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Cellular polyamine profiles in cyanobacteria

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Acid-extracted cellular polyamines from 46 strains of the cyanobacteria collections IAM, NIES, MBIC and NBRC, were analyzed by HPLC and GC, and compared to the polyamine profiles of 80 strains which were previously analyzed. The cyanobacterial strains were roughly separated into spermidine-dominant type and homospermidine-dominant type in their triamine distributions. Two species of the genus *Oscillatoria* were devoid of polyamine components. High agmatine levels were found in *Acaryochloris marina*. The tetra-amines, spermine and thermospermine, were detected in the thermophilic *Thermosynechococcus elongatus* and halophilic *Arthrospira platensis*.

Key words: cyanobacteria, homospermidine, polyamine, spermine, thermospermine

Cellular polyamine distribution profiles have provided valuable information in the prokaryotic domains Bacteria (Eubacteria) and Archaea (Archaebacteria) (Hamana, 2002a; Hamana & Hosoya, 2006). Regarding cyanobacteria, two types of major cellular triamine components, spermidine-dominant and homospermidine-dominant types, have been identified within 80 strains analyzed in our previous studies (Hamana *et al*., 1983, 1988; Hosoya *et al*., 2005) and 71 strains from another report (Hegewald & Kneifel, 1983). In the present study, cellular polyamines were analyzed to evaluate polyamine distribution patterns of 13 additional axenic strains and 33 non-axenic strains (total of 46 strains as unicyanobacterial isolates), which belong to four orders of cyanobacteria: Chroococcales (Subsection I), Pleurocapsales (II), Oscillatoriales (III), and Nostocales (IV) (Castenholz, 2001; NCBI website, 2007; Watanabe, 1995).

Cyanobacterial strains were supplied from culture collections in Japan (see Table 1), and cultivated in polyamine-free synthetic media (pH 6.0-7.5) under conditions designated by the culture collections (Yokota, 2004; Kasai *et al*., 2004; NBRC, 2005). The moderately thermophilic *Thermosynechococcus elongatus* was grown at 60℃. Alkaline-halophilic *Arthrospira platensis* was cultivated in an alkaline salt medium, IAM-A12L (pH 9.2) containing 1.7% $NaHCO_3$ and 0.1% NaCl. After 2-3 weeks, stationary growing cells were harvested from the liquid cultures (100-1,000 m*l*) by centrifugation and from the 2-5 agar slants or agar plates by scraping. The pellets of organisms were homogenized in equal volumes of 10% (1M) perchloric acid ($HClO_4$, PCA). The PCA extracts were subjected to ion-exchange chromatography using columns of Dowex 50W-X8 and then analyzed by high-performance liquid chromatography (HPLC) in a column of cation-exchange resin using a Hitachi L6000 high-speed liquid chromatograph (Hamana, 2002b; Hamana *et al*., 2005). The further purified polyamine samples from the thermophile and halophile by Whatman CM23 column, were analyzed by gas chromatography (GC) on a Shimadzu GC-9A gas chromatograph and GC-mass spectrometry (GC-MS) using a JEOL JMS-700 mass spectrometer, after heptafluorobutyrization (Hamana

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Table 1 Cellular polyamine concentrations in cyanobacteria

Organism		Polyamines (μmol/g wet weight)						
		Put	Cad	Spd	HSpd	Spm	TSpm	Agm
Chroococcales (Subsection I)								
Aphanocapsa montana	NIES-416*	–	–	–	1.15	–	–	-
Chamaesiphon polymorphus	NIES-433*	0.20	–	0.94	–	–	–	–
Chamaesiphon subglobosus	NIES-434*	0.10	–	0.80	–	–	–	–
Chroogloeocystis siderophila	NIES-1031*	0.02	-	-	0.76	-	-	0.05
Cyanobacterium sp.	MBIC10216* (=NBRC 102756)	–	–	0.20	–	–	–	–
Gloeocapsa decorticans	NIES-931*	–	–	–	0.35	–	–	–
Merismopedia tenuissima	NIES-230*	0.21	–	0.29	0.19	–	–	0.02
Synechococcus sp.	MBIC10224* (=NBRC 102757)	–	–	0.10	0.40	–	–	–
Synechococcus sp.	MBIC10456* (=NBRC 102774)	–	–	0.40	–	–	–	–
Synechococcus sp.	MBIC10459* (=NBRC 102777)	–	–	0.57	–	–	–	–
Synechococcus sp.	MBIC10613* (=NBRC 102839)	–	–	0.60	–	–	–	–
Synechococcus sp.	IAM M-200	–	–	1.52	0.02	–	–	–
Synechocystis sp. (PCC)	IAM M-274	–	–	0.77	0.04	–	–	–
Synechocystis sp. (GT)	IAM M-275	–	–	0.96	–	–	–	–
Thermosynechococcus elongatus (BP-1)	IAM M-273	0.02	-	0.02	0.17	0.01	0.01	0.02
Pleurocapsales (Subsection II)								
Dermocarpa sp.	MBIC10001* (=NBRC 102690)	–	–	–	0.80	–	–	–
Myxosarcina burmensis	NIES-481*	0.03	–	–	0.81	–	–	–
Oscillatoriales (Subsection III)								
Geitlerinema sp.	MBIC10006* (=NBRC 102693)	–	–	–	0.30	–	–	–
Lyngbya digneti	IAM M-279	–	–	–	0.25	–	–	–
Lyngbya aerugineo-coerulea	IAM M-287	–	–	0.50	–	–	–	–
Oscillatoria mougeotii	IAM M-281*	–	–	–	0.70	–	–	–
Oscillatoria neglecta	IAM M-282*	–	–	0.27	–	–	–	–
Oscillatoria amphibia	NIES-361*	–	–	–	–	–	–	–
Oscillatoria animalis	NIES-206*	–	–	0.65	–	–	–	–
Oscillatoria limnetica	NIES-36*	–	–	–	–	–	–	–
Oscillatoria rosea	NIES-208	–	–	–	–	–	–	–
Oscillatoria tenuis	NIES-33	–	–	–	0.75	–	–	–
Pseudanabaena sp.	MBIC11161* (=NBRC 103014)	–	–	–	0.74	–	–	–
Arthrospira platensis (salt water)	IAM M-135	0.01	0.01	0.88	0.06	0.01	0.15	0.05
Spirulina platensis (salt water)	NIES-46	–	–	0.89	–	0.02	ND	0.05
Spirulina platensis (freshwater)	NIES-597*	–	–	0.48	–	–	–	–
Spirulina subsalsa (freshwater)	NIES-598*	–	–	0.80	–	–	–	–
Phormidium jenkelianum	NIES-507*	0.15	–	–	0.57	–	–	0.02
Phormidium ramosum	NIES-305*	0.10	–	–	0.42	–	–	–
Phormidium sp.	MBIC10003* (=NBRC 102691)	–	–	0.64	–	–	–	–
Phormidium sp.	MBIC10025* (=NBRC 102696)	–	–	0.50	–	–	–	–

Table 1 Continued

Organism		Polyamines (μmol/g wet weight)						
		Put	Cad	Spd	HSpd	Spm	TSpm	Agm
Phormidium sp.	MBIC10070* (=NBRC 102724)	-	-	0.50	-	-	-	-
LPP-group-B	MBIC10597* (=NBRC 102833)	-	-	0.60	-	-	-	-
Nostocales (Subsection IV)								
Calothrix parasitica	IAM M-226	-	-	0.59	-	-	-	-
Calothrix parasitica	NIES-267	-	-	0.40	-	-	-	-
Calothrix scopulorum	NIES-268*	-	-	0.60	-	-	-	-
Nostoc minutum	NIES-26*	0.07	-	-	0.53	-	-	0.02
Raphidiopsis curvata	NIES-932*	0.10	-	-	0.30	-	-	-
Rivularia sp.	IAM M-261*	-	-	0.25	-	-	-	-
Tolypothrix tenuis	IAM M-110	-	-	0.38	-	-	-	-
Unclassified								
Acaryochloris marina	MBIC11017*	-	-	0.15	-	-	-	0.20

Put, putrescine; Cad, cadaverine; Spd, spermidine; HSpd, homospermidine; Spm, spermine; TSpm, thermospermine; Agm, agmatine; -, not detected (<0.005). ND, TSpm was not separately determined by HPLC, however, the value for Spm shows for Spm plus TSpm. IAM, IAM Culture Collection, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan; NIES, National Institute for Environmental Studies, Tsukuba, Japan; NBRC, Biological Resource Center, National Institute of Technology and Evaluation, Kisarazu, Japan; MBIC, Marine Biotechnology Institute Culture Collection, Kamaishi, Iwate, Japan. Recently algal collections of IAM were transferred into NIES, and also some MBIC microalgal strains into NBRC. HPLC and GC data were obtained from single-triple samples and polyamine concentrations were roughly expressed per starting wet-weight of cell pellet. *, non-axenic strain and others were axenic strain.

et al., 2005; Niitsu *et al*., 1993).

In the present study, we used both axenic and non-axenic strains, because contaminated microorganisms did not seem to affect the determination of polyamine distribution as previously shown (Hosoya *et al*., 2005). Both spermidine-dominant and homospermidine-dominant types were observed in the Chroococcales, Oscillatoriales and Nostocales (Table 1). No cellular polyamines were found in *Oscillatoria limnetica* NIES-36 and *O. rosea* NIES-208, as previously found in *O. rosea* IAM M-220, within all of the cyanobacterial strains analyzed so far (Hosoya *et al*., 2005). Two strains of the Pleurocapsales were homospermidine-dominant type. A recently found chl. d containing cyanobacterium, *Acaryochloris marina*, whose phylogenetic lineage inferred from SSU rDNA diverged independently from other subgroups of the phylum Cyanobacteria (Miyashita *et al*., 2003), contained spermidine and agmatine as major polyamines (Table 1).

As shown in the present study as well as in the previous studies (Hamana *et al*., 1988; Hosoya *et al*., 2005), cyanobacterial strains analyzed were roughly divided into spermidine-dominant and homospermidine-dominant type. The distribution of these two types was variable within each of the order Chroococcales, Oscillatoriales and Nostocales, in which quite a large number of strains and species were examined. However, all species of *Anabaena* and *Nostoc* examined so far were homospermidine-dominant type. On the other hand, polyamine distribution was variable among species in several genera such as *Oscillatoria*, *Phormidium* and *Calothrix*. This variable distribution may be due to polyphyletic distribution of species and strains belonging to such genera (Ishida *et al*., 2001; Wilmotte & Herdman, 2001).

In the present study, a novel tetra-amine, thermospermine (an isomer of spermine), and spermine, as well as agmatine, were detected in large-scale cultures of *Thermosynechococcus elongatus* IAM M-273, grown at 60℃, and of halophilic *Arthrospira platensis* IAM M-135, grown in alkaline salt medium (Fig. 1 and Table 1). This is the first report on the occurrence of thermospermine in the phylum Cyanobacteria. It is known that many moderately thermophilic eubacteria belonging to the phyla Proteobacteria and Firmicutes, growing at 45-60℃,

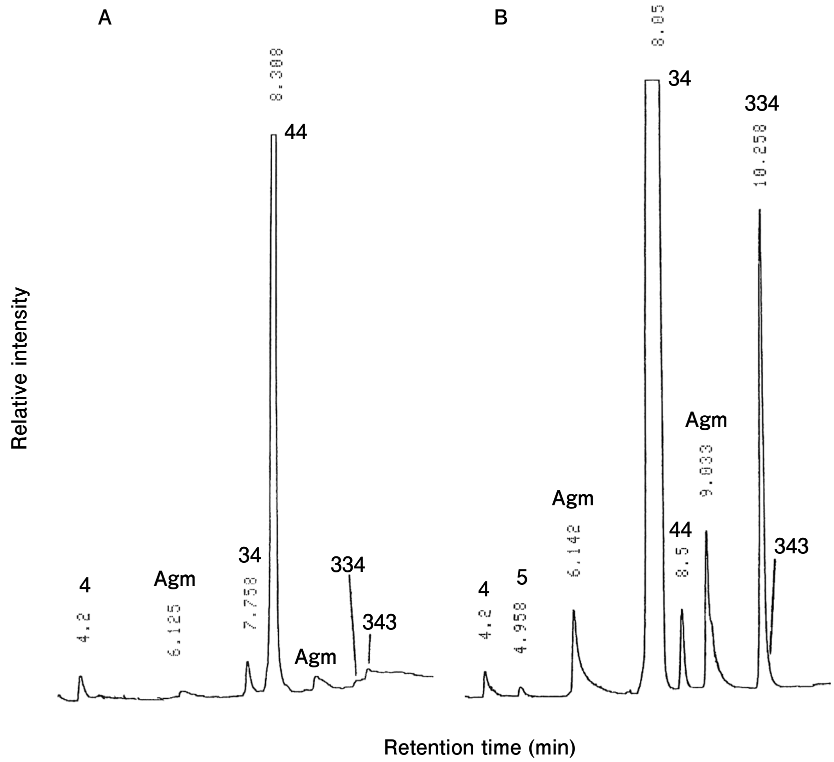

Fig. 1 GC analysis of the purified polyamine samples of *Thermosynechococcus elongatus* IAM M-273 (A) and *Arthrospira platensis* (*Spirulina platensis*) IAM M-135 (B). Agmatine was split into two peaks during GC. The peak and shoulder corresponding to 343 and 334 were identified by GC-MS. HPLC can not separate the two tetra-amine isomers. Abbreviations for polyamines: 4, putrescine (Put); 5, cadaverine (Cad); 34, spermidine (Spd); 44, homospermidine (HSpd); 343, spermine (Spm); 334, thermospermine (TSpm); Agm, agmatine. The printed numbers are the retention time at the peak.

contain a tetra-amine, spermine, as a major polyamine, in addition to spermidine (Hamana *et al.*, 2006; Hosoya *et al.*, 2004). Spermine has also been found in moderately halophilic anaerobes belonging to the order Haloanaerobiales of the phylum Firmicutes, growing in 10% NaCl (Hosoya *et al.*, 2006). Therefore, there may be a correlation between the occurrence of tetra-amines and thermotolerance and salt tolerance in cyanobacteria.

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シアノバクテリアにおける菌体内ポリアミン成分構成

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東京大学 IAM，国立環境研究所 NIES，海洋バイオテクノロジー研究所 MBIC，製品評価技術基盤機構生物遺伝資源部門 NBRC に保存されているシアノバクテリアのうち，ポリアミンが未分析であった 46 株の菌体内ポリアミン画分を過塩素酸抽出後，HPLC と GC にて分析し，分析済み 80 株のポリアミン成分構成と比較した．大多数のシアノバクテリア株は，トリアミン成分として，スペミジン優勢型とホモスペルミジン優勢型に大別された．*Oscillatoria* 属の 2 種が全ポリアミン成分を欠き，クロロフィル d を持つ特異なシアノバクテリア *Acaryochloris marina* ではグアニジノアミンであるアグマチンが高

含量であった．好熱性の *Thermosynechococcus elongatus* と好塩性の *Arthrospira platensis* ではテトラアミン成分のスペルミンとサーモスペルミンも検出された．

（担当編集委員　笠井文絵）