Polyamine Distribution Profiles within Cyanobacteria

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Cellular polyamines acid-extracted from 63 strains belonging to 25 genera of the phylum Cyanobacteria were analyzed by HPLC. Spermidine was the major polyamine of the order Chroococcales. Homospermidine was found as the major polyamine in the order Nostocales. Spermidine-dominant species and homospermidine-dominant species were found in the orders Pleurocapsales, Oscillatoriales and Stigonematales. Polyamine distribution profiles, found as different triamine types, can be used for the classification of cyanobacteria distributed within the five orders.

Key words: cyanobacteria, homospermidine, polyamine, spermidine

INTRODUCTION

Analyses of cellular polyamines have already provided valuable chemotaxonomic information on the classification of the domain Bacteria (Eubacteria) (3, 5). The oxygenic photosynthetic eubacteria comprise the phylum Cyanobacteria and have been separated into the five orders, Chroococcales (Subsection I), Pleurocapsales (II), Oscillatoriales (III), Nostocales (IV) and Stigonematales (V) (1, 12, 13,18). Two types of triamine profiles, spermidine-dominant type and homospermidine-dominant type, could be distinguished and appeared to be of chemotaxonomic significance within the 17 cyanobacterial strains tested in our previous reports (6, 7) and another report on some cyanobacterial polyamines (8). Polyamine catalogues of additional 29 axenic strains and 34 non-axenic strains (total of 63 strains as unicyanobacterial isolates belonging to 25 genera), distributed within the five orders, were analyzed in the present study to evaluate the usefulness of polyamine pattern as a phenotypic marker in cyanobacteria.

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MATERIALS AND METHODS

Cyanobacteria were supplied from the major culture collections of cyanobacteria in Japan, IAM (Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan) and NIES (National Institute for Environmental Studies, Tsukuba, Japan). The organisms were cultivated phototrophically in the light at 20-25℃ in the culture media designated by IAM and NIES (Table 1). After 2 weeks, stationary growing cells were harvested by centrifugation. The pellets of organisms were washed with 0.8% NaCl and then homogenized in equal volumes of 10% perchloric acid ($HClO_4$, PCA). Polyamines in the PCA extract were analyzed by high-performance liquid chromatography (HPLC) on a column of cation-exchange resin in a Hitachi L6000 high-speed liquid chromatograph, developed in our laboratory (3, 4).

RESULTS AND DISCUSSION

Concentrations of cellular polyamines estimated from the HPLC analyses are shown in Table 1. It is known that cellular polyamine levels of bacteria altered but major triamine type did not exchange under different culture conditions (3, 5). Differences of polyamine pattern between

Table 1. Polyamine contents of cyanobacteria

Cyanobacteria			Medium	Polyamine (μ mol/g wet wt. cell)					
				Put	Cad	Spd	Hspd	Spm	Agm
Chroococcales (Subsection I)									
Anacystis marina	IAM M-122 *		A-1A	0.05	–	0.70	0.41	–	–
Aphanocapsa holsatica	IAM M-258 *		A-33L	–	–	0.65	–	–	–
Aphanothece halophytica (halophile)	ATCC 43922	(b)		–	–	0.30	–	–	–
Aphanothece elabens	IAM M-257 *		A-33L	–	–	0.50	–	–	–
Aphanothece sacrum	Amagi *	(a)		0.11	–	0.23	0.45	–	–
Microcystis aeruginosa	IAM M-228		A-33L	–	–	1.15	–	–	–
Microcystis aeruginosa	IAM M-176	(a)		0.22	–	7.83	0.01	–	–
Microcystis aeruginosa	IAM M-178 *		A-44L	–	–	1.10	–	–	–
Microcystis aeruginosa	IAM M-247 *		A-44L	0.12	–	1.33	–	–	–
Synechococcus leopoliensis	IAM M-6		A-44A	0.05	–	0.50	–	–	–
			A-44L	–	–	0.57	–	–	0.05
Synechococcus sp. (thermophile)	Tokyo	(b)		0.05	–	0.02	0.06	–	–
Synechococcus sp. (*Anacystis nidulans*)	ATCC 27144	(a)		–	–	0.23	–	–	–
Synechocystis sp.	IAM M-208 *		A-44A	–	–	1.50	–	–	–
Pleurocapsales (II)									
Hydrococcus rivularis	NIES-593 *		CB	0.05	0.21	0.80	–	–	0.02
Myxosarcina burmensis	IAM M-246 *		A-1A	–	–	–	0.87	–	–
Oscillatoriales (III)									
Limnothrix redekei	NIES-847		CB	–	–	–	0.40	–	0.32
Oscillatoria laetevirens	IAM M-242 *		A-1A	–	–	1.35	0.25	–	–
Oscillatoria neglecta	IAM M-83	(a)		0.08	–	1.20	0.10	0.01	–
Oscillatoria rosea	IAM M-220		A-45L	–	–	–	–	–	–
Oscillatoria sp.	IAM M-117 *		A-1A	–	–	0.82	–	–	0.15
Oscillatoria tenuis	IAM M-243 *		A-1A	–	–	–	0.80	–	–
Phormidium ambiguum	IAM M-71		A-1A	–	–	–	0.50	–	–
			A-1L	0.01	–	–	1.65	–	0.05
Phormidium anqustissimum	IAM M-21 *		A-1A	0.04	–	0.92	0.20	–	–
Phormidium foveolarum	IAM M-59		A-1A	0.02	–	0.35	0.32	–	–
			A-1L	–	–	0.80	0.25	–	–
Phormidium henninqsii	IAM M-88 *		A-1A	–	–	–	0.78	–	0.02
Phormidium luridum	IAM M-84 *		A-1A	0.06	0.11	0.18	0.40	–	–
Phormidium molle	IAM M-77 *		A-1A	–	–	–	0.48	–	–
Phormidium mucicola	IAM M-221 *		A-47L	–	–	–	0.59	–	–
Phormidium sp.	IAM M-99 *		A-1A	–	–	1.45	–	–	0.18
Phormidium tenue	NIES-512		12CT	–	–	–	0.50	–	0.30
Phormidium tenue	IAM M-40 *		A-1A	–	–	0.08	0.40	–	0.04
Planktothrix agardhii	IAM M-244		A-48	–	–	0.62	–	–	0.20
Planktothrix rubescens	NIES-610		CB	–	–	1.32	–	–	–
Plectonema boryanum	IAM M-101	(a)		0.04	–	2.71	–	0.01	–
	IAM M-129	(a)		0.03	–	1.94	–	–	–
Plectonema calothrichoides	IAM M-120	(a)		0.01	–	1.83	–	–	–
Plectonema radiosum	NIES-515		CSi	–	–	–	0.57	–	–
Spirulina platensis	IAM M-135	(a)		–	–	0.59	–	0.05	–
Spirulina platensis	IAM M-184	(a)		–	–	0.60	–	0.02	–
Spirulina siamese	DIC	(a)		–	–	0.80	–	0.02	–
Spirulina subsalsa	IAM M-223		A-33L	–	–	0.85	–	–	–
Symploca muscorum	IAM M-133 *		A-1A	0.03	–	1.45	0.20	–	–
Tychonema bourrellyi	NIES-846		CB	–	–	0.87	–	–	0.14

Nostocales (IV)									
Anabaena circinalis	NIES-41		CB	0.20	−	−	0.57	−	−
Anabaena compacta	NIES-806		CT	−	−	−	1.50	−	−
Anabaena cylindrica	IAM M-253		A-1A	−	−	−	1.55	−	−
			A-1L	−	−	−	0.90	−	0.10
Anabaena cylindrica	IAM M-1	(a)		0.01	−	0.01	2.18	−	−
Anabaena fols-aquae	NIES-73		MA	−	−	−	0.50	−	−
Anabaena planktonica	NIES-814		CT	−	−	−	0.85	−	−
Anabaena solitaria	NIES-80		CB	0.05	−	−	0.65	−	−
Anabaena spiroides	NIES-78		CT	−	−	−	0.49	−	−
Anabaena spiroides	NIES-79		CB	0.01	−	−	0.35	−	−
Anabaena variabilis	IAM M-3		A-1A	−	−	−	0.77	−	−
			A-1L	−	−	−	0.79	−	−
Anabaena variabilis	DIC	(a)		0.02	−	0.04	3.85	0.02	−
Anabanopsis circularis	IAM M-4		A-1A	−	−	−	0.40	−	−
			A-1L	−	−	−	0.70	−	−
Aphanizomenon fos-aquae	NIES-81		CB	0.05	−	−	0.90	−	−
Aulosira laxa	NIES-50		MDM(S)	−	−	−	1.20	−	−
Aulosira laxa	IAM M-128 *		A-1A	−	−	−	0.80	−	−
Calothrix brevissima	IAM M-249		A-1A	−	−	−	0.66	−	−
			A-1L	−	−	−	1.45	−	−
Calothrix brevissima	IAM M-7	(a)		0.03	−	0.02	1.00	−	−
Calothrix elenkinii	IAM M-61 *		A-1A	−	−	−	0.90	−	−
Calothrix gracilis	IAM M-56		A-1A	−	−	0.02	1.35	−	−
			A-1L	−	−	−	0.95	−	−
Calothrix parasitica	IAM M-226		A-45L	−	−	1.01	−	−	−
Calothrix parietina	IAM M-121 *		A-1A	−	−	0.65	0.10	−	−
Calothrix sp. (*Fremyella diplosiphon*)	IAM M-100	(a)		0.10	−	0.03	0.88	−	−
Cylindrospermopsis sp.	NIES-991		CB	−	−	−	0.69	−	−
Cylindrospermum muscicola	IAM M-32 *		A-1A	−	−	−	0.75	−	−
Hapaloshiphon delicatulus	IAM M-266 *		A-1A	−	−	0.45	0.55	−	−
Nostoc carneum	IAM M-35 *		A-1A	−	−	−	1.35	−	−
Nostoc commune	IAM M-13 *		A-1A	−	−	−	0.69	−	−
Nostoc entophytum	IAM M-267 *		A-1A	−	−	0.16	0.75	−	−
Nostoc linckia	IAM M-251		A-1L	−	−	−	9.98	−	−
Nostoc linckia var. arvense	IAM M-30 *		A-1A	−	−	−	1.25	−	−
Nostoc muscorum	IAM M-131	(a)		0.10	−	0.11	4.78	0.09	−
Nostoc punctiforme	IAM M-15 *		A-1A	0.10	−	−	1.50	−	−
Tolypothrix tenuis	IAM M-29	(a)		0.02	−	0.05	0.48	−	−
Stigonematales (V)									
Fischerella major	NIES-592 *		CB	0.10	−	1.30	−	−	0.05
Fischerella muscicola	IAM M-125 *		A-14A	−	−	0.75	−	−	−
Scytonema sp.	IAM M-262 *		A-14A	−	−	1.25	0.20	−	−
Stigonema hormoides	IAM M-268 *		A-14A	−	−	0.40	0.42	−	−
Stigonema ocellatum	IAM M-252 *		A-14L	−	−	0.85	0.20	−	−

Note: Put, putrescine; Cad, cadaverine; Spd, spermidine; HSpd, homospermidine; Spm, spermine; Agm, agmatine; IAM, IAM Culture Collection, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan; DIC, Dainippon Ink and Chemicals Co. Inc., Tokyo; ATCC, American Type Culture Collection, Manassas, Virginia, USA; NIES, National Institute for Environmental Studies, Tsukuba, Japan; Tokyo, isolated by The University of Tokyo, Tokyo; Amagi, isolated in Amagi city, Fukuoka; *, non-axenic strain; −, not detected (<0.005). Former names are shown in parentheses. (a), cited from Hamana et al. (7), (b), cited from Hamana et al. (6).

axenic strain and non-axenic strain were not detected in *Microcystis aeruginosa*, *Phormidium tenue* and *Aulosira laxa*, indicating that contaminated microorganisms were not useful to determine the major triamine type of phototrophic cultures of cyanobacteria (Table 1).

All species of *Anacystis*, *Aphanocapsa*, *Aphanothece*, *Microcystis*, *Synechococcus* and *Synechocystis* of Chroococcales (I) contained spermidine as a major polyamine. The distribution of homospermidine in addition to spermidine was limited in *Anacystis marina*, *Aphanothece sacrum* and *Synechococcus* sp. Tokyo within the six genera.

Within the two species analyzed in Pleurocapsales (II), *Hydrococcus rivularis* contained spermidine as the major polyamine whereas the major polyamine of *Myxosarcina burmensis* was homospermidine.

Major triamine type was heterogeneous among five *Oscillatoria* species of Oscillatoriales (III). *O. rosea* IAM M-220 was devoid of polyamines. In eight *Phormidium* species except for *Phormidium* sp. IAM M-99, homospermidine was found ubiquitously as a major polyamine. Spermidine was the major polyamine in all two *Planktothrix* species, all three *Spirulina* species and a species of *Tychonema* that were analyzed. *Limnothrix redekei* contained homospermidine alone. *Symploca muscorum* as well as some *Oscillatoria* and *Phormidium* species contained spermidine and homospermidine. Among three *Plectonema* species, one contained homospermidine alone and the others contained spermidine alone.

In Nostocales (IV), all of the 32 strains belonging to the ten genera *Anabaena*, *Anabanopsis*, *Aphanizomenon*, *Aulosira*, *Calothrix*, *Cylindrospermopsis*, *Cylindrospermum*, *Hapaloshiphon*, *Nostoc* and *Tolypothrix*, except for a species of *Calothrix*, contained homospermidine as the major polyamine. Spermidine coexisted in *C. parietina* and *H. delicatulus* as a major polyamine.

In Stigonematales (V), spermidine was the major polyamine of the two species of *Fischerella*. *Scytonema* and *Stigonema* species contained spermidine and homospermidine as the major polyamine.

Spermidine and/or homospermidine were the major polyamine in the cyanobacteria including a thermophile, *Synecococcus* sp. Tokyo, growing at 60℃ and a halophile, *Aphanothece halophytica* ATCC 43922, growing in 10% NaCl (Table 1). Homospermidine was also distributed as the major polyamine in some α-proteobacteria, γ-proteobacteria and flavobacteria (3, 5). A triamine, norspermidine, found as the major polyamine in some γ-proteobacteria and extreme thermophiles (3, 5), has never been detected in cyanobacteria.

Recently, based on phylogenetic analyses on 16S rRNA sequences a phylogenetic tree divided into 14 clusters was proposed (2, 9-11, 14-17, 19). *Microcystis* and *Synechococcus* species of Chroococcales belong to Cluster XI and were spermidine-dominant type. *Synechococcus* strains tentatively included in Chroococcales are located in the divergent Clusers II and X, however, one contained spermidine/homospermidine and two others were spermidine-dominant type. *Myxosarcina* of Pleurocapsales located in Cluster VIII was homospermidine type. *Limnothrix* and some *Phormidium* species of Oscillatoriales included in Clusters V and VI were homospermidine type. *Oscillatoria* species are distributed within Clusters IV, V, VI, X and XII and polyamine profiles of the five species analyzed were heterogeneous. *Plectonema* species of Oscillatoriales are found in Cluster IV and contained homospermidine. *Spirulina* species of Oscillatoriales belonging to Cluster IX had a spermidine-dominant profile. *Fischerella* species of Stigonematales are located in Cluster I (*Nostoc/Calothrix/Phormidium/Anabaena/Cylindrospermum/Cylindrospermopsis/Tolypothrix/Aphanizomenon/Anabanopsis*). *Fischerella* species contained spermidine and others belonging to Nostocales ubiquitously contained homospermidine, suggesting that it is a *Fischerella*-specific polyamine profile.

Three different synthetic pathways of the triamines from putrescine have been found in eubacteria including cyanobacteria (3, 5). Aminopropyltransfer mediated by aminopropyltransferases produce spermidine. Aminopropyltransfer mediated by Schiff-base complex formation with L-aspartic β-semialdehyde and aminobutyltransfer mediated by Schiff-base complex formation with γ-aminobutylaldehyde produce spermidine and homospermidine, respectively.

Since species names and phylogenetic analyses of some strains in the total 81 cyanobacteria determined in our studies are conflicting, the correspondence of their polyamine type to their phylogenetic positions within 14 clusters is not yet clear. However, cellular triamine type can be used for the future classification of cyanobacterial taxa as a chemotaxonomic marker.

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シアノバクテリアにおけるポリアミン構成

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真正細菌のシアノバクテリア門の25属に属する63株よりポリアミンを酸抽出し，HPLCにて分析した．Chroococcales目ではスペルミジンが主ポリアミンとして存在した．ホモスペルミジンはNostocales目で主ポリアミンであった．Pleurocapsales目，Oscillatoriales目およびStigonematales目ではスペルミジン優先種とホモスペルミジン優先種が認められた．異なるトリアミンを含有するポリアミン構成は，5目に分布しているシアノバクテリアの属および種レベルでの分類マーカーとして有益である．