Microbes Environ. Vol. 21, No. 2, 129–133, 2006
http://wwwsoc.nii.ac.jp/jsme2/

Short Communication

Up-regulated Gene Expression during Dehydration in a Terrestrial Cyanobacterium, *Nostoc* sp. Strain HK-01

HIDEHISA YOSHIMURA[1]*, MASAHIKO IKEUCHI[2] and MASAYUKI OHMORI[1,3]

[1] *Center of Systems Biology & Ecology, Faculty of Science, Toho University, 2–2–1 Miyama, Funabashi, Chiba 274–8510, Japan*
[2] *Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, 3–8–1 Komaba, Meguro-ku, Tokyo 153–8902, Japan*
[3] *Department of Biochemistry and Molecular Biology, Faculty of Science, Saitama University, 255 Shimo-Ohkubo, Sakura-ku, Saitama-shi, Saitama 338–8570, Japan*

(Received November 10, 2005—Accepted March 17, 2006)

Genes expressed more intensively during dehydration were screened in the terrestrial cyanobacterium *Nostoc* sp. HK-01, which is phylogenetically very similar to the aquatic *Anabaena* sp. PCC 7120. A DNA microarray for *Anabaena* was used to determine the gene expression of *Nostoc* sp. HK-01 cells. The results showed that genes homologous with those of *Anabaena* were expressed increasingly more during dehydration. The expression was transient in *Anabaena* while in *Nostoc* it increased until the wet weight decreased to 10% of that before drying. It was concluded that the higher desiccation-tolerance of *Nostoc* was supported by the more intensive gene expression than in *Anabaena*.

Key words: cyanobacteria, *Nostoc*, drought-tolerance, gene expression

Cyanobacteria, the photosynthetic, O_2-evolving prokaryotes, grow in diverse habitats ranging from tropical to polar regions. In terrestrial cyanobacteria, the cells can survive under desiccated conditions for a long time. Such desiccated cells rapidly recover their physiological functions immediately after rewetting[8]. It is proposed that there are three phases in this drought tolerance[9]. The first is the drying phase which initiates dehydration from inside and outside of the cells. In this phase, cells avoid lysis from hyper osmotic-down shock and protect DNA, RNA, proteins and membranes from salt stress, oxidative stress, and UV stress. The second is the desiccation phase in which the cells dry up and no physiological activity occurs. The third is a rehydration phase when water is again supplied. In this phase, the cellular metabolism promptly recovers, and the damage from desiccation is repaired. Despite many studies on the mechanism of drought tolerance, the molecular responses of cells to drying, desiccation and rehydration remains unclear.

The terrestrial cyanobacterium *Nostoc* sp. strain HK-01 (hereafter *Nostoc* HK-01) was isolated from a cyanobacterial mat on soil and was found to be very similar to the aquatic *Anabaena* sp. strain PCC 7120 (hereafter *Anabaena* PCC 7120) in the 16S rRNA gene and *trnL*(UAA) intron sequences[4]. The genomic sequence of *Anabaena* PCC 7120 (6.4 Mbp and 6 plasmids) has been determined[3]. Recently, we have made a DNA microarray for *Anabaena* PCC 7120 using sequencing clones with 3 to 4 kbp[10]. The gene expression of terrestrial cyanobacteria during dehydration has not yet been investigated.

Nostoc HK-01 was grown at 30°C in WK medium[4] with 5 mM *N*-tris-(hydroxymethyl)methyl-2-aminoethanesulfon-

* Corresponding author; E-mail: h-yoshimura@sci.toho-u.ac.jp, Tel: +81–47–472–1365, Fax: +81–47–472–1365

Table 1. Primers used for RT-PCR and number of PCR cycles

Gene	Forward primer	Reverse primer	Number of PCR cycles
16S rRNA	GCGTAGAGATCAGGAAGA	CCATGCACCACCTGTGTT	20 (positive control) 30 (negative control)
alr0894	CGTTATTGGCACAACTTT	AATTTGCTGTTCTGGTTC	25
alr3090	GGGCCTCATTTTCTAGAT	TTTGAATGCTGGCTCAGA	30
all4050	GGATGACCAGAGACCAAG	GCCAACAGCTACTTCACC	25
all3197	CCGAATATTCCCCCAGTG	TGTGGCGATAGGAGAGTT	30
alr3199	CGCTCACCAAGAACAACT	GGGCTGATAGCCTTGATT	25
alr5182	GCTAAAGAAGGTGCAGAT	GAGGGAATTAAAGGTGTC	25
asr5183	GTAGATTGTGCTAGAGCA	CAGGTGCAATAATTTCCC	30

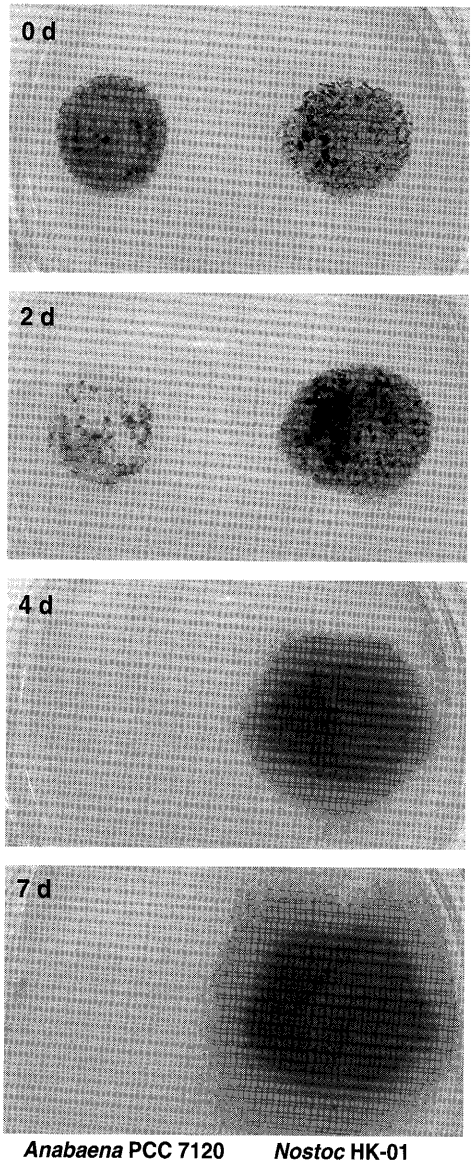

Fig. 1. Drought-test for *Anabaena* sp. PCC 7120 and *Nostoc* sp. HK-01. Cells dried for 8 months were soaked with sterile water for 10 min, streaked on a WK agar plate, and incubated under continuous light (35 μE $m^{-2}s^{-1}$) at 30°C. In each panel, the cells on the left are *Anabaena* sp. PCC 7120 and the right side cells are *Nostoc* sp. HK-01 after soaking and streaking.

ic acid (TES)-NaOH (pH 8.0) at 35 μE $m^{-2}s^{-1}$ under continuous fluorescent illumination. *Anabaena* PCC 7120 was grown at 30°C in BG11 medium[11] with 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)-NaOH (pH 8.0) at 35 μE $m^{-2}s^{-1}$ under continuous fluorescent illumination. Cells were grown in a volume of 50 ml in a culture tube and bubbled with air containing 1% CO_2. The concentration of chlorophyll extracted by methanol was measured using a spectrophotometer (model UV-160A, Shimadzu, Kyoto, Japan) and calculated with A_{665} 1=13.4 μg chlorophyll/ml[6].

A 30-ml portion of cell culture was harvested by filtration on filter paper at a chlorophyll concentration of 15 μg chlorophyll/ml and dried on a plastic dish in an incubator under light (35 μE $m^{-2}s^{-1}$) at 30°C and 30~40% relative humidity. The cells were harvested when the wet weight decreased to 50%, 30% and 10%. As a control, cells were used immediately before drying. Total RNA was isolated from the cells by the hot-phenol method[7]. Crude total RNA was treated with 0.1 U/μl DNase I (Takara, Shiga, Japan) at 37°C for 3 h. After phenol:chloroform:isoamyl alcohol extraction and ethanol precipitation, total RNA was suspended in RNase-free ultrapure water.

The DNA microarray experiment was performed as described by Katoh *et al.*[5]. Hybridization was performed overnight at 60°C. Washing was performed as follows: (i) one wash in 2x SSC and 0.2% SDS at room temperature for 10 min; (ii) two washes in 0.2x SSC and 0.2% SDS at room temperature for 10 min; (iii) two washes in 0.2x SSC and 0.2% SDS at 55°C for 10 min; and (iv) two washes in 0.2x SSC at room temperature for 5 min. Then, the microarray was dried using a spin dryer mini 2350 (Wakenyaku, Kyoto, Japan). Microarrays were scanned by a laser fluorescent scanner (Scanarray 4000, GSI Lumonics, Billerica, MA, USA). The expression level of each spot on the DNA microarray during drying was indicated by the drying/control

ratio.

The genes whose expression was up-regulated in *Nostoc* HK-01 were determined by RT-PCR using a RNA PCR Kit (Takara). The DNA sequence of those genes conserved among *Anabaena* sp. PCC 7120, *Anabaena variabilis* ATCC29413 and *Nostoc punctiforme* was chosen as a primer (Table 1). PCR amplifications were performed for 20–30 cycles each consisting of 30 s at 94°C, 30 s at 50°C and 1 min at 72°C in the case of *Nostoc* HK-01. RT-PCR experiments of positive and negative controls for *Nostoc* sp. HK-01 were performed using 16S rRNA gene primers (Table 1). Since the amount of transcript of the 16S rRNA gene had decreased as dehydration progressed for *Anabaena* PCC 7120, the quantitative determination of the total amount of RNA was made only with a spectrophotometer.

The cells of *Nostoc* HK-01 and *Anabaena* PCC 7120 were harvested after culturing for 2 weeks. Each cell suspension with 7.5 μg of chlorophyll per milliliter was then filtered on a cellulose acetate membrane filter (Toyo Roshi Co, Ltd., Tokyo, Japan), dried for a week, and stored for 8 months in the dark at 30°C and 30–40% relative humidity. These dried strains were soaked with sterile water for 10 min, streaked on an agar plate with WK medium, and incubated under light (35 $\mu E\ m^{-2}s^{-1}$) at 30°C. Figure 1 shows that once dried, *Nostoc* HK-01 cells exhibited a recovery of growth while *Anabaena* PCC 7120 cells were unable to recover from desiccation and disappeared after 4 days. The results show that even though *Nostoc* HK-01 and *Anabaena* PCC 7120 are very similar phylogenetically, they are quite different in their drought-tolerant capacity.

The genome-wide gene expression of *Nostoc* HK-01 was determined using the DNA microarray of *Anabaena* PCC 7120. The profile of the down-regulated gene expression in the *Nostoc* cells (data not shown) was almost the same as that in the *Anabaena* cells during drying[5]. We focused on genes whose expression levels were still high even if the wet weight decreased to 10% (Table 2).

To confirm the expression of genes observed in the DNA microarray, RT-PCR was carried out on almost all genes up-regulated. Results of RT-PCR experiments showed that the expression of the genes encoding homologs of alr0894, alr3090, all3197, alr3199, all4050, alr5182 and asr5183 increased during drying (Fig. 2). In the cells just before drying, no expression of genes homologous with alr0894, alr3090 and all4050 was observed. The increase in gene expression lasted until wet weight decreased to 10%. The gene expression of the all3197 homolog was detected when wet weight reached 10%. These results showed that the microar-

Table 2. List of genes in *Nostoc* sp. HK-01 clearly detected with the Anabaena DNA microarray. Names used are those of the corresponding genes of *Anabaena* sp. PCC 7120

Anabaena sp. PCC 7120 ORF contained in DNA-fragment[a]	drying/control ratio[b]		
	wet-weight 50%	wet-weight 30%	wet-weight 10%
	ratio std	ratio std	ratio std
alr0893: hypothetical protein, alr0894: hypothetical protein, alr0895: alcohol dehydrogenase, alr0896: unknown protein	4.87±0.36	2.60±0.41	3.45±0.30
alr0896: unknown protein, alr0897: alcohol dehydrogenase, alr0898: hypothetical protein, all0899: hypothetical protein, alr0900: serine/threonine kinase with two-component sensor domain	4.56±0.49	2.73±1.47	1.96±0.37
alr3088: nifS protein, class-V aminotransferase, asr3089: transglycosylase-associated protein, alr3090: hypothetical protein	1.89±0.22	2.13±0.65	1.67±0.28
alr3790: hypothetical protein, all3791: ribonuclease D, all3792: hypothetical protein, all3793: unknown protein	3.03±1.02	2.21±0.27	2.38±0.56
alr5180: unknown protein, alr5181: unknown protein, alr5182: oxidoreductase, asr5183: unknown protein, all5184: Mg-transport protein	1.64±0.07	1.78±0.24	2.24±0.49
asl3196: hypothetical protein, all3197: hypothetical protein, all3198: hypothetical protein, alr3199: unknown protein	2.09±0.14	1.78±0.27	2.01±0.80
asl3360: unknown protein, alr3361: hypothetical protein, alr3362: hypothetical protein, alr3363: hypothetical protein	2.72±0.27	2.17±0.40	2.02±0.56
asr4048: unknown protein, all4049: unknown protein, all4050: hypothetical protein, all4051: hypothetical protein	4.86±2.05	2.59±0.44	3.05±0.36

[a] Cyanobase (http:/www.kazusa.or.jp/cyanobase/) was referred to for the ORF and description.

[b] The values were calculated as a ratio of the signal intensity of drying to control. All expression rates are shown as averages with standard deviations of three independent experiments.

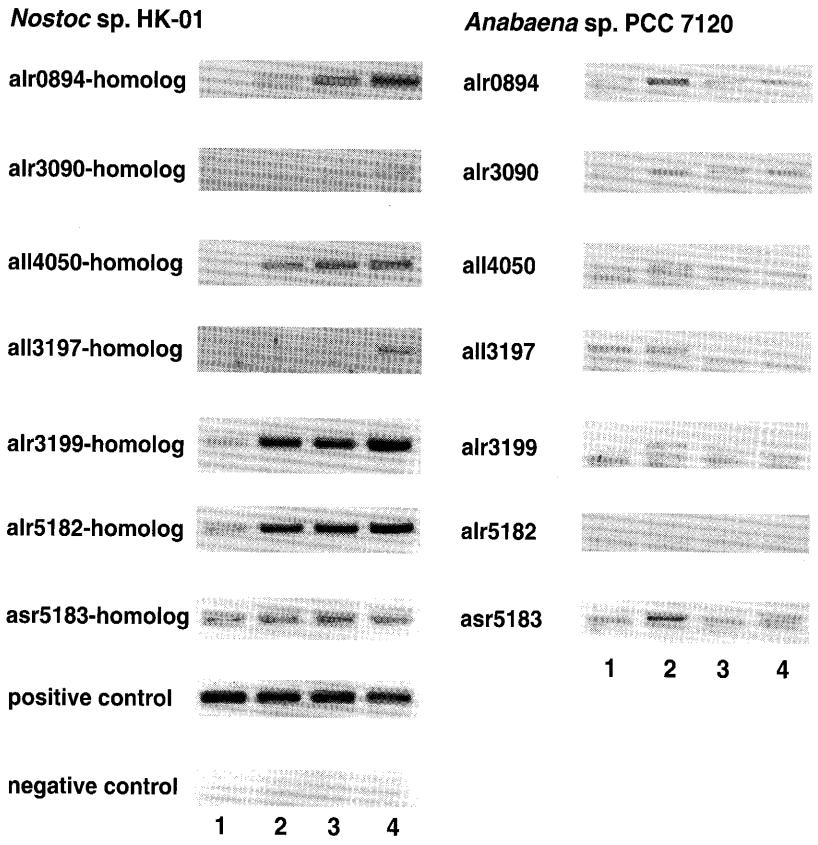

Fig. 2. RT-PCR analysis for up-regulated genes in *Nostoc* sp. HK-01 homologous with those in *Anabaena* sp. PCC 7120. Numbers show template conditions. 1–4: reverse-transcribed RNA samples when wet weight was 100%, 50%, 30% and 10% during drying, respectively. RT-PCR experiments of positive and negative controls for *Nostoc* sp. HK-01 were performed using 16S rRNA gene primers (Table 1).

ray of *Anabaena* PCC 7120 can be used to determine the gene expression of *Nostoc* HK-01.

To compare the differences of gene expression between *Nostoc* HK-01 and *Anabaena* PCC 7120 cells, RT-PCR was carried out for genes of *Anabana* PCC 7120. alr0894, alr3090, all3197, alr3199, all4050, alr5182 and asr5183, during drying. In contrast to *Nostoc* HK-01, all these genes except alr5182 were expressed most actively when wet weight reached 50% or had decreased as dehydration progressed (Fig. 2). The gene expression of alr5182 was not at all detected in the *Anabaena* PCC 7120 cells before and during drying.

Proteins homologous with alr0894, alr3199, all4050 and alr5182 reportedly accumulated in response to UV-B shock in the cells of *Nostoc commune* DRH1[2]. The expression of genes is considered indispensable to drought tolerance. The alr5182 product is predicted to be an oxidoreductase and the products of alr0894, all3197, alr3199 and asr5183 are unknown proteins. The alr3090 product was homologous with manganese-containing catalase, an antioxidant catalyzing the conversion of hydrogen peroxide to water and molecular oxygen. Hydrogen peroxide would be converted to the highly reactive hydroxyl radical which is able to damage a wide variety of molecules within a cell, leading to cell death. Thus, the alr3090 product might function to protect the cell from oxidative stress. all4050, named *anaK*, has been reported as a homolog of the akinete marker gene in *Anabaena variabilis*[12]. Akinetes are resistant to desiccation and coldness, and are able to recover cellular metabolism and germinate to produce new filaments when cells are returned to ordinary growth conditions[1].

Why did *Anabaena* PCC 7120 cells stop expressing certain genes while *Nostoc* cells kept expressing them during dehydration? Presumably, the *Anabaena* PCC 7120 cells can live only in aquatic conditions whereas *Nostoc* HK-01 cells acquired the ability to live under desiccated environmental conditions. It is extremely important that *Nostoc* HK-01 cells have a mechanism to maintain active gene expression even if wet weight decreases to 10%. More studies are needed to elucidate this molecular mechanism of drought tolerance. However, the present work provides new evidence concerning genes necessary for drought tolerance

in cyanobacteria.

Acknowledgements

This work is supported by Japan Space Forum.

References

1) Adams, D.G. and P.S. Duggan. 1999. Heterocyst and akinete differentiation in cyanobacteria. New Phytol. **144**: 3–33.
2) Ehling-Schulz, M., S. Schulz, R. Wait, A. Gorg and S. Scherer. 2002. The UV-B stimulon of the terrestrial cyanobacterium *Nostoc commune* comprises early shock proteins and late acclimation proteins. Mol. Microbiol. **46**: 827–843.
3) Kaneko, T., Y. Nakamura, C.P. Wolk, T. Kuritz, S. Sasamoto, A. Watanabe, M. Iriguchi, A. Ishikawa, K. Kawashima, T. Kimura, Y. Kishida, M. Kohara, M. Matsumoto, A. Matsuno, A. Muraki, N. Nakazaki, S. Shimpo, M. Sugimoto, M. Takazawa, M. Yamada, M. Yasuda and S. Tabata. Complete genomic sequence of the filamentous nitrogen-fixing cyanobacterium *Anabaena* sp. strain PCC 7120. 2001. DNA Res. **8**: 205–213, 227–253.
4) Katoh, H., Y. Shiga, Y. Nakahira and M. Ohmori. 2003. Isolation and Characterization of a Drought-Tolerant Cyanobacterium, *Nostoc* sp. HK-01. Microbes Environ. **18**: 82–88.
5) Katoh, H., R.K. Asthana and M. Ohmori. 2004. Gene expression in the cyanobacterium *Anabaena* sp. PCC 7120 under desiccation. Microb. Ecol. **47**: 164–174.
6) Mackinney, G. 1941. Absorption of light by chlorophyll solutions. J. Biol. Chem. **140**: 315–322.
7) Mohamed, A. and C. Jansson. 1989. Influence of light on accumulation of photosynthesis-specific transcripts in the cyanobacterium *Synechocystis* 6803. Plant Mol. Biol. **13**: 693–700.
8) Potts, M. 1994. Desiccation tolerance of prokaryotes. Microbiol. Rev. **58**: 755–805.
9) Potts, M. 2001. Desiccation tolerance: a simple process? Trends Microbiol. **9**: 553–559.
10) Sato, N., M. Ohmori, M. Ikeuchi, K. Tashiro, C.P. Wolk, T. Kaneko, K. Okada, M. Tsuzuki, S. Ehira, H. Katoh, S. Okamoto, H. Yoshimura, T. Fujisawa, A. Kamei, S. Yoshihara, R. Narikawa, T. Hamano, S. Tabata and S. Kuhara. 2004. Use of segment-based microarray in the analysis of global gene expression in response to various environmental stresses in the cyanobacterium *Anabaena* sp. PCC 7120. J. Gen. Appl. Microbiol. **50**: 1–8.
11) Stanier, R.Y., R. Kunisawa, M. Mandel and G. Cohen-Bazire. 1971. Purification and properties of unicellular blue-green algae (order Chroococcales). Bacteriol. Rev. **35**: 171–205.
12) Zhou, R. and C.P. Wolk. 2002. Identification of an akinete marker gene in *Anabaena variabilis*. J. Bacteriol. **184**: 2529–2532.