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Group 3 sigma factor gene, *sigJ*, a key regulator of desiccation tolerance, regulates the synthesis of extracellular polysaccharide in cyanobacterium *Anabaena* sp. strain PCC 7120

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Abstract

The changes in the expression of sigma factor genes during dehydration in terrestrial *Nostoc* HK-01 and aquatic *Anabaena* PCC 7120 were determined. The expression of the *sigJ* gene in terrestrial *Nostoc* HK-01, which is homologous to *sigJ* (alr0277) in aquatic *Anabaena* PCC 7120, was significantly induced in the mid-stage of dehydration. We constructed a higher-expressing transformant of the *sigJ* gene (HE0277) in *Anabaena* PCC 7120, and the transformant acquired desiccation tolerance. The results of *Anabaena* oligonucleotide microarray experiments showed that a comparatively large number of genes relating to polysaccharide biosynthesis were upregulated in the HE0277 cells. The extracellular polysaccharide released into the culture medium of the HE0277 cells was as much as 3.2-fold more than that released by the control cells. This strongly suggests that the group 3 sigma factor gene *sigJ* is fundamental and conducive to desiccation tolerance in these cyanobacteria.

Key words: desiccation tolerance; sigma factor; exopolysaccharide; cyanobacteria

1. Introduction

Cyanobacteria grow in diverse habitats on earth, ranging from the tropics to polar regions.[1] Terrestrial cyanobacteria are subjected to repeated cycles of dehydration, desiccation, and rehydration, and can survive under desiccated conditions for long periods. It has been reported that a terrestrial cyanobacterium *Nostoc commune*, which was stored in a desiccated state for more than 100 years, retained its ability to grow.[2,3] *Nostoc commune* cells actively deactivate their photosynthetic systems on sensing water loss,[4] and can recover the activities of photosystems I and II readily after rehydration.[5] *Nostoc commune* cells produce a large amount of extracellular polysaccharide (EPS), which contributes to the stabilization of the cells during desiccation.[6] Light intensity and the availability of combined nitrogen affect the synthesis of soluble EPSs in *Nostoc* strains.[7,8] Despite many studies of the mechanism of desiccation tolerance, the molecular responses of cells to dehydration, desiccation, and rehydration remain unclear.

A sigma factor for RNA polymerase is capable of specific promoter recognition and the efficient initiation of transcription. Environmental or developmental signals change gene expression by inducing sigma factors in diverse bacterial species.[9] There are two basic families of eubacterial sigma factors based on sequence similarities: the σ^{70} and σ^{54} families. The σ^{70} family is divided into three groups. Group 1 contains the primary sigma factors, which are essential for cell growth. Group 2 contains the sigma factors that are not essential for cell growth, but are similar to the primary sigma factors in the amino acid sequences of their

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DNA-binding regions. Group 3 includes the sigma factors that are only slightly homologous to groups 1 and 2 in their amino acid sequences. They regulate the transcription of specific regulons expressed in response to changes in environmental or developmental conditions. No sigma factor of the σ^{54} family has been found in the cyanobacteria. Twelve putative sigma factor genes exist in the genome and plasmids in *Anabaena* sp. PCC 7120.[10] However, there have been few reports of the corresponding functions of the sigma factors. *sigA*, which encodes a primary sigma factor, was isolated and characterized under conditions of nitrogen limitation.[11] The transcripts of the *sigB* and *sigC* genes, which encode group 2 sigma factors, are detectable under nitrogen-limited conditions.[12] However, neither gene is essential for nitrogen fixation or heterocyst differentiation.[13] The functions of the group 3 sigma factors of the PCC 7120 strain have not been reported at all.

We recently reported a comparative analysis of the differences in gene expression during dehydration between the terrestrial desiccation-tolerant cyanobacterium *Nostoc* sp. HK-01 and the aquatic cyanobacterium *Anabaena* PCC 7120.[14] The gene expression changes were transient in *Anabaena* PCC 7120, whereas in *Nostoc* sp. HK-01, they were maintained or increased until the wet weight decreased to 10% of that before drying. Therefore, we inferred that sigma factors play important roles in gene expression during dehydration, because sigma factors play key roles in the initiation of transcription and gene expression. Here, we analysed the changes in the expression levels of the sigma factor genes in both *Nostoc* sp. HK-01 and *Anabaena* PCC 7120 during dehydration. We present significant evidence that *sigJ* gene plays an important role in desiccation tolerance.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Transformant cells of *Anabaena* PCC 7120 were grown in BG11 medium[15] at 30 °C under continuous illumination at 30 μE m^{-2} s^{-1} provided by fluorescent lamps. *Nostoc* sp. HK-01 was grown at 30 °C in WK medium[16] with 5 mM *N*-tris-(hydroxymethyl)methyl-2-aminoethanesulphonic acid (TES)–NaOH (pH 8.0) under continuous illumination at 30 μE m^{-2} s^{-1} provided by fluorescent lamps. Liquid cultures were bubbled with air containing 1% CO_2.

2.2. Drought stress conditions

A 10-mL portion of cell culture was harvested by filtration on a filter paper at a chlorophyll concentration of 15 μg chlorophyll/mL and dried on a plastic dish in an incubator under light (30 μE m^{-2} s^{-1}) at 30 °C and 30–40% relative humidity. The cells were cryopreserved when the wet weight had decreased to 50, 30, and 10%, and cells harvested immediately before drying (100% wet weight) were used as the control.

2.3. Total RNA preparation

Total RNA was isolated from prepared cells by the hot-phenol method.[17] Crude total RNA was treated with 0.1 U/μL DNase I (Takara Bio, Shiga, Japan) at 37 °C for 3 h. After phenol–chloroform–isoamyl alcohol extraction and ethanol precipitation, the total RNA was suspended in RNase-free ultrapure water.

2.4. Real-time quantitative reverse transcriptase–polymerase chain reaction

Aliquots (1 μg) of total RNA were reverse-transcribed with an RNA PCR Kit (Takara Bio). An aliquot (1/20) of the reaction mixture was taken for real-time quantitative polymerase chain reaction (PCR) with a set of gene-specific primers (Table 1). Reactions were performed with a DNA Engine Opticon® 2 System (Bio-Rad, CA, USA) using SYBR® Premix Ex Taq™ (Takara Bio) in the presence of 250 nM primers under the following conditions: 1 min at 95 °C, followed by 40 cycles of 5 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C. The fluorescence intensity of the SYBR Green dye was measured after each amplification step. The relative ratios were presented as the means of data from duplicate or triplicate experiments. The primers used were designed using Primer3.[18] Each primer for the sigma factors of *Anabaena* PCC 7120 was designed based on information from CyanoBase. Each specific primer for the sigma factors of *Nostoc* sp. HK-01 was designed on the basis of partial sequencing data using PCR products amplified with primers based on the conserved DNA sequence of the corresponding gene among *Anabaena* PCC 7120, *Anabaena variabilis* ATCC 29413, and *Nostoc punctiforme* ATCC 29133. Each datum for *Nostoc* sp. HK-01 was normalized to the value for 16S rRNA. Because the amount of transcript product of the 16S rRNA gene decreases as dehydration progresses in *Anabaena* PCC 7120, the quantitative determination was based on the amount of total RNA measured with a spectrophotometer. The primers are listed in Table 1. Product identification was confirmed by melting-curve analysis.

2.5. Construction of transformants

The plasmid used for transformation in this study was based on pRL490, a stable shuttle vector in *Anabaena* species derived from a hybrid of pBR322 and the plasmid pDU1.[19–23] pRL490 contains the *tac* promoter derived from *Escherichia coli*, the *luxAB* genes derived from *Vibrio fischeri* downstream from the promoter, and a gene conferring resistance to neomycin. The *luxAB* genes were removed from the plasmid with *Bam*HI

Table 1. Primers used in real-time quantitative RT–PCR

16S rRNA	5′-TGTAGCGGTGAAATGCGTAG-3′	5′-TTCACACTTGCGTGCGTACT-3′
Anabaena PCC 7120		
all5263 (*sigA*)	5′-GGTAGCCTTGGTTTGATTCG-3′	5′-CCATGCGAGTAGCGATTTCT-3′
alr3800 (*sigB2*)	5′-CACCCACACAGAAGACACAAA-3′	5′-CGTGGTCAGCCCACTCTG-3′
all1692 (*sigC*)	5′-CAGATGCCGCCTACAATACC-3′	5′-TTCGTCCCGTCCTAACAAAC-3′
alr3810 (*sigD*)	5′-CCACAACGTTGCAAGAAATG-3′	5′-CGCAAGTTGGCTTCTACCAT-3′
alr4249 (*sigE*)	5′-CGCTAATGATGCCAGAACCA-3′	5′-CCGACACGATGGTTTAAAGA-3′
all3853 (*sigF*)	5′-CGCTACTTGGCGATACAACA-3′	5′-CAATTCTCCCAGGCTAGTGG-3′
alr3280 (*sigG*)	5′-GTTGATGCGAGGTGTCCAG-3′	5′-CACGCGAATCCAAACTTCTT-3′
alr0277 (*sigJ*)	5′-AACTAAGTTGGCTGCCCAAA-3′	5′-TAGCAGCATCTTTGCGAGAA-3′
Nostoc HK-01		
sigA	5′-TTGCTTCTCGTTGATGATGG-3′	5′-CGCGCTAATTCGATTTCTTC-3′
sigB2	5′-AACCCGTGAGCAAGAAATTG-3′	5′-TCCGAGTGTTCCTTCTTGGA-3′
sigC	5′-GCCACCGTCCATCTTTAGAA-3′	5′-GAAACCACAAGACGCAGGTT-3′
sigD	5′-AGATCGGCCGTGTACCACT-3′	5′-CATTCTCCGTTTCGCAATTT-3′
sigE	5′-GCTCAACGCGAACTTAAACA-3′	5′-TCTTTACCGACACGGTGGTT-3′
sigF	5′-TAAAGAATCTCGCTCGGAAA-3′	5′-AAGGAACTGAAAGCGTGTCC-3′
sigG	5′-TAGGATTGCGTCCAGATCGT-3′	5′-CCGATACACTCGAATCCACA-3′
sigJ	5′-GCGTTTAGTTCCTTTGCTGTG-3′	5′-CTGAATCTTTGGGAGGACGA-3′

restriction endonuclease. The resultant plasmid was named pRL490–luxAB. DNA fragments of the *sigJ* gene were amplified by PCR with the set of primers 5′-GGATCCATGGCAGCAAGTGAGTCC-3′ and 5′-GGATCCCTATGAACCAGTAGGCAT-3′ using the genomic DNA of *Anabaena* PCC 7120 as the template. The primer set was designed to allow the introduction of a *Bam*HI restriction site. The PCR products were cloned into pGEM-T Easy (Promega) according to the manufacturer's instructions. Cloning of the DNA fragments was verified by DNA sequencing. The fragment digested with *Bam*HI was cloned into the *Bam*HI restriction site of pRL490–luxAB to construct pRHE0277. pRHE0277 was transferred by conjugation into *Anabaena* PCC 7120 according to Elhai and Wolk[24] and the transformant HE0277 was selected for resistance to neomycin. The control transformant P490 was obtained by transferring pRL490–luxAB into *Anabaena* PCC 7120 as described above.

2.6. Test for recovery from desiccation

P490 and HE0277 cells grown in liquid culture to stationary phase ($OD_{750} = 3.0 \pm 0.3$) were filtered on a cellulose acetate membrane filter (Toyo Roshi Co., Ltd., Tokyo, Japan), dried for 10 h under light ($30\ \mu E\ m^{-2}\ s^{-1}$) at 30 °C and 30–40% relative humidity in an incubator, and stored for 1 week in the dark in the incubator. These dried cells were soaked with BG11 medium for 10 min. After the cell densities were adjusted ($OD_{750} = 1.0$, 0.1, and 0.01), the cell solutions were spread on an agar plate with BG11 medium and incubated under continuous light ($30\ \mu E\ m^{-2}\ s^{-1}$) at 30 °C.

2.7. DNA microarray analysis

DNA microarray analysis was performed according to Ehira and Ohmori.[25] The analysis was conducted using three sets of total RNA samples extracted independently from the cells of P490 and HE0277. The ratio of the transcript level of the HE0277 strain relative to that of P490 strain was calculated from three measurements. Open reading frames (ORFs) whose spots showed signal intensities of more than twice the average intensity were analysed.

2.8. Extraction of EPSs

Cells ($OD_{750} = 3.3 \pm 0.3$) and culture media were separated by centrifugation. This separation step was repeated three times. The harvested cells were resuspended in 0.05% sodium tetrahydridoborate solution and boiled at 100 °C for 1 h. The polysaccharide contents of the culture media and cell surfaces of the P490 and HE0277 cells were determined by the phenol–sulphuric acid method, using glucose as the standard.[26]

3. Results

3.1. Sigma factors responsive to drought stress

To determine the relationships of the sigma factors of *Anabaena* sp. PCC 7120 to those of other cyanobacteria, we aligned the sigma factor amino acid sequences from

Anabaena sp. PCC 7120, *Synechocystis* sp. PCC 6803, *Synechococcus* sp. PCC 7942, and *A. variabilis* (ATCC 29413) and constructed a phylogenetic tree (Fig. 1). *Anabaena* sp. PCC 7120 has one representative of the group 1 sigma factors, SigA; group 2 sigma factors, SigC, SigD, and SigE; and group 3 sigma factors, SigF, SigG, and SigI. The multiple paralogues of SigB, a group 2 sigma factor, have been classified as SigB (All7615), SigB2 (Alr3800), SigB3 (All7608), and SigB4 (All7179). *sigB2* is encoded on the chromosome, whereas *sigB*, *sigB3*, and *sigB4* are encoded on plasmids. A novel type of group 3 sigma factor was isolated and designated SigJ. *Anabaena* sp. PCC 7120 possesses a SigJ-type sigma factor, Alr0277, but not SigH-type sigma factor.

We analysed the expression of the sigma factor genes during dehydration in *Anabaena* PCC 7120 (hereafter *Anabaena*) and *Nostoc* sp. HK-01 (hereafter *Nostoc*) using a real-time quantitative reverse transcriptase–polymerase chain reaction (RT–PCR) method (Table 2). *Anabaena* has 12 putative genes for sigma factors on its chromosome and plasmids: nine genes exist on the chromosome and three genes on plasmids. We analysed the expression of the *sigA* (all5263), *sigB2* (alr3800), *sigC* (all1692), *sigD* (alr3810), *sigE* (alr4249), *sigF* (all3853), *sigG* (alr3280), and *sigJ* (alr0277) genes on

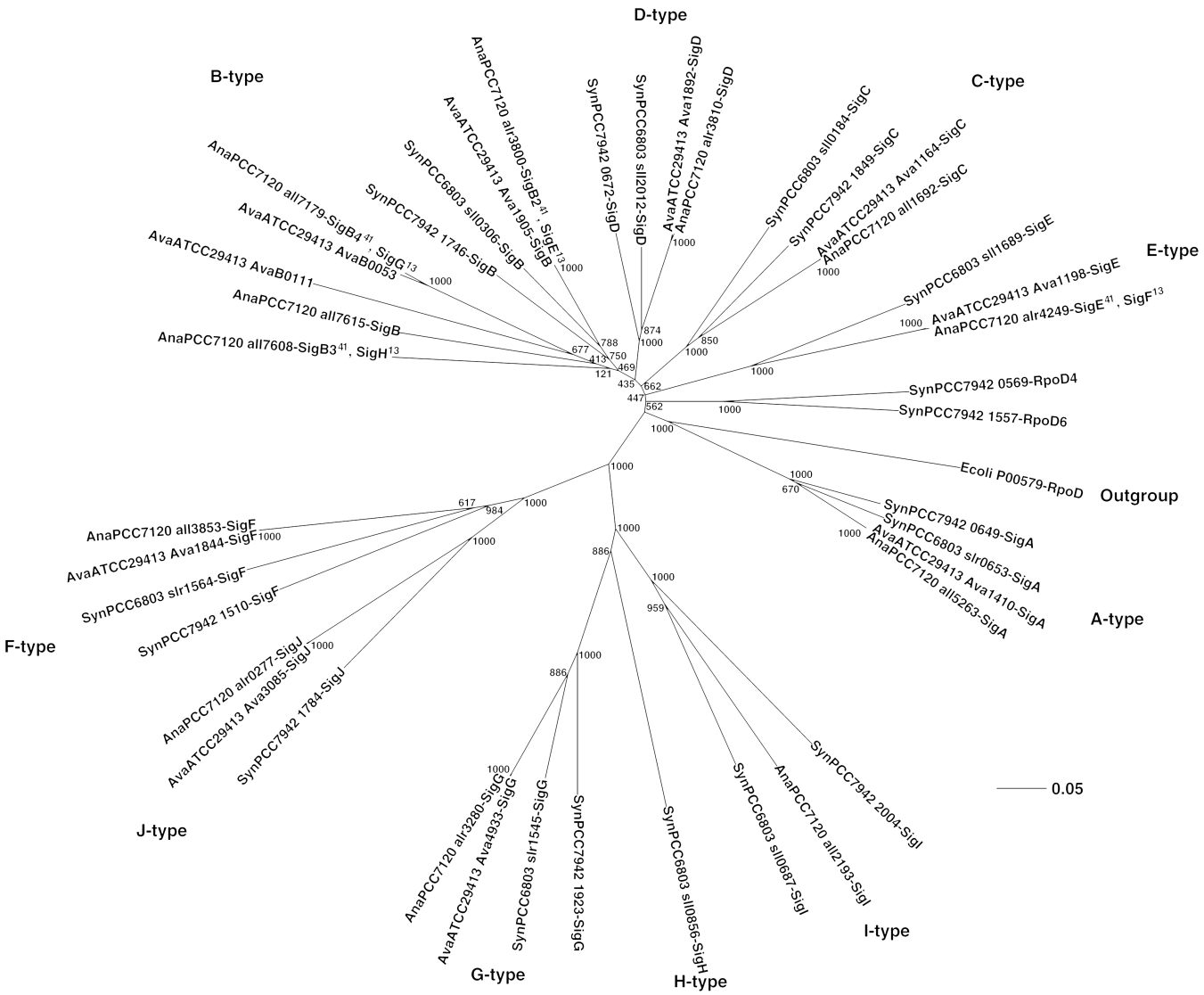

Figure 1. Phylogenetic analysis of the sigma factors of *Anabaena* sp. PCC 7120, *A. variabilis* ATCC 29413, *Synechocystis* sp. PCC 6803, and *Synechococcus* sp. PCC 7942 with these full-length amino acid query sequences. The principal sigma factor (RpoD) sequence of *E. coli* is included as an outgroup. Multiple alignment analysis was performed with ClustalW software, and the phylogenetic tree was drawn with TreeView software. Numbers at the nodes are bootstrap values calculated with 1000 replications. Designations and accession numbers for amino acid sequences of the sigma factors are as follows: *E. coli* RpoD (P00579) from GenBank; the sigma factors of *Anabaena* sp. PCC 7120 from CyanoBase (http://www.kazusa.or.jp/cyano/); the sigma factors of *A. variabilis* ATCC 29413 from CYORF (http://cyano.genome.jp/) and JGI; the sigma factors of *Synechocystis* sp. PCC 6803 from CyanoBase; and the sigma factors of *Synechococcus* sp. PCC 7942 from CYORF.

Table 2. Alteration of sigma factor gene expression during dehydration evaluated by real-time quantitative RT–PCR

Wet weight change	50%		30%		10%	
	Ratio (50%/100%)	SD	Ratio (30%/100%)	SD	Ratio (10%/100%)	SD
N. HK-01 *sigA*	0.13	±0.04	0.20	±0.03	0.12	±0.01
N. HK-01 *sigB2*	1.17	±0.11	2.92	±0.04	2.17	±0.27
N. HK-01 *sigC*	1.27	±0.12	1.95	±0.36	1.90	±0.10
N. HK-01 *sigD*	0.94	±0.01	2.17	±0.15	1.20	±0.29
N. HK-01 *sigE*	1.27	±0.16	2.68	±0.12	1.29	±0.02
N. HK-01 *sigF*	0.16	±0.03	0.17	±0.01	0.03	±0.01
N. HK-01 *sigG*	1.16	±0.08	2.30	±0.16	2.18	±0.04
N. HK-01 *sigJ*	2.47	±0.22	1.43	±0.23	0.80	±0.03
A. PCC 7120 *sigA*	0.53	±0.10	0.10	±0.03	0.07	±0.02
A. PCC 7120 *sigB2*	1.83	±0.01	0.55	±0.10	0.52	±0.02
A. PCC 7120 *sigC*	0.51	±0.004	0.10	±0.0001	0.09	±0.02
A. PCC 7120 *sigD*	0.57	±0.04	0.12	±0.02	0.08	±0.002
A. PCC 7120 *sigE*	1.63	±0.07	0.50	±0.01	0.34	±0.01
A. PCC 7120 *sigF*	1.17	±0.21	0.23	±0.01	0.18	±0.04
A. PCC 7120 *sigG*	1.28	±0.09	0.16	±0.02	0.16	±0.02
A. PCC 7120 *sigJ*	1.02	±0.42	0.13	±0.01	0.13	±0.02

The ratio values were obtained by calculating the ratio of the transcript level at each wet weight to the transcript level at the wet weight (100%) immediately before drying. Each datum is an average (±standard deviation) of three independent experiments.

the chromosome of *Anabaena* and that of the corresponding genes of *Nostoc*. No expression of *sigB* (all7615), *sigB3* (all7608), or *sigB4* (all7179) was detected before or during dehydration in either species. *sigI* (all2193), which encodes a probable sigma factor, was not analysed because it is not a common gene in filamentous cyanobacteria. No orthologue exists in the genome of *A. variabilis* ATCC 29413 or *N. punctiforme* ATCC 29133. The transcript levels of *sigA* decreased as dehydration progressed in both *Anabaena* and *Nostoc*. However, the transcript level decreased to only about 53% in *Anabaena*, whereas it decreased to 13% in *Nostoc* when the wet weight had decreased to 50% of that before dehydration (Table 2).

The transcript levels of several group 2 sigma factors decreased totally as dehydration progressed in *Anabaena*. The transcript levels of *sigC* and *sigD* continued to decrease during dehydration. Transcript levels of *sigB2* and *sigE* finally decreased, although they increased transiently at a wet weight of 50%. Conversely, the transcript levels of group 2 sigma factors increased significantly in *Nostoc*. The transcript levels of *sigB2* and *sigC* were maintained at approximately twofold higher than the control level until the late stage of dehydration. The transcript levels of *sigD* and *sigE* were significantly increased, although transiently, at a wet weight of 30%.

In *Anabaena*, the transcript levels of several group 3 sigma factors did not change until the mid-stage of dehydration and decreased markedly in the late stage of dehydration. In *Nostoc*, the transcripts of *sigF* were strongly repressed until the mid-stage of dehydration. The transcript level of *sigG* was maintained at a level over twofold higher than that of the control in the late stage of dehydration. The transcript level of *sigJ* increased significantly in the mid-stage of dehydration, and then decreased gradually in the late stage of dehydration. It is noteworthy that among the sigma factor genes, the expression of *sigJ* was upregulated first when the cells were exposed to drought stress.

3.2. Comparison of the transcript levels of each sigma factor in Anabaena and Nostoc during dehydration

The relative ratios of the transcript levels for each sigma factor in *Anabaena* and *Nostoc* were determined during dehydration using real-time quantitative RT–PCR (Table 3). The ratios for *sigC*, *sigE*, and *sigG* transcripts in *Nostoc* to those in *Anabaena* were approximately 100-fold, 10-fold, and 30-fold, respectively, at the late stage of dehydration. The relative ratio for *sigJ* was approximately 120-fold, even before drying, and the maximum ratio was approximately 1550-fold at a wet weight of 30%. The intensive expression of the *sigJ* gene in *Nostoc* should positively influence desiccation tolerance.

3.3. Recovery from desiccation of P490 and HE0277 cells

A transformation method for cells of *Nostoc* sp. HK-01 has not been established. Therefore, we produced clones of the *Anabaena* cell strain HE0277, which strongly express the *Anabaena sigJ* gene, and tested their recovery from desiccation based on the facts that the expression of

Table 3. Relative ratios of the transcript level of each sigma factor gene at each wet weight in both *Anabaena* sp. PCC 7120 and *Nostoc* sp. HK-01

Wet weight change	100%	50%	30%	10%
Relative ratio				
N. *sigA*/A. *sigA*	1.79	0.64	5.34	3.75
N. *sigB2*/A. *sigB2*	0.40	0.23	2.76	1.52
N. *sigC*/A. *sigC*	6.19	14.74	103.25	95.54
N. *sigD*/A. *sigD*	0.15	0.26	2.13	2.66
N. *sigE*/A. *sigE*	2.69	2.29	13.44	10.77
N. *sigF*/A. *sigF*	58.8[a]	5.51[a]	43.05[a]	5.82[a]
N. *sigG*/A. *sigG*	2.86	2.59	34.18	33.31
N. *sigJ*/A. *sigJ*	123.30	411.86	1548.87	869.47

The value was calculated as the ratio of the transcript level of each sigma factor gene in *Nostoc* sp. HK-01 to its transcript level in *Anabaena* sp. PCC 7120. Relative ratios are the means of data from two independent experiments.

[a]Transcript levels were very low in both species and the error margins of the calculated values were large.

sigJ was the first among the *Nostoc* sigma factor genes to be upregulated by drought stress and that *sigJ* transcripts exist more abundantly in *Nostoc* than in *Anabaena*. The cell strain transformed with a non-insertional pRL490–luxAB vector was used as the control strain, P490. As the result of Western blotting with anti-SigJ sera, the translation product level of *sigJ* in HE0277 cells was four-fold higher than the control level (data not shown). The HE0277 cells were better able to recover from the desiccated state than were the control cells (Fig. 2). Therefore, the numbers of colonies formed, as an indicator of recovery from the desiccated state, were determined for both strains when spread on a BG11 agar plate, after cell density was adjusted ($OD_{750} = 0.01$). From the results of eight measurements, the number of colonies formed by the HE0277 strain was 3.1-fold higher than that of the P490 strain (data not shown). Thus, the HE0277 cells were more desiccation-tolerant than the P490 cells.

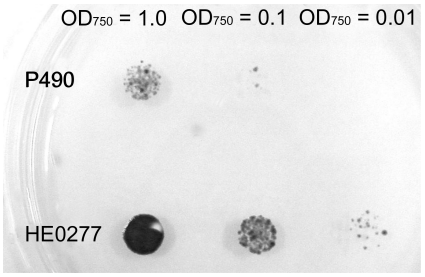

Figure 2. Recovery from desiccation of P490 and HE0277 cells. P490 and HE0277 cells were dried as described in Section 2. After the cell densities were adjusted ($OD_{750} = 1.0$, 0.1, and 0.01) using BG11 liquid medium, the cell solutions were spread on agar plates with BG11 medium and incubated for 7 days under continuous light (30 $\mu E\, m^{-2}\, s^{-1}$) at 30 °C.

3.4. Effects of higher expression of the sigJ gene in Anabaena

The effects of the elevated expression of the *sigJ* gene were determined with an *Anabaena* oligonucleotide microarray. We found that the expression of 112 genes, including *sigJ*, was upregulated and that the expression of 42 genes was downregulated in HE0277 cells relative to that of P490 cells. Among the upregulated genes, 5% or more of the entire number of genes were included in the categories 'cell envelope', 'photosynthesis and respiration', 'transcription and regulatory functions', and 'other categories' (Table 4). The genes encoding probable glycosyl transferase or glucosyl transferase in 'other categories' accounted for 14.4% of the total number of

Table 4. Functional categories of genes differentially expressed in P490 and HE0277 cell strains

Categories[a]	Number of upregulated genes	Number of downregulated genes
Amino acid biosynthesis	0	2
Biosynthesis of cofactors, prosthetic groups, and carriers	0	3
Cell envelope	6	0
Cellular processes	1	0
Central intermediary metabolism	3	0
Energy metabolism	1	1
Fatty acid, phospholipid, and sterol metabolism	0	0
Photosynthesis and respiration	6	2
Nitrogen assimilation and fixation	0	0
Purines, pyrimidines, nucleosides, and nucleotides	0	0
Transcription and regulatory functions	9	1
DNA replication, recombination, and repair	1	1
Translation	2	1
Transport and binding proteins	2	2
Other categories (glucosyl transferase or glycosyl transferase)	21 (16)	6 (1)
Hypothetical and unknown	59	23
Total	111	42

[a]Gene categories were defined according to the CyanoBase database (http://www.kazusa.or.jp/cyano/cyano.html). *sigJ* is excluded from this table.

upregulated genes. There were no particular features of the downregulated genes (Table 4 and Supplementary Table 1).

The details of the upregulated genes in HE0277 cells compared with those in the P490 cells are shown in Table 5. There are two gene clusters upregulated in the HE0277 cells. First, the gene cluster encoding ORFs all4003–asl3998 was uniformly upregulated. The *isiA* (all4001) encodes the chlorophyll-*a*-binding protein CP43′. *isiB* (alr2405), which encodes flavodoxin, an electron carrier that can substitute for iron-rich soluble ferredoxin, was also upregulated.[27] Second, the expression of the gene cluster from alr3057 to alr3074 was uniformly upregulated in HE0277 cells. Because the upregulated expression of alr3074 was not statistically significant, it is not included in Table 5. However, the fold value (HE0277/P490) was 1.79 ± 0.27, indicating a tendency to upregulation. This region contains 18 ORFs and is 22.1 kbp long. According to CyanoBase, many genes relating to polysaccharide biosynthesis are included in this cluster.

3.5. Analysis of the EPSs of HE0277 and P490 cells

Because a proportion of the genes were categorized as 'cell envelope' encoding, and the number designated glycosyl/glucosyl transferases was comparatively large relative to the entire number of genes upregulated (approximately 20%), we considered that the amount of EPS was increased in HE0277 cells. Each EPS in the P490 and HE0277 cells was collected from the cell surface and liquid culture, and analysed as described in Section 2. Significant differences were detected in the amounts of polysaccharides released into the culture medium, although no difference was detected in the amounts of cell-surface polysaccharides (Table 6). The amount of polysaccharides released by HE0277 cells was as much as 3.2-fold higher than that released by P490 cells.

4. Discussion

4.1. Role of sigma factors in drought stress

The expression of the *sigA* gene was repressed as dehydration progressed in both *Anabaena* and *Nostoc*. The *sigA* product regulates the transcription of a number of housekeeping genes and is principally involved in cell viability. Genes related to photosynthesis, carbon dioxide fixation, adenosine triphosphate (ATP) synthesis, transcription, and translation are downregulated under desiccation.[28] The transcript level of *sigA* in *Nostoc* was strongly repressed at the mid-stage of dehydration (Table 2). It is necessary to stop cell processes during dehydration to conserve the energy needed to survive under desiccation. It is reasonable that *Nostoc* strictly controls the transcription level of the *sigA* gene to ensure survival.

Expression of the group 3 sigma factor gene, *sigJ*, was responsive to drought stress at the earliest stage of dehydration in *Nostoc*. The relative ratio of the number of transcripts of the *sigJ* gene in *Nostoc* to that in *Anabaena* was more than a hundred (Tables 2 and 3). The elevated expression of the *sigJ* gene must enable the cells of *Nostoc* to respond to drought stress in a timely manner. The higher expression of the *sigJ* gene in *Anabaena* resulted in the upregulation of genes related to polysaccharide biosynthesis (Tables 4 and 5), suggesting that SigJ governs the regulons of EPS biosynthesis. The expression of the gene cluster from alr3057 to alr3074 was upregulated in the HE0277 cells during dehydration, whereas it was downregulated in the desiccation-sensitive *Anabaena*.[28] This gene cluster might be responsible for desiccation tolerance.

In the drying phase, cells avoid lysis from hyperosmotic-down shock and protect their DNA, RNA, proteins, and membranes from water stress, oxidative stress, and other stresses.[29,30] It was considered that the upregulation of *isiA* and *isiB* expression in HE0277 cells, which are induced under conditions of light excess, iron-limited conditions, and oxidative conditions,[31–34] is an example for desiccation tolerance. Genes regulated by the group 2 sigma factor RpoS of *E. coli* and the group 3 sigma factor SigB of *Bacillus subtilis*, which are general stress-responsive sigma factors, encode various functions that are involved in the prevention and repair of DNA damage, cell morphology, modulation of virulence genes, osmoprotection, thermotolerance, glycogen synthesis, membranes, and the cell envelope.[9,35] However, *sigJ* gene is not the only sigma factor gene that responds to drought stress. Other group 2 or group 3 sigma factor genes of *Nostoc* are also responsive to drought stress in the late stage of dehydration (Tables 2 and 3). The function of the sigma factors in drought stress seems to differ from that of SigJ. Further analysis of the sigma factors is required to elucidate the desiccation tolerance of *Nostoc* species.

4.2. Significance of exopolysaccharides in desiccation tolerance

In the HE0277 strain, genes putatively concerning polysaccharide biosynthesis were upregulated, and at the same time, polysaccharides were excreted increasingly into the culture medium (Table 6). We investigated the monosaccharidic composition of the released polysaccharides produced by HE0277 and P490 cells. The dried polysaccharides were hydrolysed, neutralized, and desalted, and the monosaccharides were then quantified by high-performance anion-exchange chromatography, as described previously.[36] The polysaccharides released

Table 5. Effects of higher expression of *sigJ* gene in *Anabaena* sp. PCC 7120

ORF[a]	Product[a]	Fold (HE0277/P490)[b]	SD
all0394	Hypothetical protein	2.58	±2.41
all0596	Unknown protein	2.10	±0.06
all0721	Unknown protein	2.48	±1.61
all0726	Unknown protein	3.61	±1.24
all1009	Unknown protein	2.05	±0.50
all1475	Hypothetical protein	2.01	±1.41
all1708	Unknown protein	3.08	±0.02
all1943	Unknown protein	2.13	±1.92
all2200	Unknown protein	2.35	±2.13
all2238	Unknown protein	2.42	±2.33
all2290	Similar to polysaccharide biosynthesis export protein	2.43	±0.09
all2573	Unknown protein	2.98	±1.82
all2642	Multifunctional peptide synthetase	2.45	±2.15
all2649	Probable non-ribosomal peptide synthetase	2.03	±1.51
all2703	Hypothetical protein	2.24	±2.10
all2770	Dolichyl-phosphate-mannose synthase	2.32	±0.17
all2961	Similar to reverse transcriptase	2.10	±1.88
all3317	Unknown protein	2.04	±0.22
all3531	Hypothetical protein	2.13	±1.93
all3743	Transcriptional regulator	2.44	±0.75
all3999	Unknown protein	4.60	±1.71
all4000	Photosystem II CP43 protein PsbC homologue	3.71	±0.88
all4001	Photosystem II chlorophyll-*a*-binding protein IsiA	2.06	±0.18
all4002	Photosystem II CP43 protein PsbC homologue	2.27	±1.52
all4003	Photosystem II CP43 protein PsbC homologue	2.07	±0.14
all4342	Mannose-6-phosphate isomerase	2.77	±0.53
all4375	Unknown protein	2.01	±0.48
all4420	Glucosyl transferase	2.00	±0.26
all4422	UDP-*N*-acetyl-D-mannosamine transferase	2.26	±0.88
all4459	Unknown protein	3.18	±0.91
all4719	Similar to glucosyl transferase	13.14	±0.26
all5091	Unknown protein	2.05	±1.45
all5113	Unknown protein	2.07	±1.01
all5221	Unknown protein	3.46	±0.23
all5244	Unknown protein	2.46	±0.38
all5245	Unknown protein	2.88	±0.28
all5246	Unknown protein	3.27	±2.34
all5247	Unknown protein	14.18	±3.95
all5337	Similar to TRK system potassium uptake protein	2.43	±0.56
alr0074	Putative glycosyl transferase	2.33	±0.23
alr0242	Hypothetical protein	6.33	±0.84
alr0248	Unknown protein	2.06	±0.09
alr0277	Group 3 sigma factor, SigJ	144.17	±30.69
alr0280	Ribonuclease III	2.60	±0.42
alr0369	Unknown protein	5.56	±0.67
alr0370	Unknown protein	10.21	±2.18

Continued

Table 5. Continued

ORF[a]	Product[a]	Fold (HE0277/P490)[b]	SD
alr0429	Two-component response regulator	5.66	± 0.41
alr0430	Probable short-chain dehydrogenase	2.94	± 0.70
alr0564	Hypothetical protein	2.35	± 1.02
alr0655	Hypothetical protein	2.08	± 1.57
alr0692	Similar to NifU protein	2.77	± 0.81
alr1016	Hypothetical protein	2.41	± 2.01
alr1183	Unknown protein	2.76	± 1.30
alr1372	Hypothetical protein	2.27	± 1.56
alr1534	Hypothetical protein	2.09	± 0.80
alr2071	Unknown protein	2.13	± 2.03
alr2142	Oxidoreductase	2.09	± 1.36
alr2174	Transcriptional regulator	2.10	± 0.38
alr2256	Unknown protein	2.95	± 1.51
alr2310	Similar to agmatinase	2.15	± 0.99
alr2405	Flavodoxin, IsiB	3.36	± 0.74
alr2407	Hypothetical protein	2.38	± 0.41
alr2447	DnaJ protein	2.15	± 2.09
alr2522	Unknown protein	2.07	± 0.37
alr2539	Branched-chain amino acid ABC transporter, ATP-binding protein	2.61	± 2.29
alr3057	Probable glycosyl transferase	2.63	± 0.34
alr3058	Probable glycosyl transferase	2.50	± 0.21
alr3059	Similar to polysaccharide export protein	2.86	± 0.21
alr3060	Unknown protein	3.20	± 0.47
alr3061	Similar to acetyl transferase	3.34	± 0.51
alr3062	Probable glycosyl transferase	4.74	± 0.01
alr3063	Probable glycosyl transferase	3.61	± 0.29
alr3064	Probable glycosyl transferase	3.57	± 1.30
alr3065	Probable polysaccharide biosynthesis protein	2.47	± 0.20
alr3066	Polysaccharide polymerization protein	2.53	± 0.69
alr3067	Probable glycosyl transferase	2.37	± 0.55
alr3068	Probable glycosyl transferase	3.09	± 0.65
alr3069	Probable glycosyl transferase	2.06	± 0.11
alr3070	Probable glycosyl transferase	3.18	± 0.34
alr3071	Probable glycosyl transferase	3.09	± 0.16
alr3072	Probable polysaccharide biosynthesis protein	3.36	± 0.19
alr3073	Probable glycosyl transferase	2.95	± 0.92
alr3092	Two-component hybrid sensor and regulator	2.19	± 1.58
alr3485	Unknown protein	2.09	± 0.63
alr3507	Hypothetical protein	3.40	± 0.79
alr3547	Two-component sensor histidine kinase	2.02	± 1.54
alr3548	Hypothetical protein	2.18	± 2.06
alr3554	Unknown protein	4.48	± 2.54
alr3594	Two-component response regulator	2.10	± 0.35
alr3666	Urease accessory protein D	2.30	± 1.75
alr3775	Unknown protein	2.11	± 1.33
alr3816	Unknown protein	15.90	± 2.44

Continued

Table 5. Continued

ORF[a]	Product[a]	Fold (HE0277/P490)[b]	SD
alr3817	Unknown protein	5.53	± 0.94
alr3997	Serine/threonine kinase	2.04	± 0.25
alr4057	Similar to peptide synthetase	2.49	± 0.29
alr4132	Hypothetical protein	2.44	± 0.83
alr4800	Similar to anti-sigma factor antagonist	2.65	± 0.10
alr4818	Similar to Bpu10I restriction endonuclease beta subunit	2.49	± 2.23
alr4823	Similar to glucosyl-1-phosphate transferase	3.00	± 1.05
alr4965	Hypothetical protein	4.32	± 1.68
alr5223	Glycosyl transferase	3.68	± 0.04
alr5224	Hypothetical protein	3.33	± 0.77
asl0597	Hypothetical protein	2.21	± 0.00
asl2078	Unknown protein	2.01	± 1.59
asl3998	Hypothetical protein	3.42	± 0.61
asl5041	Unknown protein	2.51	± 2.00
asr0431	Unknown protein	2.05	± 0.85
asr0941	Photosystem II protein PsbX	3.20	± 2.16
asr1185	Unknown protein	2.06	± 1.26
asr1734	Unknown protein	3.20	± 1.57
asr2206	Hypothetical protein	2.67	± 1.02
asr4951	Unknown protein	3.51	± 2.05

The fold values that exceeded 2.0 were considered significantly different between the two strains.
Each datum is the average (± standard deviation) calculated from three independent experiments.
The gene clusters relevant to putative polysaccharide biosynthesis and the ORFs encoding glycosyl/glucosyl transferase are shaded in grey.
[a]'ORF' and 'description' are defined according to the CyanoBase database.
[b]The fold value was calculated as the ratio of the signal intensity of each gene of HE0277 to that of P490.

by both HE0277 and P490 cells were composed of eight kinds of identified monosaccharides (galactose, glucose, fucose, rhamnose, arabinose, xylose, galacturonic acid, and glucuronic acid) and several unknown monosaccharides (data not shown). No difference in monosaccharidic composition was detected between the HE0277 and P490 strains. The amount of each monosaccharide in the polysaccharides released by HE0277 was two- to threefold greater than that released by P490. Many studies have been carried out to determine the function of EPSs in the capacity of some polysaccharide-producing cyanobacteria to overcome desiccation in the desert.[37] It has been proposed that EPSs provide a repository for water, thereby acting as a buffer between cells and the atmosphere, and represent the key component of the mechanism used by cyanobacteria to tolerate desiccation.[38] The EPSs of *N. commune* account for more than 60% of its dry weight.[6] The polysaccharides, together with trehalose, may play a key role in dehydration tolerance.[39] It has been reported that photosynthetic O_2 evolution in EPS-depleted *N. commune* cells is sensitive to desiccation treatment, whereas that of normal *N. commune* cells is rapidly recovered after rehydration.[40] Because HE0277 cells produced and released polysaccharides in increased amounts (Table 6), the polysaccharides must be a critical factor in desiccation tolerance. The group 3 sigma factor gene *sigJ* is fundamental to desiccation tolerance. This discovery will expedite our understanding of the mechanism of desiccation tolerance and EPS biosynthesis in cyanobacteria.

Table 6. Comparative analysis of EPSs in P490 and HE0277 cell strains

Cell strain	Carbohydrate production			
	Cell surface (mg L^{-1})	SD	Medium (mg L^{-1})	SD
P490	864.42	± 24.16	516.86	± 84.83
HE0277	778.65	± 40.56	1651.69	± 70.17

Each datum is an average (± standard deviation) of three independent experiments.
EPS, extracellular polysaccharide.

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