

Polyamine Profiles of Some Members of the Gamma Subclass of the Class *Proteobacteria* : Polyamine Analysis of Twelve Recently Described Genera

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Cellular polyamines of 65 recently described species (12 genera) belonging to the gamma subclass of the class *Proteobacteria* were analyzed by HPLC. *Thiothrix* species belonging to the gamma-1 subgroup and *Xanthomonas* species belonging to the gamma-2 subgroup ubiquitously contained spermidine alone. A gamma-2 proteobacterium, *Stenotrophomonas*, contained cadaverine and spermidine. In the gamma-3 subgroup, *Photorhabdus* and *Xenorhabdus* located in the family *Enterobacteriaceae*, ubiquitously contained putrescine and spermidine and sporadically contained cadaverine. Putrescine and spermidine were the major polyamines in all 21 authentic species of *Pseudomonas* and cadaverine was found in a half of them. *Psychrobacter* and *Moraxella* species are located in the family *Moraxellaceae* and the former ubiquitously contained spermidine as the major polyamine, however, the polyamine patterns within the latter were heterogeneous, showing the occurrence of diaminopropane and / or norspermidine as the major polyamine in seven species among the 13 species tested. Putrescine alone, spermidine alone, 2-hydroxyputrescine / putrescine, putrescine / cadaverine, and putrescine / spermidine were found as the polyamine profiles of *Shewanella* species, indicating heterogeneous polyamine patterns within the genus. *Oceanomonas baumannii* and *O. doudoroffi* contained putrescine, cadaverine and spermidine. *Cardiobacterium* and *Suttonella* belonging to the family *Cardiobacteriaceae* contained diaminopropane and spermidine. The present data serve for the polyamine catalogues of gamma proteobacteria, as a chemotaxonomic marker.

Key words : polyamine, 2-hydroxyputrescine, cadaverine, spermidine, proteobacteria

INTRODUCTION

Cellular polyamine compositions have been shown to serve as chemotaxonomic markers within the alpha, beta, gamma, delta and epsilon subclasses of the class *Proteobacteria* (6, 7, 12-18, 20-22). The polyamine profiles of the gamma proteobacteria, which is composed of three subgroups based on the 16S rDNA phylogenetic analysis, were correlated with their phylogenetic classifications, however, they

were more complicated than the polyamine patterns within other subclasses (12, 13, 15, 17, 18, 22).

In the present study, we analyzed polyamines of 65 recently established or reclassified gamma proteobacteria belonging to the 12 genera, *Thiothrix* (subgroup-1), *Xanthomonas*, *Stenotrophomonas* (subgroup-2), *Photorhabdus*, *Xenorhabdus*, *Pseudomonas*, *Psychrobacter*, *Moraxella*, *Oceanomonas*, *Shewanella*, *Cardiobacterium* and *Suttonella* (subgroup-3), to elucidate variations in their polyamine profiles, and to evaluate the usefulness of polyamine pattern as a

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phenotypic marker.

MATERIALS AND METHODS

The gamma proteobacteria analyzed are shown in Table 1. They were grown at the optimum growth temperature in the media designated by the culture collections as shown by Medium No., organic complex media such as Marine broth (MB) (DIFCO Ltd, Detroit, USA), Nutrient broth (NB) (Nissui Pharmaceutical Co Ltd., Tokyo, Japan), Brain heart infusion broth (BHIB) (Nissui), Trypticase soy broth (TSB) (BBL, Bectone Dickinson and Company, Cockeysville, MD, USA) or peptone-yeast extract medium (PY). Stationary growing cells were harvested by centrifugation. The pellets of organisms were homogenized in 0.5 M perchloric acid (HClO₄) at 2 °C. Polyamines were extracted into HClO₄ and analyzed by high-performance liquid chromatography (HPLC) on an L6000 High-Speed Liquid Chromatograph (Hitachi Ltd., Tokyo, Japan) (14, 18).

RESULTS AND DISCUSSION

Typical chromatograms of the polyamine fraction by HPLC are shown in Fig. 1. 2-Hydroxyputrescine, 1,3-diaminopropane (diaminopropane), putrescine, cadaverine, spermidine, norspermidine, homospermidine, agmatine and spermine were separately eluted in the HPLC. Estimated cellular concentrations of the polyamines are listed in Table 1.

Gamma subgroup-1

Filamentous sulfur bacteria of the families *Chromatiaceae*, *Thiothrix unzii*, *T. fructosivorans* and *T. eikelboomii*, ubiquitously contained spermidine as the major polyamine. This pattern is similar to that found in four other genera (*Allochromatium*, *Thermochromatium*, *Thiocapsa* and *Thiohalocapsa*) of the family *Chromatiaceae* (22). In other gamma proteobacteria of subgroup-1, *Halorhodospira* and *Ectothiorhodospira* belonging to the family *Ectothiorhodospiraceae* (of purple sulfur bacteria) contained spermidine and spermine, *Thiomicrospira* (of sulfur-oxidizing bacteria) contained putrescine and spermidine, and *Beggiatoa* belonging to the family *Beggiatoaceae* (a group of sulfur-depositing bac-

teria) contained putrescine, spermidine and homospermidine (18, 22).

Gamma subgroup-2

The phytopathogenic genus *Xanthomonas* is phylogenetically divided into three clusters (23, 30). Except for *X. oryzae* IAM 1657, all the *Xanthomonas* strains examined contained spermidine alone as the major polyamine, as previously reported in some species (16, 33). *X. oryzae* IAM 1657 showed the occurrence of 2-hydroxyputrescine and putrescine, and the pattern was the typical profile of beta proteobacteria. Therefore, precise taxonomic investigation is necessary for the correct assignment of the strain IAM 1657. *Xanthomonas* species was distinguished from a related gamma proteobacterium, *Stenotrophomonas maltophilia* (formerly *X. maltophilia*), which contains cadaverine and spermidine in its polyamine profiles.

Gamma subgroup-3

Photorhabdus and *Xenorhabdus* species belong to the family *Enterobacteriaceae* and form entomopathogenic symbioses with soil nematodes (10, 11). Putrescine and spermidine were the major polyamines in the three *photorhabdus* species and five *Xenorhabdus* species. A significant amount of cadaverine distributed in *X. japonicus* and *X. poinarii* among them. Although *Photorhabdus* and *Xenorhabdus* are located in a branch together with the genus *Proteus*, the *Proteus* species lack spermidine (12). Sporadic occurrence of spermidine in the family *Enterobacteriaceae* has been reported (11). Diaminopropane, which is widespread within this family (11, 33), was not detected in the *Photorhabdus* and *Xenorhabdus* species.

Pseudomonas species are phylogenetically divided into seven subclusters, *P. aeruginosa* group, *P. chlororaphis* group, *P. fluorescens* group, *P. pertucinogena* group, *P. putida* group, *P. stutzeri* group and *P. syringae* group (1,34). The *Pseudomonas* strains of 21 species distributing over the seven subclusters ubiquitously contained putrescine and spermidine. Cadaverine was sporadically detected in 12 species but was widely distributed within the seven sub-

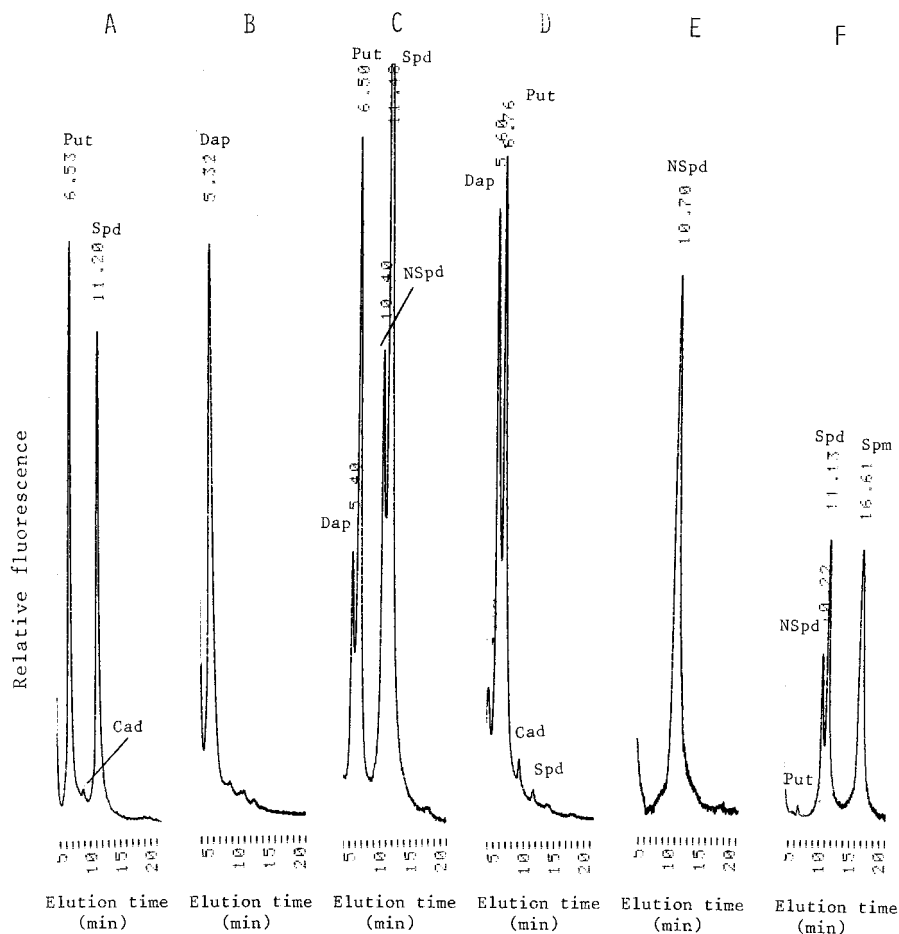


Fig. 1. HPLC analysis of polyamines extracted from *Moraxella lincolnii* ATCC 51388(A), *M. caprae* ATCC 700019(B), *M. catarrhalis* ATCC 25238(C), *M. osloensis* ATCC 19976(D), *M. ovis* ATCC 33078(E) and *M. cuniculi* ATCC 14688 (F).

Printed numbers on elution peaks indicate the elution time (min) of the peak. Abbreviations for polyamines are shown in Table 1.

clusters.

The family *Moraxellaceae* formed a distinct clade consisting of four phylogenetic groups, I (*Moraxella osloensis*, *Moraxella atlantae*), II (nine species of *Moraxella*), III (*Psychrobacter* species) and IV (*Acinetobacter* species) (2, 4, 8, 25, 27–29). Polyamine profiles among 13 species of the genus *Moraxella* were heterogeneous, as shown in Fig. 1 and Table 1. Seven members contained diaminopropane and some of them contained norspermidine as a major triamine. Two *Moraxella* species belonging to group I and *Moraxella lincolnii*, located in another separate group within the family (28), con-

tained putrescine and spermidine. Four species of the eight *Moraxella* species of group II (*M. canis*, *M. catarrhalis*, *M. nonliquefaciens*, *M. equi*, *M. lacunata*, *M. caviae*, *M. ovis*, *M. cuniculi*), analyzed in this study, contained norspermidine in addition to putrescine and spermidine. In *Moraxella cuniculi* in group II, a significant amount of spermine was found. *Moraxella* species of group II are phylogenetically separated into two clusters (28). *M. boevrei* contained diaminopropane and spermidine, and *M. caprae* contained diaminopropane alone, however, a phylogenetic analysis of the two has not been conducted. *Acinetobacter* species (group IV) contained

Table 1. Polyamine concentration within the gamma subclass of *Proteobacteria*

Organism	Medium	Temp (°C)	Polyamines (μmol/g wet cell)							
			Dap	H-Put	Put	Cad	Spd	NSpd	Spm	Agm
Gamma proteobacteria subgroup-1										
<i>Thiothrix eikelboomii</i>	ATCC 49788 ^T	1820-20	-	-	-	-	1.10	-	-	-
<i>Thiothrix fructosivorans</i>	ATCC 49748 ^T	1820-20	-	-	-	-	1.12	-	-	-
<i>Thiothrix unzii</i>	ATCC 49747 ^T	1820-10	-	-	-	-	1.10	-	-	-
Gamma proteobacteria subgroup-2										
<i>Xanthomonas arboricola</i>	IFO 13557	(b)	-	-	-	-	0.93	-	-	-
<i>Xanthomonas campestris</i>	IFO 13551	(b)	-	-	-	-	0.76	-	-	-
" <i>Xanthomonas physalidicola</i> "	IFO 13555	(b)	-	-	-	-	1.36	-	-	-
<i>Xanthomonas pisi</i>	IFO 13556	(b)	-	-	-	-	1.04	-	-	-
<i>Xanthomonas axonopodis</i>	IFO 12213	PY-30	-	-	-	-	1.40	-	-	-
	IFO 13553	PY-30	-	-	-	-	1.28	-	-	-
<i>Xanthomonas oryzae</i>	IFO 3312	NB-25	-	-	-	-	1.66	-	0.01	-
	IFO 3510	NB-25	-	-	-	-	1.63	-	0.01	-
	IFO 3827	NB-25	-	-	-	-	1.57	-	0.02	-
	IFO 3828	NB-25	-	-	-	-	1.65	-	0.06	0.03
	IFO 3895	NB-25	-	-	-	-	1.40	-	-	-
	IAM 1657	NB-25	-	0.50	0.98	-	-	-	-	-
<i>Xanthomonas translucens</i>	IFO 13559	PY-30	-	-	-	-	1.10	-	-	-
	IFO 13558	(b)	-	-	-	-	0.55	-	-	-
<i>Stenotrophomonas maltophilia</i> (<i>Xanthomonas maltophilia</i>)	IAM 12423 ^T	(b)	-	-	-	0.70	4.00	-	-	-
Gamma proteobacteria subgroup-3										
<i>Photorhabdus asymbiotica</i>	ATCC 43950 ^T	PY-26	-	-	1.74	-	0.08	-	-	-
<i>Photorhabdus luminescens</i>	NCIMB 12670	BHIB-30	-	-	0.85	0.01	0.02	-	-	-
<i>Photorhabdus temperata</i>	ATCC 29304 ^T	NB-26	-	-	1.30	-	0.05	-	-	-
<i>Xenorhabdus beddingii</i>	ATCC 49542 ^T	NB-26	-	-	1.40	0.02	0.27	-	-	-
<i>Xenorhabdus bovienii</i>	ATCC 35271 ^T	PY-26	-	-	1.45	-	0.37	-	-	-
<i>Xenorhabdus japonicus</i>	IAM 14265 ^T	NB-28	-	-	1.40	0.08	0.45	-	-	-
<i>Xenorhabdus nematophilus</i>	NCIMB 9965 ^T	NB-25	-	-	0.57	0.01	0.28	-	-	-
<i>Xenorhabdus poinarii</i>	ATCC 35272 ^T	PY-26	-	-	1.50	0.95	0.55	-	-	-
<i>Pseudomonas aeruginosa</i> group										
<i>Pseudomonas aeruginosa</i>	IAM 1514 ^T	NB-30	-	-	0.80	0.51	0.40	-	-	-
<i>Pseudomonas alcaligenes</i>	IAM 12411 ^T	NB-30	-	-	0.92	0.25	0.40	-	-	-
<i>Pseudomonas alcaliphila</i>	IAM 14884 ^T	NB-25	-	-	1.25	0.34	1.02	-	-	-
<i>Pseudomonas mendocina</i>	JCM 5966 ^T	NB-37	-	-	0.74	0.50	1.46	-	-	-
<i>Pseudomonas nitroreducens</i>	IAM 1439 ^T	NB-25	-	-	0.85	-	0.70	-	-	-
<i>Pseudomonas oleovorans</i>	IAM 1508 ^T	NB-25	-	-	0.90	-	0.80	-	-	-
<i>Pseudomonas pseudoalcaligenes</i>	IAM 12410	NB-25	-	-	0.54	-	0.46	-	-	-
<i>Pseudomonas straminea</i>	IAM 1598 ^T	NB-25	-	-	0.27	0.28	0.62	-	-	-
<i>Pseudomonas chlororaphis</i> group										
<i>Pseudomonas chlororaphis</i>	IAM 12354 ^T	NB-25	-	-	1.02	0.26	0.57	-	-	-
<i>Pseudomonas fragi</i>	IAM 1650	NB-25	-	-	0.45	-	0.27	-	-	-
<i>Pseudomonas taetrolens</i>	IAM 1653 ^T	NB-25	-	-	0.60	-	0.50	-	-	-
<i>Pseudomonas fluorescens</i> group										
<i>Pseudomonas fluorescens</i>	IAM 12022 ^T	NB-25	-	-	0.90	-	0.58	-	-	-
<i>Pseudomonas azotoformans</i>	IAM 1603 ^T	NB-25	-	-	0.76	0.04	0.52	-	-	-
<i>Pseudomonas mucidolens</i>	IAM 12406 ^T	NB-25	-	-	0.82	-	0.84	-	-	-
<i>Pseudomonas pertucinogena</i> group										
<i>Pseudomonas pertucinogena</i>	IFO 14163 ^T	PY-30	-	-	1.10	1.05	0.35	-	-	-
<i>Pseudomonas putida</i> group										
<i>Pseudomonas putida</i>	IAM 1236 ^T	NB-25	-	-	0.72	-	0.52	-	-	-
<i>Pseudomonas fluva</i>	IAM 1529 ^T	NB-25	-	-	0.50	-	0.50	-	-	-
<i>Pseudomonas stutzeri</i> group										
<i>Pseudomonas stutzeri</i>	IAM 12668 ^T	NB-25	-	-	0.69	0.05	0.60	-	-	-

Pseudomonas syringae group

<i>Pseudomonas syringae</i>	IFO 3310	PY-30	-	-	0.90	-	0.40	-	-	-
<i>Pseudomonas ficuserectae</i>	JCM 2440 ^T	NB-28	-	-	0.56	-	0.44	-	-	-
<i>Pseudomonas synxantha</i>	IAM 12356 ^T	NB-25	-	-	0.72	0.02	0.42	-	-	-
<i>Psychrobacter phenylpyruvicus</i> (<i>Moraxella phenylpyruvica</i>)	IAM 12282 ^T	(d)	-	-	-	0.20	1.35	-	-	-
<i>Psychrobacter urativorans</i> (<i>Micrococcus cryophilus</i>)	ATCC 15174 ^T	(a)	-	-	0.61	0.14	0.07	-	-	-
<i>Psychrobacter frigidicola</i>	ATCC 700361 ^T	1849-15	-	-	-	-	1.08	-	-	-
<i>Psychrobacter glacincola</i>	ATCC 700754 ^T	MB-15	-	-	0.01	0.06	0.86	-	0.02	-
<i>Psychrobacter immobilis</i>	IAM 12280 ^T	NB-25	-	-	0.25	-	1.30	-	-	-
<i>Psychrobacter pacificensis</i>	IFO 16270	MB-25	-	-	0.10	-	1.15	-	-	-
<i>Psychrobacter</i> sp. (<i>Micrococcus cryophilus</i>)	IAM 12030	PY-10	-	-	0.10	0.04	0.85	-	0.04	-
<i>Psychrobacter</i> sp. (<i>Iizukaella psychrophila</i>)	IFO 15753	PY-30	-	-	0.37	0.04	0.60	-	0.01	-
<i>Moraxella atlantae</i>	IFO 14588 ^T	(d)	-	-	0.15	-	2.88	-	-	-
<i>Moraxella equi</i> (<i>Moraxella bovis</i>)	IAM 12313 ^T	(d)	-	-	-	-	1.10	-	-	-
<i>Moraxella nonliquefaciens</i>	IAM 12281 ^T	(d)	-	-	-	-	1.30	-	-	-
<i>Moraxella boevrei</i>	ATCC 700022 ^T	BHIB-30	0.32	-	-	-	0.84	-	-	-
<i>Moraxella canis</i>	ATCC 51391 ^T	BHIB-30	0.20	-	0.50	-	1.35	-	-	-
<i>Moraxella caprae</i>	ATCC 700019 ^T	BHIB-30	1.10	-	-	-	-	-	-	-
<i>Moraxella catarrhalis</i>	ATCC 25238 ^T	BHIB-30	0.35	-	0.75	-	0.96	0.35	-	-
<i>Moraxella caviae</i>	ATCC 14659 ^T	BHIA-30	0.20	-	0.01	-	0.02	0.75	0.04	-
<i>Moraxella cuniculi</i>	ATCC 14688 ^T	BHIA-30	-	-	0.02	-	0.32	0.57	0.52	-
<i>Moraxella lacunata</i>	ATCC 17967 ^T	260-30	0.56	-	0.02	-	0.04	-	-	-
<i>Moraxella lincolni</i>	ATCC 51388 ^T	260-30	-	-	1.15	0.01	0.95	-	-	-
<i>Moraxella osloensis</i>	ATCC 19976 ^T	BHIB-30	1.05	-	1.25	0.03	0.02	-	-	-
<i>Moraxella ovis</i>	ATCC 33078 ^T	BHIB-30	-	-	-	-	-	1.05	-	-
<i>Oceanomonas baumannii</i>	NCIMB 13685 ^T	TSB-25	-	-	0.20	0.01	0.46	-	-	-
<i>Oceanomonas doudoroffii</i> (<i>Pseudomonas doudoroffii</i>)	IAM 14168 ^T	MB-25	-	-	0.38	0.04	0.90	-	-	-
<i>Shewanella algae</i>	IAM 14159 ^T	(c)	-	-	1.45	0.50	-	-	-	-
<i>Shewanella baltica</i>	NCIMB 1733	(e)	-	-	1.20	1.50	-	-	-	-
<i>Shewanella benthica</i>	JCM 10176	(e)	-	0.25	0.45	-	-	-	-	-
	JCM 10264	(a)	-	-	0.77	-	-	-	-	-
	ATCC 43992 ^T	(e)	-	-	0.25	-	-	-	-	-
<i>Shewanella frigidimarina</i>	NCIMB 400	(e)	-	-	0.80	-	0.02	-	-	-
<i>Shewanella hanedai</i>	IAM 12641 ^T	(c)	-	-	1.50	-	-	-	-	-
<i>Shewanella putrefaciens</i>	IAM 12079 ^T	(c)	-	-	3.00	-	-	-	-	-
<i>Shewanella violacea</i>	JCM 10179 ^T	(e)	-	0.60	0.45	-	-	-	-	-
<i>Shewanella woodyi</i>	NCIMB 13526 ^T	(e)	-	-	0.50	-	0.25	-	-	-
<i>Shewanella amazonensis</i>	ATCC 700329 ^T	1065-30	-	-	1.05	-	0.08	-	-	-
<i>Shewanella pealeana</i>	ATCC 700345 ^T	MB-30	-	-	0.67	-	-	-	-	-
<i>Shewanella oneidensis</i>	ATCC 700550 ^T	TSB-30	-	-	1.50	1.10	-	-	-	-
<i>Shewanella gelidimarina</i>	ATCC 700752 ^T	MB-30	-	-	0.01	0.01	1.12	-	-	-
<i>Cardiobacterium hominis</i>	ATCC 15826 ^T	BHIB-37	0.02	-	-	-	0.40	-	-	-
<i>Suttonella indologenes</i>	ATCC 25869 ^T	BA-37	0.18	-	0.04	-	0.52	-	-	-

Note : Dap, 1, 3-diaminopropane ; H-Put, 2-hydroxyputrescine ; Put, putrescine ; Cad, cadaverine ; Spd, spermidine ; NSpd, norspermidine ; Spm, spermine ; Agm, agmatine ; IAM, IAM Culture Collection, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan ; IFO, Institute for Fermentation, Osaka, Japan ; JCM, Japan Collection of Microorganisms, RIKEN, Saitama, Japan ; NCIMB, The National Collections of Industrial and Marine Bacteria, Aberdeen, Scotland, UK ; ATCC, American Type Culture Collection, Manassas, Virginia, USA ; ^T, Type strain ; -, not detectable (<0.005). Former names are shown in parentheses. Quotation marks indicate the scientific name has not been validly published. Abbreviations for media are shown in MATERIALS AND METHODS. a, cited from Hamana et al. (18). b, cited from Hamana and Matsuzaki (15). c, cited from Hamana et al. (12). d, cited from Hamana and Takeuchi (17). e, cited from Hamana et al. (21).

diaminopropane alone (15). Spermidine was ubiquitously detected in the six psychrophilic *Psychrobacter* species (group III). Cellular concentration of putrescine and cadaverine as well as spermidine level varied among the six species.

Reclassification of *Pseudomonas doudoroffii* into a new genus *Oceanomonas* and a description of *Oceanomonas baumannii* were presented (5). The genus *Oceanomonas* is phylogenetically located near the three families *Vibrionaceae*, *Enterobacteriaceae* and *Aeromonadaceae* (5) and contained putrescine, cadaverine and spermidine. This polyamine pattern is different from the profiles of the three families containing diaminopropane in addition to spermidine and/or norspermidine.

Since four different polyamine patterns have been reported in eight *Shewanella* species (13, 18, 22), polyamine profiles of four newly validated species (3, 26, 31) were analyzed. In the previous studies, putrescine and cadaverine were found in *S. algae* and *S. baltica*, 2-hydroxyputrescine and putrescine in *S. baltica* and *S. violacea*, putrescine alone in *S. frigidimarina*, *S. hanedai* and *S. putrefaciens*, and putrescine and spermidine in *S. woodyi*. In the four new species, *S. amazonensis* and *S. pealeana* contained putrescine alone, *S. oneidensis* putrescine and cadaverine, and *S. gelidimarina* contained spermidine alone. Thus, variation in polyamine profile has been observed within the genus *Shewanella*; however, diaminopropane, norspermidine and homospermidine have never been found in the genus.

Cardiobacterium hominis and *Suttonella indologenes* contained diaminopropane in addition to spermidine. Norspermidine was not produced from diaminopropane in the organisms. They are phylogenetically placed near the branching point between the beta and gamma subclasses, however, 2-hydroxyputrescine, a good chemotaxonomic marker for beta proteobacteria, was not detected in the two gamma proteobacteria.

Polyamine profiles as a chemotaxonomic marker

In the previous studies on gamma proteobacterial polyamines, homospermidine was selectively dis-

tributed in *Legionella* and *Beggiatoa* species (18, 22) and norspermidine was selectively distributed within the genera *Vibrio* and *Photobacterium* of the family *Vibrionaceae* and the genus *Moritella* (13, 22). The occurrence of norspermidine in some *Moraxella* species was first found in the present study. In contrast to these triamines, diaminopropane was widely distributed, although sporadically, within the genera *Moraxella*, *Cardiobacterium* and *Suttonella*, as shown in this study, and the families *Enterobacteriaceae* (12, 35), *Pasteurellaceae* (13, 17), *Aeromonadaceae* (13) and *Vibrionaceae* (14, 22), and the genus *Acinetobacter* (15) of the gamma subclass of *Proteobacteria*. Although norspermidine is produced from diaminopropane, norspermidine has not been found in gamma proteobacteria other than the families *Vibrionaceae* and *Moraxellaceae*. The distribution of the two triamines, norspermidine and homospermidine, may be idiosyncratic, however, it may serve as a phenotypic marker for certain genera within the gamma subclass of *Proteobacteria*.

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REFERENCES

1. Anzai, Y., Kim, H., Park, J.-Y., Wakabayashi, H. and Oyaizu, H. Phylogenetic affiliation of the pseudomonas based on 16S rRNA sequence. *Int. J. Syst. Evol. Microbiol.* **50** : 1563–1589 (2000).
2. Bowman, J.P., Cavanagh, J., Austin, J.J. and Sanderson, K. Novel *Psychrobacter* species from Antarctic ornithogenic soils. *Int. J. Syst. Bacteriol.* **46** : 841–848 (1996).
3. Bowman, J.P., McCammon, S.A., Nichols, D.S., Skerratt, J.H., Rea, S.M., Nichols, P.D. and McMeekin, T.A. *Shewanella gelidimarina* sp. nov., and *Shewanella frigidimarina* sp. nov., novel Antarctic species with the ability to produce eicosapentaenoic acid (20:5 ω 3) and grow anaerobically by dissimilatory Fe(III) reduction. *Int. J. Syst. Bacteriol.* **47** : 1040–1047 (1997).
4. Bowman, J.P., Nichols, D.S. and McMeekin, T.A. *Psychrobacter glacincola* sp. nov., a halotolerant,

- psychrophilic bacterium isolated from Antarctic sea ice. *Syst. Appl. Microbiol.* **20**: 209–215 (1997).
5. Brown, G.R., Sutcliffe, I.C. and Cummings, S.P. Reclassification of [*Pseudomonas*] *doudoroffii* (Baumann et al. 1983) into the genus *Oceanomonas* gen. nov. as *Oceanomonas doudoroffii* comb. nov., and description of a phenol-degrading bacterium from estuarine water as *Oceanomonas baumannii* sp. nov. *Int. J. Syst. Evol. Microbiol.* **51**: 67–72 (2001).
 6. Busse, J. and Auling, G. Polyamine pattern as a chemotaxonomic marker within the *Proteobacteria*. *System. Appl. Microbiol.* **11**: 1–8 (1988).
 7. Busse H.-J., Denner, E.B.M. and Lubitz, W. Classification and identification of bacteria: current approaches to an old problem. Overview of methods used in bacterial systematics. *J. Biotechnol.* **47**: 3–38 (1996).
 8. De Baere, T., Muylaert, A., Everaert, E., Wauters, G., Claeys, G., Verschaegen, G. and Vanechoutte, M. Bacteria due to *Moraxella atlantae* in a cancer patient. *J. Clin. Microbiol.* **40**: 2693–2695 (2002).
 9. Dewhirst, F.E., Paster, B.J., La Fontaine, S. and Rood, J.I. Transfer of *Kingella indologenes* (Snell and Lapage 1976) to the genus *Suttonella* gen. nov. as *Suttonella indologenes* comb. nov. ; transfer of *Bacteroides nodosus* (Beveridge 1941) to the genus *Dichelobacter* gen. nov. as *Dichelobacter nodosus* comb. nov. ; and assignment of the genera *Cardiobacterium*, *Dichelobacter*, and *Suttonella* to *Cardiobacteriaceae* fam. nov. in the gamma division of *Proteobacteria* on the basis 16S rRNA sequence comparisons. *Int. J. Syst. Bacteriol.* **40**: 426–433 (1990).
 10. Fischer-Le Saux, M., Viillard, V., Brunel, B., Normand, P. and Boemare, N. E. Polyphasic classification of the genus *Photothabdus* and proposal of new taxa: *P. luminescens* subsp. *luminescens* subsp. nov., *P. luminescens* subsp. *akhurstii* subsp. nov., *P. luminescens* subsp. *laumondii* subsp. nov., *P. temperata* sp. nov., *P. temperata* subsp. *temperata* subsp. nov. and *P. asymbiotica* sp. nov. *Int. J. Syst. Bacteriol.* **49**: 1645–1656 (1999).
 11. Forst, S., Dowds, B., Boemare, N. and Stackebrandt, E. *Xenorhabdus* and *Photothabdus* spp. : Bugs that kill bugs. *Annu. Rev. Microbiol.* **51**: 47–72 (1997).
 12. Hamana, K. Distribution of diaminopropane and acetylspermidine in *Enterobacteriaceae*. *Can. J. Microbiol.* **42**: 107–114 (1996).
 13. Hamana, K. Polyamine distribution patterns within the families *Aeromonadaceae*, *Vibrionaceae*, *Pasteurellaceae*, and *Halomonadaceae*, and related genera of the gamma subclass of the *Proteobacteria*. *J. Gen. Appl. Microbiol.* **43**: 49–59 (1997).
 14. Hamana, K. and Matsuzaki, S. Polyamines as a chemotaxonomic marker in bacterial systematics. *Crit. Rev. Microbiol.* **18**: 261–283 (1992).
 15. Hamana, K. and Matsuzaki, S. Diaminopropane occurs ubiquitously in *Acinetobacter* as the major polyamine. *J. Gen. Appl. Microbiol.* **38**: 191–194 (1992).
 16. Hamana, K. and Matsuzaki, S. Polyamine distribution patterns serve as a phenotypic marker in the chemotaxonomy of the *Proteobacteria*. *Can. J. Microbiol.* **39**: 304–310 (1993).
 17. Hamana, K. and Nakata, K. Distribution of diamino-propane, putrescine and cadaverine in *Haemophilus* and *Actinobacillus*. *Microbios* **103**: 43–51 (2000).
 18. Hamana, K. and Takeuchi, M. Polyamine profiles as chemotaxonomic marker within alpha, beta, gamma, delta, and epsilon subclasses of class *Proteobacteria* : Distribution of 2-hydroxyputrescine and homospermidine. *Microbiol. Cult. Coll.* **14**: 1–14 (1998).
 19. Hamana, K., Matsuzaki, S., Niitsu, M. and Samejima, K. Synthesis of novel polyamines in *Paracoccus*, *Rhodobacter* and *Micrococcus*. *FEMS Microbiol. Lett.* **67**: 267–274 (1990).
 20. Hamana, K., Saito, T. and Okada, M. Polyamine profiles within the beta subclass of the class *Proteobacteria* : Distribution of 2-hydroxyputrescine. *Microbiol. Cult. Coll.* **16**: 63–69 (2000).
 21. Hamana, K., Saito, T. and Okada, M. Distribution of profiles spermidine and homospermidine within the alpha subclass of the class *Proteobacteria*. *Microbiol. Cult. Coll.* **17**: 3–12 (2001).
 22. Hamana, K., Okada, M., Saito, T. and Nogi, Y. Polyamine distribution profiles among some members of the gamma subclass of the class *Proteobacteria*. *Microbiol. Cult. Coll.* **16**: 51–61 (2000).
 23. Hauben, L., Vauterin, L., Swings, J. and Moore, E.R.B. Comparison of 16S Ribosomal DNA sequences of all *Xanthomonas* species. *Int. J. Syst.*

- Bacteriol. **47** : 328–335 (1997).
24. Howarth, R., Unz, R.F., Seviour, E.M., Seviour, R.J., Blackall, L.L., Pickup, R.W., Jones, J.G., Yaguchi, J. and Head, I.M. Phylogenetic relationships of filamentous sulfur bacteria (*Thiothrix* spp. and Eikelboom type 021N bacteria) isolated from wastewater-treatment plants and description of *Thiothrix eikelboomii* sp. nov., *Thiothrix unzii* sp. nov., *Thiothrix fructosivorans* sp. nov. and *Thiothrix defluvi* sp. nov. Int. J. Syst. Bacteriol. **49** : 1817–1827 (1999).
 25. Kodjo, A., Tonjum, T., Richard, Y. and Bovre, K. *Moraxella caprae* sp. nov., a new member of the classical *Moraxellae* with very close affinity to *Moraxella bovis*. Int. J. Syst. Bacteriol. **45** : 467–471 (1995).
 26. Leonardo, M.R., Moser, D.P., Barbieri, E., Brantner, C.A., MacGregor, B.J., Paster, B.J., Stackebrandt, E. and Nealon, K.H. *Shewanella pealeana* sp. nov., a member of the microbial community associated with the accessory nidamental gland of the squid *Loligo pealei*. Int. J. Syst. Bacteriol. **49** : 1341–1351 (1999).
 27. Maruyama, A., Honda, D., Yamamoto, H., Kitamura, K. and Higashihara, T. Phylogenetic analysis of psychrophilic bacteria isolated from the Japan Trench, including a description of the deep-sea species *Psychrobacter pacificensis* sp. nov. Int. J. Syst. Evol. Microbiol. **50** : 835–846 (2000).
 28. Pettersson, B., Kodji, A., Ronaghi, M., Uhlen, M. and Tonjum, T. Phylogeny of the family *Moraxellaceae* by 16S rDNA sequence analysis, with special emphasis on differentiation of *Moraxella* species. Int. J. Syst. Bacteriol. **48** : 75–89 (1998).
 29. Vandamme, P., Gillis, M., Vancanneyt, M., Hoste, B., Kersters, K. and Falsen, E. *Moraxella lincolnii* sp. nov. isolated from the human respiratory tract, and reevaluation of the taxonomic position of *Moraxella osloensis*. Int. J. Syst. Bacteriol. **43** : 474–481 (1993).
 30. Vauterin, L., Hoste, B., Kersters, K. and Swings, J. Reclassification of *Xanthomonas*. Int. J. Syst. Bacteriol. **45** : 472–489 (1995).
 31. Venkateswaran, K., Dollhopf, M.E., Aller, R., Stackebrandt, E. and Nealon, K.H. *Shewanella amazonensis* sp. nov., a novel metal-reducing facultative anaerobe from Amazonian shelf muds. Int. J. Syst. Bacteriol. **48** : 965–972 (1998).
 32. Venkateswaran, K., Moser, D.P., Dollhopf, M.E., Lies, D.P., Saffarini, D.A., MacGregor, B.J., Ringelberg, D.B., White, D.C., Nishijima, M., Sano, H., Burghardt, J., Stackebrandt, E. and Nealon, K.H. Polyphasic taxonomy of the genus *Shewanella* and description of *Shewanella oneidensis* sp. nov. Int. J. Syst. Bacteriol. **49** : 705–724 (1999).
 33. Yang, P., De Vos, P., Kersters, K. and Swings, J. Polyamine patterns as chemotaxonomic markers for the genus *Xanthomonas*. Int. J. Syst. Bacteriol. **43** : 709–714 (1993).
 34. Yumoto, I., Yamazaki, K., Hishinuma, M., Nodasaka, Y., Suemori, A., Nakajima, K., Inoue, N. and Kawasaki, K. *Pseudomonas alcaliphila* sp. nov., a novel facultatively psychrophilic alkaliphile isolated from seawater. Int. J. Syst. Evol. Microbiol. **51** : 349–355 (2001).
 35. Zhrebilo, O.E., Kucheryava, N., Gvozdyak, R.I., Ziegler, D., Scheibner, M. and Auling, G. Diversity of polyamine patterns in soft rot pathogens and other plant-associated members of the *Enterobacteriaceae*. System. Appl. Microbiol. **24** : 54–62 (2001).

γ サブクラスに属する数種のプロテオバクテリアにおけるポリアミン構成：近年記載された12属のポリアミン分析

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γ サブクラスに属するプロテオバクテリアとして近年記載された65種(12属)のポリアミンをHPLCにより分析し、分析済みの同属および近縁属の種と比較し、 γ プロテオバクテリア内のポリアミン構成を考察した。

γ -1 サブグループに属する *Thiothrix* 属 3 種および γ -2 サブグループに属する *Xanthomonas* 属 7 種は、スベルミジンのみを有していた。 γ -2 サブグループの *Stenotrophomonas* 属 1 種はカダベリンとスベルミジンを含有した。 γ -3 サブグループでは以下のような結果であった。 *Enterobacteriaceae* 科の *Photorhabdus* 属と *Xenorhabdus* 属では全 8 種がプトレスシンとスベルミジンを有し、カダベリンは一部の種に含まれていた。プトレスシンとスベルミジンが *Pseudomonas* 属の 21 種の全てにおける主ポリアミンとなっていたが、カダベリンはその半数の種にのみしか含まれていなかった。 *Moraxellaceae* 科を構成する *Psychrobacter* 属と *Moraxella* 属では、 *Psychrobacter* 全 8 種と 6 種の *Moraxella* の主ポリアミンはスベルミジンであった。他 7 種の *Moraxella* は、主ポリアミンとしてジアミノプロパンとノルスベルミジンの両方か、どちらか一つを含むことから、 *Moraxella* 種のポリアミン構成は不均一であった。 *Shewanella* 属 12 種では、プトレスシンのみ、スベルミジンのみ、2-ヒドロキシプトレスシンとプトレスシン、プトレスシンとカダベリン、プトスシンとスベルミジン、のいずれかとなり、ポリアミン構成は種間で異なっていた。 *Oceanomonas baumannii* と *O. doudoroffii* はプトレスシン、カダベリン、スベルミジンを含有していた。 *Cardiobacteriaceae* 科の *Cardiobacterium* 属 1 種と *Suttonella* 属 1 種はジアミノプロパンとスベルミジンを含んでいた。

上記のデータは γ プロテオバクテリアにおける化学分類マーカーとしてのポリアミン構成をカタログ化する上で役立つものである。