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EXPERIMENTAL ARTICLES

The First Protein Map of *Synechococcus* sp. Strain PCC 7942

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Abstract—The first protein map was developed of *Synechococcus* sp. strain PCC 7942, a model organism for studies of photosynthesis, prokaryotic circadian rhythms, cell division, carbon-concentrating mechanisms, and adaptive responses to a variety of stresses. The proteome was analyzed by two-dimensional gel electrophoresis with subsequent MALDI-TOF mass spectroscopy and database analysis. Of the 140 analyzed protein spots, 110 were successfully identified as 62 different proteins, many of which occurred as multiple spots on the gel. The identified proteins participate in the major metabolic and cellular processes in cyanobacterial cells during the exponential growth phase. In addition, 14 proteins which were previously either unknown or considered to be hypothetical were shown to be true gene products in *Synechococcus* sp. strain PCC 7942. These results may be helpful for the annotation of the recently sequenced genome of this cyanobacterium, as well as for biochemical and physiological studies of *Synechococcus.*

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The unicellular cyanobacterium *Synechococcus* sp. strain PCC 7942 is an obligate photoautotroph, belonging to an ancient group of prokaryotes [1]. *Synechococcus* sp. strain PCC 7942, previously known as *Anacystis nidulans* R2, was the first cyanobacterium demonstrated to be transformable by exogenously added DNA [2]. A variety of molecular genetic tools are presently available for a number of cyanobacterial species; information concerning their genomic sequences is being accumulated [3]. *Synechococcus* sp. strain PCC 7942 has been extensively used in genetic and physiological research as a model organism.

Proteomics deals with extensive sets of proteins and applies biochemical techniques for their separation (two-dimensional electrophoresis in polyacrylamide gel) and identification. This rapidly developing field of knowledge can promote deeper understanding of the functions of genes and of the reactions of organisms to physiological factors. Few works have been published on two-dimensional (2-D) electrophoresis and identification of *Synechococcus* sp. PCC 7942 proteins characteristic for specific growth conditions [4–6]. More active proteomic work involving *Synechococcus* sp. strain PCC 7942 is now possible due to the availability of the genome sequence database at http://genome.jgi-psf.org/finished_microbes/synel/synel.home.html. The existence of proteins annotated as "hypothetical" can be verified by proteomic methods, and such verification of predicted gene products is an important step in correct annotation of its genome. Post-translational modifications of proteins are often not apparent from DNA sequence analysis; they are usually revealed by proteomic research based on 2-D gel electrophoresis data analysis. The level of such modifications can be monitored by identifying protein spots encoded by the same gene but occurring at different positions on the 2-D gels. These modifications might eventually be related to the function or activity of a protein [7].

The aim of the present work was to develop a procedure optimized for the proteomic research on *Synechococcus* sp. PCC 7942 and to build the first protein map of this organism.

MATERIALS AND METHODS

Cultivation of *Synechococcus* sp. PCC 7942. The wild-type strain of Synechococcus sp. PCC 7942 was grown in BG11 medium [1] in 100-ml Erlenmeyer flasks at 25°C under continuous illumination (18 μmol quanta $m^{-2}s^{-1}$) on a rotary shaker for three days.

Protein extraction and separation. The cells from 50 ml of the culture (OD_{665} 0.4–0.5) were collected by centrifugation (3000 *g*) at 4°C for 5 min and kept frozen at –75°C until use. The cells were resuspended in 300 μl of protein extraction buffer of the following composition: 8M urea; 0.5% (wol/vol) IPG buffer (Amersham Biosciences, Sweden) pH 3–10; 2% (wt/vol) CHAPS, bromophenol blue (traces); protease

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inhibitors (Protease Inhibitor Cocktail Tablets, Roche Diagnostics Scandinavia AB, Sweden); and 0.28% DTT (wt/vol). The samples were then subjected to five cycles of freezing in liquid nitrogen and thawing, sonicated (BANDELIN Sonopuls HD 2070 MS72, DPC Scandinavia AB, Sweden) for six cycles of two seconds each, and cooled on ice for 40 sec in order to prevent carbamylation [8]. Cell debris was pelleted by centrifugation in a bench top centrifuge at 15800 *g* for 10 min at room temperature. The supernatants with total proteins were retrieved and stored at –20°C until use. Protein concentration was measured with the RC DC Protein Assay kit (Bio-Rad, Richmond, CA, United States).

Two-dimensional gel electrophoresis. Overnight rehydration of 18-cm immobiline gel strips (Amersham Biosciences, Sweden) with a pH range 3–10 in extraction buffer (see protein extraction) at room temperature was performed for electrophoresis in the first direction. Prior to isoelectric focusing, 800 μg of protein extracts were loaded onto the gel strips with the application cups positioned at the acidic end of the strip. Electrophoresis in the first direction was performed on an IPGphore (Amersham Biosciences, Sweden) with voltage settings of 500 V for 1 h, 1000 V for 1 h, and 8000 V for approximately 10 h. The last focusing step was stopped when the run reached a total of 80000 Vh. Before electrophoresis in SDS-PAGE, the IPG strips were equilibrated for 15 min in a solution containing 50 mM *Tris*–HCl buffer (pH 8.8), 6 M urea, 30% (vol/vol) glycerol [87%], 2% SDS, and 10 mg ml^{-1} DTT, with subsequent 15-min incubation in the same solution with 25 mg ml^{-1} iodoacetamide instead of DTT. After equilibration, the strips were placed on top of 12% polyacrylamide gel and embedded by the addition of heated 0.5% (wt/vol) low-melting agarose in the SDS electrophoresis running buffer (25 mM *Tris*, 192 mM glycine, 0.5% SDS). SDS-PAGE was performed in a PROTEAN II® xi cell gel electrophoresis unit (BioRad). After separation (10 mA/gel for 15 min followed by 20 mA/gel until the dye front reached the bottom edge of the gel), the gel was incubated in the fixing solution (20% (vol/vol) methanol and 10% (vol/vol) acetic acid) for 4 h and stained for proteins with Coomassie Brilliant Blue R250 (Fluka Chemie AG, Switzerland) for 24 h. Destaining was accomplished by overnight incubation in a mixture of 20% (vol/vol) methanol and 10% (vol/vol) acetic acid at 4°C, followed washing with distilled water. Gel imaging was performed on a CanoScan 5000F (Canon, Japan). BenchMark™ Pre-Stained Protein Ladder (Invitrogen, United States) was used as the protein molecular weight standard.

In-gel digestion and peptide extraction. Spots were excised from the gel, destained with 20% methanol and 10% acetic acid and in-gel digested with trypsin according to Wilm et al. [9].

MALDI-TOF mass spectrometry analysis and protein identification. Mass spectra were recorded in the positive reflection mode by using an Applied Biosystems MALDI-TOF Voyager-DE STR mass spectrometer equipped with delayed ion registration technology. The peptide mass profiles produced by MS were internally calibrated using the MoverZ software package (http://www.genomicsolutionscanada.com), using the known autolysis peaks from porcine trypsin. After subtraction of the known background peaks derived from the matrix, trypsin, and traces of keratin, the lists of peptide masses were compared to the databases with the help of the Mascot software package (http://www.matrixscience.com).

RESULTS AND DISCUSSION

Of the 140 protein spots which were revealed on the gel (Figure), 110 were identified as representing 62 different proteins. These proteins can be placed in 15 different functional groups (the table). This protein chart shows a snapshot of the cellular and metabolic processes in the exponentially grown culture. Twenty-three proteins have been found as sets of several spots (isomorphs), while 14 unknown and hypothetical proteins have been localized on the protein map of *Synechococcus* sp. PCC 7942 for the first time. The identified proteins represent soluble and membrane proteins. Some of the identified proteins are described in further detail below.

Proteins involved in cell morphogenesis (Group 1). Several spots (1; 11a–c, and 24) represent cell division proteins. Spot 1 represents a molecular chaperone containing two overlapping regions. The first region contains the amino acid sequence characteristic of MreB, which is involved in cell morphogenesis (cell division and chromosome partitioning) [10]. The second region contains the amino acid sequence characteristic of the ATP-dependent chaperone Hsp70.

Spot 24 represents an actinlike ATPase (MreB) (Figure, Table, Group 1). It is notable that MreB is a shape-determining protein, homologous to the Hsp70 and FtsA proteins, which are involved in bacterial cell division [11]. One of the key proteins of cell division, FtsZ, was recovered as three spots (11a–11c) with slightly different values of pI (isoelectric point) and mw (molecular weight). This protein is a GTPase and participates in the formation of the septum in the course of bacterial cell division [12].

An actively grown cyanobacterial culture, like the one used in the present work, requires continuous synthesis of the new structural components of the cell wall. The proteins responsible for these processes are therefore present in its proteome. Spot 5 represents an outer membrane efflux protein. The members of this protein family, the OEP proteins, form three-dimensional channels which permit the export of a variety of substrates by gram-negative bacteria [13]. Another protein (spots 13 a and b) was similar to the SomA protein from the closely related cyanobacterial strain, *Synechococcus*

Proteins identified in *Synechococcus* sp. PCC 7942

Spot no.	Protein identification	Putative Mw/pI	NCBI Accession no.
	Group 1. Cell morphogenesis		
1	Molecular chaperone	67.7/4.7	46130530
11a	Cell division GTPase	40.2/4.9	46130477
11b	Cell division GTPase	40.2/4.9	46130477
11c	Cell division GTPase	40.2/4.9	46130477
24	Actin-like ATPase	35.0/5.1	53763074
5	Outer membrane protein	57.6/5.2	45513235
13a	PPE-repeat protein	57.2/5.4	46129905
13b	PPE-repeat protein	57.2/5.4	46129905
14a	Type II secretory pathway, component HofQ	80.3/8.4	46130521
14b	Type II secretory pathway, component HofQ	80.3/8.4	46130521
14c	Type II secretory pathway, component HofQ	80.3/8.4	46130521
46	Organic solvent tolerance protein OstA	15.2/9.3	45511847
7a	UDP-N-acetylmuramyl tripeptide synthase	53.1/5.1	46129915
7b	UDP-N-acetylmuramyl tripeptide synthase	53.1/5.1	46129915
7c	UDP-N-acetylmuramyl tripeptide synthase	53.1/5.1	46129915
33a	Diaminopimelate epimerase	30.3/4.8	46130191
33b	Diaminopimelate epimerase	30.3/4.8	46130191
	Group 2. Protein synthesis		
15a	Asp-tRNAAsn/Glu-tRNAGln amidotrans-ferase B subunit (PET112 homolog)	54.6/5.4	53763184
15b	Asp-tRNAAsn/Glu-tRNAGln amidotrans-ferase B subunit (PET112 homolog)	54.6/5.4	53763184
28	Ribosomal protein S1	31.8/5.2	22002498
29a	Translation elongation factor Ts	24.4/5.3	46130565
29b	Translation elongation factor Ts	24.4/5.3	46130565
40	Ribosome recycling factor	19.2/6.4	53762955
53	RNA-binding protein (RRM domain)	10.7/4.9	45513450
59	RNA-binding protein (RRM domain)	11.3/5.5	53762887
	Group 3. Posttranslational protein processing, modification, maturation, protein turnover, chaperones		
2a	Chaperonin GroEL (HSP60 family)	58.2/4.7	53762838
2b	Chaperonin GroEL (HSP60 family)	58.2/4.7	53762838
4a	Chaperonin GroEL	58.0/5.1	46130438
4b	Chaperonin GroEL	58.0/5.1	46130438
4c	Chaperonin GroEL	58.0/5.1	46130438
4d	Chaperonin GroEL	58.0/5.1	46130438
4e	Chaperonin GroEL	58.0/5.1	46130438
4f	Chaperonin GroEL	58.0/5.1	46130438
18a	Periplasmic protease	46.2/8.9	53762820
18b	Periplasmic protease	46.2/8.9	53762820
34	Molecular chaperone GrpE	23.0/4.7	45513516
42a	Cyclophilin; peptidylprolyl isomerase; rotamase	15.8/5.7	46489
42b	Cyclophilin; peptidylprolyl isomerase; rotamase	15.8/5.7	46489
54	GroES protein	10.7/4.7	97572

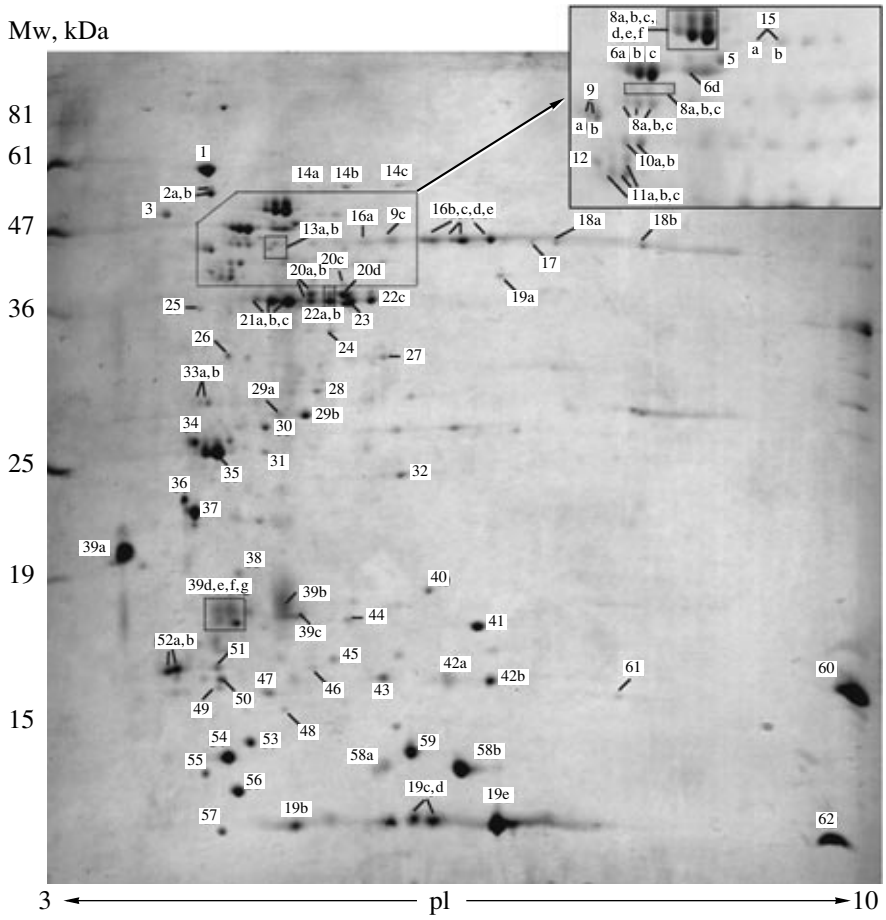
Table. (Contd.)

Spot no.	Protein identification	Putative Mw/pI	NCBI Accession no.
61	FKB-type peptidyl-prolyl *cis-trans* isomerase 1	18.2/5.3	56686717
12	FOG: TPR repeat	31.7/5.4	53762940
	Group 4. Regulatory functions		
3	Methyl-accepting chemotaxis protein	45.9/4.4	46129853
	Group 5. Energy production and conversion		
6a	Unnamed protein	52.2/5.0	48024
6b	Unnamed protein	52.2/5.0	48024
6c	Unnamed protein	52.2/5.0	48024
6d	Unnamed protein	52.2/5.0	48024
37	Inorganic pyrophosphatase	19.1/4.6	46129844
38	Adenylate kinase	20.3/5.0	45513641
	Group 6. Amino acids metabolism		
8a	*S*-adenosylmethionine synthetase	45.4/5.0	46130527
8b	*S*-adenosylmethionine synthetase	45.4/5.0	46130527
8c	*S*-adenosylmethionine synthetase	45.4/5.0	46130527
27	Cystein synthase	34.1/5.5	45512974
	Group 7. Glucose metabolism		
9a	Enolase	45.4/4.7	53762871
9b	Enolase	45.4/4.7	53762871
9c	Enolase	45.4/4.7	53762871
21a	Fructose-1,6-bisphosphatase/sedoheptulose 1,7-bisphosphatase	37.3/5.1	45511855
21b	Fructose-1,6-bisphosphatase/sedoheptulose 1,7-bisphosphatase	37.3/5.1	45511855
21c	Fructose-1,6-bisphosphatase/sedoheptulose 1,7-bisphosphatase	37.3/5.1	45511855
22a	Fructose/tagatose bisphosphate aldolase	39.1/5.3	46129889
22b	Fructose/tagatose bisphosphate aldolase	39.1/5.3	46129889
22c	Fructose/tagatose bisphosphate aldolase	39.1/5.3	46129889
	Group 8. DNA synthesis		
10a	DNA polymerase III beta subunit	40.5/4.9	974615
10b	DNA polymerase III beta subunit	40.5/4.9	974615
	Group 9. Sulfate metabolism		
16a	Sulfite reductase, alpha subunit (flavoprotein)	44.4/5.7	45512552
16b	Sulfite reductase, alpha subunit	44.4/5.7	45512552
16c	Sulfite reductase, alpha subunit	44.4/5.7	45512552
16d	Sulfite reductase, alpha subunit	44.4/5.7	45512552
16e	Sulfite reductase, alpha subunit	44.4/5.7	45512552
	Group 10. Lipid metabolism		
17	Predicted deinelactone hydrolase	56.0/6.3	53763021
	Group 11. Carbon dioxide concentrating mechanism and carbon dioxide fixation		
19a	Carbon concentrating mechanism/carboxysome shell protein	10.9/5.9	46129870
19b	Carbon concentrating mechanism/carboxysome shell protein	10.9/5.9	46129870
19c	Carbon concentrating mechanism/carboxysome shell protein	10.9/5.9	46129870
19d	Carbon concentrating mechanism/carboxysome shell protein	10.9/5.9	46129870
19e	Carbon concentrating mechanism/carboxysome shell protein	10.9/5.9	46129870
58a	Ribulose bisphosphate carboxylase, small subunit	13.3/5.6	46129874
58b	Ribulose bisphosphate carboxylase, small subunit	13.3/5.6	46129874

Table. (Contd.)

Spot no.	Protein identification	Putative Mw/pI	NCBI Accession no.
	Group 12. Nucleotide transport and metabolism		
20a	IMP dehydrogenase/GMP reductase	40.6/5.3	46130119
20b	IMP dehydrogenase/GMP reductase	40.6/5.3	46130119
20c	IMP dehydrogenase/GMP reductase	40.6/5.3	46130119
20d	IMP dehydrogenase/GMP reductase	40.6/5.3	46130119
	Group 13. Photosynthesis		
39a	Phycocyanin beta-subunit	18.4/5.2	38900
39b	Phycocyanin beta-subunit	18.4/5.2	38900
39c	Phycocyanin beta-subunit	18.4/5.2	38900
39d	Phycocyanin beta-subunit	18.4/5.2	38900
39e	Phycocyanin beta-subunit	18.4/5.2	38900
39f	Phycocyanin beta-subunit	18.4/5.2	38900
39g	Phycocyanin beta-subunit	18.4/5.2	38900
57	Plastocyanin	13.4/5.5	45512645
60	PsaD protein	15.6/9.3	226392
62	Hypothetical protein Selo03000602	8.1/8.0	45512850
	Group 14. Oxidative stress protection		
30	Glutathion S-transferase	29.1/5.1	45512374
35	Peroxidase	23.7/4.8	81301258
43	Peroxiredoxin	16.6/5.5	45513396
45	Peroxiredoxin	17.6/5.4	45513279
52a	Peroxiredoxin	15.8/4.5	46130348
52b	Peroxiredoxin	15.8/4.5	46130348
	Group 15. Unknown and hypothetical proteins		
23	Hypothetical protein	43.6/6.4	24414820
25	Hypothetical protein Selo03001742	33.5/4.7	45513861
26	Hypothetical protein	20.8/5.0	56750131
32	Uncharacterized conserved protein	25.0/6.6	53762884
36	Hypothetical protein Selo03002321	11.4/4.5	53763101
41	Hypothetical protein	20.8/5.0	56750131
44	Unknown protein	23.0/5.5	24251256
47	Hypothetical protein Selo03002005	20.4/8.0	53762906
48	Unknown protein	16.9/5.1	24251259
49	Predicted membrane protein	18.3/4.9	45512224
50	Hypothetical protein Selo03000898	20.9/6.2	46129994
51	Hypothetical protein Selo03000735	16.6/5.1	45512960
55	Unknown protein	14.0/4.7	17220757
56	Unknown protein	11.8/4.9	24251253
12	Hypothetical protein	46.4/4.9	56750625

sp. PCC 6301 (89% sequence identity) [14]. This protein is a porin localized in the cyanobacterial outer membrane [15]. Similar to the protein represented by spots 13a and 13b, SomA contains a domain specific to the proteins of the surface S layer of the cell wall. The S layers are formed by the glycoprotein monolayers on the cell surface. Some of the S layer proteins and certain other cell wall proteins include one or more copies of the SLH (S Layer Homology) domain containing 50–60 amino acids. This domain was shown to anchor



Two-dimensional electrophoresis protein index (Coomassie-stained gel) of total protein of *Synechococcus* sp. strain PCC 7942. The protein molecular weight standard (Mw) (*y* axis) and isoelectric points (pI) (*x* axis) are shown. The identification of each labeled spot is presented in the table.

the macromolecules to peptidoglycan [16]. The component of the type II secretory pathway, HofQ, was found as three separate spots with different pI values (spots 14a–c). This protein is involved in the general (type II) secretion pathway (GSP) of gram-negative bacteria [17].

The protein OstA (spot 46) determines the resistance to organic solvents. In *Escherichia coli*, OstA determines its resistance to *n*-hexane and is involved in maintaining the outer membrane permeability. This protein is essential for the envelope biogenesis and could be a part of the targeting system for outer membrane components [18].

Two enzymes involved in peptidoglycan synthesis were identified, UDP-*N*-acetylmuramyl tripeptide synthase (spots 7a–c) and diaminopimelate epimerase (spots 33a, b). The former enzyme belongs to the Mur ligase family and possesses a Mur ligase C domain. The latter is involved in lysine biosynthesis; it catalyzes the formation of meso-diaminopimelate (meso-DAP), the direct precursor of lysine and an essential component of cell-wall peptidoglycan in gram-negative bacteria [19].

Protein synthesis and post-translational protein processing (Groups 2 and 3). Two different RNA-binding proteins (spots 53 and 59) were identified; they contain a RRM (RNA Recognition Motif) domain, which is involved in RNA binding and the regulation of transcription termination. Cyanobacterial RNA-binding proteins (Rbp) and eukaryotic glycine-rich RNA-binding proteins (GRPs) were shown to be similar in both structure and regulation; this is a result of convergent evolution [20]. Cyanobacterial *rpb* genes are expressed under stress conditions. For example, in *Anabaena variabilis* strain M3, the expression of *rpb* genes is induced by low temperature (similar to the synthesis of GRP in plants and mammals). Moreover, the transcription of *rbp* genes in *Anabaena* sp. PCC 7120 was also shown to be under osmotic stress regulation [21, 22].

The ribosomal protein S1, identified as spot 28, possesses three S1-like RNA-binding domains. This protein is essential for cell viability and is part of the 70S ribosome in bacteria [23]. Furthermore, ribosome-recycling factor was identified as spot 40 (the figure). A translation elongation factor, Ts, was identified as two

spots (29a, b) of different pI values. An Asp-tRNAAsn/Glu-tRNAGln amidotransferase B subunit is present on the protein map as two spots (15a, b), also with different pI values.

Seven proteins involved in post-translational protein processing and protein folding were identified (Table, group 5). From the HSP60 chaperonin family, two different chaperonin GroEL (spots 2a, b and spots 4a–f) proteins were identified. One protein was also identified as a GroES chaperone (spot 54). The GroEL/GroES system is a major chaperone system in all bacteria, and the mechanisms by which this system responds to stress conditions have been extensively studied in cyanobacteria [24–26]. The fourth identified chaperone is a molecular chaperone GrpE (spot 34). This protein stimulates, jointly with DnaJ, the ATPase activity of the DnaK chaperone in prokaryotes [27]. A periplasmic protease (spots 18a, b) possessing an N-terminal transmembrane domain was identified. This protein contains a PDZ domain, which promotes protein–protein interactions by binding to internal or C-terminal sequences of the partner proteins.

FKB-type peptidyl-prolyl-*cis-trans*-isomerase 1 was revealed (spot 61); it accelerates protein folding by catalyzing the cis-trans isomerization of proline imide peptide bonds in oligopeptides. Another isomerase, cyclophilin, was identified in spots 42a, b. Cyclophilins participate in protein folding and represent a family within the enzyme class of peptidyl-prolyl-*cis-trans*-isomerases [28].

Protein–protein interactions and regulatory functions (Groups 3 and 4). Two different proteins (spots 12 and 31) were identified as TPR repeat-containing proteins. The tetratricopeptide repeat (TPR) is a degenerate sequence (34 amino acids) that participates in protein–protein interactions and often in the assembly of multiprotein complexes. Extensive evidence indicates the role of TPR motifs in the functioning of chaperone proteins, cell cycle proteins, transcription, and protein-transport complexes [29]. TPR repeats were recently revealed in the cell division protein FTN2 of *Synechococcus* sp. PCC 7942 [30] and in a protein (gene *sll0886*) required for light-activated heterotrophic growth of *Synechocystis* sp. PCC 6803 [31]. In the present work, we have identified two new TPR-repeat-containing proteins in *Synechococcus* sp. PCC 7942; their function is unknown.

A methyl-accepting chemotaxis protein (spot 3) was identified and classified as a regulatory protein, homologous to the methyl-accepting chemotaxis protein required for the biogenesis of thick pili, and to the phytochrome-like photoreceptor protein involved in positive phototaxis in *Synechocystis* sp. PCC 6803 [32, 33].

Proteins of general cell metabolism (Groups 5–13). This group of proteins is involved in cyanobacterial cell metabolism. Many of these proteins were identified as sets of two or more spots on the 2-D gel. Two proteins (Group 14, spots 19a–e and 58a, b) are involved in the carbon-concentrating mechanism and carbon dioxide fixation. A small carboxysome shell protein was identified as a set of five spots, four of which have the predicted molecular weight of approximately 11 kDa (spots 19b–e, the figure and the table) and differ in their pI values. Spot 19a has a higher molecular weight (approximately 44 kDa) and may represent a tetramer of this small protein, which has not been dissociated during the electrophoretic separation.

Photosynthesis-related proteins (Group 16) were represented by several proteins, including a phycocyanin beta subunit (spots 39a–g), plastocyanin (spot 57), and two components of photosystem I, namely PsaD (spot 60) and PsaE (spot 62).

All the proteins involved in glucose metabolism (Group 10) were revealed on the 2-D map as sets of multiple spots. For example, enolase (spots 9a–c) and fructose-1,6-bisphosphatase/sedoheptulose 1,7 bisphosphatase (spots 21a–c) were both present as triple spots mainly differing in pI. Fructose/tagatose bisphosphate aldolase was also identified in three different spots (spots 22a–c), two of which differed slightly in molecular weight and the third one in pI (the figure).

Oxidative stress protection proteins (Group 14). Being a photosynthetic organism, *Synechococcus* sp. PCC 7942 encounters an excess of free radicals as a byproduct of oxygenic photosynthesis. Five proteins possibly involved in oxidative stress protection were identified: a glutathione S-transferase (spot 30) and four different peroxiredoxins (spots 35, 43, 45, and 52a, b). Peroxiredoxins are thioredoxin- or glutaredoxin-dependent peroxidases. Anti-oxidative stress systems in cyanobacteria have been studied more extensively in *Synechocystis* sp. PCC 6803 [34–36].

Unknown and hypothetical proteins (Group 15). An unclassified group of proteins (the table, group 18) was represented by 14 unknown and hypothetical proteins. One example is the hypothetical protein Selo03002321 (spot 36); its gene had been found previously only in the genome sequence of *Synechococcus* sp. PCC 7942. An uncharacterized conservative protein (spot 32) possesses a region specific for a protein of unknown function (DUF541). Proteins of this family have only been found in bacteria and in mice; in the latter case, they were identified as SIMPL (signaling molecule that associates with mouse pelle-like kinase); their function in bacteria is still unknown.

The membrane protein (spot 49) contains a region specific for proteins of unknown function (DUF1269). This protein family includes several bacterial and archaeal proteins [37]. The function of this family is unknown.

The protein of spot 55 possesses an FAS1 region. It has been suggested that the FAS1 domain represents an ancient cell adhesion domain [38]. This protein has been annotated in the *Synechococcus elongatus* PCC 7942 genome project (ZP_00165104) as COG2335: secreted and surface protein containing fascilin-like

repeats. The closest known homologous proteins are the ones found in *Gloeobacter violaceus* PCC 7421 (hypothetical protein *glr1006*, 67% sequence identity) and in *Anabaena variabilis* ATCC 29413 (COG2335: secreted and surface protein, 62% sequence identity). An unknown protein (spot 56) contains a region characteristic for thioredoxins. Thioredoxins are a class of small (approximately 12 kDa) redox-active proteins that maintain the intracellular redox potential. Thioredoxins are important regulatory proteins in carbon assimilation by the cells of oxygenic phototrophs. The regulatory functions of chloroplast thioredoxins have recently been described [39] using a proteomic approach. Future work on targeted inactivation of the genes encoding unknown and hypothetical proteins will promote the study of their functions.

In conclusion, we have optimized the experimental conditions for proteomic research and developed the first protein map of *Synechococcus* sp. PCC 7942. A total of 62 soluble and membrane-bound were identified; these can be organized into 15 different functional groups. Twenty-three proteins were found as sets of several spots that could represent posttranslational protein modifications and might be involved in the regulation of their function or activity (isomorphs). The obtained results give insight into the functional and metabolic processes occurring in an important model organism, and may be significant for subsequent proteomic research on *Synechococcus* sp. PCC 7942. Fourteen unknown and hypothetical proteins have been identified on the protein map of *Synechococcus* sp. PCC 7942 for the first time. Such verification of the existence of these protein products will be an important and useful step in annotating the genome. The results obtained in the present work can be used as controls for future experiments dealing with the response of *Synechococcus* sp. PCC 7942 to various stress factors.

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REFERENCES

1. Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M., and Stanier, R.Y., Generic Assignments, Strain Histories and Properties of Pure Cultures of Cyanobacteria, *J. Gen. Microbiol.*, 1979, vol. 111, pp. 1–61.
2. Shestakov, S.V. and Khyen, N.T., Evidence for Genetic Transformation in Blue-Green Alga *Anacystis Nidulans, Mol. Gen. Genet.*, 1970, vol. 107, pp. 372–375.
3. Koksharova, O.A. and Wolk, C.P., Genetic Tools for Cyanobacteria, *Appl. Microbiol. Biothechnol,* 2002, vol. 58, pp. 123–137.
4. Görl, M., Sauer, J., Baier, T., and Forchhammer, K., Nitrogen-Starvation-Induced Chlorosis in *Synechococcus* PCC 7942: Adaptation To Long-Term Survival, *Microbiology (UK)*, 1998, vol. 144, pp. 2449–2458.
5. Pandey, R., Chauhan, S., Singal, G.S., and Kale, R.K., Spectral Light Quality Affects Protein Profile of *Synechococcus* sp. PCC 7942: a Comparative 2-Dimensional Gel Electrophoresis (2-DGE) Analysis, *Curr. Microbiol.*, 2000, vol. 40, pp. 297–301.
6. Cha, E-Y., Park, J.S., Jeon, S., Kong, J.S., Choi, Y.K., Ryu, J.-Y., Park, Y.-II, and Park, Y. S. Functional Characterization of the Gene Encoding UDP-Glucose: Tetrahydrobiopterin -Glucosyltransferase in *Synechococcus* sp. PCC 7942, *J. Microbiol.*, 2005, vol. 43, pp. 191–195.
7. VanBogelen, R.A., Schiller, E.E., Thomas, J.D., and Neidhardt, F.C., Diagnosis of Cellular States of Microbial Organisms Using Proteomics, *Electrophoresis*, 1999, vol. 20, pp. 2149–2159.
8. McCarthy, J., Hopwood, F., Oxley, D., Laver, M., Castagna, A., Righetti, P.G., Williams, K., and Herbert, B., Carbamylation of Proteins in 2-D Electrophoresis - Myth Or Reality?, *J. Proteome Res*, 2003, vol. 2, pp. 239–242.
9. Wilm, M., Shevchenko, A., Houthaeve, T., Breit, S., Schweigerer, L., Fotsis, T., and Mann, M., Femtomole Sequencing of Proteins from Polyacrylamide Gels by Nano-Electrospray Mass Spectrometry, *Nature*, 1996, vol. 379, pp. 466–469.
10. Doolittle, R.F. and York, A.L., Bacterial Actins? An Evolutionary Perspective, *BioEssays*, 2002, vol. 24, pp. 293–296.
11. Bork, P., Sander, C., and Valencia, A., An ATPase Domain Common To Prokaryotic Cell Cycle Proteins, Sugar Kinases, Actin, and hsp70 Heat Shock Proteins, *Proc. Natl. Acad. Sci. USA*, 1992, vol. 89, pp. 7290–7294.
12. Bi, E. and Lutkenhaus, J., FtsZ Ring Structure Associated with Division in *Escherichia coli, Nature*, 1991, vol. 354, pp. 161–164.
13. Johnson, J.M. and Church, G.M., Alignment and Structure Prediction of Divergent Protein Families: Periplasmic and Outer Membrane Proteins of Bacterial Efflux Pumps, *J. Mol. Biol.*, 1999, vol. 287, pp. 695–715.
14. Hansel, A., Pattus, F., Jurgens, U.J., and Tadros, M.H., Cloning and Characterization of the Genes Coding for Two Porins in the Unicellular Cyanobacterium *Synechococcus* PCC 6301, *Biochim. Biophys. Acta*, 1998, vol. 1399, pp. 31–39.
15. Umeda, H., Aiba, H., and Mizuno, T., *Som*A, a Novel Gene That Encodes a Major Outer-Membrane Protein of *Synechococcus* sp. PCC 7942, *Microbiology (UK), 1996*, vol. 142, pp. 2121–2128.
16. Mesnage, S., Fontaine, T., Mignot, T., Delepierre, M., Mock, M., and Fouet, A., Bacterial SLH Domain Proteins Are Non-Covalently Anchored To the Cell Surface Via a Conserved Mechanism Involving Wall Polysaccharide Pyruvylation, *EMBO J.*, 2000, vol. 19, pp. 4473–4484.
17. Genin, S. and Boucher, C.A., A Superfamily of Proteins Involved in Different Secretion Pathways in Gram-Negative Bacteria: Modular Structure and Specificity of the N-Terminal Domain, *Mol. Gen. Genet.*, 1994, vol. 243, pp. 112–118.
18. Braun, M. and Silhavy, T.J., Imp/OstA Is Required for Cell Envelope Biogenesis in *Escherichia coli, Mol. Microbiol.*, 2002, vol. 45, pp. 1289–1302.

19. Mirelman, D, Biosynthesis and Assembly of Cell Wall Peptidoglycan, *Bacterial outer membranes*, Inouye, M., Ed., New York: Wiley, 1979, pp. 115–166.
20. Maruyama, K., Sato, N., and Ohta, N., Conservation of Structure and Cold-Regulation of RNA-Binding Proteins in Cyanobacteria: Probable Convergent Evolution with Eukaryotic Glycine-Rich RNA-Binding Proteins, *Nucleic Acid Res.*, 1999, vol. 27, pp. 2029–2036.
21. Ehira, S., Hamano, T., Hayashida, T., Kojima, K., Nakamoto, H., Hiyama, T., Ohmori, M., Shivaji, S., and Sato, N., Conserved Temperature-Dependent Expression of RNA-Binding Proteins in Cyanobacteria with Different Temperature Optima, *FEMS Microbiol. Letts.*, 2003, vol. 225, pp. 137–142.
22. Mori, S., Castoreno, A., Mulligan, M.E., and Lammers, P.J., Nitrogen Status Modulates the Expression of RNA-Binding Proteins in Cyanobacteria, *FEMS Microbiol. Letts.*, 2003, vol. 227, pp. 203–210.
23. Roberts, M.W. and Rabinowitz, J.C., The Effect of *Escherichia coli* Ribosomal Protein S1 on the Translational Specificity of Bacterial Ribosomes, *J. Biol. Chem.*, 1989, vol. 264, pp. 2228–2235.
24. Hihara, Y., Kamei, A., Kanehisa, M., Kaplan, A., and Ikeuchi, M., DNA Microarray Analysis of Cyanobacterial Gene Expression During Acclimation To High Light, *Plant Cell*, 2001, vol. 13, pp. 792–806.
25. Kovacs, E., van der Vies, S.M., Glatz, A., Torok, Z., Varvasovszki, V., Horvath, I., and Vigh, L., The Chaperonins of *Synechocystis* PCC 6803 Differ in Heat Inducibility and Chaperone Activity, *Biochem. Biophys. Res. Commun.*, 2001, vol. 289, pp. 908–915.
26. Mary, I., Tu, C-J., Grossman, A., and Vaulot, D., Effects of High Light on Transcripts of Stress-Associated Genes for the Cyanobacteria *Synechocystis* sp. PCC 6803 and *Prochlorococcus* MED4 and MIT9313, *Microbiology (UK)*, 2004, vol. 150, pp. 1271–1281.
27. Bremer, D., Gassler, C., Rist, W., Mayer, M.P., and Bukau, B., Influence of GrpE on DnaK-Substrate Interactions, *J. Biol. Chem.*, 2004, vol. 279, pp. 27957–27964.
28. Konno, M., Sano, Y., Okudaira, K., Kawaguchi, Y., Yamagishi-Ohmori, Y., Fushinobu, S., and Matsuzawa, H., *Escherichia coli* Cyclophilin B Binds a Highly Distorted Form of Trans-Prolyl Peptide Isomer, *Eur. J. Biochem.*, 2004, vol. 271, pp. 3794–3803.
29. Blatch, G.L. and Lässle, M., The Tetratricopeptide Repeat: a Structural Motif Mediating Protein-Protein Interactions, *BioEssays*, 1999, vol. 21, pp. 932–939.
30. Koksharova, O.A. and Wolk, C.P., A Novel Gene That Bears a DnaJ Motif Influences Cyanobacterial Cell Division, *J. Bacteriol.*, 2002, vol. 184, pp. 5524–5528.
31. Kong, R., Xu, X., and Hu, Z., A TPR-Family Membrane Protein Gene Is Required for Light-Activated Heterotrophic Growth of the Cyanobacterium *Synechocystis* sp. PCC 6803, *FEMS Microbiol. Letts.*, 2003, vol. 219, pp. 75–79.
32. Bhaya, D., Takahashi, A., and Grossman, A.R., Light Regulation of Type IV Pilus-Dependent Motility by Chemosensor-Like Elements in *Synechocystis* PCC 6803, *Proc. Natl. Acad. Sci. USA*, 2001, vol. 98, pp. 7540–7545.
33. Yoshihara, S., Suzuki, F., Fujita, H., Geng, X.X., and Ikeuchi, M., Novel Putative Photoreceptor and Regulatory Genes Required for the Positive Phototactic Movement of the Unicellular Motile Cyanobacterium *Synechocystis* sp. PCC 6803, *Plant Cell Physiol.*, 2000, vol. 41, pp. 1299–1304.
34. Hosoya-Matsuda, N., Motohashi, K., Yoshimura, H., Nozaki, A., Inoue, K., Ohmori, M., and Hisabori, T., Anti-Oxidative Stress System in Cyanobacteria: Significance of Type II Peroxiredoxin and the Role of 1-Cys Peroxiredoxin in *Synechocystis* sp. Strain PCC 6803, *J. Biol. Chem.*, 2005, vol. 280, pp. 840–846.
35. Kobayashi, M., Ishizuka, T., Katayama, M., Kanehisa, M., Bhattacharyya-Pakrasi, M., Pakrasi, H.B., and Ikeuchi, M., Response To Oxidative Stress Involves a Novel Peroxiredoxin Gene in the Unicellular Cyanobacterium *Synechocystis* sp. PCC 6803, *Plant Cell Physiol.,* 2004, vol. 45, pp. 290–299.
36. Li, H., Singh, A.K., McIntyre, L.M., and Sherman, L.A., Differential Gene Expression in Response To Hydrogen Peroxide and the Putative PerR Regulon of *Synechocystis* sp. Strain PCC 6803, *J. Bacteriol.*, 2004, vol. 186, pp. 3331–3345.
37. Bateman, A., The Structure of a Domain Common To Archaebacteria and the Homocystinuria Disease Protein, *Trends. Biochem. Sci*, 1997, vol. 22, pp. 12–13.
38. Huber, O. and Sumper, M., Algal-CAMs: Isoforms of a Cell Adhesion Molecule in Embryos of the Alga *Volvox* with Homology To *Drosophila* Fasciclin I, *EMBO J.*, 1994, vol. 13, pp. 4212–4222.
39. Balmer, Y., Koller, A., del Val, G., Manieri, W., Schürmann, P., and Buchanan, B.B., Proteomics Gives Insight Into the Regulatory Function of Chloroplast Thioredoxins, *Proc. Natl. Acad. Sci. USA*, 2003, vol. 100, pp. 370–375.