Cellular Polyamine Catalogues of the Five Classes of the Phylum Proteobacteria: Distributions of Homospermidine within the Class Alphaproteobacteria, Hydroxyputrescine within the Class Betaproteobacteria, Norspermidine within the Class Gammaproteobacteria, and Spermine within the Classes Deltaproteobacteria and Epsilonproteobacteria

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Abstract：Cellular polyamines extracted from reclassified or newly validated 47 alphaproteobacteria, 46 betaproteobacteria, 96 gammaproteobacteria, 12 deltaproteobacteria and 10 epsilonproteobacteria were analyzed by high-performance liquid chromatography. Homospermidine was widely distributed within the class Alphaproteobacteria, however, homospermidine-dominant type, spermidine-dominant type and homospermidine/spermidine-dominant type were found and the three triamine profiles were genus-specific. The all genera belonging to the class Betaproteobacteria, ubiquitously contained putrescine and 2-hydroxyputrescine. Triamines were absent in almost betaproteobacteria. Many genera, including psychrophilic species, of the class Gammaproteobacteria, contained putrescine and spermidine as the major polyaminenes. Diaminopropane and norspermidine were selectively distributed in several genera of the class Gammaproteobacteria. Spermidine was the major polyamine in the classes Deltaproteobacteria and Epsilonproteobacteria. Spermine was found in some thermophiles within Betaproteobacteria, Deltaproteobacteria and Epsilonproteobacteria, suggesting that the occurrence of spermine correlate to their thermophily. Additional these polyamine catalogues serve for the classification of the phylum Proteobacteria, as a chemotaxonomic marker.

Key words：Homospermidine, Hydroxyputrescine, Norspermidine, Polyamine, Proteobacteria, Spermidine, Spermine

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INTRODUCTION

The phylum Proteobacteria (formerly the class Proteobacteria) of the domain Bacteria contains the five classes (formerly subclasses) Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria and Epsilonproteobacteria[1, 2]. Diamines such as diaminopropane (1,3-diaminopropane), putrescine, cadaverine and hydroxyputrescine (2-hydroxyputrescine), triamines such as spermidine, norspermidine, homospermidine, hydroxyspermidine (2-hydroxyspermidine) and acetylspermidine (N^1-acetylspermidine), a triamine, spermine, and a guanidinoamine, agmatine, have been found as their cellular polyamine components. Our studies on polyamine distribution profiles within proteobacteria already provided valuable chemotaxonomic informations on their classifications[3-12]. Afterward, many new members of proteobacteria have been isolated and validated in Bergey' Manual of Systematic Bacteriology[1], National Centre for Biotechnology Information (NCBI)[2] and Validation Lists of International Journal of Systematic and Evolutionary Microbiology[13].

As shown in the previous studies, a novel triamine, homospermidine, was selectively distributed in the class Alphaproteobacteria and a group of the class Gammaproteobacteria within Proteobacteria[6, 9, 11, 14-16]. Homospermidine distribution in the reclassified and 47 newly validated alphaproteobacteria (belonging to 39 genera), divided into five orders, were detarmined to elucidate variations in their polyamine profiles, and to evaluate the usefulness of polyamine pattern as a phenotypic marker.

A novel hydroxydiamine, hydroxyputrescine, widespread within the class Betaproteobacteria[6, 8, 14, 17]. Its distribution was limited in betaproteobacteria within Proteobacteria. To determine the occurrence of hydroxypolyamines, cellular polyamines of the reclassified and 46 newly validated betaproteobacteria belonging to 21 genera were analyzed.

Norspermidine was widespread within the gammaproteobacteria belonging to the order Vibrionales of the class Gammaproteobacteria[6, 7, 10, 18, 19]. This uncommon triamine was found in this class and it has never been detected in other four classes within Proteobacteria. Polyamine analyses were carried out in the reclassified and 96 newly validated gammaproteobacteria belonging to eight orders (42 genera) including various marine psychrophiles.

Within the class Deltaproteobacteria, some myxobacteria contained homospermidine, however, it was absent in the other deltaproteobacteria containing spermidine[6, 12]. Spermidine was the major polyamine in the members of the class Epsilonproteobacteria, previously analyzed[6, 12]. To survey spermine distribution in thermophilic proteobacteria, polyamines of 12 newly published deltaproteobacteria including a novel thermophile and 10 newly published epsilonproteobacteria including five novel thermophiles were analyzed.

MATERIALS AND METHODS

The proteobacteria were grown at the optimum growth temperature in the media designated by the culture collections (listed in Tables 1-4) as shown by Medium No., Marine broth (MB) (DIFCO Ltd., Detroit, USA), Nutrient broth (NB) (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan), NB supplemented with 5% NaCl (NB-NaCl), Trypticase soy broth (TSB) (BBL, Bectone Dickinson and Company, Cockeysville, MD, USA), Brain heart infusion broth (BHIB) (Nissui), yeast extract-mannitol medium (YM), peptone-yeast extract medium (PY) and PY dissolved in seawater (PYSW). Polyamine-free synthetic 199 medium (Nissui Pharmaceutical Co. Ltd), 199 medium supplemented with 5% NaCl (199NaCl) and 199 medium dissolved in seawater (199SW) were also used. Organisms at the stationary phase were harvested by centrifugation. The pellets were homogenized in 0.5 M perchloric acid ($HClO_4$). Polyamines were extracted into $HClO_4$ and analyzed by high-performance liquid chromatography (HPLC) on a Hitachi L6000 High-Speed Liquid Chromatograph[20]. Estimated cellular concentrations of polyamines are listed in Tables 1, 2, 3 and 4.

RESULTS AND DISCUSSION

Class Alphaproteobacteria (Table 1)

Order Sphingomonadales

The wide distribution of homospermidine in this order has been known. Homospermidine was found in

Table 1. Cellular concentrations of polyamines of alphaproteobacteria

Organism	Medium-Temp(℃)	Polyamine (μ mol/g wet wt cell): Dap	Put	Cad	Spd	HSpd	Spm	Agm
Order Sphingomonadales								
Sphingomonas soli IAM 15213^{T}	NB-30	-	-	-	0.69	0.40	-	0.10
(*Sphingomonas composta*)								
Sphingomonas oligophenolica JCM 12082^{T}	NB(1/10)-30	-	-	-	-	1.04	-	-
Sphingomonas yabuuchiae JCM 11416^{T}	TSB-30	-	-	-	0.05	0.96	-	-
Sphingomonas azotifigens IFO 15497^{T}	PY-30[e]	-	-	-	-	1.20	-	-
Sphingomonas trueperi IFO 16157^{T}	PY-30[e]	-	-	-	-	1.10	-	-
Sphingomonas adhaesiva IFO 15099^{T}	PY-30[e]	-	-	-	-	1.90	-	-
Sphingomonas asaccharolytica IFO 15499^{T}	PY-30[e]	-	-	-	-	1.30	-	-
Sphingomonas echinoides IFO 15742^{T}	PY-30[e]	-	-	-	-	1.76	-	-
Sphingomonas mali IFO 15500^{T}	PY-30[e]	-	-	-	-	0.75	-	-
Sphingomonas parapaucimobilis IFO 15100^{T}	PY-30[e]	-	-	-	-	2.35	-	-
Sphingomonas paucimobilis IFO 13953^{T}	PY-30[e]	-	-	-	-	2.55	-	-
Sphingomonas pruni IFO 15498^{T}	PY-30[e]	-	-	-	-	1.42	-	-
Sphingomonas sanguinis IFO 13937^{T}	PY-30[e]	-	-	-	-	1.97	-	-
Sphingomonas roseiflava IAM 14823^{T}	IAMB38-25[f]	-	-	-	-	1.20	-	-
Sphingomonas aquatilis IFO 16141^{T}	PY-30[f]	-	-	-	1.30	-	-	0.04
Sphingomonas koreensis IFO 16142^{T}	PY-30[f]	-	-	-	0.90	-	-	0.02
" *Sphingomonas azotoformans* " IAM 15131	NB-30	-	-	-	0.88	-	-	0.20
Sphingobium chungbukense JCM 11454^{T}	JCM75-30	-	-	-	1.45	-	-	0.15
Sphingobium amiense IAM 15006^{T}	NB-30	-	-	-	0.66	-	-	0.02
	199-30	-	-	-	0.97	-	-	0.05
Sphingobium japonicum NBRC 101211	PY-30	-	-	-	1.20	-	-	-
Sphingopyxis taejonensis IFO 16144^{T}	PY-30	-	0.04	-	1.37	-	-	0.04
(*Sphingomonas taejonensis*)								
Novosphingobium pentaromativorans JCM 12182^{T}	MB-30	-	-	-	1.30	-	-	-
Novosphingobium taihuense JCM 12465^{T}	JCM443-30	-	-	-	1.20	-	-	-
Novosphingobium tardaugens JCM 11434T^{T}	JCM12-25	-	0.02	1.45	0.75	-	-	-
Sphingosinicella microcystinivorans JCM 13185^{T}	NB-30[f]	-	-	-	-	1.15	-	-
	199-30[f]	-	-	-	-	0.82	-	-
Erythrobacter aquimaris JCM 12189^{T}	MB-30	-	-	-	0.74	-	-	-
Erythrobacter luteolus JCM 12599^{T}	MB-30	-	-	-	1.06	-	-	-
Erythrobacter litoralis IAM 14332^{T}	MB-30[e]	-	-	-	0.95	-	-	-
Erythrobacter longus IFO 14126^{T}	MB-30[e]	-	-	-	0.43	-	-	-
Porphyrobacter sanguineus IAM 12620^{T}	NB-24[g]	-	0.34	-	0.85	-	0.02	-
(*Agrobacterium sanguineum*)								
Shinella granuli JCM 13254^{T}	JCM346-30	-	0.25	-	0.75	0.67	-	-
Shinella zoogloeoides IAM 12669^{T}	199-30[h]	-	0.01	-	0.54	0.02	0.02	-
(*Crabtreella sacharophila, Zoogloea ramigera*)								
Order Rhodobacterales								
Dinoroseobacter shibae NCIMB 14021^{T}	199SW-30	-	0.30	-	0.05	0.25	-	-
	MB-30	-	0.60	-	0.70	0.05	-	-
Paracoccus denitrificans IAM 12479^{T}	199-30[d]	-	0.49	0.02	1.50	-	-	-
Paracoccus thiocyanatus IAM 12816^{T}	NB-30[c]	0.05	1.45	0.28	2.54	-	0.04	-
Paracoccus versutus IFO 14567^{T}	NB-30[c]	-	0.28	-	0.34	-	-	-
Paracoccus alkenifer NCIMB 13527^{T}	NB-30[e]	-	1.45	0.02	2.20	-	-	-
Paracoccus pantotrophus ATCC 35512^{T}	NB-30[b]	-	1.60	0.49	2.05	-	-	-
Paracoccus alcaliphilus JCM 7364^{T}	199-30[d]	-	2.40	0.05	2.10	-	0.01	0.06
Paracoccus aminophilus JCM 7686^{T}	199-30[d]	-	0.52	0.04	1.98	-	0.01	0.01
Paracoccus aminovorans JCM 7686^{T}	199-30[d]	-	0.88	0.30	1.61	-	-	-
Paracoccus kocurii JCM 7684^{T}	199-30[d]	-	0.38	0.41	3.10	-	0.01	0.52
Paracoccus seriniphilus NBRC 100798^{T}	MB-30	0.03	0.26	0.60	0.75	-	-	-
Paracoccus zeaxanthinifaciens MBIC 03966	MB-25	-	0.33	0.14	1.66	-	-	-
Paracoccus koreensis IAM 15216^{T}	NB-30	-	0.75	0.08	0.40	-	-	-
Pelagibaca bermudensis JCM 13377^{T}	MB-25	-	0.70	0.49	2.45	-	-	-
Catellibacterium nectariphilum NBRC 100046^{T}	NBRC388-3	-	1.40	-	0.92	-	-	-
Jannaschia cystaugens NBRC 100362^{T}	MB-25	-	0.45	0.10	0.43	-	-	-
Pseudovibrio denitrificans JCM 12308^{T}	MB-30	-	-	-	0.60	-	-	-
Pseudovibrio ascidiaceicola IAM 15084^{T}	MB-30	-	0.10	-	0.89	-	-	0.07
Roseovarius tolerans IAM 14840^{T}	IAMB141SW-20	-	0.25	0.80	0.42	-	-	-
Pseudorhodobacter ferrugineus IAM 12616^{T}	199SW-30	-	0.33	-	1.80	-	-	-
(*Agrobacterium ferrugineum*)	MB-24[b]	-	0.34	-	0.85	-	0.02	-

Roseibacterium elongatum JCM 11220[T]	MB-27	-	0.15	0.35	0.82	-	-	-
Yangia pacifica JCM 12573[T]	MB-30	-	0.10	0.08	0.44	-	-	-
Order Rhodospirillales								
Defluvicoccus vanus NCIMB 13612[T]	NCIMB481-30	-	-	-	0.80	-	-	-
Saccharibacter floricola JCM 12116[T]	JCM97-28	0.02	0.05	-	1.40	-	-	-
Azospirillum oryzae IAM 15130[T]	NB-30	-	1.88	-	0.36	0.24	-	-
Azospirillum irakense ATCC 51182[T]	199-30[c]	-	1.45	-	0.10	2.55	-	-
Azospirillum amazonense ATCC 35119[T]	199-30[c]	-	0.75	-	0.02	0.70	-	-
Azospirillum halopraeferens ATCC 43709[T]	199-30[c]	-	0.15	-	0.05	0.95	-	-
Azospirillum brasilense JCM 1224[T]	NM-30[c]	-	0.07	-	0.23	0.31	-	-
Azospirillum lipoferm JCM 1247[T]	NM-30[c]	-	0.07	-	0.08	0.60	-	-
Tistrella mobilis IAM 14872[T]	PY-30	-	0.06	0.54	0.96	1.15	-	-
Kozakia baliensis JCM 11301[T]	JCM90-30	-	0.05	-	0.80	-	-	-
Asaia krungthepensis NBRC 100057[T]	PYG-30	-	0.30	-	1.40	-	-	-
Neoasaia chiangmaiensis NBRC 101099[T]	PYG-30	-	0.88	-	0.96	-	-	-
Gluconobacter thailandicus JCM 12310[T]	JCM97-30	-	0.46	-	1.50	-	-	-
Acetobacter cibinongensis JCM 11196[T]	JCM97-30	-	0.01	-	0.98	-	-	-
Acetobacter orientalis JCM 11195[T]	JCM97-30	-	0.10	-	1.12	-	-	-
Acetobacter syzygii JCM 11197[T]	JCM97-30	-	0.01	-	1.05	-	-	-
Order Rhizobiales								
Methylocapsa acidiphila NCIMB 13765[T]	NCIMB472-30	-	-	-	0.70	-	-	-
Kaistia adipata IAM 15023[T]	NB-30	-	-	-	0.46	-	-	-
Rhodopseudomonas faecalis JCM 11668[T]	JCM358-30	-	-	-	-	1.10	-	-
Rhodopseudomonas palustris NCIMB 8252[T]	NCIMB27-30[b]	-	0.02	-	0.10	1.30	-	-
Rhodopseudomonas acidophila NCIMB 11761[T]	NCIMB153-30[b]	-	0.15	-	-	2.40	-	-
Pseudaminobacter defluvii IAM 12817[T]	IAMB60-30	-	0.99	-	1.43	1.02	-	-
Roseomonas lacus JCM 13283[T]	JCM346-30	-	0.10	-	1.50	1.10	-	-
Hoeflea phototrophica NCIMB 14078[T]	MB-30	-	-	-	1.15	-	-	-
Order Caulobacterales								
Asticcacaulis taihuensis JCM 12463[T]	JCM443-30	-	-	0.10	0.90	0.40	-	0.20
Brevundimonas nasdae JCM 11415[T]	TSB-30	-	-	-	0.60	-	-	-
Brevundimonas bacteroides NCIMB 9790[T]	NCIMB58-30[b]	-	-	-	0.20	1.25	-	-
Brevundimonas subvibrioides IFO 16660[T]	199-30[b]	-	-	-	-	1.40	-	-
Brevundimonas bullata IAM 13153[T]	199-30[b]	-	-	-	2.20	1.80	-	-
Brevundimonas diminuta IFO 12697[T]	199-30[b]	-	-	-	-	0.65	-	-
Brevundimonas vesicularis IFO 12165[T]	199-30[b]	-	-	-	1.17	0.20	-	-
Phenylobacterium koreense IAM 15119[T]	NB-30	-	-	-	1.10	-	-	-
Incertae sedis								
Pleomorphomonas oryzae IAM 15079[T]	NB-30	-	0.60	-	0.06	0.81	-	-
Terasakiella pusilla IFO 13613[T]	199SW-30[a]	-	0.24	-	1.19	-	-	-
(*Oceanospirillum pusillum*)								
Kordiimonas gwangyangensis JCM 12864[T]	MB-30	-	0.70	-	0.35	0.20	-	-

Dap, diaminopropane; Put, putrescine; Cad, cadaverine; Spd, spermidine; HSpd, homospermidine; Spm, spermine; Agm, agmatine; -, not detected (<0.005); [T], Type strain; IAM, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan; JCM, Japan Collection of Microorganisms, RIKEN, Wako, Saitama, Japan; IFO, Institute for Fermentation, Osaka, Japan; NBRC, Biological Resource Center, National Institute of Technology and Evaluation, Kisarazu, Japan; MBIC, Marine Biotechnology Institute Culture Collection, Kamaishi, Iwate, Japan; ATCC, American Type Culture Collection, Manassas, Virginia, USA; NCIMB, National Collections of Industrial, Foods and Marine Bacteria, Scotland, UK. Former names are shown in parentheses. Quotation marks indicate that the scientific name has not been validly published. [a] was cited from Hamana *et al.*, 1994, [b] Hamana *et al.*, 2001, [c] Hamana & Takeuchi, 1998, [d] Hamana & Matsuzaki, 1992, [e] Takeuchi *et al.*, 2001, [f] Hamana *et al.*, 2003, [g] Hamana & Matsuzaki, 1990, [h] Hamana & Matsuzaki 1993, [i] Maruyama *et al.*, 2006.

the newly analyzed three *Sphingomonas* species as well as *Sphingosinicella microcystinivorans*[21], in the present study. The major polyamine was spermidine in *Sphingomonas azotoformans*, *Sphingobium chungbukense*, *Sphingobium amiense*, *Sphingobium japonicum*, *Sphingopyxis taejonensis* and three *Novosphingobium* species. *Sphingomonas soli* and two *Shinella* species contained spermidine and homospermidine as the major polyamines, indicating a unique triamine profile within this order. Other 13 authentic *Sphingomonas* species contained homospermidine. It has been reported that three *Sphingobium* species, six *Novosphingobium* species and three *Sphingopyxis* species contained spermidine[22]. New *Erythrobacter* and *Porphyrobacter* species contained spermidine alone. These results suggest that the distribution of homospermidine in this order is genus-specific, however, are chemotaxonomically conflicting with the classification of some *Sphingomonas* species.

Order Rhodobacterales

Dinoroseobacter shibae contained spermidine and homospermidine. Spermidine was the major polyamine in the new alphaproteobacteria located in the genera, *Paracoccus*, *Pelagibaca*, *Catallibacterium*, *Jannaschia*, *Pseudovibrio*, *Roseovarius*, *Pseudorhodobacter* and *Yagia* species, analyzed in the present study.

Order Rhodospirillales

Homospermidine and spermidine were found in *Azospirillum* and *Tistrella*, whereas spermidine was detected in *Defluvicoccus*, *Saccharibacter*, *Kozakia*, *Asaia*, *Neoasaia*, *Gluconobacter* and *Acetobacter* species, analyzed in the present study.

Order Rhizobiales

Methylocapsa acidiphila, *Kaistia adipata* and *Hoeflea phototrophica* contained spermidine alone. *Rhodopseudomonas faecalis* contained homospermidine alone. *Pseudaminobacter defluvii* and *Roseomonas lacus* contained spermidine and homospermidine. Three types on triamine profile were observed in this order.

Order Caulobacterales

Asticcacaulis taihuensis contained spermidine, homospermidine and agmatine. *Phenylobacterium koreense* contained spermidine alone. Distribution profiles of spermidine and homospermidine are heterogeneous in the six *Brevundimonas* species.

Others

Homospermidine was distributed in the newly validated *Pleomorphomonas* and *Kordiimonas* species, however, their phylogenetic positions within the class Alphaproteobacteria are unknown. The major triamine in *Terasakiella pusilla* was spermidine.

Class Betaproteobacteria (Table 2)

It has been demonstrated that 66 betaproteobacteria, consisting the six orders Burkholderiales, Hydrogenophilales, Methylophilales, Neisseriales, Nitrosomonadales and Rhodocyclales, ubiquitously contained hydroxyputrescine in addition to putrescine[6, 8]. Hydroxyputrescine has not been detected in almost other four classes of the phylum Proteobacteria. Therefore, it has been proposed that the occurrence of this hydroxydiamine served as a chemotaxonomic marker for the class Betaproteobacteria.

Azohydromonas, *Burkholderia*, *Caldimonas* , *Cupriavidus*, *Comamonas* , *Curvibacter*, *Delftia* , *Derxia* , *Diaphorobacter* , *Herbaspirillum* , *Hydrogenophaga* , *Hylemonella*, *Janthinobacterium*, *Malikia*, *Mitsuaria*, *Ottowia*, *Pelomonas*, *Ralstonia* and *Wautersia* belonging to Burkholderiales, *Zoogloea* and "*Pseudomonas butanovora*" belonging to Rhodocyclales, *Hydrogenophilus* belonging to Hydrogenophilales, and *Alysiella*, *Conchiformibium*, *Bergeriella*, *Simonsiella* and *Microvirgula* belonging to Neisseriales, analyzed in the present study, ubiquitously contained putrescine and hydroxyputrescine as the major polyamines.

Hydroxyputrescine was found also in *Thiobacter subterraneus*, *Sivamonas terrae* and "*Pseudomonas jianii*" classified into this class. Spermidine was found in thermophiles and some mesophilic species analyzed in the present study, however, phylogenetic significance of this triamine was not clear. Hydroxyspermidine was produced by aminopropyltransfer into hydroxyputrescine, and coexisted with hydroxyputrescine in the betaproteobacteria containing spermidine as a major

Table 2. Cellular concentrations of polyamines of betaproteobacteria

Organism	Medium-Temp(℃)	Polyamine (μ mol/g wet wt cell): Dap	H-Put	Put	Cad	H-Spd	Spd	Spm
Order Burkholderiales								
Burkholderia kururiensis JCM 10599[T]	NB-30	-	1.75	0.52	0.02	-	0.12	-
Burkholderia glathei JCM 10563[T]	NB-37	-	0.45	0.04	-	-	0.06	-
Brukholderia sordidicola JCM 11778[T]	TSB-30	-	0.15	0.61	-	-	-	-
Ralstonia mannitolilytica JCM 11284[T]	TSB-30	-	0.44	1.15	-	-	-	-
Ralstonia pickettii JCM 10171[T]	NB-30[d]	-	0.70	1.30	-	-	-	-
Cupriavidus eutropha IAM 13533[T]	199-30[a]	-	2.40	3.50	-	-	-	-
(*Ralstonia eutropha, Wautersia eutropha*)								
Cupriavidus oxalaticus IFO 13593[T]	NB-30	-	0.70	0.65	0.02	-	-	-
(*Ralstonia oxalatia, Wautersia oxalatica*)								
Cupriavidus gilardii JCM 11283[T]	TSB-30	-	0.40	0.75	-	-	-	-
(*Ralstonia gilardii, Wautersia gilardii*)								
Cupriavidus pauculus JCM 11286[T]	TSB-30	-	0.52	1.10	-	-	-	-
(*Ralstonia paucula, Wautersia paucula*)								
"*Wautersia metalotorelans*" IAM 14785	NB-30	-	1.12	0.66	-	-	-	-
("*Ralstonia metalophila*")	199-30	-	0.77	0.26	-	-	-	-
"*Wautersia silverii*" IAM 14786	NB-30	-	0.62	0.48	-	-	-	-
("*Ralstonia silverii*")	199-30	-	0.50	0.32	-	-	-	-
"*Wautersia tsushimaensis*" IAM 14787	NB-30	-	0.65	0.37	-	-	-	-
("*Ralstonia tsushimae*")	199-30	-	0.70	0.46	-	-	-	-
Delftia acidovorans IAM 12409[T]	PY-30	-	0.30	1.55	-	-	-	-
Delftia tsuruhatensis NBRC 16741[T]	PY-30	-	0.42	2.20	-	-	-	-
Diaphorobacter nitroreducens JCM 11421[T]	JCM22-30	-	0.16	1.85	-	-	-	-
Caldimonas manganoxidans JCM 10698[T]	JCM74-45	-	0.49	0.54	-	-	-	-
Ottowia thiooxydans JCM 11629[T]	JCM346-26	-	0.65	1.05	-	-	-	-
Hylemonella gracilis IFO 14920[T]	PY-30[b]	-	1.40	1.10	-	-	-	-
(*Aquaspirillum gracile*)								
Herbaspirillum autotrophicum IFO 15327[T]	199-30[c]	-	0.65	1.45	-	-	-	-
(*Aquaspirillum autotrophicum*)								
Herbaspirillum seropedicae ATCC 35892[T]	ATCC8838-20[b]	-	1.35	2.10	-	-	-	-
Herbaspirillum chlorophenolicum IAM 15024[T]	NB-25	-	0.05	0.44	-	-	-	-
Herbaspirillum huttiense IAM 14941[T]	NB-25	-	0.35	1.40	-	-	-	-
(*Pseudomdonas huttiensis*)								
Herbaspirillum putcus IAM 15032[T]	NB-25	-	0.38	0.80	-	-	-	-
Herbaspirillum frisingense IAM 14974[T]	NB-30	-	0.55	0.99	-	-	0.01	-
Herbaspirillum rubrisubalbicans IAM 14876[T]	BHIB-25	-	0.15	0.35	-	-	-	-
Curvibacter gracilis IAM 15033[T]	NB-25	-	0.50	1.22	-	-	-	-
Curvibacter lanceolatus IAM 14947[T]	MB-25	-	0.14	1.03	-	-	-	-
(*Pseudomonas lanceolata*)								
Curvibacter delicatus IFO 14919[T]	PY-30[c]	-	0.02	0.58	-	-	-	-
(*Aquaspirillum delicatum*)								
Comamonas badia IAM 14839[T]	NB-25	-	0.42	0.56	0.03	-	0.19	-
Comamonas terrigena IFO 13299[T]	199-30[a]	-	0.81	0.35	-	-	-	-
Comamonas testosteroni IAM 12419[T]	199-30[a]	-	0.32	0.95	-	-	0.02	-
Malikia spinosa IAM 14918[T]	NB-25	-	0.37	1.26	0.02	-	0.10	-
(*Pseudomonas spinosa, Hydrogenophaga spinosa*)								
Pelomonas saccharophila IAM 14368[T]	NB-30	-	0.49	1.02	-	-	-	-
(*Pseudomonas saccharophila*)								
Janthinobacterium lividum IAM 13948[T]	199-25[b]	-	1.74	2.50	-	-	-	-
Janthinobacterium agaricidamnosum IAM 14973[T]	NB-25	-	0.15	0.96	-	-	-	-
Hydrogenophaga flava IAM 14931[T]	NB-30	-	0.65	1.00	-	-	-	-
Hydrogenophaga intermedia IAM 14919[T]	NB-27	-	0.15	0.40	-	-	0.03	-
Hydrogenophaga palleronii IAM 14930[T]	NB-30	-	1.15	1.90	-	-	-	-
Hydrogenophaga pseudoflava IAM 14928[T]	NB-37	-	0.45	0.97	-	-	-	-
Hydrogenophaga taeniospiralis IAM 14929[T]	NB-30	-	0.40	1.28	-	-	-	-
Derxia gummosa ATCC 15994[T]	ATCC165-30	-	1.45	1.50	0.03	-	-	-
IAM 13946[T]	IAMB1-25	-	1.02	1.60	-	-	0.10	0.02
IAM 14990	IAMB1-27	-	0.90	1.08	0.02	-	-	-
Mitsuaria chitosanitabida IAM 14711[T]	NB-30	-	0.09	1.17	-	-	-	-
Azohydromonas lata IAM 12599[T]	199-30[a]	-	0.70	0.95	0.02	-	0.10	-
(*Alcaligenes latus*)								
Azohydromonas australica IAM 12664[T]	199-30[a]	-	0.35	0.45	-	-	0.18	-

(*Alcaligenes latus*)								
Giesbergeria anulus IFO 14917[T]	PY-30[b]	-	1,15	2.00	-	-	-	-
(*Aquaspirillum anulus*)								
Giesbergeria giesbergeri IFO 13959[T]	PY-30[b]	-	0.25	0.50	-	-	-	-
(*Aquaspirillum giesbergeri*)								
Giesbergeria sinuosa IFO 14925[T]	PY-30[b]	-	0.10	0.64	-	-	-	-
(*Aquaspirillum sinuosum*)								
Simplicispira metamorpha IFO 13960[T]	199-30[b]	-	0.77	0.49	-	-	-	-
(*Aquaspirillum metamorphum*)								
Simplicispira psychrophila IFO 13611[T]	PY-30[b]	-	0.25	1.54	-	-	0.08	-
(*Aquaspirillum psychrophilum*)								
Order Rhodocyclales								
"*Pseudomonas butanovora*" IAM 12574	NB-30	-	0.65	2.20	-	-	0.38	0.02
Zoogloea ramigera IAM 12136[T]	199-30[d]	-	0.35	0.12	-	-	-	-
Zoogloea resiniphila ATCC 700687[T]	ATCC2136-30[d]	-	0.60	1.45	-	-	-	-
Zoogloea oryzae IAM 15218[T]	NB-30	-	0.17	0.25	-	-	-	-
Order Hydrogenophilales								
Hydrogenophilus thermoluteolus IFO 14978[T]	IFO820-50[c]	-	1.32	0.80	0.02	0.02	0.54	0.02
Hydrogenophilus hirschii JCM 10831[T]	JCM279-60	-	1.70	0.50	0.15	0.02	0.24	0.67
Order Neisseriales								
Alysiella crassa IAM 14969[T]	IAMB127-37	-	0.50	0.35	0.07	-	-	-
(*Simonsiella crassa*)								
Alysiella filiformis IAM 14895[T]	IAMB127-37	-	0.30	0.02	-	-	-	-
Conchiformibium steedae IAM 14972[T]	IAMB127-37	-	1.07	0.60	1.40	-	-	-
(*Simonsiella steedae*)								
Conchiformibium kuhniae IAM 15037[T]	199-30	-	0.25	0.95	-	-	-	-
Bergeriella denitrificans IAM 14975[T]	NB-30	-	0.04	0.15	0.03	-	1.18	-
	119-30	-	0.04	0.10	-	-	0.35	-
Microvirgula aerodenitrificans IAM 14991	IAMB104-30	-	0.55	0.10	0.16	-	-	-
IAM 14943[T]	IAMB33-27	-	0.15	0.04	1.88	-	0.05	-
(*Aquaspirillum disper*) IFO 15328	199-25[c]	0.20	0.95	0.01	1.30	-	-	-
Incertae sedis								
Thiobacter subterraneus JCM 12421[T]	JCM 419-55	-	0.10	0.20	1.40	-	0.10	-
"*Pseudomonas jianii*" IAM 12403	NB-30	-	0.19	0.40	-	-	-	-
Sivamonas terrae IAM 15217[T]	NB-30	-	0.50	1.10	-	-	-	-

Dap, diaminopropane; H-Put, 2-hydroxyputrescine; Put, putrescine; Cad, cadaverine; H-Spd, 2-hydroxyspermidine; Spd, spermidine; Spm, spermine; -, not detected (<0.005); [T], Type strain; IAM, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan; JCM, Japan Collection of Microorganisms, RIKEN, Wako, Saitama, Japan; IFO, Institute for Fermentation, Osaka, Japan; NBRC, Biological Resource Center, National Institute of Technology and Evaluation, Kisarazu, Japan; ATCC, American Type Culture Collection, Manassas, Virginia, USA. Former names are shown in parentheses. Quotation marks indicate that the scientific name has not been validly published. [a] was cited from Hamana *et al.*, 1992, [b] Hamana *et al.*, 1994, [c] Hamana *et al.*, 1991, [d] Hamana *et al.*, 2000.

polyamine. Norspermidine and homospermidine have not been found in the betaproteobacteria analyzed in the previous[6, 8] and present studies.

Spermidine and spermine were detected in a moderately thermophilic species of *Hydrogenophilus*, *H. hirschii*, growing at 60℃ as a major polyamine, suggesting that the occurrence of spermine, a tetra-amine, correlate to its thermophily upper 60℃. On the other hand, significant amount of spermine was not found in slightly thermophilic (thermotolerant) *Caldimonas manganoxidans* growing at 45℃, *Hydrogenophilus thermoluteolus* growing at 50℃ and *Thiobacter subterraneus* growing at 55℃. Uptake of spermine by other mesophilic species from media is not excluded.

Class Gammaproteobacteria (Table 3)

Order Alteromonadales

Norspermidine was detected in two newly analyzed species of *Psychromonas* in the present study and a *Moritella* species previously analyzed[7]. Four types of polyamine profiles, putrescine alone, putrescine plus cadaverine, hydroxyputrescine plus putrescine, and putrescine plus spermidine, were found in *Shewanella* species including newly analyzed species. Cadaverine was the major polyamine in the two new species of *Moritella*, however, spermidine had not been found in this genus. Two species of *Colwellia* contained no cellular polyamines. Polyamine disribution profiles were varied within the mesophilic/psychrophilic marine proteobacteria belonging to the genera *Psychromonas*, *Shewanella*, *Colwellia* and *Moritella*, indicating that these genera are phylogenetically and chemotaxonomically conflicting taxa. Hydroxyputrescine was detected in several *Shewanella* and a *Colwellia* species belonging to this order. The occurrence of this hydroxydiamine related to branching point of the two classes Betaproteobacteria and Gammaproteobacteria, therefore, serve as a chemotaxonomic marker for betaproteobacteria within the phylum Proteobacteria. Spermidine was the major polyamine in *Alteromonas*, *Idiomarina*, *Marinobacter*, *Marinobacterium*, *Pseudoalteromonas* and *Ferrimonas* species. *Pseudidiomarina taiwanensis* contained putrescine alone. The occurrence of spermine in *Pseudoalteromonas phenolica* and *Psychromonas marina* growing at 15℃ is interesting, however, spermine has been detected ordinarily in thermophilic proteobacteria as shown in the present study. Spermine was not found in the psychrophilic *Psychromonas*, *Shewanella*, *Colwellia* and *Moritella* species growing under 10℃.

Order Vibrionales

Norspermidine was found in three new species of *Vibrio* and three new species of *Photobacterium*. This triamine had been detected in almost species of these two genera previously analyzed[19].

Order Oceanospirillales

Former *Oceanospirillum* species have been devided into the three genera *Oceanospirillum*, *Pseudospirillum* and *Oceanobacter* belonging to the order Oceanospirillales and the genus *Marinobacter* belonging to the order Alteromonadales[1, 2, 13]. However, the analyzed species belonging to the four genera as well as the related genera *Marinomonas*, *Halomonas* and *Chromohalobacter* belonging to this order contained putrescine and spermidine as the major polyamine. *Microbulbifer* and *Agarivorans* contained cadaverine and spermidine. Occurrence of cadaverine seems to be species-specific in the genera *Halomonas* and *Marinomonas*.

Order Pseudomonadales

Almost species of the genera *Azotobacter*, *Azorhizophilus*, *Psychrobacter*, *Moraxella* and *Pseudomonas* contained putrescine, cadaverine and spermidine. Two *Cellvibrio* species contained putrescine alone. A new species as well as previously analyzed species of *Acinetobacter* contained diaminopropane alone[23].

Order Thiotrichales

Five *Thiothrix* species of the order Thiotrichales were heterogeneous in their polyamine profiles. *Thiomicrospira* and *Methylophaga* had same polyamine profile, putrescine plus spermidine.

Order Xanthomonadales

Among *Pseudoxanthomonas*, *Rhodanobacter* and *Aquimonas* species analyzed in the present study, a species of *Pseudoxanthomonas*, *P. koreensis*, lacked polyamines. Others contained spermidine as the major polyamine. Chemotaxomomic significance of the absence of cellular polyamines, found in *P.*

Table 3. Cellular concentrations of polyamines of gammaproteobacteria

Organism	Medium-Temp(℃)	Polyamine (μ mol/g wet wt cell): Dap	H-Put	Put	Cad	NSpd	Spd	Spm	Agm
Order Alteromonadales									
Alteromonas macleodii IAM 12920[T]	199NaCl-25	-	-	-	0.14	-	1.25	-	0.04
	MB-25	-	-	-	0.05	-	0.45	-	0.08
Alteromonas marina JCM 11804[T]	MB-30	-	-	-	0.39	-	0.92	-	0.17
Alteromonas litorea JCM 12188[T]	MB-30	-	-	-	0.30	-	1.80	-	0.15
Pseudoalteromonas peptidolytica MBIC 01416[T]	MB-30	-	-	-	-	-	1.10	-	0.15
Pseudoalteromonas spongiae JCM 12884[T]	MB-30	-	-	-	-	-	1.05	-	0.05
Pseudoalteromonas phenolica IAM 14989[T]	MB-15	-	-	-	-	-	0.60	0.30	-
	MBagar-15	-	-	-	0.07	-	0.50	0.30	-
Pseudoalteromonas sagamiensis JCM 11461[T]	MBagar-27	-	-	-	-	-	0.10	-	-
Psychromonas marina IAM 14899[T]	MB-15	-	-	0.01	0.22	0.80	0.04	0.65	-
Psychromonas kaikoae JCM 11054[T]	MB-10	-	-	0.04	1.25	0.25	-	-	-
Psychromonas profunda JCM 11437[T]	MB-4	-	-	0.15	1.10	-	-	-	-
Idiomarina seosinensis JCM 12526[T]	MB-30	-	-	-	-	-	0.75	-	0.20
Pseudidiomarina taiwanensis JCM 13360[T]	PY-30	-	-	0.70	-	-	-	-	-
Shewanella algae IAM 14159[T]	199SW-20[e]	-	-	1.45	0.50	-	-	-	-
Shewanella baltica NCIMB 1733	MB-20[f]	-	-	1.20	1.50	-	-	-	-
Shewanella bentica ATCC 43992[T]	MB-2[e]	-	-	0.25	-	-	-	-	-
Shewanella frigidimarina NCIMB 400	MB-30[f]	-	-	0.80	-	-	0.02	-	-
Shewanella hanedai IAM 12641[T]	199SW-20[e]	-	-	1.50	-	-	-	-	-
Shewanella putrefaciens IAM 12079[T]	199SW-30[e]	-	-	3.00	-	-	-	-	-
Shewanella violacea JCM 10179[T]	MB-4[f]	-	0.60	0.45	-	-	-	-	-
Shewanella woodyi NCIMB 13526[T]	MB-20[f]	-	-	0.50	-	-	0.25	-	-
Shewanella amazonensis ATCC 700329[T]	ATCC1065-20[g]	-	-	1.05	-	-	0.08	-	-
Shewanella pealeana ATCC 700345[T]	MB-30[g]	-	-	0.67	-	-	-	-	-
Shewanella oneidensis ATCC 700550[T]	TSB-30[g]	-	-	1.50	1.10	-	-	-	-
Shewanella gelidimarina ATCC 700752[T]	MB-30[g]	-	-	0.01	0.01	-	1.12	-	-
Shewanella aquimarina JCM 12193[T]	MB-30	-	-	1.08	-	-	-	-	-
Shewanella decolorationis IAM 15094[T]	MB-25	-	-	1.11	-	-	-	-	-
Shewanella gaetbuli JCM 11814[T]	MB-30	-	1.25	0.30	-	-	-	-	-
Shewanella japonica IAM 14956[T]	MB-25	-	-	0.30	0.75	-	-	-	-
Shewanella marinintestina JCM 11558[T]	MB-25	-	1.42	1.25	-	-	-	-	-
Shewanella marisflavi JCM 12192[T]	MB-30	-	-	1.06	-	-	0.08	-	-
Shewanella profunda JCM 12080T	MB-25	-	-	0.73	0.05	-	-	-	-
Shewanella sairae JCM 11563[T]	MB-25	-	-	0.72	-	-	-	-	-
Shewanella sediminis NCIMB 14036[T]	MB-10	-	0.75	1.10	-	-	0.02	-	-
Shewanella schlegeliana JCM 11561[T]	MB-25	-	1.44	0.25	-	-	-	-	-
Shewanella hafniensis NBRC 100975[T]	MB-25	-	0.87	0.02	-	-	-	-	-
Shewanella halifaxensis NCIMB 14093[T]	MB-15	-	0.60	0.30	-	-	-	-	-
Shewanella morhuae NBRC 100978[T]	MB-25	-	1.27	-	-	-	-	-	-
Shewanella pneumatophori JCM 13187[T]	MB-27	-	-	0.75	-	-	-	-	-
Colwellia piezophila JCM 11831[T]	MB-10	-	-	-	-	-	-	-	-
Colwellia maris JCM 10085[T]	JCM253-10[f]	-	0.16	0.56	-	-	0.34	-	-
Colwellia psychrerythrae ATCC 27364[T]	MB-10[f]	-	-	-	-	-	-	-	-
Moritella profunda JCM 11435[T]	MB-2	-	-	-	0.40	-	-	-	-
Moritella abyssi JCM 11436[T]	MB-4	-	-	-	0.38	-	-	-	-
Moritella marina ATCC 15381[T]	PYSW-25[f]	-	-	-	1.10	-	-	-	-
Moritella japonica JCM 10249[T]	NB-10[f]	-	-	-	0.50	-	-	-	-
Moritella yayanosii JCM 10263[T]	MB-10[f]	-	-	0.02	-	1.50	-	-	-
Marinobacter litoralis JCM 11547[T]	MB-30	-	-	-	-	-	1.10	-	-
Marinobacter lutaoensis JCM 11179[T]	MBagar-37	-	-	-	0.33	-	1.16	-	-
Marinobacter hydrocarbonoclasticus ATCC 49640[T]	199SW-20[e]	-	-	-	-	-	0.98	-	-
(*Pseudomonas nautica*) IAM 12929(=ATCC 27132)	MB-25	-	-	0.03	0.10	-	1.35	-	0.03
Marinobacter flavimaris JCM 12323[T]	MB-30	-	-	0.05	-	-	0.69	-	0.03
Marinobacter daepoensis JCM 12324[T]	MB-30	-	-	0.07	-	-	1.20	-	-
Marinobacter alkaliphilus JCM 12291[T]	JCM392-30	-	-	0.10	0.12	-	0.65	0.01	0.05
Marinobacter maritimus JCM 12521[T]	JCM443-22	-	-	0.18	0.04	-	0.56	-	0.02
Marinobacterium georgiense ATCC 700074[T]	MB-25[i]	-	-	0.04	0.10	-	1.90	0.03	-
(*Pseudomonas iners*) IAM 1419	NB-25	-	-	0.33	0.10	-	0.75	0.01	-
Marinobacterium jannaschii IFO 15466[T]	199SW-30[i]	-	-	0.02	-	-	0.80	-	-
(*Oceanospirillum jannaschii*)									
Ferrimonas marina MBIC 06486[T]	MB-30	-	-	-	-	-	1.05	-	-

Order Vibrionales									
Photobacterium ganghwense JCM 12487^{T}	MB-25	-	-	-	-	0.60	0.40	-	-
Photobacterium frigidiphilum JCM 12947^{T}	MB-10	-	-	-	-	0.24	0.48	-	-
Photobacterium indicum IFO 14233^{T}	MB-30[b]	-	-	0.01	-	0.52	0.62	-	-
("*Hyphomicrobium indicum*")									
Vibrio metschnikovii IAM 14406^{T}	MB-37	-	-	0.08	-	0.50	0.48	-	-
Vibrio superstes IAM 15009^{T}	MB-15	-	-	-	-	0.02	0.65	0.10	-
	MBagar-15	-	-	0.04	-	0.20	0.38	-	-
Vibrio ruber JCM 11486^{T}	TSB-30	-	-	-	0.55	0.62	-	0.02	-
	TSBagar-30	-	-	-	0.20	0.50	-	-	-
Order Oceanospirillales									
Oceanobacter kriegii IFO 15467^{T}	199SW-30[j]	-	-	0.03	-	-	0.84	-	-
(*Oceanospirillum kriegii*)									
Pseudospirillum japonicum IFO 15446^{T}	199SW-30[j]	-	-	0.01	-	-	1.11	-	-
(*Oceanospirillum japonicum*)									
Halomonas alimentaria JCM 10888^{T}	MB-30	-	-	0.02	-	-	0.83	-	-
Halomonas glaciei JCM 11692^{T}	MB-20	-	-	0.07	0.15	-	0.89	-	-
Halomonas marisflavi JCM 10873^{T}	MB-20	-	-	-	-	-	1.26	-	-
Halomonas koreensis JCM 12237^{T}	MB-35	-	-	0.04	-	-	1.32	-	0.10
Chromohalobacter salexigens NCIMB 13768^{T}	NCIMB219-30	-	-	-	-	-	0.80	-	-
Chromohalobacter canadensis NCIMB 13767^{T}	NCIMB473-30	-	-	0.02	-	-	1.32	-	-
(*Halomonas canadensis*)									
Chromohalobacter israelensis NCIMB 13766^{T}	NCIMB473-30	-	-	-	-	-	0.77	-	-
(*Halomonas israelensis*)									
Marinomonas communis IAM 12914^{T}	199SW-30[a]	-	-	1.05	-	-	0.35	-	-
Marinomonas vaga IAM 12923^{T}	199SW-30[d]	-	-	0.10	-	-	0.50	-	-
Marinomonas mediterranea IAM 14944^{T}	MB-25	-	-	0.04	0.16	-	0.48	-	-
Marinomonas primoryensis IAM 15010^{T}	MB-30	-	-	0.35	0.50	-	0.64	-	-
Marinomonas ushuaiensis JCM 12170^{T}	MB-30	-	-	0.36	0.02	-	0.37	-	-
Microbulbifer salipaludis JCM 11542^{T}	MB-37	-	-	-	0.20	-	0.60	-	0.15
Microbulbifer hydrolyticus ATCC 700072^{T}	199SW-25[j]	-	-	0.02	1.65	-	0.25	-	0.06
(*Microbulbifer hydrolytica*)									
Agarivorans albus IAM 14998^{T}	MB-25	0.01	-	0.05	0.37	-	0.98	0.03	-

Order Pseudomonadales									
Azotobacter beijerinckii IAM 12683^{T}	YM-30[h]	-	-	0.11	0.01	-	0.04	-	-
Azotobacter chroococcum IAM 12994^{T}	YM-30[a]	-	-	1.44	0.22	-	0.50	-	-
Azotobacter nigricans IAM 15005^{T}	NB-28	-	-	0.10	0.10	-	0.44	-	-
Azotobacter vinelandii IAM 15004^{T}	IAMB138-28	-	-	1.30	0.12	-	0.32	-	-
	IAMB138agar-28	-	-	0.70	0.10	-	0.12	-	-
IAM 1078	YM-30[a]	-	-	0.94	0.27	-	0.28	0.03	-
Azotobacter armeniacus IAM 15047^{T}	IAMB129-30	-	-	0.80	0.08	-	0.28	-	-
Azotobacter salinestris AM 15020^{T}	IAMB129-26	-	-	0.75	0.35	-	0.30	-	-
Azorhizophilus paspali IAM 12667^{T}	IAMB13-30	-	-	1.37	-	-	0.34	-	-
(*Azotobacter paspali*)	199-30[a]	-	-	2.16	-	-	0.85	-	-
Psychrobacter phenylpyruvicus IAM 12282^{T}	199-25[a]	-	-	-	0.20	-	1.35	-	-
Psychrobacter urativorans ATCC15174^{T}	199-25[g]	-	-	0.61	0.14	-	0.07	-	-
Psychrobacter frigicola ATCC 700361^{T}	ATCC1849-15[g]	-	-	-	-	-	1.08	-	-
Psychrobacter glacincola ATCC 700754^{T}	MB-15[g]	-	-	0.01	0.06	-	0.86	0.02	-
Psychrobacter immobilis IAM 12280^{T}	NB-25[g]	-	-	0.25	-	-	1.30	-	-
Psychrobacter pacificensis IFO 16270^{T}	MB-25[g]	-	-	0.10	-	-	1.15	-	-
Psychrobacter jeotgali JCM 11463^{T}	MB-30	-	-	0.04	0.20	-	1.60	-	-
Psychrobacter okhotskensis JCM 11840^{T}	MB-20	-	-	0.10	0.07	-	1.26	-	-
Psychrobacter alimentaris JCM 12315^{T}	MB-30	-	-	0.15	-	-	0.96	-	-
Psychrobacter celer JCM 12601^{T}	MB-30	-	-	0.10	0.05	-	1.10	-	-
Moraxella oblonga IAM 14971^{T}	646+SE-37	-	-	0.02	-	-	1.02	-	-
Pseudomonas azotifigens JCM 12708^{T}	JCM443-30	-	-	0.50	0.40	-	0.40	-	-
Pseudomonas beijerinckii IAM 14940^{T}	NB-NaCl-25	-	-	0.01	-	-	1.95	-	-
Pseudomonas pachastrellae JCM 12285^{T}	MB-30	-	-	1.20	-	-	0.80	-	-
Pseudomonas psychrophila JCM 10889^{T}	NB-30	-	-	1.05	-	-	0.65	-	-
Cellvibrio vulgaris NCIMB 8633^{T}	NCIMB16-20	-	-	0.40	-	-	-	-	-
Cellvibrio fulvus NCIMB 8634^{T}	NCIMB16-20	-	-	0.30	-	-	-	-	-
Acinetobacter venetianus MBIC 01332	MB-20	1.50	-	-	-	-	-	-	-
Acinetobacter calcoaceticus IAM 12087^{T}	199-30[j]	2.41	-	-	-	-	-	-	-
Acinetobacter baumannii IAM 12088^{T}	199-30[j]	1.34	-	-	-	-	-	-	-
Acinetobacter johnsonii IAM 1517	199-30[j]	1.35	-	-	-	-	-	-	-
Acinetobacter lwoffii GIFU 1951T	199-30[j]	2.30	-	-	-	-	-	-	-

Acinetobacter radioresistens ATCC 43998^{T}	199-30[j]	2.50	-	-	-	-	-	-	-
Order Thiotrichales									
Thiothrix disciformis JCM 11364^{T}	JCM269-30	-	-	0.77	0.30	-	0.75	-	-
Thiothrix flexilis JCM 11135^{T}	JCM 296-25	-	-	1.72	0.25	-	-	-	-
Thiothrix eikelboomii ATCC 49788^{T}	ATCC1820-20[g]	-	-	-	-	-	1.10	-	-
Thiothrix fructosivorans ATCC 49748^{T}	ATCC1820-20[g]	-	-	-	-	-	1.12	-	-
Thiothrix unzii ATCC 49747^{T}	ATCC1820-20[g]	-	-	-	-	-	1.10	-	-
Thiomicrospira thermophila JCM 12397^{T}	JCM423-30	-	-	0.20	-	-	0.40	-	-
Thiomicrospira crunogena ATCC 35932^{T}	ATCC 1422-26[f]	-	-	0.18	-	-	0.70	-	-
Thiomicrospira pelophila ATCC 27801^{T}	ATCC1036-26[f]	-	-	0.27	-	-	0.22	-	-
Thiomicrospira thyasirae ATCC 51452^{T}	ATCC1891-30[f]	-	-	0.57	-	-	0.03	-	-
Methylophaga sulfidovorance IAM 15035^{T}	MB+methanol	-	-	0.05	-	-	0.10	-	-
Methylophaga marina JCM 6886^{T}	199NaCl-30[k]	-	-	0.20	-	-	3.70	-	-
Methylophaga thalassica JCM 6887^{T}	1999-30[k]	-	-	0.20	-	-	2.00	-	-
Order Xanthomonadales									
Dyella japonica IAM 15069^{T}	NB-25	-	-	0.18	1.30	-	1.25	-	0.47
Pseudoxanthomonas mexicana JCM 11524^{T}	TSB-30	-	-	-	-	-	1.44	-	-
Pseudoxanthomonas japonensis JCM 11525^{T}	TSB-30	-	-	-	-	-	1.37	-	-
Pseudoxanthomonas koreensis IAM 15116^{T}	TSB-30	-	-	-	-	-	-	-	-
	199-30	-	-	-	-	-	-	-	-
Pseudoxanthomonas daejeonensis IAM 15115^{T}	TBS-30	-	-	-	-	-	1.42	-	-
Rhodanobacter fulvus IAM 15025^{T}	NB-25	-	-	-	-	-	0.45	-	0.05
Aquimonas voraii JCM 12896^{T}	TSB-30	-	-	-	-	-	0.80	-	-
Order Chromatiales									
Thiovirga sulfuroxydans JCM 12417^{T}	JCM414-30	-	-	-	-	-	0.80	-	-
Halothiobacillus halophilus ATCC 49870^{T}	ATCC1846-30[c]	-	-	0.60	-	-	1.90	-	-
Halothiobacillus hydrothermalis ATCC 51453^{T}	ATCC1901-37[c]	-	-	0.02	-	-	0.80	-	-
Halothiobacillus neapolitanus JCM 3861^{T}	JCM90-30[c]	-	-	0.88	0.11	-	0.05	-	-
Thioalkalivibrio jannaschii JCM 11372^{T}	JCM314-30	-	-	-	-	-	1.00	-	-
Thioalkalivibrio thiocyanoxidans JCM 11368^{T}	JCM314-30	-	-	-	-	-	1.45	-	-
Thioalkalivibrio paradoxus JCM 11367^{T}	JCM310-30	-	-	-	-	-	1.03	-	-
Rheinheimera pacifica IAM 15043^{T}	MB-15	-	-	1.25	-	-	0.50	-	-
Incertae sedis									
Kangiella aquimarina JCM 12318^{T}	MB-30	-	-	0.47	0.36	-	0.50	-	-
Kangiella koreensis JCM 12317^{T}	MB-30	-	-	0.65	0.42	-	1.08	-	0.60
"*Pseudomonas auricularis*" IAM 12037	NB-30	-	-	0.52	-	-	-	-	-
"*Mesophilobacter marinus*" IAM 13185	199SW-37	-	-	0.60	1.25	-	1.00	-	-
Salinisphaera shabanensis JCM 11575^{T}	JCM339-30	-	-	0.17	-	-	0.82	-	0.60

Order Enterobacteriales

		Dap	H-Put	Put	Cad	NSpd	Spd	Spm	Agm	Ac-Spd
Aquamonas fontana IAM 15072^{T}	BHIB-30	-	-	-	0.10	-	1.10	-	-	-
Erwinia aphidicola IAM 14479^{T}	NB-25	0.50	-	0..60	0.19	-	0.32	-	-	0.05
	199-25	0.57	-	0.85	0.60	-	0.10	-	0.05	0.20
Erwinia chrysanthemi MAFF 30176	199-25[l]	-	-	1.42	0.15	-	0.57	-	-	0.14
Erwinia nigrifluens MAFF 301435	199-25[l]	0.70	-	0.55	0.27	-	0.15	-	0.34	0.01
Erwinia persicinus IAM 12843^{T}	199-25[l]	0.07	-	1.10	0.32	-	0.57	-	-	0.03
Erwinia rhapontici MAFF 301331	199-25[l]	0.90	-	0.30	0.15	-	0.12	-	0.03	-
Pectobacterium carotovorum IAM 12633										
(*Erwinia carotovora*)	NB-25[l]	0.35	-	1.50	0.05	-	0.75	-	-	-

Dap, diaminopropane; H-Put, 2-hydroxyputrescine; Put, putrescine; Cad, cadaverine; Spd, spermidine; HSpd, homospermidine; Spm, spermine; Agm, agmatine; Ac-Spd, N^1–acetylspermidine; -, not detected (<0.005); ^{T}, Type strain; IAM, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan; JCM, Japan Collection of Microorganisms, RIKEN, Wako, Saitama, Japan; IFO, Institute for Fermentation, Osaka, Japan; NBRC, Biological Resource Center, National Institute of Technology and Evaluation, Kisarazu, Japan; MBIC, Marine Biotechnology Institute Culture Collection, Kamaishi, Iwate, Japan; ATCC, American Type Culture Collection, Manassas, Virginia, USA; NCIMB, National Collections of Industrial, Foods and Marine Bacteria, Scotland, UK. Former names are shown in parentheses. Quotation marks indicate that the scientific name has not been validly published. [a] was cited from Hamana & Takeuchi, 1998, [b] Hamana *et al.*, 2001, [c] Hamana, 2000, [d] Hamana & Matsuzaki, 1993, [e] Hamana, 1997, [f] Hamana *et al.*, 2000, [g] Hamana *et al.*, 2003, [h] Hamana *et al.*, 1988, [i] Hamana *et al.*, 1994, [j] Hamana & Matsuzaki, 1992, [k] Hamana & Kishimoto, 1996, [l] Hamana, 1996.

koreensis as well as two *Colwellia* species within the Gammaproteobacteria, is unclear.

Order Chromatiales

Three *Thioalkalivibrio* species and *Thiovirga sulfuroxydans* ubiquitously contained spermidine alone. *Rheinheimera pacifica* as well as *Halothiobacillus* species contained putrescine and spermidine.

Order Enterobacteriales

A new *Erwinia* species analyzed in the present study, as well as other four spcies and a related *Pectobacterium* species, contained acetylspermidine and diaminopropane. It has been reported that these two polyamines were serectively distributed in the family Enterobacteriaceae of this order of the class Gammaproteobacteria[18]. However, *Aquamonas fontana* located in this order contained putrescine and spermidine.

Others

Salinisphaera species analyzed in the present study contained putrescine, cadaverine and agmatine.

Class Deltaproteobacteria (Table 4)

Within the class Deltaproteobacteria, many species belonging to the heterotrophic order Myxoccocales contained homospermidine, whereas previously analyzed other deltaproteobacteria belonging to other six orders Desulfobacterales, Desulfovibrionales, Desulfuromonadales, Syntrophobacterales, Desulfurellales and Bdellovibrionales contained spermidine as the major polyamine[6, 12]. In the present analysis, sulfate-reducing *Desulfobacterium cetonicum* belonging to the order Desulfobacterales contained cadaverine and homospermidine whereas other two *Desulfobacterium* species contained cadaverine and spermidine. The occurrence of homospermidine in *D. cetonicum* is the first datum and is unique within this order.

In the order Desulfuromonadales, thermophilic *Geothermobacter ehrlichii* belonging to the Fe(Ⅲ)-reducing family Geobacteraceae contained spermidine and spermine as the major polyamines. On the other hand, psychrotolerant *Geopsychrobacter electrodiphilus* belonging to Geobacteraceae as well as mesophilic *Geobacter* and *Pelobacter* species, contained spermidine alone. New species of the sulfate-reducing genus *Desulfovibrio* of the order Desulfovibrionales, *D. brasiliensis*, *D. aerotolerans*, *D. alkalitolerans*, *D. frigidus* and *D. ferrireducens*, as well as other four *Desulfovibrio* species analyzed previously[6, 12], contained spermidine. Although thermophilic *Desulfurella acetivorans* (the order Desulfurellales) and *Desulfacinum infernum* (the order Syntrophobacterales) were poor in spermine, the major occurrence of spermine was found in a thermophile, *Geothermobacter ehrlichii*, in the present study. Distribution of cadaverine and agmatine seems to be genus- or species-specific in this class.

Class Epsilonproteobacteria (Table 4)

Hydrogen-oxidizing *Hydrogenimonas thermophila*, a novel thermophile growing at 55℃, is located in the order Campylobacterales, and contained spermidine and spermine as the major polyamines. Mesophilic sulfur-oxidizing *Sulfurimonas autotrophica* and *Sulfuricurvum kujiense* located in the order Campylobacterales, contained spermidine alone. Other various heterotrophic, mesophilic epsilonproteobacteria belonging to the families Campylobacteraceae and Helicobacteraceae of the order Campylobacterales, contained also spermidine as the major polyamine[6, 12].

Spermine was found as a major polyamine in two thermophilic nitrate-ammonifying *Caminibacter* species and a thermophilic, acidophilic hydrogen-oxidizing *Lebetimonas* species of the new order Nautiliales. Mesophilic nitrate-reducing *Nitratifractor salsuginis* and mesophilic sulfur-oxidizing *Sulfurovum lithotrophicum* and sulfur-reducing *Thioreductor micantisoli* contained spermidine alone. Thermophilic nitrate-reducing *Nitratiruptor tergarcus* contained spermine as the main polyamine component. Polyamines of various chemolithoautotrophs belonging to deltaproteobacteria and epsilonproteobacteria have been analyzed. However, polyamine profiles related to their optimum growth temperature rather than their chemolithoautotrophic type. The occurrence of spermine in the chemolithotrophic , thermophilic deltaproteobacteria growing at 50-60℃ and epsilonproteobacteria growing at 50-55℃ was

Table 4. Cellular concentrations of polyamines of deltaproteobacteria and epsilonproteobacteria

Organism	Medium-Temp (℃)	Polyamine (μ mol/g wet wt cell): Dap	Put	Cad	Spd	HSpd	Spm	Agm
Deltaproteobacteria								
Order Desulfobacterales								
Desulfobacterium cetonicum JCM 12296[T]	JCM395-30	-	-	0.20	-	0.72	-	-
Desulfobacterium vacuolatum JCM 12295[T]	JCM394-30	-	-	0.75	0.85	-	-	-
Desulfobacterium niacini JCM 12294[T]	JCM393-30	-	-	0.79	0.65	-	-	-
Desulfococcus multivorans NCIMB 12965[T]	NCIMB295-30[b]	-	0.02	-	0.70	-	0.04	-
Desulfobulbus propionicus NCIMB 12907[T]	NCIMB17-37[b]	-	0.02	-	0.88	-	0.04	-
Order Desulfuromonadales								
Geothermobacter ehrlichii JCM 12418[T]	JCM416-50	-	-	-	0.40	-	0.90	-
Geobacter sulfurreducens ATCC 51573[T]	ATCC1957-30[b]	-	-	-	1.10	-	-	0.03
Geobacter hydrogenophilus ATCC 51590[T]	ATCC1957-30[b]	-	-	-	0.92	-	-	0.10
Geopsychrobacter electrodiphilus JCM 12469[T]	JCM425-22	-	-	-	0.52	-	-	-
Pelobacter acidigallici ATCC 49970[T]	ATCC1852-30[b]	-	-	-	1.45	-	-	0.24
Pelobacter masseliensis ATCC 49973[T]	ATCC1852-30[b]	-	-	-	1.20	-	-	0.20
Desulfuromonas svalbardensis JCM 12927[T]	JCM463-15	-	-	-	0.44	-	-	-
Desulfuromusa ferrireducens JCM 12926[T]	JCM463-15	-	-	-	0.60	-	-	-
Order Desulfurellales								
Desulfurella acetivorans ATCC 51451[T]	ATCC 1920-55[b]	-	-	-	0.97	-	0.02	-
Order Desulfovibrionales								
Desulfovibrio aerotolerans JCM 12613[T]	JCM439-30	-	-	-	0.40	-	-	-
Desulfovibrio alkalitolerans JCM 12612[T]	JCM438-40	-	-	-	0.42	-	-	-
Desulfovibrio brasiliensis JCM 12178[T]	JCM383-30	-	-	-	0.52	-	-	-
Desulfovibrio ferrireducens JCM 12925[T]	JCM462-20	-	-	-	0.29	-	-	-
Desulfovibrio frigidus JCM 12924[T]	JCM462-20	-	-	-	0.44	-	-	-
Desulfovibrio africanus NCIMB 8401[T]	NCIMB17-37[a]	0.01	0.12	0.02	0.95	-	0.02	0.03
Desulfovibrio desulfuricans NCIMB 8307[T]	NCIMB17-30[b]	-	0.24	1.98	0.40	-	-	-
Desulfovibrio salexigens NCIMB 8403[T]	NCIMB104-30[b]	-	0.05	0.04	0.65	-	-	0.20
Desulfovibrio vulgaris NCIMB 9442[T]	NCIMB17-30[b]	-	0.02	0.01	1.50	-	0.10	0.03
Order Syntrophobacterales								
Desulfacinum infernum NCIMB 13416[T]	NCIMB109-60[b]	-	0.02	-	0.68	-	0.06	-
Epsilonproteobacteria								
Order Campylobacterales								
Sulfurimonas autotrophica JCM 11897[T]	JCM365-24	-	-	-	0.75	-	-	-
Sulfurimonas denitrificans ATCC 33889[T]	ATCC1255-26[b]	-	-	0.40	2.10	-	-	-
(*Thiomicrospira denitrificans*)								
Sulfuricurvum kujiense JCM 11577[T]	JCM340-25	-	-	-	0.50	-	-	-
Hydrogenimonas thermophila JCM 11971[T]	JCM369-55	-	0.02	-	0.70	-	0.17	-
Sulfurospirillum barnesii ATCC 700032[T]	ATCC2034-30[b]	-	-	-	1.10	-	0.01	-
Sulfurospirillum arsenophilum ATCC 700056[T]	ATCC2018-30[b]	0.02	-	0.85	0.10	-	0.03	-
Sulfurospirillum deleyianum ATCC 51133[T]	ATCC1842-30[a]	-	-	-	1.25	-	-	-
Order Nautiliales								
Caminibacter profundus JCM 12957[T]	JCM367-55	-	0.27	-	0.12	-	0.75	-
Caminibacter mediatlanticus JCM 12641[T]	JCM440-55	-	0.04	-	0.04	-	0.70	0.04
Lebetimonas acidiphila JCM 12420[T]	JCM417-50	-	-	0.10	0.40	-	0.70	-
Incertae sedis								
Nitratiruptor tergarcus JCM 12459[T]	JCM421-55	-	-	-	0.02	-	1.00	-
Nitratifractor salsuginis JCM 12458[T]	JCM421-37	-	-	-	0.54	-	-	-
Sulfurovum lithotrophicum JCM 12117[T]	JCM374-28	-	-	-	0.25	-	-	-
Thioreductor micantisoli JCM 12457[T]	JCM420-32	-	-	-	0.40	-	-	-

Dap, diaminopropane; Put, putrescine; Cad, cadaverine; Spd, spermidine; HSpd, homospermidine; Spm, spermine; Agm, agmatine; -, not detected (<0.005); [T], Type strain; ATCC, American Type Culture Collection, Manassas, Virginia, USA; JCM, Japan Collection of Microorganisms, RIKEN, Wako, Saitama, Japan; NCIMB, National Collection of Industry, Marine and Food Bacteria, Scotland, UK. [a] was cited from Hamana & Takeuchi, 1998, [b] Hamana *et al.*, 2004.

observed.

Polyamine distribution profiles in the phylum Proteobacteria

Homospermidine was distributed as the major polyamine in the alphaproteobacteria belonging to the nine genera, *Sphingomonas* and *Sphingosinicella* within the order Sphingomonadales, *Dinoroseobacter* within the order Rhodobacterales, *Azospirillum* and *Tistrella* within the order Rhodospirillales, *Rhodopseudomonas* and *Pseudaminobacter* within the order Rhizobiales, and *Asticcacaulis* and *Brevundimonas* within the order Caulobacterales. The three triamine profiles, homospermidine-dominant type, spermidine-dominant type and homospermidine/spermidine-dominant type, were genus-specific. These results suggest the varieties of their triamine profiles and the usefulness of triamine pattern as a phenotypic marker in the class Alphapoteobacteria. The occurrence of homospermidine instead of spermidine was not ubiquitous in the genera *Sphingomonas* and *Brevundimonas*, indicating that chemotaxonomic usefulness of polyamine profile is not agree with the criteria of these genera. Spermidine and homospermidine are produced by the different pathways, aminopropyl transfer or aminobutyl transfer, however, the two biosynthetic activities correlate to their phylogenetic locations at genus level within the class Alphaproteobacteria.

All betaproteobacteria analyzed ubiquitously contained hydroxyputrescine and putrescine. Spermidine was sporadicaly distributed in some members. Norspermidine and homospermidine were not found. Since hydroxyputrescine was almost selectively distributed in the class Betaproteobacteria including 66 species previously analyzed[6, 8], the occurrence of hydroxyputrescine in 46 strains also serve as a chemotaxonomic marker for betaproteobacteria within the phylum Proteobacteria. Putrescine is produced by ornithine decarboxylation and then hydroxylated. When spermidine was synthesized from putrescine, hydroxyspermidine was also produced from hydroxyputrescine. In addition to spermidine, spermine was dominant in a thermophilic *Hydrogenophilus* species within this class.

Norspermidine was the major polyamine in the *Photobacterium* and *Vibrio* species analyzed, belonging to the order Vibrionales, and was detected as a major polyamine in two species of *Psychromonas* and one species of *Moritella*, within the order Alteromonadales. In the order Alteromonadales, the genera *Shewanella*, *Colwellia* and *Marinobacter* were heterogeneous in their hydroxyputrescine, cadaverine and spermidine levels. Spermidine was the major polyamine in the other six orders Oceanospirillales, Pseudomonadales, Thiotrichales, Xanthomonadales, Chromatiales and Enterobacteriales. Homospermidine was not found in the all gammaproteobacteria analyzed. Diaminopropane was the main component in *Acinetobacter* of Pseudomonadales[23] and a major polyamine in *Erwinia* and *Pectobacterium* of Enterobacteriales[18]. Diaminopropane and norspermidine, produced from diaminopropane by an aminopropylation, were selectively detected in some genera. These polyamine distribution profiles serve for the chemotaxonomy of gammaproteobacteria, however, the profiles are conflicting in some genera.

Desulfobacterium cetanicum contained homospermidine and other deltaproteobacteria, *Desulfococcus*, *Geothermobacter*, *Geopsychrobacter* and *Desulfovibrio* species, contained spermidine as the major polyamine. Cadaverine distributed in *Desulfobacterium*, *Desulfococcus* and *Desulfovibrio* species. Spermidine was found as a major polyamine in epsilonproteobacteria. Spermine was detected in a novel thermophile of *Geothermobacter* within the class Deltaproteobacteria and in the five novel thermophiles belonging to the genera *Hydrogenimonas*, *Caminibacter*, *Lebetimonas* and *Nitratiruptor* of the class Epsilonproteobacteria, suggesting that the occurrence of spermine correlate to their thermophily. The widespread distribution of moderate thermophiles containing spermine within the phylum Proteobacteria is suggested. Additional data on cellular polyamine catalogues, shown in the present study, serve as a chemotaxonomy for further classification of the phylum Proteobacteria.

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