

Polyamine analysis within the eubacterial thirteen phyla *Acidobacteria*, *Actinobacteria*, *Chlorobi*, *Chloroflexi*, *Chrysiogenetes*, *Deferribacteres*, *Fibrobacteres*, *Firmicutes*, *Fusobacteria*, *Gemmatimonadetes*, *Nitrospirae*, *Planctomycetes*, and *Verrucomicrobia*

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Cellular polyamines of the recently described 51 eubacteria distributed within 13 phyla were analyzed by HPLC, to demonstrate polyamine profiles in numerous eubacterial taxa of different lines of descent. Homospermidine or norspermidine was found within the phyla *Acidobacteria*, *Chloroflexi* and *Chlorobi*. In the phylum *Nitrospirae*, mesophilic *Nitrospira* and *Leptospirillum* contained spermidine alone, whereas thermophilic *Thermodesulfovibrio* contained norspermine, spermine and homocaldopentamine. The phylum *Chrysiogenetes* contained spermidine alone, and the phylum *Deferribacteres* cadaverine, spermidine and spermine, and the phylum *Fibrobacteres* spermidine. Spermidine and spermine were distributed within the phylum *Fusobacteria*. The phylum *Gemmatimonadetes* contained homospermidine alone. Within the phylum *Planctomycetes*, homospermidine was the major polyamine. The phylum *Verrucomicrobia* contained spermidine alone. In the phylum *Firmicutes*, the mesophiles of the order *Clostridiales* contained low levels of cadaverine, spermidine, spermine and agmatine and thermophilic *Heliobacterium* was rich in spermine. The order *Halanaerobiales* contained spermidine and spermine. In the orders *Bacillales* and *Lactobacillales*, many mesophiles contained spermidine and agmatine and some lacked polyamines. In the phylum *Actinobacteria*, the orders *Rubrobacterales*, *Coriobacteriales* and *Sphaerobacterales* contained no cellular polyamines whereas thermophilic *Thermoleophilum* contained branched pentaamines. The eubacteria tested can be characterized by major triamine profiles (spermidine, norspermidine and/or homospermidine), roughly corresponding to their phylogenetic positions at class or order level. Tetra-amines and penta-amines appeared in thermophiles. Psychrophily, halophily, acidophily and alkaliphily found in some eubacteria within the 13 phyla seem to be not related to their polyamine profiles.

Key words: polyamine, *Acidobacteria*, *Actinobacteria*, *Chlorobi*, *Chloroflexi*, *Chrysiogenetes*, *Deferribacteres*, *Fibrobacteres*, *Firmicutes*, *Fusobacteria*, *Gemmatimonadetes*, *Nitrospirae*, *Planctomycetes*, *Verrucomicrobia*

INTRODUCTION

Studies on cellular polyamine distribution profiles have already provided valuable chemotaxonomic information on the classification of the domain *Bacteria* (*Eubacteria*) (Hamana, 2002a; Hamana & Matsuzaki, 1992). Polyamine analysis has been established in the systematics of the phyla *Proteobacteria* (Hamana *et al.*, 2000a, 2000b, 2001, 2003a, 2003b, 2004; Hamana & Takeuchi, 1998) and *Bacteroidetes* (Hamana & Matsuzaki, 1990; Hamana & Nakagawa, 2001a, 2001b; Hosoya & Hamana,

2004), and eubacterial Gram-positives (Hamana, 1994, 1999b, 1999c; Hamana & Satake, 1995; Hamana *et al.*, 2002) and thermophiles (Hamana *et al.*, 1996, 1997, 1998; Hosoya *et al.*, 2004). The significance of this approach for the recently validated eight phyla *Acidobacteria*, *Chloroflexi*, *Nitrospirae*, *Chlorobi*, *Chrysiogenetes*, *Deferribacteres*, *Fusobacteria* and *Gemmatimonadetes*, phylogenetically deeply branching in *Eubacteria* (Boone & Castenholz, 2001), was investigated in the present study. Polyamines of some novel species belonging to the two large Gram-positive groups, the two phyla *Firmicutes* and *Actinobacteria* (Boone & Castenholz, 2001), have not been clarified. Polyamine data in the related minor three phyla *Fibrobacteres*, *Planctomycetes*

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and *Verrucomicrobia*, tentatively published in a local report (Hamana, 1999a), were cited in the present study. Polyamine distributions within 51 newly described (mostly validated) strains of these taxa (total 13 phyla) were analyzed to fill up polyamine distribution catalogues in numerous so far not investigated eubacterial taxa of different lines of descent. Polyamine profiles in thermophiles, psychrophiles, halophiles, acidophiles and alkaliphiles found within the 13 phyla were also analyzed.

MATERIALS AND METHODS

Fifty-one newly available strains were aerobically or anaerobically grown at the optimum growth temperature in the medium No. designed by the culture collections (ATCC Bacteria Collection 2005, IAM Catalogue of Strains 2004, JCM Catalogue of Strains 2005, and NCIMB Catalogue of Strains 2002), and the media NB, Nutrient Broth (Nissui); NB-U, Nutrient Broth plus 2% urea; MB, Marine Broth 2216 (DIFCO); TSB, Trypticase Soy Broth (BBL); GAM, GAM Broth (Nissui); BA, Blood Agar Medium containing heart extract and peptone (Eiken Chemical); 199, polyamine-free synthetic 199 medium, ordinarily used for tissue culture (Nissui); YTA, Yeast extract/Tryptone/Sodium acetate and PYE, Peptone/Yeast extract. Exponentially growing cells or living cells in the early stationary growth phase (200–1000 mg wet weight) were harvested by centrifugation from 200–1000 ml liquid cultures or 2–4 agar plates. The pellets of organisms were homogenized in 0.5 M perchloric acid (PCA, HClO₄). Polyamines were extracted into 0.5 M PCA and analyzed by high-performance liquid chromatography (HPLC) on a L6000 High-Speed Liquid Chromatograph (Hitachi) (Hamana, 2002a, 2002b; Hamana *et al.*, 1998).

When no major polyamine (detected at the level of 2.00–0.10 $\mu\text{mol/g}$ wet weight cells) was found and polyamines were nondetectable (<0.005 $\mu\text{mol/g}$ wet weight cells) in the PCA extracts, cell residues after the PCA extraction were hydrolyzed with 6 M HCl at 110°C, for 20 h. By HPLC of the hydrolyzates to confirm complete polyamine extraction into 0.5M PCA, the two nondetectable polyamine data could be expressed as that the organism (grown under the used condition) lacks cellular polyamines. It is well known that the composition of medium, culture temperature and growth stage can influence cellular polyamine content, but cannot change the major polyamine profile. Therefore, the present investiga-

tion focused on identifying major cellular polyamine components.

RESULTS AND DISCUSSION

Acidobacteria

A novel Fe (III)-reducing bacterium, *Geothrix fermentans* (Coates *et al.*, 1999), newly analyzed in the present study, contained homospermidine as the major polyamine (Table 1). This organism as well as *Acidobacterium* and *Holophaga* species belongs to the phylum *Acidobacteria* (Coates *et al.*, 1999). Two acidophilic *Acidobacterium capsulatum* strains also contained homospermidine alone as reported previously (Hamana, 1999a), suggesting a wide distribution of this triamine within this phylum.

Chloroflexi

Two *Chloroflexus* species and two *Herpetosiphon* species belonging to the class *Chloroflexi* contained homospermidine as the major polyamine, as previously reported (Hamana *et al.*, 1997). In this study, three newly reported species were subjected to polyamine analysis: one was a slightly thermophilic photosynthetic bacterium, *Roseiflexus castenholzii*, growing at 50°C, belonging to the class *Chloroflexi* (Hanada *et al.*, 2002) and the other two were filamentous slight-thermophiles growing at 55°C, *Anaerolinea thermophila* and *Caldilinea aerophila* belonging to the class *Anaerolineae* (Sekiguchi *et al.*, 2003). The major polyamine of *R. castenholzii* was homospermidine, suggesting the ubiquitous occurrence of homospermidine within the class *Chloroflexi* (Table 1). Although tetra-amines were detected as minor polyamines in the thermophilic *Chloroflexus* species, tetra-amines were not found in this slight-thermophile growing at 55°C.

In the class *Anaerolineae*, the major polyamine of *A. thermophila* was norspermidine, however, its profile was similar to that of an extreme thermophile, *Thermomicrobium roseum* containing norspermine, caldopentamine and caldohexamine of the class *Thermomicrobia* (Table 1). On the other hand, *C. aerophila* as well as five slight-thermophiles of the class *Chloroflexi* contained homospermidine. In *A. thermophila* and *C. aerophila*, the major polyamine type did not change in the cultures harvested at different growth stages (Table 1).

Slightly thermophilic *Sphaerobacter thermophilus* growing at 55°C (Demharter *et al.*, 1989; Hugenholtz & Stackebrandt, 2004) as a single species of the

order *Sphaerobacterales* of the class *Thermomicrobia* lacked polyamines when the organism was grown in both organic and synthetic media (Hamana, 1999c). The one species, *Thermomicrobium roseum* belonging to the order *Thermomicrobiales* of the class *Thermomicrobia*, was validated in this phylum (Yakimov *et al.*, 2003). This extreme thermophile, growing at 70°C, contained three penta-amines and a hexa-amine synthesized from spermine, thermospermine and/or norspermine (Hamana *et al.*, 1998). Homospermidine and branched polyamine were not detected.

Two different polyamine patterns observed in the phylum *Chloroflexi* suggest heterogeneous polyamine distribution profiles within this phylum.

Nitrospirae

The phylum *Nitrospirae* phylogenetically comprises *Nitrospira*, *Leptospirillum*, *Magnetobacterium* and *Thermodesulfovibrio* species (Boone & Castenholz, 2001; Garrity & Holt, 2001b; Hippe, 2000). We reported that the major polyamine of *Leptospirillum ferrooxidans* was spermidine and extremely (moderately) thermophilic *Thermodesulfovibrio yellowstonii*, growing at 60°C, contained norspermidine, norspermine, spermine and homocaldopentamine in addition to spermidine (Hamana *et al.*, 1997, 1998). *Nitrospira marina*, newly analyzed in the present study, contained spermidine alone (Table 1). The two mesophiles, acidophilic *L. ferrooxidans* and neutrophilic *N. marina*, have same polyamine profiles.

Chlorobi

The major polyamine of two photosynthetic *Chlorobium* species, slightly thermophilic *C. tepidum* (growing at 45°C) and mesophilic *C. linicola* belonging to the phylum *Chlorobi*, was homospermidine (Hamana *et al.*, 1997). Two strains of *C. linicola*, available in the present study, contained also homospermidine as the major polyamine (Table 2). Since other novel genera belonging to this phylum were not available, the chemotaxonomic significance of the occurrence of homospermidine is unclear.

Chrysiogenetes

The phylum *Chrysiogenetes* is currently represented by a single species, which was reportedly distinct from members of other phyla (Boone & Castenholz, 2001; Garrity & Holt, 2001a). *Chrysiogenes arsenatis* is mesophilic, exhibiting anaerobic respiration

in which arsenate serves as the electron acceptor (Macy *et al.*, 1996). This organism contained spermidine alone as the major polyamine, as shown in the present study (Table 2).

Deferribacteres

Chemoorganotrophic heterotrophs, *Deferribacter*, *Flexistipes*, *Geovibrio* and *Denitrovibrio* species in this phylum, respire anaerobically with terminal electron acceptors including sulfur, nitrate, Fe(III) and arsenate (Boone & Castenholz, 2001; Garrity & Holt, 2001c; Miroshnichenko *et al.*, 2003; Takai *et al.*, 2003). The two moderately thermophilic *Deferribacter* species, *D. desulfuricans* and *D. abyssi*, growing at 60–62°C, available in the present study, contained cadaverine, spermidine and spermine (Table 2).

Fibrobacteres

Fibrobacter species only comprise the phylum *Fibrobacteres* (Boone & Castenholz, 2001). It has been reported previously that three *Fibrobacter* species contained spermidine as the major polyamine (Hamana, 1999a). These results were shown again in the present study that compared polyamine patterns among some related phyla (Table 2).

Fusobacteria

The genera *Fusobacterium*, *Ilybacter*, *Leptotrichia*, *Propionigenium*, *Sebaldella* and *Streptobacillus* species comprise the phylum *Fusobacteria* (Boone & Castenholz, 2001). In the present study, polyamines of seven *Fusobacterium* species available, including newly analyzed *F. equinum* (Dorsch *et al.*, 2001), were described. Spermidine and spermine were detected in all the mesophilic species (Table 2). Putrescine and/or agmatine were found in three species, however, the significance of the diamine and guanidinoamine distribution is unclear.

Gemmatimonadetes

Gemmatimonas aurantiaca was isolated as a representative of the new eubacterial phylum, *Gemmatimonadetes* (Zhang *et al.*, 2003). This organism contained homospermidine alone (Table 2). The occurrence homospermidine instead of spermidine in this deeply branching phylum as well as the other early branching different lines of descent, *Acidobacteria*, *Chloroflexi* and *Chlorobi*, is phylogenetically interesting.

Table 2. Cellular concentration of polyamines in the phyla *Chlorobi*, *Chrysiogenetes*, *Deferribacteres*, *Fibrobacteres*, *Fusobacteria*, *Gemmatimonadetes*, *Planctomycetes* and *Verrucomicrobia*

Organism	Strain	Culture condition			Polyamines ^a ($\mu\text{mol/g}$ wet cells)					
		Medium	pH	T _m ($^{\circ}\text{C}$)	4	5	34	44	343	A _{gm}
Phylum <i>Chlorobi</i>										
<i>Chlorobium tepidum</i>	ATCC 49652 ^{T b}	ATCC 37	6.7	45	0.01	-	-	2.35	0.04	-
<i>Chlorobium limicola</i>	ATCC 17092 ^b	ATCC 37	6.7	30	0.01	-	-	2.26	-	-
<i>Chlorobium limicola</i>	NCIMB 8325	NCIMB 10	6.0	30	-	-	-	0.60	-	-
<i>Chlorobium limicola</i>	NCIMB 8346	NCIMB 10	7.0	30	-	-	-	0.65	-	-
Phylum <i>Chrysiogenetes</i>										
<i>Chrysiogenes arsenatis</i>	ATCC 700172 ^T	ATCC 2139	7.0	28	-	-	0.55	-	-	-
Phylum <i>Deferribacteres</i>										
<i>Deferribacter desulfuricans</i>	JCM 11476 ^T	JCM 324	6.5	62	-	0.65	0.30	-	0.15	-
<i>Deferribacter abyssi</i>	JCM 11955 ^T	JCM 365	6.5	60	-	0.65	0.55	-	1.10	-
Phylum <i>Fibrobacteres</i>										
<i>Fibrobacter intestinalis</i>	ATCC 43854 ^{T c}	ATCC 1943	6.6	37	-	-	1.50	-	-	-
<i>Fibrobacter succinogenes</i> subsp. <i>elongata</i>	ATCC 43856 ^{T c}	ATCC 1943	6.6	37	0.17	-	1.75	-	-	1.05
<i>Fibrobacter succinogenes</i> subsp. <i>succinogenes</i>	ATCC 19169 ^{T c}	ATCC 1943	6.6	37	0.19	-	2.10	-	-	1.13
Phylum <i>Fusobacteria</i>										
<i>Fusobacterium equinum</i>	JCM 11174 ^T	GAM	7.3	37	0.20	-	0.60	-	1.30	-
<i>Fusobacterium necrophorum</i> subsp. <i>funduliforme</i>	JCM 3724 ^T	GAM	7.3	37	-	-	0.10	-	0.66	-
<i>Fusobacterium necrophorum</i> subsp. <i>necrophorum</i>	JCM 3718 ^T	GAM	7.3	37	-	-	0.48	-	0.45	-
<i>Fusobacterium nucleatum</i> subsp. <i>nucleatum</i>	JCM 8532 ^T	GAM	7.3	37	-	-	0.04	-	1.20	-
<i>Fusobacterium nucleatum</i> subsp. <i>vincentii</i>	JCM 11023 ^T	GAM	7.3	37	0.30	0.05	0.20	-	0.04	-
<i>Fusobacterium pseudonecrophorum</i>	JCM 3721 ^T	GAM	7.3	37	1.51	-	0.15	-	0.25	0.25
<i>Fusobacterium varium</i>	JCM 3722 ^T	GAM	7.3	37	1.75	-	0.04	-	0.22	0.05
Phylum <i>Gemmatimonadetes</i>										
<i>Gemmatimonas aurantiaca</i>	JCM 11422 ^T	JCM 211	7.0	30	-	-	-	0.97	-	-
Phylum <i>Planctomycetes</i>										
<i>Planctomyces brasiliensis</i>	NCIMB 13185 ^{T c}	ATCC 1732	7.0	30	-	-	0.22	0.74	-	-
<i>Planctomyces limnophilus</i>	ATCC 43296 ^{T c}	MB	7.6	30	2.80	-	0.90	-	-	-
<i>Planctomyces maris</i>	NCIMB 2232 ^{T c}	ATCC 1521	7.2	30	-	-	0.06	0.72	-	-
<i>Blastopirellula marina</i>	ATCC 49069 ^{T c}	ATCC 1657	7.5	30	0.01	-	0.15	1.50	-	-
<i>Pirellula staleyi</i>	ATCC 27377 ^{T c}	ATCC 36	7.0	30	-	-	0.30	1.62	-	-
<i>Isosphaera pallida</i>	ATCC 43644 ^{T c}	ATCC 1962	7.6	45	0.02	-	0.02	1.48	-	-
<i>Rhodopirellula baltica</i>	NCIMB 13988 ^T	NCIMB 1540	7.0	28	-	-	0.05	0.67	-	-
Phylum <i>Verrucomicrobia</i>										
<i>Prostheco bacter dejongeii</i>	NCIMB 12777 ^{T c}	ATCC 462	7.5	30	-	-	1.30	-	-	-
<i>Prostheco bacter fusiformis</i>	NCIMB 12778 ^{T c}	ATCC 462	7.5	30	-	-	2.10	-	-	-
<i>Prostheco bacter vanneervinii</i>	ATCC 700199 ^{T c}	ATCC 462	7.5	30	-	-	2.30	-	-	-
<i>Verrucomicrobium spinosum</i>	ATCC 43997 ^{T c}	ATCC 1661	7.5	30	0.01	-	2.25	-	-	-

^a See Table 1. ^{b, c} data from Hamana *et al.*, 1997, Hamana, 1999a.

Planctomycetes

The phylum *Planctomycetes* includes the genera *Planctomyces*, *Gemmata*, *Isosphaera*, *Pirellula*, *Blastopirellula* and *Rhodopirellula* (Boone & Castenholz, 2001; Schlesner *et al.*, 2004; Ward *et al.*, 2000). Homospermidine was found in *Rhodopirellula*

species, analyzed here, in addition to the previously analyzed four genera, as shown in Table 2. Spermine was not detected in *Isosphaera pallida* growing at 45 $^{\circ}\text{C}$ as well as other mesophilic species. Among them, *Planctomyces limnophilus* contained spermidine instead of homospermidine. The previous results were cited from our report (Hamana,

1999a), however, similar polyamine data were reported simultaneously elsewhere (Gripenburd *et al.*, 1999).

Verrucomicrobia

The phylum *Verrucomicrobia* includes the genera *Prostheco bacter* and *Verrucomicrobium* (Boone & Castenholz, 2001; Ward *et al.*, 2000) and their four species contained spermidine alone, as previously reported (Hamana, 1999a) (Table 2).

Firmicutes

Polyamine analyses of two novel anoxygenic phototrophs and two novel halophilic anaerobes located in the large phylum *Firmicutes* were added in the present study. The family *Heliobacteriaceae* located in the order *Clostridiales* of this phylum comprising the genera *Heliobacterium*, *Heliobacillus*, *Heliophilum* and *Heliorestis*, is a group of anoxygenic phototrophs (Boone & Castenholz, 2001; Madigan, 2001). Three heliobacteria analyzed previously contained low levels of cadaverine, spermidine, spermine and agmatine (Hamana, 1999c). Uptake of these polyamines from the media was not excluded. In the present study, slightly thermophilic *Heliobacterium modesticaldum* and mesophilic *Heliophilum fasciatum* were analyzed. Polyamine profiles were identical within the five heliobacteria, however, slightly thermophilic *H. modesticaldum*, growing at 50°C, was rich in a tetra-amine, spermine (Table 3). In the family *Clostridiaceae* of this order, thermophiles often show a tendency for tetra- and penta- amines instead of di- and tri- amines (Hamana, 1999c; Hamana *et al.*, 1996; Hosoya *et al.*, 2004).

Recently, two newly reported halophilic anaerobes *Halanaerobium fermentans* and *Selenihalanaerobacter shriftii* belonging to the order *Halanaerobiales* of this phylum were published (Blum *et al.*, 2001; Boone & Castenholz, 2001; Kobayashi *et al.*, 2000; Oren, 2000). The two species growing at 10% concentration of NaCl as well as eight other halophilic anaerobes (Hamana, 1999c), ubiquitously contained spermidine and spermine (Table 3).

In the previous study on aerobic Gram-positive cocci located in the order *Bacillales* of this phylum, the presence or absence of spermidine was conflicting (Hamana, 1994). Polyamines of new members belonging to two families of this order (Boone & Castenholz, 2001) were analyzed. All species of the genera *Planococcus* (Nakagawa *et al.*, 1996; Yoon

et al., 2003), *Sporosarcina* (except for *S. pasteurii*) (Yoon *et al.*, 2001b), *Planomicrobium* (Dai *et al.*, 2005; Yoon *et al.*, 2001a), *Filibacter* (Clausen *et al.*, 1985) and *Kurthia* (Boone & Castenholz, 2001) of the family *Planococcaceae* contained spermidine as the major polyamine (Table 3). *Caryophanon* species of the family *Caryophanaceae* (Farrow *et al.*, 1994) contained spermidine (Table 3). *Alkalibacterium*, *Carnobacterium*, *Desemzia* and *Marinilactibacillus* species are members of the family *Carnobacteriaceae* of the order *Lactobacillales* (Ishikawa *et al.*, 2003; Ntougias & Russell, 2001; Toffin *et al.*, 2005; Yumoto *et al.*, 2004). Two *Carnobacterium* species and two *Marinilactibacillus* species contained spermidine as the major polyamine. Polyamines were not detected in a *Desemzia* species and two *Alkalibacterium* species grown in organic media used for their good growth. Further polyamine analysis of these taxa is needed to evaluate chemotaxonomic usefulness.

Actinobacteria

We reported the absence of appreciable amounts of polyamines in slightly thermophilic *Rubrobacter radiotolerans* growing at 60°C (Hamana, 1994). In the present study, significant amounts of polyamines were not detected in the moderately thermophilic *Rubrobacter xylanophilus* growing at 60°C (Ferreira *et al.*, 1999) and mesophilic *Conexibacter woesei* (Monciardini *et al.*, 2003) of the order *Rubrobacterales* (the subclass *Rubrobacteridae*) (Boone & Castenholz, 2001) (Table 4). On the other hand, extremely thermophilic *Thermoleophilum* species, growing at 70°C, phylogenetically located in the order, contained norspermidine, spermidine and quaternary branched penta- amines (Hamana *et al.*, 1998).

Atopobium, *Collinsella*, *Coriobacterium*, *Eggerthella*, *Olsenella* and *Slackia* comprise the order *Coriobacteriales* (the subclass *Coriobacteridae*) of the class *Actinobacteria* (Boone & Castenholz, 2001; Dewhirst *et al.*, 2001; Kageyama *et al.*, 1999; Wade *et al.*, 1999). Three *Collinsella* species, *Coriobacterium glomerans*, *Slackia exigua*, *Eggerthella lenta* and *Olsenella uli* analyzed in the present study, as well as *Atopobium rimae* previously reported (Hamana & Satake, 1995), lacked appreciable amounts of cellular polyamines (Table 4).

Slightly (moderately)-thermophilic (growing at 60°C), acidophilic *Acidothermus cellulolyticus*, grown in both organic and synthetic media, belonging to the order *Actinomycetales* (Boone & Castenholz,

Table 3. Cellular concentration of polyamines in the phylum *Firmicutes*

Organism	Strain	Culture condition			Polyamines ^a ($\mu\text{mol/g}$ wet cells)						
		Medium	pH	Tm(°C)	4	5	34	44	343	Agm	Pea
Phylum <i>Firmicutes</i>											
Order <i>Clostridiales</i>											
Family <i>Heliobacteriaceae</i>											
<i>Heliobacterium chlorum</i>	ATCC 35205 ^{T b}	ATCC 112	7.0	27	-	0.03	0.04	-	0.07	0.05	-
<i>Heliobacterium gestii</i>	ATCC 43375 ^{T b}	ATCC 1552	7.1	37	-	0.03	0.15	-	0.04	0.20	-
<i>Heliobacterium modesticaldum</i>	ATCC 51547 ^T	PYE	6.9	50	-	-	0.07	-	0.30	0.02	-
<i>Heliobacillus mobilis</i>	ATCC 43427 ^{T b}	ATCC 1552	7.1	37	-	0.09	0.06	-	0.08	0.06	-
<i>Heliophilum fasciatum</i>	ATCC 51790 ^T	PYE	6.9	37	-	-	0.04	-	0.07	0.02	-
Order <i>Halanaerobiales</i>											
Family <i>Halanaerobiaceae</i>											
<i>Halanaerobium acetoethylicum</i>	ATCC 43120 ^{T b}	ATCC 1476	7.3	30	0.01	-	0.08	-	0.16	-	-
<i>Halanaerobium praevalens</i>	ATCC 33744 ^{T b}	ATCC 1275	7.0	30	0.01	-	0.07	-	0.02	-	-
<i>Halanaerobium salsulgo</i>	ATCC 51327 ^{T b}	ATCC 1879	6.5	30	0.03	-	0.50	-	0.30	-	-
<i>Halanaerobium fermentans</i>	JCM 10494 ^T	JCM 260	7.6	35	-	-	0.10	-	0.02	-	-
Family <i>Halobacteroidaceae</i>											
<i>Halobacteroides halobius</i>	ATCC 35273 ^{T b}	ATCC 1342	6.8	30	0.17	-	0.24	-	0.06	-	-
<i>Halanaerobacter lacunaris</i>	ATCC 49944 ^{T b}	ATCC 1824	7.0	30	0.01	-	0.04	-	0.14	-	-
<i>Acetohalobium arabaticum</i>	ATCC 49924 ^{T b}	ATCC 1871	7.8	30	0.02	-	0.10	-	0.42	-	-
<i>Orenia marismortui</i>	ATCC 35420 ^{T b}	ATCC 1342	6.8	30	0.02	-	0.36	-	0.07	-	-
<i>Sporohalobacter lortetii</i>	ATCC 35059 ^{T b}	ATCC 1302	7.0	30	0.01	-	0.12	-	0.26	-	-
<i>Selenihalanaerobacter shriftii</i>	ATCC BAA-73 ^T	ATCC 2264	7.0	30	-	-	0.10	-	0.02	-	-
Order <i>Bacillales</i>											
Family <i>Planococcaceae</i>											
<i>Planococcus maritimus</i>	JCM 11543 ^T	MB	7.6	28	-	0.08	1.45	-	0.08	-	-
<i>Planococcus citrens</i>	JCM 2532 ^{T c}	199	7.2	30	0.01	-	2.10	-	-	0.28	-
<i>Planococcus kocurii</i>	JCM 2569 ^{T c}	199	7.2	30	0.02	-	1.50	-	-	0.20	-
<i>Sporosarcina aquimarina</i>	JCM 10887 ^T	TSB	7.3	30	-	-	1.20	-	-	-	-
<i>Sporosarcina pasteurii</i>	NCIMB 8841 ^T	NB-U	7.0	30	-	-	-	-	-	-	-
<i>Sporosarcina globispora</i>	IAM 14760 ^T	NB	7.2	30	-	-	1.35	-	-	-	-
<i>Sporosarcina ureae</i>	IAM 13053 ^{T c}	199	7.2	30	-	-	0.65	-	-	-	-
<i>Sporosarcina psychrophila</i>	IAM 12468 ^{T b}	199	7.2	15	-	-	1.24	-	-	-	-
<i>Planomicrobium chinense</i>	JCM 12466 ^T	MB	7.6	30	-	-	0.66	-	-	-	-
<i>Planomicrobium koreense</i>	JCM 10704 ^T	MB	7.6	28	-	-	0.75	-	-	-	-
<i>Planomicrobium okeanokoites</i>	IFO 12536 ^{T d}	199	7.2	30	0.01	-	1.41	-	-	0.18	-
<i>Filibacter limicola</i>	NCIMB 11923 ^T	TSB	7.3	20	-	-	1.47	-	-	-	-
<i>Kurthia gibsonii</i>	JCM 6105 ^T	NB	7.0	30	-	-	0.75	-	-	-	-
<i>Kurthia sibirica</i>	JCM 8563 ^T	NB	7.0	17	-	-	1.10	-	-	-	-
<i>Kurthia zopfii</i>	JCM 6101 ^T	NB	7.0	30	-	-	0.95	-	-	-	-
Family <i>Caryophanaceae</i>											
<i>Caryophanon latum</i>	NCIMB 9533 ^T	YTA	7.5	30	-	-	1.80	-	-	0.35	-
<i>Caryophanon tenue</i>	NCIMB 9535 ^T	YTA	7.5	30	-	-	1.50	-	-	0.60	-
Order <i>Lactobacillales</i>											
Family <i>Carnobacteriaceae</i>											
<i>Alkalibacterium olivoapovlenticus</i>	NCIMB 13710 ^T	NCIMB 473	7.2	25	-	-	-	-	-	-	-
<i>Alkalibacterium psychrotolerans</i>	JCM 12281 ^T	JCM 455	10.0	27	-	-	-	-	-	-	-
<i>Carnobacterium divergens</i>	JCM 5816 ^T	JCM 1 (MRS)	6.5	30	-	-	1.17	-	-	-	-
<i>Carnobacterium piscicola</i>	JCM 5348 ^T	JCM 1 (MRS)	6.5	25	-	-	0.50	-	-	-	-
<i>Desemzia incerta</i>	JCM 1969 ^T	BA	7.4	37	-	-	-	-	-	-	-
<i>Marinilactibacillus piezotolerans</i>	JCM 12337 ^T	JCM 404	7.2	37	-	-	0.90	-	-	-	-
<i>Marinilactibacillus psychrotolerans</i>	IAM 14980 ^T	IAM B-133	8.5	5	-	-	0.53	-	-	-	-
			8.5	35	-	-	0.78	-	-	-	-

^a See Table 1. ^{b, c, d} data from Hamana, 1999c, Hamana, 1994, Hamana & Matsuzaki, 1990.

Table 4. Cellular concentration of polyamines in the phylum *Actinobacteria*

Organism	Strain	Culture condition					Polyamines ^a ($\mu\text{mol/g}$ wet cells)									
		Medium	pH	T _m (°C)	4	5	33	34	44	343	344	444	Agm	3(3)4	3(3)4	3(3)3
<i>Phylum Actinobacteria</i>																
<i>Order Rubrobacteriales</i>																
<i>Rubrobacter radiotolerans</i>	JCM 2153 ^{T b}	199	7.3	46	-	-	-	-	-	-	-	-	-	-	-	-
<i>Rubrobacter xylanophilus</i>	JCM 11954 ^T	TSB	7.3	60	-	-	-	-	-	-	-	-	-	-	-	-
		199	7.3	60	-	-	-	-	-	-	-	-	-	-	-	-
<i>Conexibacter woesei</i>	JCM 11494 ^T	TSB	7.3	28	-	-	-	-	-	-	-	-	-	-	-	-
		199	7.3	28	-	-	-	-	-	-	-	-	-	-	-	-
<i>Thermoleophilum album</i>	ATCC 35263 ^{T c}	ATCC 949	7.2	70	0.05	-	0.01	0.02	-	-	-	-	-	0.02	2.20	0.01
<i>Thermoleophilum minutum</i>	ATCC 35265 ^{T c}	ATCC 949	7.2	70	0.01	-	0.01	0.02	-	-	-	-	-	0.02	1.35	0.01
<i>Order Coriobacteriales</i>																
<i>Atopobium rimae</i>	IFO 15546 ^{T d}	GAM	7.3	37	-	-	-	0.01	-	-	-	-	-	-	-	-
<i>Collinsella aerofaciens</i>	JCM 10188 ^T	GAM	7.3	37	-	-	-	-	-	-	-	-	-	-	-	-
		BA	7.4	37	-	-	-	-	-	-	-	-	-	-	-	-
<i>Collinsella intestinalis</i>	JCM 10643 ^T	GAM	7.3	37	-	-	-	-	-	-	-	-	-	-	-	-
<i>Collinsella stercoris</i>	JCM 10641 ^T	GAM	7.3	37	-	-	-	-	-	-	-	-	-	-	-	-
		BA	7.4	37	-	-	-	-	-	-	-	-	-	-	-	-
<i>Coriobacterium glomerans</i>	JCM 10262 ^T	GAM	7.3	30	-	-	-	-	-	-	-	-	-	-	-	-
<i>Eggerthella lenta</i>	JCM 9979 ^T	GAM	7.6	37	-	-	-	-	-	-	-	-	-	-	-	-
<i>Olsenella ulii</i>	JCM 12494 ^T	GAM	7.3	37	-	-	-	-	-	-	-	-	-	-	-	-
		BA	7.4	37	-	-	-	-	-	-	-	-	-	-	-	-
<i>Slackia exigua</i>	JCM 11022 ^T	GAM	7.6	37	-	-	-	-	-	-	-	-	-	-	-	-
<i>Order Actinomycetales</i>																
<i>Acidothermus cellulolyticus</i>	ATCC 43068 ^{T e}	199	5.0	60	-	-	-	-	-	-	-	-	-	-	-	-
		ATCC 1473	5.0	60	-	-	-	-	-	-	-	-	-	-	-	-

^a See Table 1. ^{b, c, d, e} data from Hamana, 1994, Hamana *et al.*, 1998, Hamana & Satake, 1995, Hamana, 1999c.

2001; Demharter *et al.*, 1989) lacked polyamines (Hamana, 1999c), as shown as a reference datum (Table 4). Polyamines were not detected in various Gram-positives belonging to the two orders *Actinomycetales* and *Bifidobacteriales* of this phylum including many slight- or moderate-thermophiles growing at 45–60°C (Hamana, 1999c). On the other hand, thermophilic *Thermoactinomyces* species belonging to the order *Bacillales* of the phylum *Firmicutes* contained spermidine and spermine as the major polyamines (Hamana, 1999c).

Polyamine profiles of the thermophiles, psychrophiles, halophiles, acidophiles and alkaliphiles in the 13 phyla

Homospermidine or norspermidine was the major polyamine in the early deeply branched five phyla *Acidobacteria*, *Chloroflexi*, *Chlorobi*, *Gemmatimonadetes* and *Planctomycetes*. Mesophilic species, slightly thermophilic species and acidophilic species in each phyla (or classes) show the same triamine profile, indicating that slight thermophily and acidophily do not affect polyamine distributions.

Thermophilic *Thermomicrobium* species within the class *Thermomicrobia* in which slightly alkaliphilic *Sphaerobacter* was isolated, and thermophilic *Thermodesulfovibrio* species within the phylum *Nitrospira* in which acidophilic *Leptospirillum* was isolated, contained norspermine, penta-amines and a hexa-amine. These results suggested that the occurrence of these long polyamines coincided with their extreme/moderate thermophily and had no direct correlation to their optimum alkaline or acidic growth conditions.

Within the order *Rubrobacterales*, extremely thermophilic *Thermoleophilum* species contained quaternary branched penta-amines whereas polyamines were not detected in slight/moderate thermophiles of *Rubrobacter* and a mesophile, *Conexibacter woesei*. Polyamines were not detected in slightly-thermophilic, slightly-acidophilic *Actinotherrmus* of the order *Actinomycetales* as well as mesophilic, neutrophilic species of the order *Coriobacteriales*.

Halophilic anaerobes in the order *Clostridiales* and halococci in the order *Bacillales* had no unique polyamine profiles within the orders, suggesting that their halophily does not affect their polyamine profiles.

Within the family *Carnobacteriaceae* in the order *Lactobacillales*, polyamines were not detected in

the two alkaliphiles, *Alkalibacterium olivoapovliticus* and *A. psychrotolerans*. The absence of major polyamines was also found in a mesophilic species, *Desemzia incerta*, in this order. Alkaliphilic, psychrotolerant *Marinilactibacillus psychrotolerans* as well as neutrophilic, mesophilic *M. piezotolerans* contained spermidine alone. Although psychrophilic or psychrotolerant species were found in the orders *Bacillales* and *Lactobacillales*, they were similar to the mesophilic species in the two orders, in their polyamine profiles.

The occurrence of a tetra-amine, spermine, seems to be correlated to moderate thermophily in many eubacterial thermophiles. Penta-amines were selectively produced in extreme thermophiles. Slight-thermophily as well as psychrophily, halophily, acidophily and alkaliphily within the 13 phyla did not correlate directly to their cellular polyamine components as a phylum-, class-, order-, family- or genus-specific chemotaxonomic marker.

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Acidobacteria, Actinobacteria, Chlorobi, Chloroflexi, Chrysiogenetes, Deferribacteres, Fibrobacteres, Firmicutes, Fusobacteria, Gemmatimonadetes, Nitrospirae, Planctomycetes, および Verrucomicrobia の真正細菌 13 門におけるポリアミン分析

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ポリアミンが未分析であった真正細菌 51 種 (13 門) についてポリアミンを HPLC により分析し, 化学分類マーカーとしてのポリアミン構成を考察した. *Acidobacteria* 門, *Chloroflexi* 門, *Chlorobi* 門では主ポリアミンとしてホモスペルミジンかノルスペルミジンを含有していた. *Nitrospirae* 門では, *Nitrospira* と *Leptospirillum* がスペルミジンを, 好熱性 *Thermodesulfobivrio* がスペルミジンに加え, ノルスペルミジン, ノルスペルミン, スペルミン, カダベリン, ホモカルドペンタアミンを有していた. *Chrysiogenetes* 門は現在 1 種のみであるが, スペルミジンを含有していた. *Deferribacteres* 門ではカダベリン, スペルミジン, スペルミンを含有し, *Fibrobacteres* 門はスペルミジンを有していた. *Fusobacteria* 門では全 6 種においてスペルミジン, スペルミンが検出された. *Gemmatimonadetes* 門はホモスペルミジンを含有した. *Planctomycetes* 門では 1 種を除き主ポリアミンはホモスペルミジンであった. *Verrucomicrobia* 門ではスペルミジンを有していた. *Firmicutes* 門では *Clostridiales* 目で低濃度のカダベリン, スペルミジン, スペルミン, アグマチンを有し, 好熱性 *Heliobacterium* はスペルミンが高濃度であった. *Halanaerobiales* 目ではすべての種でスペルミジンとスペルミンを含有していた. *Bacillales* 目, *Lactobacillales* 目では, ホモスペルミジンやスペルミンは検出されず, スペルミジンとアグマチンの存在に科や属間の差が認められた. *Actinobacteria* 門では, *Rubrobacterales* 目, *Conexibacteriales* 目, *Actinomycetales* 目でポリアミンを検出せず, 好熱性 *Thermoleophilum* が例外として分岐 4 級ペンタアミンを含有した. トリアミンのスペルミジン, ノルスペルミジン, ホモスペルミジン, のいずれかの存在が綱や目レベルでの系統分類と対応していた. 分析した菌群には好熱菌, 好冷菌, 好塩菌, 好酸菌, 好アルカリ菌を含んでおり, テトラアミン (スペルミン) の含量とペンタアミンの存在は好熱性と相関している場合が多く, 好冷性, 好塩性, 好酸性, 好アルカリ性はポリアミン構成に影響を与えているようには思われなかった.