

Additional cellular polyamine data in the twenty bacterial phyla,
Acidobacteria, *Armatimonadetes*, *Atribacterota*, *Balneolaeota*,
Chlorobi, *Chloroflexi*, *Chrysiogenetes*, *Deferribacteres*,
Elusimicrobia, *Fusobacteria*, *Gemmatimonadetes*, *Ignavibacteriae*,
Kiritimatiellaeota, *Lentisphaerae*, *Nitrospirae*, *Planctomycetes*,
Rhodothermaeota, *Spirochaetes*, *Synergistetes*, and *Verrucomicrobia*

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To evaluate cellular polyamine distribution profiles as a chemotaxonomic marker to phylogenetic classification in bacteria, acid-extracted cellular polyamines from 134 strains (species) located in the 20 phyla, *Armatimonadetes*, *Atribacterota*, *Balneolaeota*, *Elusimicrobia*, *Ignavibacteriae*, *Kiritimatiellaeota*, *Lentisphaerae*, *Rhodothermaeota*, and *Synergistetes*, furthermore *Acidobacteria*, *Chlorobi*, *Chloroflexi*, *Chrysiogenetes*, *Deferribacteres*, *Fusobacteria*, *Gemmatimonadetes*, *Nitrospirae*, *Planctomycetes*, *Spirochaetes*, and *Verrucomicrobia* were additionally analyzed by HPLC and HPGC. In 29 strains (species) of the 9 phyla first analyzed, *Armatimonas* and *Fimbriimonas* were absent (undetectable) in polyamines and *Capsulimonas* contained homospermidine in the phylum *Armatimonadetes*. *Atribacter* of the *Atribacterota* contained putrescine, spermidine and spermine. *Alifodinibius* lacked polyamines and *Gracilimonas* contained homospermidine in the phylum *Balneolaeota*. *Elusimicrobium* of the phylum *Elusimicrobia* contained homospermidine. Homospermidine was found in *Igmavibacterium*, and putrescine, spermidine, homospermidine and spermine were found in *Melioribacter* in the phylum *Ignavibacteriae*. *Kiritimatiella* of the phylum *Kiritimatiellaeota* contained spermidine and spermine. In the phylum *Lentisphaerae*, *Lentisphaera* contained spermidine and homospermidine, and *Victivallis* and *Oligosphaera* contained spermidine alone. In the phylum *Rhodothermaeota*, *Rubricoccus* was absent in polyamines, *Rubrivirga* lacked polyamines or contained homospermidine, and *Longimonas* contained spermidine. Spermidine and spermine were major polyamines in *Acetomicrobium*, *Aminiphilus*, *Aminovibrio*, *Aminobacterium*, *Aminomonas*, *Cloacibacillus*, *Dethiosulfovibrio*, *Fretibacterium*, *Lactivibrio*, *Pyramidobacter*, and *Thermoanaerovibrio* of the phylum *Synergistetes*. Although the numbers of analyzed species was not enough to the evaluation of phylogenetic significance in the 9 phyla. In the 105 additionally analyzed strains (species) belonging to 11 other phyla, correlation of their phylogenetic locations and spermidine, homospermidine, and spermine distributions were discussed.

Key words: *Armatimonadetes*, *Atribacterota*, *Balneolaeota*, chemotaxonomy, *Elusimicrobia*, *Ignavibacteriae*, *Kiritimatiellaeota*, *Lentisphaerae*, *Rhodothermaeota*, polyamine, *Synergistetes*

INTRODUCTION

Subkingdom, infrakingdom, and superphylum are not validly introduced in the domain (kingdom) Bacteria, therefore, phylum is the first taxonomic rank in bacterial classification in the present report. At the present time, 36-42 phylum-level lineages have been proposed by the description of pure cul-

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tures as the total phylogenetic taxa of Bacteria (Castelle & Banfield, 2018; Parks *et al.*, 2018; Waite *et al.*, 2020), however, identification of newly isolated bacteria and reclassification of previously validated bacteria are continuing. We referred to the taxonomy database of NCBI (National Center for Biotechnology Information, US National Library of Medicine, USA) (NCBI, 2021).

To characterize cellular polyamine components of bacteria, various linear and branched polyamine components such as aliphatic diamines, triamines, tetra-amines, penta-amines, and hexa-amines are acid-extracted from cultured bacterial cells and are able to be fully analyze by the liquid chromatography and gas chromatography developed in our laboratory. Therefore, endogenous cellular polyamine distribution profiles within bacteria have already provided valuable chemotaxonomic information (Hamana, 2002; Hamana & Hosoya, 2006; Hamana & Matsuzaki, 1992; Michael, 2018; Tindall *et al.*, 2010). We have reported polyamine analyses in the class, order, family, and species levels (ranks) of the six major phyla *Actinobacteria* (Hosoya *et al.*, 2006), *Bacteroidetes* (Hamana & Nakagawa, 2001a, 2001b; Hamana *et al.*, 1995, 2008d), *Cyanobacteria* (Hamana *et al.*, 2008a, 2016b; Hosoya *et al.*, 2005), *Deinococcus-Thermus* (Hamana *et al.*, 2009, 2016a), *Firmicutes* (Hamana, 1999b; Hamana & Niitsu, 1999; Hamana *et al.*, 2012, 2014; Hosoya *et al.*, 2006), and *Proteobacteria* (Hamana 2002; Hamana & Matsuzaki, 1992, 1993; Hamana & Takeuchi, 1998; Hamana *et al.*, 2006).

In the present study, to propose polyamine distributions of so far not investigated bacterial taxa of different lineages and to describe an additional chemotaxonomic evaluation of polyamine profiles in bacteria, we newly analyzed cellular polyamines in the 9 phyla, *Armatimonadetes*, *Atribacterota*, *Balneolaeota*, *Elusimicrobia*, *Ignavibacteriae*, *Kiritimatiellaeota*, *Lentisphaerae*, *Rhodothermaeota*, and *Synergistetes*. Furthermore, we tried additional polyamine analyses in the 11 phyla *Acidobacteria*, *Chlorobi*, *Chloroflexi*, *Chrysiogenetes*, *Deferribacteres*, *Fusobacteria*, *Gemmatimonadetes*, *Nitrospirae*, *Planctomycetes*, and *Verrucomicrobia* listed in Hosoya *et al.* (2006), and *Spirochaetes* listed in Hamana *et al.* (2009), and show alphabetically in Table 1. In this study, we added the polyamine data by analyzing the strains available from domestic culture collections JCM and NBRC.

Since ionic interaction of high basic polyamines to nucleic acids DNA and RNA and other acidic components in bacterial cells affect growth environments, cellular levels of some polyamines in bacteria are realized to depend roughly on growth temperature, pH, and salt concentration, in addition to their phylogenetically classified locations (Hamana, 2002; Hamana & Hosoya, 2006; Hamana & Matsuzaki, 1992). Psychrophiles (grown at 10–20°C), slight thermophiles (grown at 45–50°C), moderate thermophiles (grown at 55–65°C), extreme thermophiles (grown at 70–80°C), acidophiles (grown at pH 4.0–5.0), alkaliphiles (grown at pH 9.0–10.0), and moderate halophiles (grown in the presence of 3–10% NaCl) were included to survey the effect of their growth conditions to their polyamine profiles.

MATERIALS AND METHODS

Bacterial strains were provided by JCM (Japan Collection of Microorganisms, RIKEN BioResource Research Center, Tsukuba, Ibaraki, Japan. <https://jcm.brc.riken.jp/ja/>) and NBRC (Biological Resource Center, National Institute of Technology and Evaluation, Kisarazu, Chiba, Japan. <https://www.nite.go.jp/nbrc/>) and cultivated in the liquid media (100–1000 mL) or on the agar media (2–5 petri-dish plates) as designated by the culture collections for optimum growth under aerobic or anaerobic conditions. The organisms in the stationary phase were harvested. When bacterial growth was poor in the first culture, re-cultures under same conditions or different culture temperature, medium pH, or in different culture media, were performed (Culture No. 1–3 in Table 1). Although it is natural that cellular polyamine concentrations vary under different culture conditions, distribution profiles of major cellular polyamine components in optimum growth cells were determined and described in our previous and present studies. Since incorporation of the common polyamines, putrescine, spermidine and spermine from media components such as yeast extract and peptone, used for the most cultures in the present study, are not excluded, minor their cellular levels are possible by their uptake.

The pellets of organisms were homogenized in equal volumes of 1 M (10%) perchloric acid (PCA) (HClO₄). The 0.5 M (5%) PCA extract was subjected to a Dowex 50W column to concentrate polyamines. The concentrated polyamine fractions were analyzed by high-performance liquid chromatography

Table 1 Cellular concentrations of polyamines in twenty bacterial phyla

Taxon	Bacteria (Property)	Strain	References	Culture		Polyamines (μmol/g wet wt. cell)																						
				No.	°C	pH	4	5	33	34	44	333	303	343	334	304	344	444	3333	3343	3334	30304	33333	33343	33433	Agm		
Phylum Acidobacteria																												
Class Acidobacteria																												
Order Acidobacteriales																												
<i>Acidiphila rosea</i>	(acidophile)	NBRC 107607 [†]		25	4.5	-	-	-	-	1.35	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acidicapsa aciditoli</i>	(acidophile)	NBRC 111227 [†]		25	5.0	-	-	-	-	0.50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acidicapsa aliphensis</i>	(acidophile)	NBRC 113252 [†]		28	4.5	-	-	-	-	0.90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Edaphobacter bradus</i>	(acidophile)	NBRC 113250 [†]		28	4.5	-	-	-	-	0.95	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Edaphobacter flagellatus</i>	(acidophile)	NBRC 113251 [†]		28	4.5	0.05	-	-	-	0.99	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Terriglobus agnaticus</i>		JCM 17517 [†]		30	7.2	0.08	-	-	-	0.66	0.05	-	-	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Terriglobus tenax</i>		NBRC 109677 [†]		30	7.2	-	-	-	-	0.16	0.80	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Terracidiphilus gabretensis</i>		NBRC 111238 [†]		25	5.5	-	-	-	-	0.03	0.88	-	-	0.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Granulicella cerasi</i>		NBRC 107139 [†]		30	7.0	-	-	-	-	0.80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Class Holophagae																												
Order Acanthopleuribacteriales																												
<i>Acanthopleuribacter pedis</i>		NBRC 101209 [†]		25	7.0	-	-	-	0.30	-	-	-	0.82	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.10
Order Thermotomaculales																												
<i>Thermotomaculum hydrothermale</i>	(thermophile)	JCM 17643 [†]		55	6.6	0.40	0.02	-	0.50	-	-	-	0.63	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Class Thermoanaerobactulia																												
Order Thermoanaerobactulales																												
<i>Thermoanaerobactulum agnaticum</i>	(thermophile)	JCM 18256 [†]		60	7.0	0.02	-	-	-	0.99	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phylum Armatimonadetes																												
Class Armatimonadiales																												
Order Armatimonadales																												
<i>Armatimonas rosea</i>		NBRC 105658 [†]		1	35	7.2	-	0.01	-	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				2	30	7.2	-	0.05	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Order Fimbrimonadales																												
<i>Fimbrimonas ginsengisoli</i>		JCM 17079 [†]		30	7.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Order Capsulimonadales																												
<i>Capsulimonas corticalis</i>		NBRC 113044 [†]		1	30	6.0	-	-	-	0.64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				2	30	6.0	0.05	-	-	0.50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phylum Atribacterota																												
Class Atribacteria																												
Order Atribacterales																												
<i>Atribacter laminatus</i>	(thermophile)	NBRC 112890 [†]		45	7.3	0.20	-	-	0.65	-	-	-	0.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phylum Balneolota																												
Order Balneolales																												
<i>Gracilimonas rosea</i>		JCM 18898 [†]		35	7.0	-	-	-	-	0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.02
<i>Alifodtibiobius salicampi</i>		NBRC 112531 [†]		1	37	7.2	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				2	37	7.2	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phylum Chlorobi																												
Class Chlorobia																												
Order Chlorobiales																												
<i>Prosthecochloris indica</i>		JCM 13299 [†]		30	6.8	-	-	-	0.35	0.67	-	-	0.38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.12
<i>Chlorohomolulotum</i>		NBRC 105664 [†]		25	8.0	0.05	-	-	-	0.50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phylum Chloroflexi																												
Class Anaerolineae																												
Order Anaerolineales																												
<i>Aggregatilinea lenta</i>		JCM 32065 [†]		30	7.5	0.10	-	-	0.25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Anaerolinea thermophila</i>	(thermophile)	NBRC 100420 [†]		55	7.0	-	0.02	-	0.48	-	0.05	0.02	1.16	0.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Flexilinea flocculi</i>		JCM 30897 [†]		37	7.0	-	-	-	0.66	-	0.01	-	1.18	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Flexilinea tardivalis</i>		JCM 12579 [†]		37	6.5	0.03	-	-	0.20	-	-	-	0.50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Levilinea saccharolytica</i>		JCM 12578 [†]		37	6.5	0.15	-	-	0.55	-	-	-	0.14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pelolinea submarina</i>		JCM 17238 [†]		1	30	7.0	0.05	-	0.37	-	-	-	0.24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				2	30	7.0	0.03	-	0.30	-	-	-	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Thermanaerobrix daxensis</i>	(thermophile)	JCM 16980 [†]		60	7.0	0.01	-	-	0.15	-	-	-	0.59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Class Ardentocantia																												
Order Ardentocantiales																												
<i>Ardentocantia maritima</i>	(thermophile)	JCM 17282 [†]		65	7.2	-	-	-	0.53	-	-	-	0.62	0.02	-	-	-	-	-	-	-	-	-	-	-	0.60	-	-
Class Caldilineae																												
Order Caldilineales																												
<i>Caldilinea tarbellica</i>	(thermophile)	JCM 16120 [†]		60	7.0	-	-	-	0.91	0.22	-	-	0.60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Caldilinea aerophila</i>	(thermophile)	JCM 11387 [†]	Hosoya et al., 2006	55	7.2	-	0.01	-	0.05	1.25	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		NBRC 104270 [†]		1	45	7.2	-	-	0.06	1.05	-	-	0.38	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				2	60	7.2	0.25	-	0.33	1.12	-	-	0.45	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Class Chloroflexia																												
Order Chloroflexales																												
Family Chloroflexaceae																												
<i>Chloroflexus islandicus</i>	(thermophile)	JCM 30533 [†]		55	7.5	0.06	-	-	0.02	1.50	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Family Roseiflexaceae																												
<i>Kouletothrix aurantiaca</i>		JCM 19913 [†]		1	25	7.6	0.04	-	-	0.20	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				2	25	7.6	-	-	-	0.40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Order Kallotenuales																												
Family Kallotenuaceae																												
<i>Kallotenua papyrolyticum</i>	(thermophile)	JCM 19132 [†]		1	55	7.5	-	-	-	0.60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				2	55	7.5	-	-	-	0.45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Family Herpetosiphonaceae																												
<i>Herpetosiphon giganteus</i>		NBRC 112828 [†]		30	7.0	-	-	-	0.05	0.50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	
<i>Herpetosiphon ginsensis</i>		NBRC 112829 [†]		30	7.0	-	-	-	0.05	0.72	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	
<i>Herpetosiphon sp.</i>		JCM 19918		1	25	7.0	-	-	0.15	0.80	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
				2	25	7.0	-	-	0.04	0.97	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Class Ktedonobacteria																												
Order Ktedonobacteriales																												
Family Dictyobacteraceae																												
<i>Dictyobacter kobayashii</i>		NBRC 113153 [†]		30	7.2	0.03	-	-	0.75	0.50	-	-	0.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Dictyobacter alpinus</i>		NBRC 113154 [†]		30	7.2	0.10	-	-	0.80	0.34	-	-	0.11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tenguoibacter tsumagoiensis</i>		NBRC 113152 [†]		30	7.2	0.03	-	-	0.15	0.52	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Phylum Chrysiogenetes																												
Order Chrysiogenales																												
<i>Desulfurispirillum albaliphilum</i>	(alkaliphile)	JCM 15827 [†]		30	9.0	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phylum Deferribacteres																												
Order Deferribacteriales																												
<i>Deferribacter sp.</i>	(thermophile)	NBRC 102158		60	6.5	-	0.02	-	0.45	-	-	-	0.30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Dentritrobia aciphilus</i>		NBRC 114555 [†]		35	7.0	-	-	-	1.18																			

(HPLC) on a column of cation-exchange resin in a Hitachi L6000 high-speed liquid chromatograph (Hamana *et al.*, 2016a, b). High performance gas chromatography (HPGC) in a SHIMADZU GC-17A or HPGC-GC-mass spectrometry (HPGC-MS) in a JEOL JMS-700 was performed for some polyamine samples (Hamana *et al.*, 2016a, b). Using authentic polyamine standards, identification and quantification of polyamine peaks were completed, and molar concentrations of per gram of wet cell pellet ($\mu\text{mol/g}$ wet wt. cell) estimated from the HPLC and HPGC analyses, are shown in Table 1.

Numeric codes of polyamines abbreviated as number of methylene (CH_2) groups between amino (NH_2) or imino (NH) groups: 4, putrescine; 5, cadaverine; 33, norspermidine; 34, spermidine; 44, homospermidine; 333, norspermine; 343, spermine; 334, thermospermine; 3(3)4, N^{r} -aminopropylspermidine; 344, aminopropylhomospermine; 444, homospermine; 3333, caldopentamine; 3334, homocaldopentamine; 3343, thermopentamine; 33333, caldohexamine, are used in Table 1. Agmatine, a guanidinoamine, was listed as a member of polyamines and abbreviated as Agm.

RESULTS AND DISCUSSION

Phylum *Acidobacteria*

This phylum is one of the most abundantly distributed bacterial groups in the environments and 26 subdivisions (corresponding to classes or orders) currently existed in this phylum; however, the classification of acidobacteria is conflicting (Dedysh & Yilmaz, 2018; NCBI, 2021). Only four strains have been analyzed in our previous studies (Hamana 1999a, Hamana *et al.*, 2009; Hosoya *et al.*, 2006). Eleven acidobacteria belonging to five different orders were newly available to the present polyamine analyses. Heterogeneous polyamine profiles were found in the acidobacteria, in which spermidine (34) or homospermidine (44) was the major polyamine, and the presence or absence of spermine (343) and agmatine (Agm) were varied (Table 1). Although without statistical analysis, dominance of spermidine and homospermidine as a major triamine appeared in order levels within this phylum.

Spermine, a tetra-amine, was found as the major polyamine in methophilic *Acanthopleuribacter pedis* and thermophilic *Thermotomaculum hydrothermale* containing spermidine as the major triamine (Table 1). Spermine was not found in the thermophilic *Thermoanaerobaculum aquaticum* grown at 60°C,

because the acidobacterium contained homospermidine and is absent in spermidine as the precursor for spermine. Other tetra-amines aminopropylhomospermidine (344) and homospermine (444) were not detected in the thermophile.

Phylum *Armatimonadetes*

Armatimonas rosea of the phylum formally called the candidate phylum OP10 was isolated in Japan and was located in the order *Armatimonadales* (Tamaki *et al.*, 2011). Three orders *Capsulimonadales*, *Chthonomonadales*, and *Fimbriimonadales* were added into the phylum (Im *et al.*, 2012; Lee *et al.*, 2011; Li *et al.*, 2019; NCBI, 2021). No significant amount of polyamine component was detected in *A. rosea* or *Fimbriimonas ginsengisoli*, as shown in Table 1. *Capsulimonas corticalis* contained homospermidine (44) as the major polyamine. Although *Chthonomonas calidirosea* belonging to the order *Chthonomonadales* have never been available to our polyamine analysis, the absence of major polyamines or the occurrence of homospermidine (44) alone is judged as a unique polyamine profile in the phylum.

Phylum *Atribacterota*

A new species, *Atribacter laminatus* that has an intracytoplasmic membrane apparently surrounding the nucleoid, was isolated and a new phylum, *Atribacterota*, was proposed (Katayama *et al.*, 2020). The slightly thermophilic *Atribacter* species contained putrescine, spermidine and spermine. It is suggested that the occurrence of spermine relate to its thermophily growing at 45°C.

Phylum *Balneolaeota*

In the genome-based taxonomic classification of the phylum *Bacteroidetes*, separation of the family *Balneolaceae* as a new phylum *Balneolaeota* from the phylum *Rhodothermaeota* was proposed (Hahnke *et al.*, 2016). *Gracilimonas rosea* contained homospermidine (44) as the major polyamine. PCA extract (No. 1 sample in Table 1) and HCl hydrolyzate (No. 2 sample in Table 1) of *Alifodinibius salicampi* contained a trace amount (0.01–0.02 $\mu\text{mol/g}$ wet weight of cells) of spermidine (34), indicating the absence of a significant amount of cellular polyamines in the bacteria. If spermidine is covalently binding in the cell and not extracted into the PCA extract, spermidine is found in the HCl hydrolyzate.

Phylum *Chlorobi*

Four species belonging to the genera *Chlorobium*, *Chlorobaculum* (Hamana *et al.*, 1997; Hosoya *et al.*, 2006), and *Prosthecochloris* ubiquitously contained homospermidine (44), as shown in Table 1. *Prosthecochloris indica* (Anil Kumar *et al.*, 2009) contained spermidine (34) and spermine (343) in addition to homospermidine. Although other species of the three genera and *Pelodictyon*, *Chloroherpeton* and *Clathrochloris* species (NCBI, 2021) have never been analyzed, the occurrence of homospermidine within the class *Chlorobia* is suggested.

Phylum *Chloroflexi*

The phylum is consisted with the eight classes *Anaerolineae*, *Ardenticatenia*, *Caldilineae*, *Chloroflexia*, *Ktedonobacteria*, *Dehalococcoidetes*, *Thermoflexus*, and *Thermomicrobia* (NCBI, 2021). Members of the class *Anaerolineae* are cosmopolitan bacteria found in various ecosystems, 10 *Anaerolineae* species have been isolated, and their genome sequences have been available (Matsuura *et al.*, 2015). Cellular polyamines of some species belonging to this phylum have been reported (Hamana *et al.*, 1997, 2008b, 2009; Hosoya *et al.*, 2006). Polyamine profiles of newly analyzed strains (species) belonging to the former seven classes are shown in Table 1.

Anaelolinea thermophila contained spermidine (34) and spermine (343) as the major polyamines and norspermine (333) and thermospermine (334) as the minor polyamines, and resemble to *Flexilinea* species of the class *Anaerolineae*. Though norspermidine, norspermine, caldopentamine (3333), and caldohexamin (33333) are produced from diaminopropane (3), analyses of these polyamines under different growth phases and culture conditions may be need in the latter three classes. Mesophilic *Aggregatilinea lenta* belonging to the class *Anaerolineae* (Nakahara *et al.*, 2019) contained putrescine and spermidine alone.

Homospermidine was spread in the classes *Caldilineae* and *Chloroflexia*, as shown in Table 1. In the class *Ktedonobacteria*, a new species of *Dictyobacter* and *Tengunoibacter* (order *Ktedonobacteriales*) (Wang *et al.*, 2019) contained homospermidine in addition to putrescine and spermidine (and spermine). *Thermosporothrix* (order *Ktedonobacteriales*) (Yabe *et al.*, 2010, 2016) and *Thermogemmatispora* (order *Thermogemmatisporales*) (Zheng *et al.*, 2019) con-

tained spermidine and spermine.

A penta-amine, thermopentamine (3343), was found in *Ardenticatena maritime* grown at 65°C (Class *Ardenticatenia*) but not detected in *Thermoflexus hugenholtzii* grown at 75°C (Class *Thermoflexia*) (Table 1). The occurrence of aminopropylhomospermidine (344) and homospermine (444) in *Chloroflexus* had been reported (Hamana *et al.*, 1997). In the thermophiles grown at 50–65°C, a tetra-amine, spermine, was ubiquitously found within the phylum. However, the occurrence of the tetra- and penta-amines might be related to the thermophily of the thermophiles.

In the seventh class, *Thermomicrobia*, moderately thermophilic *Sphaerobacter thermophilus* belonging to the order *Sphaerobacterales* were absent in the cellular polyamines, whereas extremely thermophilic *Thermomicrobium roseum* contained three penta-amines, caldopentamine, thermopentamine (3343), and homocaldopentamine (3334), and a hexa-amine, caldohexamine, in addition to spermidine, norspermine, and spermine (Hosoya *et al.*, 2006). A variety of polyamine profiles found within the phylum raise further polyamine analysis of new members of this phylum.

Phylum *Chrysiogenetes*

It has been shown that *Chrysiogenes arsenatis* contained spermidine (34) alone (Hosoya *et al.*, 2006). Significance amount of polyamines was not detected in alkaliphilic *Desulfurispirillum alkaliphilum* newly validated (NCBI, 2021) (Table 1).

Phylum *Deferribacteres*

It has been known that two moderately thermophilic *Deferribacter* species grown at 60–62°C, contained cadaverine (5), spermidine (34), and spermine (343) (Hosoya *et al.*, 2006) and *Deferribacter* sp. NBRC 102158 contained same polyamines in the present study. *Petrothermobacter organivorans* (grown at 55°C) analyzed here as well as *Calditerrivibrio nitroreducens* (grown at 60°C) contained spermidine, spermine and agmatine (Agm) (Hamana *et al.*, 2009) (Table 1). *Denitrovibrio acetiphilus* contained spermidine as the major polyamine (Table 1).

Phylum *Elusimicrobia*

Elusimicrobium minutum is the first cultivated representative of the phylum (Geissinger *et al.*, 2009;

Herlemann *et al.*, 2009). The ultramicrobacterium analyzed here contained homospermidine (44) as the major polyamine (Table 1).

Phylum *Fusobacteria*

Spermidine (34) and spermine (343) have been found in the seven species of the genus *Fusobacterium* (Hosoya *et al.*, 2006). An additional five *Fusobacterium* species ubiquitously contained spermidine (34) and spermine (343) (Table 1). In the family *Fusobacteriaceae*, psychrophilic *Psychrilyobacter atlanticus* (grown at 10°C) and moderately thermophilic *Hypocyclicus thermotrophus* (grown at 50°C) had the same polyamine component, as shown in the present study (Table 1). Occurrence of spermine (343) as a major polyamine in the mesophilic fusobacteria grown at 37°C is unique within the domain Bacteria; however, their spermine levels were not stable.

Newly analyzed *Leptotrichia* and *Streptobacillus* species belonging to the family *Leptotrichiaceae* contained spermidine (34) as a major polyamine and spermine (343) as a minor polyamine (Table 1). Their cellular levels of the diamines, putrescine (4), cadaverine (5), and agmatine (Agm) varied in the phylum.

Phylum *Gemmatimonadetes*

Polyamines of *Gemmatimonas aurantiaca* isolated as the first bacterium of the phylum (order) (Zhang *et al.*, 2003) and its major polyamine was homospermidine (44) as shown previously (Hosoya *et al.*, 2006) (Table 1). When same strain was re-cultured in a different medium in the present study, significant amount of spermidine in addition to the lower level of homospermidine. Further polyamine analysis should be need.

Phylum *Ignavibacteriae*

Slightly thermophilic *Ignavibacterium album* was isolated as the first bacterium of this phylum (Iino *et al.*, 2010; Liu *et al.*, 2012) and its major polyamine was homospermidine (44) by HPLC and HPGC-MS analyses, as shown in Table 1. The trace amounts of spermine (343) may be incorporated from the culture medium containing spermine, because putrescine (4) and spermidine (34) as the precursor for spermine were non-detectable. Moderately thermophilic *Melioribacter roseus* was isolated as the second bacterium in the phylum (Podosokorskaya *et al.*,

2013; Kadnikov *et al.*, 2013) contained putrescine, spermidine, homospermidine and spermine. Spermine, a tetra-amine have been detected in many thermophilic bacteria containing spermidine. Significant amount of spermine in *M. roseus* seems to be correlated to its moderate thermophily. Heterogeneous polyamine distributions are suggested within the phylum.

Phylum *Kiritimatiellaeota*

Kiritimatiella glycovorans was validated as the first cultured representative of the subdivision 5 of the previous phylum *Verrucomicrobia* and located in this new phylum (Spring *et al.*, 2016; van Vliet *et al.*, 2019; NCBI, 2021). *K. glycovorans* contained spermidine and spermine. Although this species is not thermophile, the occurrence of spermine, a tetra-amine, is interested. Spermidine (and spermine)-containing type and homospermidine-containing type have been found in the related phylum *Verrucomicrobia*, as shown later in this report.

Phylum *Lentisphaerae*

Lentisphaera marina and *Lentisphaera profundus* located in the order *Lentisphaerales* of the class *Lentisphaeria* (Choi *et al.*, 2013, 2015) contained spermidine (34) and homospermidine (44), whereas *Victivallis lenta* located in the order *Victivallales* of the class *Lentisphaeria* and *Oligosphaera ethanolica* located in the order *Oligosphaerales* of another class *Oligosphaeria* (Qiu *et al.*, 2013; NCBI, 2021) contained spermidine as the major triamine (Table 1). It is suggested that heterogeneous distribution of spermidine and homospermidine within this phylum.

Phylum *Nitrospirae*

It has been reported that thermophilic *Thermodesulfobivrio* species, grown at 60–65°C, contained unusual norspermidine (33), norspermine (333), and homocaldopentamine (3334) in addition to spermidine (34) and spermine (343) (Hamana *et al.*, 1998, 2009). *Nitrospira marina* and *Leptospirillum ferrooxidans* previously analyzed contained spermidine (34) alone (Hosoya *et al.*, 2006) (Table 1). Newly available *Nitrospira inopinata* and *Nitrospira japonica* contained spermidine alone. The occurrence of tetra-amines and penta-amines seems to be limited in the thermophiles within this phylum. Homospermidine (44) has never been found in this phylum.

Phylum *Planctomycetes*

Two classes have been proposed in this phylum (Table 1). In the class *Planctomycetia*, the major polyamine of *Phycisphaera mikurensis* belonging to the order *Phycisphaerales* was spermidine (34) alone (Table 1). In the genus *Planctomyces* of the order *Planctomycetales*, homospermidine (44) was major in the two species, whereas putrescine (4) and spermidine were major in another species. *Blastopirellula*, *Pirellula*, *Isosphaera*, *Rhodopirellula*, *Thermopirellula* contained spermidine and homoapermidine as shown in the previous (Hamana 1999a; Hosoya et al., 2006) and present studies. *Thermogutta* and *Thermostilla* belonging to the order *Planctomycetales* and *Tepidisphaera* belonging to the order *Tepidisphaerales* of the class *Phycisphaerae* contained homospermidine (44) alone (Table 1). Distribution of spermidine and homospermidine were heterogeneous within the phylum. The culture strains of *Anaerohalosphaera lusitana* JCM 31926 and *Limihaloglobus sulfuriphilus* JCM 31927 were not available in the present study.

Phylum *Rhodothermaeota*

Revised phylogeny of the phylum *Bacteroidetes* showed one new phylum, *Rhodothermaeota* (Munoz et al., 2016). Culture and analysis of the two *Rubrivirga* species (Park et al., 2013; Song et al., 2016) were repeated in the different samples No. 1 and No. 2 (Table 1). In the family *Rubricoccaceae*, *Rubricoccus marinus* and *Rubrivirga profunda* were absent in cellular polyamines, while *Rubrivirga marina* contained homospermidine (44) and agmatine (Agm) or putrescine (4) as a precursor for homospermidine synthesis (Table 1). Trace amounts (0.01–0.02 $\mu\text{mol/g}$ wet weight of cells) of spermidine (34) and spermine (343) are possible by a contamination or incorporation from the medium containing polyamines. Haloalkaliphilic *Longimonas haloalkaliphila* contained a low level of spermidine and spermine while halophilic *Salinibacter ruber* contained a low level of spermidine and homospermidine. Polyamine distribution profiles were varied within the mesophilic species of this phylum. We escape from the analysis of mesophilic *Natronotalea proteinilytica* JCM 31342 under the Plant Protection Law of Japan. Polyamine profiles in moderately/extremely thermophilic *Rhodothermus* species containing tetra-amines, penta-amines and hexa-amines were discussed in a previous report (Hamana et al., 2014).

Phylum *Spirochaetes*

It has been shown that thermophilic *Exilispira thermophile*, grown at 60°C, contained spermidine (34) and spermine (343) as the major polyamines (Hamana et al., 2009). Its spermine level was higher than its spermidine level. On the other hand, significant amounts of polyamines were not detected in haloalkaliphilic *Spirochaeta dissipartiopha* or halophilic *Sediminispirochaeta smaragdinae*. *Salinispira pacifica* contained spermidine alone. *Treponema socraskii* contained spermidine and spermine. *Pleomorphochaeta multiformis* and *Treponema stenostreptum* contained putrescine (4) and spermidine. *Alkalispirochaeta* species contained spermidine alone. *Rectinema cohabitans* contained a high level of putrescine and spermidine. *Leptosira* species belonging to another order *Leptosirales* contained spermidine alone. Homospermidine (44) was not found in the ten species in the two orders of the phylum. The levels of putrescine, spermidine (produced from putrescine), and spermine (produced from spermidine) in the halophiles, alkaliphiles, haloalkaliphiles, thermophiles, and psychrophiles, indicate a variety of the levels of three by optimum growth conditions.

Phylum *Synergistetes*

The phylum comprise the genera *Acetomicrobium*, *Aminiphilus*, *Aminivibrio*, *Aminobacterium*, *Aminomonas*, *Cloacibacillus*, *Dethiosulfovibrio*, *Fretibacterium*, *Lactivibrio*, *Jonquetella*, *Pyramidobacter*, *Synergistes*, *Thermanaerovibrio*, and *Thermovirga* at the present time (Jumas-Bilak et al., 2009; NCBI, 2021). Polyamines of the four species *Acetomicrobium flavidum*, *Aminobacterium mobile*, *Aminomonas paucivorans*, and *Thermanaerovibrio acidaminovorans* (formerly belonging to the phylum *Firmicutes*) located in this new phylum have been analyzed previously (Hamana, 1999b; Hosoya et al., 2004). Thirteen species were newly analyzed in the present study. Although differences were found in their putrescine (4) and spermine (343) levels, spermidine (34) was detected ubiquitously as the major triamine in the 16 species (Table 1). Putrescine level was predominant at a low growth temperature and spermine level increased at a high growth temperature. The occurrence of thermopentamine (3343) has been found in moderate thermophile, *Acetomicrobium flavidum* grown at 60°C (Hamana, 1999b). A tertiary triamine, N^{tr} -aminopropylspermidine (3(3)4) was

found in moderately thermophilic *Acetomicrobium mobile* (formerly *Anaerobaculum mobile*) (Hania *et al.*, 2016) grown at 55°C in the present study.

Phylum *Verrucomicrobia*

The phylum *Verrucomicrobia* comprises the four orders *Opitutales*, *Puniceicoccales*, *Verrucomicrobiales*, and *Spartobacteriales* (NCBI, 2021). *Opitutus terrae*, *Ereboglobus luteus*, and *Nibricoccus aquaticus* belonging to the order *Opitutales* contained homospermidine (44) as the major polyamine, as shown in the present study (Table 1). *Nibricoccus aquaticus* contained spermidine (34) and spermine (343) as a minor polyamine. Spermidine was the major polyamine in the genera located in the orders *Puniceicoccales* and *Verrucomicrobiales* as shown previously (Hamana 1999a; Hosoya *et al.*, 2006) and additionally in Table 1. In the order *Spartobacteriales*, *Sporohalobacter salines* contained spermidine as the major polyamine, whereas *Terrimicrobium sacchariphilum* contained spermidine and homospermidine. Distribution of spermidine and homospermidine seems to be significant for polyamine distribution within this phylum. Putrescine (4), cadaverine (5), and agmatine (Agm) were sporadically distributed among different species and strains; therefore, their distribution was not connected to phylogenetically relation.

CONCLUSION AND FUTURE

Unusual polyamines, such as 1,3-diaminopropane (3), aminopropylcadaverine (35), aminopentylhomospermidine (335), *N,N*-bis(3-aminopropyl)cadaverine (353), and canavalmine (434), found sporadically in other bacterial pure cultures, were not detected in the newly and previously analyzed bacterial members of the present 20 phyla. Acetyl-, hydroxyl- and methyl-derivatives of polyamines were also not found in the bacterial species of the 20 phyla.

Lesser extent polyamine concentration was observed in alkaliphilic, halophilic and haloalkaliphilic bacteria analyzed in the present study. Hyperthermophiles (grown at 85–100°C) and extreme halophiles (grown in the presence of 20–25% NaCl) have never been isolated in the 20 phyla objected in the present study. Although it is difficult to prove perfect absence of cellular polyamines, nearly polyamine-absent species were found in the closely related two phyla *Balnealaeota* and *Rhodothermaceota*

and another phylum *Armatimonadetes*. Few polyamine-absent (polyamine-non-detected) species have been found sporadically in the phyla *Actinobacteria* and *Bacteroidetes* (Hamana, 2002), in addition to *Sphaerobacter* species of the phylum *Chloroflexi* (Table 1); however, significance for chemotaxonomy is not clear.

Few species were available in the 9 phyla *Armatimonadetes*, *Atribacterota*, *Balnealaeota*, *Elusimicrobia*, *Ignavibacteriae*, *Kiritimatiellaeota*, *Lentisphaerae*, *Rhodothermaeota*, and *Synergistetes* first analyzed in the present study; thus, their polyamine profiles have not been fully characterized. In the course of this study, it has been clear that the phyla *Acidobacteria*, *Chloroflexi*, *Planctomycetes*, *Spirochaetes* and *Verrucomicrobia* are one of the major taxonomic groups in the 36–42 phylum-level lineages of bacteria, and polyamine distribution profile is useful as a chemotaxonomic marker within these phyla.

Polyamine profiles in the thermophilic bacterial phyla *Thermotogae*, *Aquificae*, *Caldiserica*, *Dictyoglomi* and *Coprothermobacterota* (proposed by Pavan *et al.*, 2018) have been shown elsewhere (Hamana *et al.*, 2008c, 2009, 2014). Supplemented polyamine analyses for the five thermophilic phyla and the phyla *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Deinococcus-Thermus*, *Fibrobacteres*, *Firmicutes*, and the emended phylum *Proteobacteria*, including three new phyla *Desulfobacterota*, *Myxococcota* and *Bdellovibrionota* reclassified from the class *Deltaproteobacteria* of the former phylum *Proteobacteria* (Waite *et al.*, 2020), and a phylum, *Epsilonbacteraeota* by the reclassification of the class *Epsilonproteobacteria* and the order *Desulfurellales* (*Deltaproteobacteria*) (Waite *et al.*, 2017), are operating in our laboratory. Polyamine analyses of the five remaining phyla, *Calditrichaeota* (containing *Caldithrix* and *Calorithrix*), *Chlamydiae*, *Melainabacteria* (formerly a class of the phylum *Cyanobacteria*), candidatus *Omniitrophica*, and *Tenericutes* are planning. We have analyzed cellular polyamines in another prokaryotic domain Archaea (Hamana *et al.*, 2007, 2008b, 2009, 2013, 2018) and can compare polyamine distribution profiles between Archaea and Bacteria.

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細菌 20 門 *Acidobacteria*, *Armatimonadetes*, *Atribacterota*, *Balneolaeota*, *Chlorobi*, *Chloroflexi*, *Chrysiogenetes*, *Deferribacteres*, *Elusimicrobia*, *Fusobacteria*, *Gemmatimonadetes*, *Ignavibacteriae*, *Kiritimatiellaeota*, *Lentisphaerae*, *Nitrospirae*, *Planctomycetes*, *Rhodothermaeota*, *Spirochaetes*, *Synergistetes* および *Verrucomicrobia* における細胞内ポリアミンの追加データ

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細菌の細胞内ポリアミン構成分布を化学分類指標の一つとして細菌の系統分類に対応させるため、未分析であった 9 門 *Armatimonadetes*, *Atribacterota*, *Balneolaeota*, *Elusimicrobia*, *Ignavibacteriae*, *Kiritimatiellaeota*, *Lentisphaerae*, *Rhodothermaeota*, *Synergistetes* に属する 29 株 (種), および既分析の他 11 門 *Acidobacteria*, *Chlorobi*, *Chloroflexi*, *Chrysiogenetes*, *Deferribacteres*, *Fusobacteria*, *Gemmatimonadetes*, *Nitrospirae*, *Planctomycetes*, *Spirochaetes*, *Verrucomicrobia* に属する新規追加の 105 株 (種), の菌体から酸抽出したポリアミン画分を HPLC と HPGC で分析した。分析株のポリアミン含量 ($\mu\text{mol/g}$ wet weight of cells) を表として記載した。新規分析 9 門では, *Armatimonadetes* 門では *Armatimonas* と *Fimbriimonas* がポリアミンを欠き, *Capsulimonas* はホモスペルミジンを含む。 *Atribacterota* 門の *Atribacter* はプトレシン, スペルミジンとスペルミン。 *Balneolaeota* 門では, *Alifodinibius* はポリアミンを欠き, *Gracilimonas* はホモスペルミジン。 *Elusimicrobia* 門の *Elusimicrobium* はホモスペルミジン。 *Ignavibacteriae* 門の *Ignavibacterium* はホモスペルミジンのみ, *Melioribacter* はプトレシン, スペルミジン, ホモスペルミジンとスペルミン。 *Kiritimatiellaeota* 門の *Kiritimatiella* はスペルミジンとスペルミン。 *Lentisphaerae* 門の *Lentisphaera* はスペルミジンとホモスペルミジン, *Victivallis* と *Oligosphaera* はスペルミジンのみ。 *Rhodothermaeota* 門の *Rubricoccus* はポリアミンを欠き, *Rubrivirga* はポリアミンを欠くかホモスペルミジンで, *Longimonas* はスペルミジン。 *Synergistetes* 門の 11 属 *Acetomicrobium*, *Aminiphilus*, *Aminovibrio*, *Aminobacterium*, *Aminomonas*, *Cloacibacillus*, *Dethiosulfovibrio*, *Fretibacterium*, *Lactivibrio*, *Pyramidobacter*, *Thermoanaerovibrio* すべてでスペルミジンとスペルミンを含む。この 9 門での分析株数は, 化学分類指標として評価するには不十分ではあった。他の 11 門においては, スペルミジン, ホモスペルミジン, スペルミン, の分布と系統分類との相関性を追加検討した。