

Additional analysis of cyanobacterial polyamines — Distributions of spermidine, homospermidine, spermine, and thermospermine within the phylum *Cyanobacteria* —

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To further catalogue the distribution of cyanobacterial cellular polyamines, we used HPLC and HPGC to newly analyze the acid-extracted polyamines from 14 cyanobacteria. The colony-forming *Nostoc verrucosum* ("Ashitsuki") and *Nostoc commune* ("Ishikurage"), as well as *Anabaena* species (*Nostocales*), contained homospermidine. The thermo-halotolerant *Spirulina subsalsa* var. *salina* (*Spirulinales*), as well as freshwater *Spirulina* strains, contained spermidine. Putrescine, spermidine, and homospermidine were found in freshwater colony-forming *Aphanothece sacrum* ("Suizenji-nori"), whereas the halotolerant *Aphanothece halophytica* and *Microcystis* species (*Chroococcales*) contained spermidine alone. In addition to putrescine, spermidine, homospermidine and agmatine, thermospermine was found as a major polyamine in haloalkaliphilic *Arthrospira platensis* ("Spirulina") (*Oscillatoriales*). In the *Synechococcales*, chlorophyll *b*-containing *Prochlorococcus marina* contained spermidine, and chlorophyll *d*-containing *Acaryochloris marina* contained spermidine and homospermidine. Thermophilic *Thermosynechococcus vulcanus* and *Thermosynechococcus* sp. NK55a, as well as *Thermosynechococcus elongatus*, contained low levels of spermine in addition to homospermidine. The present 14 and previous 126 strains of cyanobacteria were categorized into spermidine-dominant types, homospermidine-dominant types and spermidine-homospermidine-mix types, but their polyamine profiles have not yet been established as chemotaxonomic markers.

Key words: cyanobacteria, homospermidine, polyamine, thermospermine

Bacterial cellular polyamine distribution profiles are related to the phylogenetically classified locations of the bacteria, as well as to the growth temperature, pH, and salt conditions in bacterial growth environments (Hamana, 2002a; Hamana & Hosoya, 2006; Hamana & Matsuzaki, 1992; Hamana *et al.*, 2014; Hosoya *et al.*, 2006). In the oxygen-producing photosynthetic cyanobacteria, as the origins of the plastids of photosynthetic eukaryotic algae, it is useful to consider the polyamine profiles and the chemotaxonomic usage of the polyamine components in studies of the early evolution of algae and plants. The cellular polyamines of 126 cyanobacterial strains have been roughly separated into spermidine-dominant types and homospermidine-dominant types (Hamana *et al.*, 1983, 1988, 2008; Hosoya *et al.*,

2005). Diaminopropane, putrescine, cadaverine, and agmatine have been found sporadically. Spermine and thermospermine have been tentatively detected as minor polyamines in some thermophiles and haloalkaliphiles (Hamana *et al.*, 2008). The triamine norspermidine, the tetra-amine norspermine, and pentaamines are widely distributed in algae (Hamana *et al.*, 2013, 2016) but have not been found in cyanobacteria.

Although cyanobacterial classification has undergone phylogenetic analyses based on molecular sequence data (Komarek *et al.*, 2014; MCC-NIES, 2016; NCBI, 2016), the taxonomy of cyanobacterial class, order, family, genus and species is conflicting (Oren, 2004; Watanabe, 1995). Here, we analyze the cellular polyamines of the 14 newly available cyanobacteria, including thermophilic (thermotolerant), halophilic (halotolerant), haloalkaliphilic (alkaline-halophilic; alkali- and salt-tolerant), polysaccharide-colony forming, chlorophyll (Chl) *b*-containing, and Chl *d*-containing strains, in order to add them to cyano-

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bacterial polyamine catalogues. We also re-examined some of our previous cyanobacterial polyamine data by using mass cultivation of cyanobacteria and new chromatographic techniques. Triamines (spermidine and homospermidine) are produced from the diamine putrescine or the guanidinoamine agmatine, and then the tetra-amines (spermine and thermospermine) are produced from spermidine. The cellular distribution of the triamines and tetra-amines is thus focused on the phylum *Cyanobacteria*.

Seven unique cyanobacteria supplied by NIES (National Institute for Environmental Studies, Japan) and NBRC (Biological Resource Center, National Institute of Technology and Evaluation, Japan) were cultured phototrophically in the light (10–14 h/24 h) at either 20–25°C (mesophiles), 40–50°C (thermotolerants) or 55–60°C (thermophiles) by using 1–10 L of the liquid media designed by NIES (MCC-NIES, 2016) or NBRC (NBRC, 2016). Liquid cultures (1 L) of two cyanobacterial DBT strains were purchased from DBT (Department of Biotechnology, Institute of Environmental Biology Co., Shizuoka, Japan). Although both axenic and non-axenic (i.e. not proven to be axenic) cyanobacterial strains have been used, disadvantage for the use of non-axenic strains was not found in the cyanobacterial polyamine analyses (Hamana *et al.*, 2008; Hosoya *et al.*, 2005). Two dried powder products (A grade and B grade) of “Spirulina” (*Arthrospira platensis*) cultured in open culture tanks at the Kumejima Factory of Japan Algae, Okinawa, Japan were obtained from Japan Algae Co., Ltd. (Tokyo, Japan). Another dried powder product “Spirulina” cultured in the field pools of Hainan DIC Microalgae Co., Ltd., China was obtained from DIC Lifetech Co., Ltd. (Tokyo, Japan). “Suizenji-nori” (*Aphanothece sacrum*) has been cultured by Endoukanagawa-dou Co. (Asakura, Fukuoka), Kisen-dou Co. (Asakura, Fukuoka), Tanseidou Co. (Kashima, Kumamoto), and Green Science Material Inc. (Mashiki, Kumamoto), in Japan. Dried sheets of “Suizenji-nori” were purchased from Endoukanagawa-dou Co. in the present study.

Organisms (1–10 g wet weight) harvested at the early stationary phase in our laboratory or dried powders (5–10 g) purchased, were homogenized in 5% perchloric acid (PCA). The PCA extract was passed through a column of a cation-exchange resin (Dowex 50 WX8; 1 cm I.D. × 3 cm) and eluted from the column with 6M HCl to concentrate the polyamines. We performed high-performance liquid

chromatography (HPLC) of the concentrated polyamines on a Hitachi L6000 with a column of cation-exchange resin [Hitachi 2619F (=Hitachi 2720); 4 mm I.D. × 50 mm] (Hamana, 2002b; Hamana *et al.*, 2008; Hosoya *et al.*, 2005), instead of using the old-type of HPLC with a column of Kyowa Seimitsu 62210F (4.8 mm I.D. × 80 mm) (Hamana *et al.*, 1983, 1988); samples were then quantified by using post-labeled fluorometry after heating with *o*-phthalaldehyde. After heptafluorobutyrylation of the concentrated polyamines, we performed high-performance gas chromatography (HPGC) with a SHIMADZU GC-17A and GC-mass spectrometry (HPGC-MS) on a JEOL JMS-700 equipped with a long capillary column of InertCap 1MS (0.32 mm I.D. × 30 m, df 0.25 μm) (GL Sciences, Tokyo, Japan) (Furuchi *et al.*, 2015a, b; Niitsu *et al.*, 2014), instead of using standard GC with a short packed column of 3% SE-30 Chromosorb WHP (Gasukuro Kogyo, Tokyo, Japan) (3 mm I.D. × 2.1 m) (Hamana *et al.*, 1983, 1988, 2008; Hosoya *et al.*, 2005; Niitsu *et al.*, 1993). He gas was used as the carrier gas and in the flame ionization detector. HPGC on a SHIMADZU GC-17A was operated at the column temperature 120–16°C/min–280°C. HPGC on a JEOL JMS-700 was operated at the column temperature 90–16°C/min–280°C and mass spectra were obtained by using electron impact mode at an ionization energy of 70 eV. The molar concentrations of cellular polyamines per gram of wet weight of the starting pellets, as estimated from the HPLC and HPGC analyses using authentic polyamine standards, are shown in Table 1 and are compared among those of neighboring species.

In the order *Nostocales*, some of the *Anabaena* species (*Anabaena circinalis*, *Anabaena akankoensis*, *Anabaena affinis*, and *Anabaena spiroides*=*Anabaena crassa*) were reclassified into the new genus *Dolichospermum* (NCBI Taxonomy Browser, 2016). A mass culture of the DBT strain of filamentous *A. spiroides*, as well as other *Anabaena* and *Dolichospermum* species previously analyzed, contained homospermidine as the major polyamine. The aquatic (freshwater) *Nostoc verrucosum* “Ashitsuki” (Sakamoto *et al.*, 2011) as well as the terrestrial *Nostoc commune* “Ishikurage” (Arima *et al.*, 2012) are unique as colony-forming cyanobacteria with extracellular polysaccharides; they contained homospermidine as the major polyamine. All of the *Nostoc* and *Anabaena* species analyzed had the same polyamine profile; however, the taxonomy of the two

Table 1 Cellular polyamine concentrations of cyanobacteria

Phylum <i>Cyanobacteria</i>	Ref.	Polyamines ($\mu\text{mol/g}$ wet weight)							
		Dap 3	Put 4	Cad 5	Spd 34	HSpd 44	Spm 343	TSpm 334	Agm
Order <i>Nostocales</i>									
<i>Anabaena spiroides</i> DBT		–	0.11	–	–	1.22	–	–	–
<i>Anabaena spiroides</i> NIES-79	2005	–	0.01	–	–	0.35	–	–	–
<i>Anabaena circinalis</i> NIES-41	2005	–	0.20	–	–	0.57	–	–	–
<i>Anabaena compacta</i> NEIS-806	2005	–	–	–	–	1.50	–	–	–
<i>Anabaena cylindrica</i> IAM M-1	1983	–	0.01	–	0.01	2.18	–	–	–
<i>Anabaena cylindrica</i> IAM M-253	2005	–	–	–	–	1.55	–	–	–
<i>Anabaena planctonica</i> NIES-814	2005	–	–	–	–	0.85	–	–	–
<i>Anabaena solitaria</i> NIES-80	2005	–	0.05	–	–	0.65	–	–	–
<i>Anabaena variabilis</i> IAM M-3	2005	–	–	–	–	0.79	–	–	–
<i>Anabaena variabilis</i> DIC	1983	–	0.02	–	0.04	3.85	0.02	–	–
<i>Dolichospermum crassum</i> (formerly <i>Anabaena spiroides</i>) NIES-78	2005	–	–	–	–	0.49	–	–	–
<i>Nostoc commune</i> IAM M-13 “Ishikurage”	2005	–	–	–	–	0.69	–	–	–
<i>Nostoc verrucosum</i> NIES-2539 “Ashitsuki”		–	0.02	–	–	0.75	–	–	–
<i>Nostoc carneum</i> IAM M-35	2005	–	–	–	–	1.35	–	–	–
<i>Nostoc entophytum</i> IAM M-267	2005	–	–	–	0.16	0.75	–	–	–
<i>Nostoc linckia</i> IAM M-251	2005	–	–	–	–	9.98	–	–	–
<i>Nostoc linckia</i> var. <i>arvense</i> IAM M-30	2005	–	–	–	–	1.25	–	–	–
<i>Nostoc minutum</i> NEIS-26	2008	–	0.07	–	–	0.53	–	–	0.02
<i>Nostoc punctiforme</i> IAM M-15	2005	–	0.10	–	–	1.50	–	–	–
Order <i>Spirulinales</i>									
<i>Spirulina subsalsa</i> IAM M-223 (= IAM M-183) (=NIES-27)	2005	–	–	–	0.85	–	–	–	–
NIES-598	2008	–	–	–	0.80	–	–	–	–
var. <i>salina</i> NIES-3373 (Thermo-halotolerant)		–	–	–	0.20	–	–	–	–
Order <i>Chroococcales</i>									
<i>Microcystis wesenbergii</i> DBT		–	0.02	–	1.20	–	–	–	–
<i>Microcystis aeruginosa</i> IAM M-228	2005	–	–	–	1.15	–	–	–	–
IAM M-178	2005	–	–	–	1.10	–	–	–	–
IAM M-247	2005	–	0.12	–	1.33	–	–	–	–
<i>Aphanothece sacrum</i> “Suizenji-nori”	1983	–	0.11	–	0.23	0.45	–	–	–
No.1		–	0.15	0.01	0.07	1.14	–	–	–
No.2		–	0.15	0.01	0.05	1.25	–	–	–
<i>Aphanothece elabens</i> IAM M-257	2005	–	–	–	0.50	–	–	–	–
<i>Aphanothece halophytica</i> ATCC 43922 (Halotolerant alkaliphilic)	1988	–	–	–	0.30	–	–	–	–
Order <i>Chroococciopsidales</i>									
<i>Chroogloeocystis siderophila</i> NIES-1031 (Thermotolerant, 50°C)	2008	–	0.02	–	–	0.76	–	–	0.05
Order <i>Oscillatoriales</i>									
<i>Arthrospira platensis</i> Japan Algae [A] “Spirulina” (Haloalkaliphilic)		0.03	0.05	0.01	1.23	0.07	–	0.15	0.10
(formerly salt water <i>Spirulina platensis</i>) Japan Algae [B]		0.04	0.06	0.02	1.20	0.05	–	0.13	0.05
DIC Lifetech “Spirulina”		0.01	0.08	–	1.25	0.07	0.02	0.24	0.05
IAM M-135 (=IAM M-222=NIES-39)	2008	–	0.01	0.01	0.88	0.06	0.01	0.15	0.05
NIES-46 (=IAM M-185)	2008	–	–	–	0.89	–	0.02	★	0.05
subsp. <i>siamese</i> DIC	1983	–	0.01	–	0.80	–	0.01	★	–
IAM M-135	1983	–	–	–	0.59	–	0.05	★	–
<i>Planktothrix spiroides</i> (formerly freshwater <i>Spirulina platensis</i>)	1983	–	0.01	–	0.61	–	0.02	–	–
IAM M-184 (=NIES-45)									
<i>Planktothrix agardhii</i> IAM M-244	2005	–	–	–	0.62	–	–	–	0.20
<i>Planktothrix rubescens</i> NIES-610	2005	–	–	–	1.32	–	–	–	–

Table 1 Continued

Phylum <i>Cyanobacteria</i>	Ref.	Polyamines ($\mu\text{mol/g}$ wet weight)							
		Dap 3	Put 4	Cad 5	Spd 34	HSpd 44	Spm 343	TSpm 334	Agm
Order <i>Synechococcales</i>									
<i>Prochlorococcus marina</i> NIES-2086 (Chlorophyll <i>b</i>)		–	–	–	0.25	–	–	–	0.03
NIES-2087		–	0.05	–	0.51	–	–	–	–
<i>Acaryochloris marina</i> MBIC 11017 (=NBRC 102967) (Chlorophyll <i>d</i>)	2008	–	–	–	0.15	–	–	–	0.20
NIES-2412		0.01	0.12	–	0.45	0.13	–	–	0.02
<i>Thermosynechococcus elongatus</i> (BP-1) IAM M-273 (=NIES-2133)	2008	–	0.02	–	0.02	0.17	0.01	0.01	0.02
(Thermophilic, 60°C)									
(BP-1) Tokyo Univ. (55°C)	1988	–	0.05	–	0.02	0.06	–	–	–
<i>Thermosynechococcus vulcanus</i> NIES-2134 (=IAM M-293)		–	–	–	0.02	0.04	0.01	–	–
(Thermophilic, 60°C)									
<i>Thermosynechococcus</i> sp. (NK55a) NBRC 108920		–	0.10	–	0.01	0.27	0.04	–	–
(Thermophilic, 60°C)									
<i>Limnothrix redekei</i> NIES-847	2005	–	–	–	–	0.40	–	–	0.32
<i>Pseudanabaena galeata</i> (formerly <i>Phormidium tenue</i>) NIES-512	2005	–	–	–	–	0.50	–	–	0.30

Dap (3) (=the numbers of methylene chain units (CH₂) between NH₂ or NH), diaminopropane; Put (4), putrescine; Cad (5), cadaverine; Spd (34), spermidine; HSpd (44), homospermidine; Spm (343), spermine; TSpm (334), thermospermine; Agm, agmatine; ATCC, American Type Culture Collection, Manassas, VA, USA; IAM, IAM Culture Collection, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan (IAM algal collections have been transferred to NIES); MBIC, Marine Biotechnology Institute Culture Collection, Kamaishi, Iwate, Japan (MBIC collections have been transferred to NBRC); NIES, National Institute for Environmental Studies, Tsukuba, Japan; DBT, Department of Biotechnology, Institute of Environmental Biology Co., LTD, Yaezu, Shizuoka, Japan; DIC Lifetech, DIC Lifetech Co., Ltd., Tokyo, Japan; Japan Algae, Japan Algae Co., Ltd, Tokyo, Japan; NBRC, Biological Resource Center, National Institute of Technology and Evaluation, Kisarazu, Japan; –, not detected (<0.005 $\mu\text{mol/g}$ wet weight). Citations of our data from the References (Ref.) are shown as the year of publication (Hamana *et al.* or Hosoya *et al.*). The 14 samples without Ref. year notations were analyzed in the present study. ★, analysis of thermospermine by using standard GC and HPGC was not performed.

genera is conflicting (Komarek *et al.*, 2014; MCC-NIES, 2016).

In filamentous *Spirulina subsalsa* belonging to the order *Spirulinales*, the thermo-halotolerant strain, *S. subsalsa* var. *salina* grown at 40°C in the presence of 10% NaCl was isolated (Kuroiwa *et al.*, 2014). Both the thermo-halotolerant strain and two freshwater strains of *S. subsalsa* contained spermidine; however, the cellular level of spermidine was lower in the thermo-halotolerant strain.

In the order *Chroococcales*, freshwater *Microcystis* species are single-celled; they form colonies and then form blooms called “Aoko”. *Microcystis wesenbergii* (analyzed here) and *Microcystis aeruginosa* (analyzed previously) contained spermidine as the major polyamine. The halotolerant, alkaliphilic *Aphanothece halophytica* grown at pH 7.8 in the presence of 12% NaCl (Laloknam, *et al.*, 2006) has a low spermidine content (Hamana *et al.*, 1988). Freshwater *Aphanothece sacrum* forms colonies with polysaccharides and lives naturally in a river, the Kogane-

gawa, in Asakura, Fukuoka, Japan (Fujishiro *et al.*, 2004). Our polyamine analysis of the two samples (No. 1 and No. 2) of dried “Suizenji-nori” (*A. sacrum*) by HPLC, HPGC, and HPGC-MS showed the presence of homospermidine in addition to putrescine and spermidine (Fig. 1 and Table 1), supporting previous data obtained on “Suizenji-nori” kept in saline (purchased from Endoukanagawa-dou Co.) and analyzed by using the old type of HPLC (Hamana *et al.*, 1983). Although contamination of the “Suizenji-nori” with other bacteria and algae cannot be excluded, it appears putrescine, spermidine and homospermidine are major components of *A. sacrum*.

When thermotolerant, siderophilic *Chroogloeocystis siderophila* isolated from La Duke Hot Spring, USA, and located in the order *Chroococciopsidales* (Brown *et al.*, 2005), was cultured at 50°C, homospermidine was found as the major polyamine (Hamana *et al.*, 2008).

Haloalkaliphilic, filamentous *Arthrospira platensis* (formerly saltwater *Spirulina platensis*), belonging to

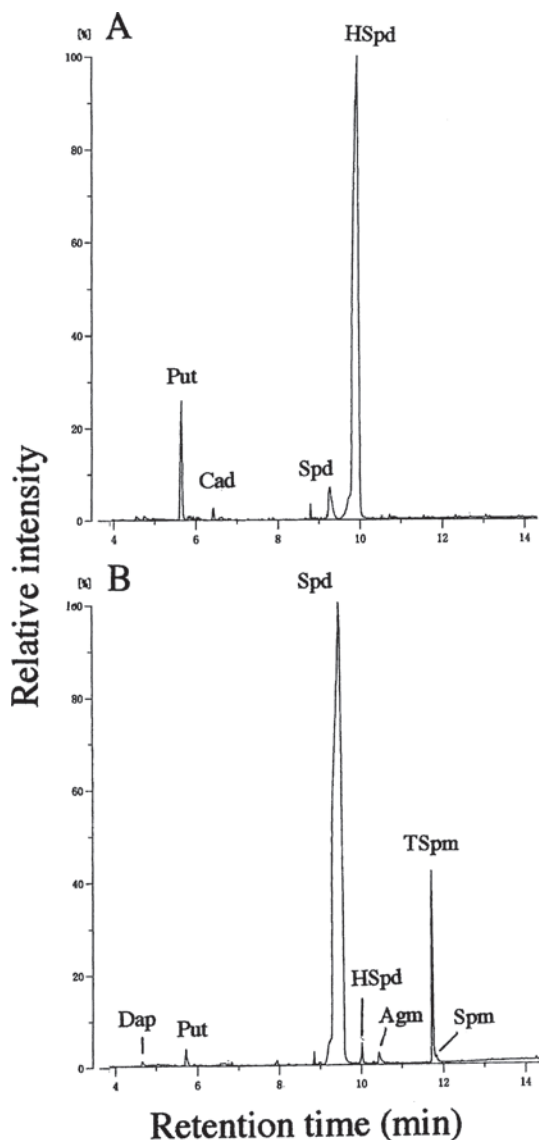


Fig. 1 Gas chromatographs from HPGC analysis of the cellular polyamines of *Aphanothece sacrum* (“Suizenji-nori”) No. 2 (A) and *Arthrospira platensis* (“Spirulina”) DIC Lifetech (B) on a JEOL JMS-700. Abbreviations for polyamines are shown in Table 1.

the order *Oscillatoriales*, was isolated from haloalkaline lakes and is phototrophically cultured as a food in outdoor artificial pools (Fujisawa *et al.*, 2010). Thermospermine, in addition to spermidine, homospermidine and agmatine, has been found by using standard GC in the axenic strain NIES-39 of *A. platensis* grown at pH 9.2 (1.7% NaHCO₃ and 0.1% NaCl) (Hamana *et al.*, 2008). Here, by HPGC and HPGC-MS, we confirmed the major occurrence of thermospermine as a unique polyamine profile in Japan

Algae’s “Spirulina” and DIC Lifetech’s “Spirulina” from *A. platensis* (Fig. 1 and Table 1). No differences in the polyamine profile was observed between an axenic-like pure culture A (grown in deep seawater) and crude culture B of Japan Algae’s “Spirulina”, indicating that contamination did not affect to the polyamine profile. We speculated that the tetra-amine, thermospermine is essential for growth of this organism at alkaline pH under a high salinity. In the order *Oscillatoriales*, three freshwater *Planktothrix* species including *Planktothrix spiroides* (formerly freshwater *Spirulina platensis*) contained spermidine as the major polyamine (Hamana *et al.*, 1983; Hosoya *et al.*, 2005).

Although most of cyanobacterial species contain Chl *a* as a major pigment, Chl *b*-containing *Prochlorococcus*, *Prochloron* and *Prochlorothrix* species (Partensky *et al.*, 1999) and Chl *d*-containing *Acaryochloris* species (Miyashita *et al.*, 2003) have been isolated and they are located in the order *Synechococcales* (Komarek *et al.*, 2014). Two strains of a tiny single-celled marine cyanobacterium, *Prochlorococcus marina* which includes divinyl derivatives of Chl *a* and Chl *b*, contained spermidine alone. In *Acaryochloris marina* containing Chl *a* and Chl *d*, we found putrescine, spermidine, and homospermidine as the major polyamines of the strain NIES-2412 isolated in Japan and analyzed by HPGC and HPGC-MS, whereas spermidine and agmatine have been detected in the strain NBRC 102967 isolated in Palau and analyzed by HPLC alone (Hamana *et al.*, 2008). To determine whether homospermidine is present as a minor polyamine, we are planning mass culture of NBRC 102967 and analysis of its polyamines by HPGC.

Moderately thermophilic *Thermosynechococcus elongatus* (belonging to the order *Synechococcales*) was isolated from a hot spring in Beppu, Japan (Nakamura *et al.*, 2002). Spermine and thermospermine have been detected by the standard GC analysis in this cyanobacterium grown at 60°C (Hamana *et al.*, 2008). When *T. elongatus* was grown at 55°C, the two tetra-amines were not detected (Hamana *et al.*, 1988). Here, we are found low levels of spermidine, homospermidine, and spermine in *Thermosynechococcus vulcanus* (formerly *Synechococcus vulcanus*) isolated from a hot spring in Wakayama, Japan (MCC-NEIS, 2016) and in *Thermosynechococcus* sp. NK55a isolated from a hot spring in Nagano, Japan (NBRC, 2016; Stolyar *et al.*,

2014). Our HPGC and HPGC-MS analyses revealed no thermospermine in these two moderate thermophiles grown at 60°C. The polyamine levels of the two species were rather lower than those of other mesophilic cyanobacteria. In other bacterial taxa, high tetra-amine levels have been observed in many moderately thermophilic species grown at 60°C (Hamana & Hosoya, 2006; Hamana *et al.*, 2014). Extremely thermophilic cyanobacterial species growing above 70°C have not been found.

It has been known that *Limnothrix redekei* and *Pseudanabaena galeata* (belonging to the order *Synechococcales*), located in an early branching clade on the phylogenetic tree of the phylum *Cyanobacteria* (Komarek *et al.*, 2014; MCC-NIES, 2016), contained homospermidine (Hamana *et al.*, 2008). *Gloeobacter* species, which lack thylakoids (Nakamura *et al.*, 2003) and are located in the most early branching cyanobacterial clade, namely the order *Gloeobacterales* (Komarek *et al.*, 2014; MCC-NIES, 2016), were not available for our analysis.

The previous results and those of our current study suggest that the environmental adaptations such as haloalkaliphily, halotolerance and thermotolerance in cyanobacteria affect to their polyamine profiles, whereas cell morphology (unicellular or filamentous), colony-forming ability, N₂-fixing ability, and chlorophyll type are not related directly to their polyamine profiles. Norspermidine (33), aminopropylcadaverine (35), norspermine (333), aminopropyl-homospermidine (344), canavalmine (434) and homospermine (444), which are found in some other bacterial taxa and algae, have not been found in cyanobacteria. For the use of polyamine profiles to chemotaxonomic information in the phylum *Cyanobacteria*, more cyanobacterial species need to be made available and their contents of minor polyamines need to be analyzed by using new HPGC.

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シアノバクテリアのポリアミンの追加分析
—シアノバクテリア門内におけるスペルミジン, ホモスペルミジン, スペルミン, サーマスペルミンの分布—

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シアノバクテリアのポリアミン構成カタログへの追加のために, 特徴的な 14 株のシアノバクテリアから酸抽出したポリアミン画分の高性能液体クロマト (HPLC) と高性能ガスクロマト (HPGC) による追加分析を行った. 群体形成 *Nostoc verrucosum* ‘アツキ’は *Nostoc commune* ‘イシクラゲ’を含む他の *Nostoc* や *Anabaena* 種 (*Nostocales* 目) と同様にホモスペルミジンを含有していた. 耐熱・耐塩性 *Spirulina subsalsa* var. *salina* (*Spirulinales* 目) は淡水産 *Spirulina subsalsa* と同じスペルミジンであった. 淡水産・群体形成 *Aphanothece sacrum* ‘スイゼンジノリ’ (*Chroococcales* 目) はプトレスシン, スペルミジンとホモスペルミジンを含有したが, 耐塩・好アルカリ性 *Aphanothece halophytica* や *Microcystis* 種はスペルミジンのみであった. プトレッシン, スペルミジン, ホモスペルミジン, アグマチンに加えてサーマスペルミンが耐塩・好アルカリ性 *Arthrospira platensis* ‘スピルリナ’ (*Oscillatoriales* 目) に検出された. *Synechococcales* 目のクロロフィル *b* 含有-海産 *Prochlorococcus marina* はスペルミジンで, クロロフィル *d* 含有-海産 *Acaryochloris marina* はスペルミジンとホモスペルミジンであった. 好熱性の *Thermosynechococcus vulcanus* と *Thermosynechococcus* sp. NK55a は *Thermosynechococcus elongatus* と同様, 低含量のホモスペルミジンの他に低含量のスペルミンを有していた. 追加分析 14 株と既分析 126 株でのポリアミン構成は系統分類の化学分類マーカーとして確立されないが, スペルミジン型, ホモスペルミジン型, スペルミジン/ホモスペルミジン混合型に区分された.