

[30] Antelmann, H., Bernhardt, J., Schmid, R., Mach, H. *et al.*, *Electrophoresis* 1997, *18*, 1451–1463.

[31] O'Farrell, P. H., *J. Biol. Chem.* 1975, *250*, 4007–4021.

[32] Görg, A., Obermaier, C., Boguth, G., Harder, A. *et al.*, *Electrophoresis* 2000, *21*, 1037–1053.

[33] Zheng, S., Schneider, K. A., Barder, T. J., Lubman, D. M., *BioTechniques* 2003, *35*, 1202–1212.

[34] Yan, F., Subramanian, B., Nakeff, A., Barder, T. J. *et al.*, *Anal. Chem.* 2003, *75*, 2299–2308.

[35] Zhang, N., Doucette, A., Li, L., *Anal. Chem.* 2001, *73*, 2968–2975.

[36] Dai, Y. Q., Whittal, R. M., Li, L., *Anal. Chem.* 1999, *71*, 1087–1091.

[37] Wang, H., Kachman, M. T., Schwartz, D. R., Cho, K. R., Lubman, D. M., *Proteomics* 2004, *4*, 2476–2495.

[38] Hohfeld, J., Hartl, F. U., *J. Cell Biol.* 1994, *126*, 305–315.

[39] Martin, J., Geromanos, S., Tempst, P., Hartl, F. U., *Nature* 1993, *366*, 279–282.

[40] Holcik, M., Sonenberg, N., *Nat. Rev. Mol. Cell Biol.* 2005, *6*, 318–327.

[41] Cavicchioli, R., Siddiqui, K. S., Andrews, D., Sowers, K. R., *Curr. Opin. Biotechnol.* 2002, *13*, 253–261.

[42] Marceau, A., Zagorec, M., Chaillou, S., Mera, T. *et al.*, *Appl. Environ. Microbiol.* 2004, *70*, 7260–7268.

[43] Bryan, P. J., Steffan, R. J., DePaola, A., Foster, J. W. *et al.*, *Curr. Microbiol.* 1999, *38*, 168–175.

[44] Horton, A. J., Hak, K. M., Steffan, R. J., Foster, J. W. *et al.*, *Antonie Van Leeuwenhoek* 2000, *77*, 13–20.

[45] Erdal, U. G., Erdal, Z. K., Randall, C. W., *Water Sci. Technol.* 2003, *47*, 123–128.

[46] Leblanc, L., Leboeuf, C., Leroi, F., Hartke, A. *et al.*, *Curr. Microbiol.* 2003, *46*, 157–162.

[47] Polissi, A., De Laurentis, W., Zangrossi, S., Briani, F. *et al.*, *Res. Microbiol.* 2003, *154*, 573–580.

[48] Thammavongs, B., Corroler, D., Panoff, J. M., Auffray, Y. *et al.*, *Lett. Appl. Microbiol.* 1996, *23*, 398–402.

[49] Bang, W., Drake, M. A., *J. Food Prot.* 2002, *65*, 975–980.

[50] Fischer, G., Schmid, F. X., *Biochemistry* 1990, *29*, 2205–2212.

[51] Asahara, H., Uhlenbeck, O. C., *Proc. Natl. Acad. Sci. USA* 2002, *99*, 3499–3504.

[52] Liberek, K., Georgopoulos, C., Zylicz, M., *Proc. Natl. Acad. Sci. USA* 1988, *85*, 6632–6636.

[53] Hoffmann, H. J., Lyman, S. K., Lu, C., Petit, M. A. *et al.*, *Proc. Natl. Acad. Sci. USA* 1992, *89*, 12108–12111.

[54] Setya, A., Murillo, M., Leustek, T., *Proc. Natl. Acad. Sci. USA* 1996, *93*, 13383–13388.

[55] Benndorf, D., Loffhagen, N., Babel, W., *FEMS Microbiol. Lett.* 2001, *200*, 247–252.

[56] Khaware, R. K., Koul, A., Prasad, R., *Biochem. Mol. Biol. Int.* 1995, *35*, 875–880.

[57] Azachi, M., Sadka, A., Fisher, M., Goldshlag, P. *et al.*, *Plant Physiol.* 2002, *129*, 1320–1329.

[58] Vreeland, R. H., Anderson, R., Murray, R. G., *J. Bacteriol.* 1984, *160*, 879–883.

[59] Svensater, G., Sjogreen, B., Hamilton, I. R., *Microbiology* 2000, *146*, 107–117.

[60] Diamant, S., Eliahu, N., Rosenthal, D., Goloubinoff, P., *J. Biol. Chem.* 2001, *276*, 39586–39591.

[61] Coker, J. A., Sheridan, P. P., Loveland-Curtze, J., Gutshall, K. R. *et al.*, *J. Bacteriol.* 2003, *185*, 5473–5482.

6 Addendum

Table 3. Global protein identification

Gene name	Protein name	Protein MW (Da)/p*I*	Experimental MW	Existence			
				22	4	22S	4S
Hyperthetical (13)							
psyc_30jun03_gene2189	Hypothetical protein, possibly involved in molybdopterin biosynthesis	29307/5.6	29501	X	X	X	X
psyc_30jun03_gene627	Hypothetical protein	20463/4.6	20477	X	X	X	X
psyc_30jun03_gene2474	Hypothetical protein	15653/6.7	15790	X	X	X	X
psyc_30jun03_gene2443	Hypothetical protein	14112/6.2	14209	X	X	X	X
psyc_30jun03_gene2289	Hypothetical protein	18850/9.5	18697	X	X	X	X
psyc_30jun03_gene403	Hypothetical protein	33466/5.9	33200	X	X	X	X
psyc_30jun03_gene525	Hypothetical protein	28631/4.5	23845	X	X	X	X
psyc_30jun03_gene298	Hypothetical protein	14779/6.3	14452	X	X	X	X
psyc_30jun03_gene2712	Hypothetical protein	7647/9.1	7670	X	X	X	X
psyc_30jun03_gene902	Hypothetical protein	22545/9.3	22848	X	X	X	X
psyc_30jun03_gene2701	Hypothetical protein	9274/4.7	9452	X	X	X	X
psyc_30jun03_gene1396	Hypothetical protein	18846/4.7	N/A	X	X	X	X
psyc_30jun03_gene709	Hypothetical protein	11577/5.6	N/A	X	X	X	X
Conserved Hyperthetical (33)							
psyc_30jun03_gene612	Haloacid dehalogenase-like hydrolase:HAD-superfamily hydrolase, subfamily IB (PSPase-like):HAD-superfamily subfamily IB hydro. . .	27362/5.2	27234	X	X	X	X
psyc_30jun03_gene889	Conserved hypothetical protein	15149/5.0	15018	X	X	X	X
psyc_30jun03_gene408	Conserved hypothetical protein	38387/4.7	38560	X	X	X	X
psyc_30jun03_gene1762	Conserved hypothetical protein	41365/5.3	41367	X	X	X	X
psyc_30jun03_gene211	Conserved hypothetical protein	24691/4.8	24693	X	X	X	X
psyc_30jun03_gene1302	Conserved hypothetical protein	9123/4.3	9124	X	X	X	X
psyc_30jun03_gene855	Conserved hypothetical protein	32493/4.6	32364	X	X	X	X
psyc_30jun03_gene1745	Conserved hypothetical protein	12387/4.9	12340	X	X	X	X

Table 3. Continued

Gene name	Protein name	Protein MW (Da)/p*I*	Experimental MW	Existence			
				22	4	22S	4S
psyc_30jun03_gene2145	Conserved hypothetical protein, probably involved in catabolism of external DNA	33089/8.8	33143	X	X	X	X
psyc_30jun03_gene202	Probable oxidoreductase, NAD binding site	46749/6.3	46758	X	X	X	X
psyc_30jun03_gene233	Conserved hypothetical protein	33930/6.1	33846	X	X	X	X
psyc_30jun03_gene1802	Conserved hypothetical protein	91530/5.9	91880	X	X	X	X
psyc_30jun03_gene185	Conserved hypothetical protein, DUF262	78994/6.1	79050	X	X	X	X
psyc_30jun03_gene1692	Conserved hypothetical protein	40873/5.4	40790	X	X	X	X
psyc_30jun03_gene1835	Conserved hypothetical protein	24898/10.7	24510	X	X	X	X
psyc_30jun03_gene81	Conserved hypothetical protein	57744/5.3	57530	X	X	X	X
psyc_30jun03_gene2753	Conserved hypothetical protein	64417/9.8	62495	X	X	X	X
psyc_30jun03_gene2392	Conserved hypothetical protein	23012/5.5	23222	X	X	X	X
psyc_30jun03_gene767	Conserved hypothetical protein	23101/5.8	23150	X	X	X	X
psyc_30jun03_gene935	Conserved hypothetical protein	47210/6.1	47890	X	X	X	X
psyc_30jun03_gene1434	Conserved hypothetical protein	50117/5.7	50170	X	X	X	X
psyc_30jun03_gene1274	Conserved hypothetical protein	58714/6.3	53259	X	X	X	X
psyc_30jun03_gene1906	Conserved hypothetical protein	32642/6.0	9846	X	X	X	X
psyc_30jun03_gene393	Protein of unknown function DUF6	27670/8.9	9571	ND	X	X	X
psyc_30jun03_gene414	Protein of unknown function DUF541	26040/9.2	9456	ND	X	X	X
psyc_30jun03_gene1764	Conserved hypothetical protein	36090/5.3	36343	X	X	X	X
psyc_30jun03_gene1906	Conserved hypothetical protein	32642/6.0	11276	X	X	X	X
psyc_30jun03_gene2351	Conserved hypothetical protein	65002/5.0	N/A	X	X	X	X
psyc_30jun03_gene751	Possible nucleotidyltransferase	73751/5.6	N/A	X	X	X	X
psyc_30jun03_gene1036	Conserved hypothetical protein	23370/6.6	N/A	X	X	X	X
psyc_30jun03_gene1490	Conserved hypothetical protein	12136/5.8	N/A	X	X	X	X
psyc_30jun03_gene1612	Conserved hypothetical (GTP-binding domain)	35971/8.9	N/A	X	X	X	X
psyc_30jun03_gene932	Conserved hypothetical protein	10907/5.8	N/A	X	X	X	X
Translation (42)							
psyc_30jun03_gene1864	Sigma 54 modulation protein/ribosomal protein S30EA	14167/6.4	14380	X	X	X	X
psyc_30jun03_gene906	Possible ribosomal protein L25	24604/4.9	24693	X	X	X	X
psyc_30jun03_gene831	Putative peptidylprolyl isomerase, FKBP-type	17426/4.6	17297	X	X	X	X
psyc_30jun03_gene1994	Elongation factor Ts	31766/4.9	31789	X	X	X	X
psyc_30jun03_gene2698	Ribosomal protein S6	15560/5.2	15438	X	X	X	X
psyc_30jun03_gene1993	30S ribosomal protein S2	28884/6.1	26846	X	X	X	X
psyc_30jun03_gene1037	GrpE protein	22125/4.6	21996	X	X	X	X
psyc_30jun03_gene1202	Ribosomal protein L7/L12	12657/4.6	12526	X	X	X	X
psyc_30jun03_gene1195	Translation elongation factor Tu (EF-Tu)	43099/5.1	43066	X	X	X	X
psyc_30jun03_gene2824	Probable PpiC-type peptidyl-prolyl *cis-trans* isomerase	50688/6.0	50469	X	X	X	X
psyc_30jun03_gene2846	Ribosomal protein L13	15787/9.6	15788	X	X	X	X
psyc_30jun03_gene2102	Ribosomal protein S11	13622/11.0	13506	X	X	X	X
psyc_30jun03_gene2089	Ribosomal protein L14	13395/10.5	13396	X	X	X	X
psyc_30jun03_gene1193	Ribosomal protein S7	17742/10.3	17612	X	X	X	X
psyc_30jun03_gene2092	Ribosomal protein S14	11754/10.8	11624	X	X	X	X
psyc_30jun03_gene2103	30S ribosomal protein S4	24252/10.0	24126	X	X	X	X
psyc_30jun03_gene2085	Ribosomal protein S3	27241/10.2	27115	X	X	X	X
psyc_30jun03_gene1201	Ribosomal protein L10	18811/9.1	18741	X	X	X	X
psyc_30jun03_gene2080	Ribosomal protein L4	21836/9.5	21915	X	X	X	X
psyc_30jun03_gene2105	50S ribosomal protein L17	13554/10.6	13556	X	X	X	X
psyc_30jun03_gene221	ATPase, ParA-type	27981/5.4	27733	X	X	X	X
psyc_30jun03_gene2744	Probable CoA-transferase family III	38760/5.6	38597	X	X	X	X
psyc_30jun03_gene501	Possible methionyl-tRNA formyltransferase	25544/6.0	25487	X	X	X	X
psyc_30jun03_gene694	Glutamyl-tRNA(Gln) amidotransferase A subunit	53798/5.6	53715	X	X	X	X
psyc_30jun03_gene2529	Possible ATP-dependent helicase, DEAD-box:DEAD/DEAH box helicase:helicase, *C*-terminal:ATP/GTP-binding site motif A (P-loop)	60613/9.5	60627	X	X	X	X

Table 3. Continued

Gene name	Protein name	Protein MW (Da)/p*I*	Experimental MW	Existence			
				22	4	22S	4S
psyc_30jun03_gene2601	Probable aspartyl-tRNA synthetase	68231/5.2	68250	X	X	X	X
psyc_30jun03_gene2079	Ribosomal protein L3	22187/9.8	22170	X	X	X	X
psyc_30jun03_gene1726	Possible pseudouridine synthase	26571/5.5	26015	X	X	X	X
psyc_30jun03_gene1746	Possible RNA methyltransferase	27267/5.9	25443	X	X	X	X
psyc_30jun03_gene2334	Probable pseudouridine synthase, Rsu:aldehyde dehydrogenase:RNA-binding S4:pseudouridine synthase	35625/10.1	35630	X	X	X	X
psyc_30jun03_gene1411	Putative tRNA-(ms(2)io(6)a)-hydroxylase	29437/5.3	28906	X	X	X	X
psyc_30jun03_gene1159	Putative arginyl tRNA synthetase	67269/5.0	66876	X	X	X	X
psyc_30jun03_gene876	Ribosomal protein S21	8524/10.7	9506	X	X	X	X
psyc_30jun03_gene2509	Peptide chain release factor 1	40513/4.8	40536	X	X	X	X
psyc_30jun03_gene1759	Ribosomal protein S15	10186/10.4	10188	X	X	X	X
psyc_30jun03_gene1450	Ribosomal protein L28	9111/11.8	8990	X	ND	ND	ND
psyc_30jun03_gene2098	Ribosomal protein L15	15516/10.9	15388	X	X	X	X
psyc_30jun03_gene426	Putative Acyl-CoA dehydrogenase A	65034/5.1	30359	X	X	X	X
psyc_30jun03_gene2082	Ribosomal protein L2	30399/11.3	30360	X	X	X	X
psyc_30jun03_gene14	tRNA synthetases, class-II (G, H, P and S):seryl-tRNA synthetase, class IIa: aminoacyl-transfer RNA synthetase, class II"	47740/5.2	47787	X	X	X	X
psyc_30jun03_gene748	Methionine aminopeptidase, Type 1	32740/5.8	N/A	X	X	X	X
psyc_30jun03_gene1141	Translation-putative tyrosyl-tRNA synthetase	44948/5.1	N/A	X	X	X	X
Transcription (11)							
psyc_30jun03_gene2312	Putative response regulator, regulatory protein	23389/5.6	23390	X	X	X	X
psyc_30jun03_gene1618	Putative transcription termination factor Rho	48621/6.5	48847	X	X	X	X
psyc_30jun03_gene1198	Putative transcription antitermination protein NusG	20506/5.1	20508	X	X	X	X
psyc_30jun03_gene1511	Putative two-component response regulator	27361/5.0	27233	X	X	X	X
psyc_30jun03_gene938	Response regulator receiver	28556/5.2	28427	X	X	X	X
psyc_30jun03_gene2104	DNA-directed RNA polymerase, 30–40 kDa subunit:RNA polymerase, alpha chain, bacterial and organelle	37373/4.7	37374	X	X	X	X
psyc_30jun03_gene2578	Putative two-component system regulatory protein	26258/5.8	26019	X	X	X	X
psyc_30jun03_gene2233	Probable bacterial transcriptional regulator	27791/5.7	27474	X	X	X	X
psyc_30jun03_gene1863	Probable sigma-54 factor family	63845/4.4	63380	X	X	X	X
psyc_30jun03_gene1064	Probable DNA helicase	88436/6.2	N/A	X	X	X	X
psyc_30jun03_gene2229	Prokaryotic transcription elongation factor GreA	17526/5.0	N/A	X	X	X	X
Unassigned (17)							
psyc_30jun03_gene2524	Ferritin:bacterioferritin	18856/4.7	18856	X	X	X	X
psyc_30jun03_gene2340	Putative pyruvate decarboxylase	60697/5.3	60627	X	X	X	X
psyc_30jun03_gene1062	Possible peptidyl-prolyl isomerase	26092/5.2	26016	X	X	X	X
psyc_30jun03_gene1277	Alkyl alkyl hydroperoxide reductase, subunit c	20603/4.7	20516	X	X	X	X
psyc_30jun03_gene1981	Unassigned-GTP-binding era protein	40870/5.1	41008	X	X	X	X
psyc_30jun03_gene1039	Heat shock protein Hsp70	69551/4.5	69419	X	X	X	X
psyc_30jun03_gene239	Probable histidine triad (HIT) protein	16130/5.7	16002	X	X	X	X
psyc_30jun03_gene1402	2-Dehydro-3-deoxyphosphooctonate aldolase	32233/5.6	32281	X	X	X	X
psyc_30jun03_gene1359	Predicted metal-dependent phosphoesterases (PHP family)	34543/6.9	34327	X	X	X	X
psyc_30jun03_gene2154	Beta-lactamase-like protein	29558/8.9	29428	X	X	X	X
psyc_30jun03_gene2379	Enoyl-CoA hydratase/isomerase family protein	27819/5.6	27733	X	X	X	X
psyc_30jun03_gene2415	Putative cold-shock protein	8111/4.9	8770	ND	X	X	X
psyc_30jun03_gene1211	Probable type IV fimbrial biogenesis protein, PilQ	80951/5.1	34710	X	X	X	X
psyc_30jun03_gene2358	Probable type 1 restriction modification system methylase	47564/5.3	47890	X	X	X	X
psyc_30jun03_gene242	Short-chain dehydrogenase/reductase SDR:glucose/ribitol dehydrogenase	32132/9.2	N/A	X	X	X	X
psyc_30jun03_gene166	Putative outer membrane protein	43506/4.7	24480	X	X	X	X
psyc_30jun03_gene239	Probable histidine triad (HIT) protein	16130/5.7	N/A	X	X	X	X

Table 3. Continued

Gene name	Protein name	Protein MW (Da)/p*I*	Experimental MW	Existence			
				22	4	22S	4S
Unassigned & others (11)							
psyc_30jun03_gene536	Alkyl hydroperoxide reductase/thiol-specific antioxidant	23573/4.9	23487	X	X	X	X
psyc_30jun03_gene2270	Bacterial stress protein, probable tellurium resitance protein TerZ	21661/5.6	21446	X	X	X	X
psyc_30jun03_gene148	Possible acetone carboxylase gamma subunit	18097/5.0	18097	X	X	X	X
psyc_30jun03_gene2265	Probable tellurium resistance protein, stress protein	20750/4.6	20620	X	X	X	X
psyc_30jun03_gene2268	Bacterial stress protein, probable tellurium resistance TerD	20381/4.4	20250	X	X	X	X
psyc_30jun03_gene538	Hypothetical protein	32452/4.6	32314	X	X	X	X
psyc_30jun03_gene2321	Peptidyl-prolyl *cis-trans* isomerase, cyclophilin type	18693/5.1	18696	X	X	X	X
psyc_30jun03_gene135	Putative PpiC-type peptidyl-prolyl *cis-trans* isomerase	68942/5.3	69259	X	X	X	X
psyc_30jun03_gene91	Putative Bacterial surface antigen (D15)	88865/4.9	88580	X	X	X	X
psyc_30jun03_gene539	Probable FxsA cytoplasmic membrane protein	22214/9.8	22380	X	X	X	X
psyc_30jun03_gene1394	Polyphosphate kinase	86619/5.8	N/A	X	X	X	X
Transposons (5)							
psyc_30jun03_gene2457	Phage terminase, large subunit, PBSX family	48271/6.0	48392	X	X	X	X
psyc_30jun03_gene471	Transposase, mutator family	46228/8.7	46126	X	X	X	X
psyc_30jun03_gene683	Putative transposase	45942/8.2	46011	X	X	X	X
psyc_30jun03_gene1486	Transposase, orfA, IS3/IS911family	19500/10.0	N/A	X	X	X	X
psyc_30jun03_gene2456	Conserved hypothetical phage protein	17399/9.4	N/A	X	X	X	X
Amino Acid Metabolism (24)							
psyc_30jun03_gene290	Putative branched-chain amino acid aminotransferase I	34666/5.8	34671	X	X	X	X
psyc_30jun03_gene823	Amino acid metabolism-gamma-glutamyl phosphate reductase	46746/5.1	46707	X	X	X	X
psyc_30jun03_gene1964	Acyl-CoA dehydrogenase	43183/5.3	43098	X	X	X	X
psyc_30jun03_gene904	Putative ribose-phosphate pyrophosphokinase	34588/5.7	34462	X	X	X	X
psyc_30jun03_gene3	Argininosuccinate synthase	46198/5.0	46074	X	X	X	X
psyc_30jun03_gene2237	Glycine cleavage T protein (aminomethyl transferase)	42972/5.0	42844	X	X	X	X
psyc_30jun03_gene2733	Probable ornithine cyclodeaminase/mu-crystallin family	35313/5.3	35186	X	X	X	X
psyc_30jun03_gene2003	D-3 phosphoglycerate dehydrogenase	44837/5.4	44820	X	X	X	X
psyc_30jun03_gene2751	Aminotransferase	44236/4.8	44325	X	X	X	X
psyc_30jun03_gene3	Argininosuccinate synthase	46198/5.0	46126	X	X	X	X
psyc_30jun03_gene144	Chorismate synthase	39241/5.9	39160	X	X	X	X
psyc_30jun03_gene306	Probable chorismate mutase/prephenate dehydratase	43208/5.7	43170	X	X	X	X
psyc_30jun03_gene1963	Hydroxymethylglutaryl-coenzyme A lyase	32489/5.4	32462	X	X	X	X
psyc_30jun03_gene1047	Putative imidazoleglycerol-phosphate dehydratase	24594/5.9	24511	X	X	X	X
psyc_30jun03_gene1952	Probable amino acid-binding component of amino acid ABC transporter	35370/5.1	35441	X	X	X	X
psyc_30jun03_gene2284	Tetrahydrodipicolinate *N*-succinyltransferase	29557/5.2	29473	X	X	X	X
psyc_30jun03_gene594	Probable Cys/Met metabolism pyridoxal-phosphate-dependent enzyme	46098/5.9	46620	X	X	X	X
psyc_30jun03_gene1657	Imidazole glycerol phosphate synthase subunit hisF (IGP synthase cyclase subunit) (IGP synthase subunit hisF) (ImGP synthase subunit hisF) (IGPS subunit hisF).	27848/5.1	27854	X	X	X	X
psyc_30jun03_gene420	*S*-Adenosyl-L-homocysteine hydrolase	52299/4.9	52530	X	X	X	X
psyc_30jun03_gene2384	Putative membrane alanine aminopeptidase zinc-dependent	98948/4.6	46690	X	X	X	X
psyc_30jun03_gene1053	Putative phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase	26466/5.0	7440	X	X	X	X
psyc_30jun03_gene1833	Possible 1-pyrroline-5-carboxylate reductase	28928/4.6	N/A	X	X	X	X
psyc_30jun03_gene2434	Glutamine synthetase class-I, adenylation site:glutamine synthetase type I: glutamine synthetase, catalytic domain: glutamine s..	52026/5.1	N/A	X	X	X	X
psyc_30jun03_gene1730	Dihydrodipicolinate reductase	29554/5.1	N/A	X	X	X	X

Table 3. Continued

Gene name	Protein name	Protein MW (Da)/p*I*	Experimental MW	Existence			
				22	4	22S	4S
Lipid Metabolism (11)							
psyc_30jun03_gene604	Acyl-CoA dehydrogenase	43411/6.0	43056	X	X	X	X
psyc_30jun03_gene320	Probable medium chain acyl-CoA ligase (AMP binding domain)	63306/5.1	63058	X	X	X	X
psyc_30jun03_gene566	Probable acyl-CoA thioester hydrolase-related protein	20831/8.6	20712	X	X	X	X
psyc_30jun03_gene319	Putative acetyl-CoA acyltransferase (thiolase)	42480/5.4	42357	X	X	X	X
psyc_30jun03_gene523	Putative GcpE protein	40164/5.9	40166	X	X	X	X
psyc_30jun03_gene868	Lipid metabolism-putative wax ester synthase/acyl-CoA:diacylglycerol acyltransferase; fatty acyl-CoA acyltransferase	54395/8.9	54224	X	X	X	X
psyc_30jun03_gene2378	Putative Acyl-CoA dehydrogenase:	42257/5.2	42262	X	X	X	X
psyc_30jun03_gene1831	Probable acetyl-CoA carboxylase, alpha subunit	29406/5.5	29278	X	X	X	X
psyc_30jun03_gene1996	Probable 1-acyl-*sn*-glycerol-3-phosphate acyltransferase	28901/9.8	28898	X	X	X	X
psyc_30jun03_gene1575	Probable phosphatidylserine decarboxylase	30614/6.4	30212	X	X	X	X
psyc_30jun03_gene1167	Putative fatty acid oxidation complex, beta subunit (3-ketoacyl-CoA thiolase)	41395/5.8	N/A	X	X	X	X
Energy Metabolism (20)							
psyc_30jun03_gene2377	Aldehyde dehydrogenase family protein	53000/5.3	53047	X	X	X	X
psyc_30jun03_gene1414	Glycolate oxidase subunit glcE	38596/5.9	38560	X	X	X	X
psyc_30jun03_gene1391	Fumarate hydratase, class II (fumarase)	50295/5.2	50210	X	X	X	X
psyc_30jun03_gene468	Putative ferrodoxin NADP+ reductase	29066/5.2	28938	X	X	X	X
psyc_30jun03_gene1098	H+-transporting two-sector ATPase, F1 sector, beta subunit	51731/4.8	51645	X	X	X	X
psyc_30jun03_gene1096	H+-transporting two-sector ATPase, F1 sector, alpha subunit	55947/5.1	55759	X	X	X	X
psyc_30jun03_gene1301	Malate dehydrogenase	35235/5.0	35106	X	X	X	X
psyc_30jun03_gene1776	Probable succinate dehydrogenase flavoprotein subunit	67485/5.7	67956	X	X	X	X
psyc_30jun03_gene1777	Probable succinate dehydrogenase	26744/6.3	26651	X	X	X	X
psyc_30jun03_gene199	Putative aldehyde dehydrogenase	53996/5.4	53987	X	X	X	X
psyc_30jun03_gene314	Putative acetyl-CoA hydrolase/transferase family protein	59962/6.0	55331	X	X	X	X
psyc_30jun03_gene2825	Putative NADH:flavin oxidoreductase/NADH oxidase: FMN/related compound-binding core	39617/5.3	39491	X	X	X	X
psyc_30jun03_gene2709	Putative iron-containing alcohol dehydrogenase	44810/5.1	44894	X	X	X	X
psyc_30jun03_gene178	Malic oxidoreductase	62676/6.0	62702	X	X	X	X
psyc_30jun03_gene2871	Aldehyde dehydrogenase family protein	55714/5.0	55761	X	X	X	X
psyc_30jun03_gene605	Probable acyl-CoA transferases/carnitine dehydratase	45296/6.0	45130	X	X	X	X
psyc_30jun03_gene1095	Putative H+-transporting two-sector ATPase, F1 sector, delta subunit	22717/5.2	22588	X	X	X	X
psyc_30jun03_gene2873	Glucose-methanol-choline oxidoreductase:GMC oxidoreductase	59458/5.7	59009	X	X	X	X
psyc_30jun03_gene1780	Probable lipoamide dehydrogenase	51143/5.4	51191	X	X	X	X
psyc_30jun03_gene2778	Possible molybdopterin oxidoreductase	88432/5.8	N/A	X	X	X	X
Carbonhydrate Metabolism (6)							
psyc_30jun03_gene1766	Probable fructose-bisphosphate aldolase	33294/5.1	33340	X	X	X	X
psyc_30jun03_gene502	NeuB family protein with antifreeze-like domains	38611/5.2	38483	X	X	X	X
psyc_30jun03_gene546	Fructose-bisphosphate aldolase	37271/5.3	37148	X	X	X	X
psyc_30jun03_gene2594	Probable transaldolase	34675/4.8	34870	X	X	X	X
psyc_30jun03_gene1407	Enolase	47200/4.9	47200	X	X	X	X
psyc_30jun03_gene1879	Inositol monophosphatase	30473/6.1	N/A	X	X	X	X
Nucleotide Metabolism (7)							
psyc_30jun03_gene62	Putative adenylate kinase	23843/5.0	23844	X	X	X	X
psyc_30jun03_gene123	Putative cytidylate kinase	26971/4.8	26982	X	X	X	X
psyc_30jun03_gene518	Nucleoside diphosphate kinase	15610/4.9	15480	X	X	X	X
psyc_30jun03_gene195	Putative inosine 5-monophosphate	52436/6.0	52531	X	X	X	X
psyc_30jun03_gene2800	Phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase	56508/5.4	56476	X	X	X	X

Table 3. Continued

Gene name	Protein name	Protein MW (Da)/p*I*	Experimental MW	Existence			
				22	4	22S	4S
psyc_30jun03_gene2650	Ribonucleotide reductase, alpha chain	85394/5.8	85840	X	X	X	X
psyc_30jun03_gene127	Putative orotidine 5-phosphate	24967/5.0	24944	X	X	X	X
psyc_30jun03_gene254	Amidophosphoribosyltransferase	56744/6.0	N/A	X	X	X	X
Metabolism of cofactors (9)							
psyc_30jun03_gene1797	Probable 3-demethylubiquinone-9 3-methyltransferase	30200/5.6	30628	X	X	X	X
psyc_30jun03_gene949	Probable 3,4-dihydroxy-2-butanone 4-phosphate synthase:GTP cyclohydrolase II	40365/5.2	40280	X	X	X	X
psyc_30jun03_gene2221	*S*-Adenosylmethionine synthetase	42136/5.3	42140	X	X	X	X
psyc_30jun03_gene2859	Delta-aminolevulinic acid dehydratase	37105/5.2	37312	X	X	X	X
psyc_30jun03_gene381	Putative 3'-phosphoadenosine 5'-phosphosulfate sulfotransferase	24335/4.8	24206	X	X	X	X
psyc_30jun03_gene1475	Bifunctional enzyme – riboflavin kinase/FAD synthetase	38322/5.9	38372	X	X	X	X
psyc_30jun03_gene2280	Putative lipoic acid synthetase	39109/7.5	39745	X	X	X	X
psyc_30jun03_gene2115	Putative acetolactate synthase, large subunit	66736/5.7	66686	X	X	X	X
psyc_30jun03_gene1975	Adenosylmethionine–8-amino-7-oxononanoate aminotransferase	47783/5.6	47886	X	X	X	X
Signal Transduction (5)							
psyc_30jun03_gene1640	Putative DnaK suppressor protein	16906/4.9	16772	X	X	X	X
psyc_30jun03_gene1100	Putative two-component response regulator (CheY-like receiver domain)	26999/4.7	27000	X	X	X	X
psyc_30jun03_gene1666	Probable two-component transcriptional response regulator (ompR family)	25220/6.8	25289	X	X	X	X
psyc_30jun03_gene1173	Putative two-component response regulator (phosphate signalling)	26437/5.4	26650	X	X	X	X
psyc_30jun03_gene645	Putative PhoH-like protein, predicted ATPase	40824/5.4	40860	X	X	X	X
Cellular processes(22)							
psyc_30jun03_gene532	Putative membrane-bound lytic murein transglycosylase	47301/6.5	47311	X	X	X	X
psyc_30jun03_gene2399	Probable ABC transport permease	21186/4.6	20918	X	X	X	X
psyc_30jun03_gene2152	Catalase	77814/5.6	77864	X	X	X	X
psyc_30jun03_gene1164	Putative superoxide dismutase (Mn/Fe binding)	23475/5.0	23346	X	X	X	X
psyc_30jun03_gene1587	Probable peptidylprolyl isomerase, FKBP-type (trigger factor)	50587/4.6	50469	X	X	X	X
psyc_30jun03_gene2137	Putative chaperonin HSP60 family	57708/4.8	57590	X	X	X	X
psyc_30jun03_gene1697	Possible ABC transporter	24662/5.6	24496	X	X	X	X
psyc_30jun03_gene1560	Putative beta-ketoacyl-ACP synthase I (fatty acid biosynthesis)	42694/5.2	42631	X	X	X	X
psyc_30jun03_gene2397	CBS domain:sugar isomerase (SIS):KpsF/GutQ family protein	35446/5.6	35495	X	X	X	X
psyc_30jun03_gene1915	Ferric-uptake regulator	16329/5.3	16196	X	X	X	X
psyc_30jun03_gene617	Chaperonin clpA/B:ATP/GTP-binding site motif ATPase with chaperone activity ATP-binding subunit	95909/5.0	95946	X	X	X	X
psyc_30jun03_gene1985	Putative outer membrane protein	42210/4.5	42180	X	X	X	X
psyc_30jun03_gene1338	chaperone protein htpG (Hsp90)	73615/4.7	73690	X	X	X	X
psyc_30jun03_gene69	Putative bacterial extracellular solute-binding protein, family 1	40471/4.9	40280	X	X	X	X
psyc_30jun03_gene2185	Probable peptidyl-prolyl *cis-trans* isomerase	37999/4.7	30272	X	X	X	X
psyc_30jun03_gene2136	Putative chaperonin HSP10	10358/4.9	10770	ND	X	X	X
psyc_30jun03_gene1504	Putative septum site determining protein (ATPase activity) MinD	29671/5.1	29574	X	X	X	X
psyc_30jun03_gene1617	Putative thioredoxin (disulfide oxidoreductase), A strand	11887/4.8	11800	X	X	X	X
psyc_30jun03_gene1186	Putative UDP-*N*-acetylglucosamine 1-carboxyvinyltransferase (cell wall biosynthesis)	44723/5.2	44700	X	X	X	X
psyc_30jun03_gene118	Chaperonin clpA/B: ATP-dependent Clp protease, ATPase subunit	93677/5.3	N/A	X	X	X	X

Table 3. Continued

Gene name	Protein name	Protein MW (Da)/p*I*	Experimental MW	Existence			
				22	4	22S	4S
psyc_30jun03_gene2401	ABC TRANSPORTER ATPase	29112/7.7	N/A	X	X	X	X
psyc_30jun03_gene29	Possible outer membrane efflux protein	57922/6.5	N/A	X	X	X	X
Replication and Repair (6)							
psyc_30jun03_gene295	Putative exodeoxyribonuclease III	31730/5.3	31603	X	X	X	X
psyc_30jun03_gene1058	Putative topoisomerase IV subunit B	69180/5.7	69000	X	X	X	X
psyc_30jun03_gene705	Putative phage terminase, large subunit	65480/5.3	65638	X	X	X	X
psyc_30jun03_gene470	Putative phage integrase	51501/9.3	51210	X	X	X	X
psyc_30jun03_gene1387	DNA topoisomerase I	100201/8.5	50102	X	X	X	X
psyc_30jun03_gene655	ATP-dependent DNA helicase, UvrD/REP family	77992/5.7	73124	X	X	X	X
Transport (3)				X	X	X	X
psyc_30jun03_gene1513	ABC transporter, ATP-binding protein	28025/6.0	23345	X	X	X	X
psyc_30jun03_gene1145	Possible periplasmic C4-dicarboxylate-binding dicarboxylate receptor (TRAP-T family, P subunit)	37867/5.0	35767	X	X	X	X
psyc_30jun03_gene1576	Putative ABC-type phosphate transport system, periplasmic solute binding component	40385/5.0	40320	X	X	X	X
Pseudo (1)							
psyc_30jun03_gene2036	Probable type I restriction-modification system, subunit M (DNA methylase)	75189/4.8	N/A	X	X	X	X

Presence indicates culture conditions at which a protein was detected. 22: at 22°C in 1/2 TSB media; 4: at 4°C in 1/2 TSB media; 22S: at 22°C in 1/2 TSB with 5% NaCl media; 4S: at 22°C in 1/2 TSB with 5% NaCl media.
ND: not detected, N/A: not available.

