Short Communication

POLYAMINE DISTRIBUTION PATTERNS IN C₁ COMPOUND-UTILIZING EUBACTERIA AND ACIDOPHILIC EUBACTERIA

KOEI HAMANA* AND NORIAKI KISHIMOTO¹

College of Medical Care and Technology, Gunma University, Maebashi 371, Japan

¹Mimasaka Women's Junior College, Tsuyama 708, Japan

(Received January 30, 1996; Accepted September 5, 1996)

C₁ compound-utilizing bacteria, the so-called methylotrophs or methanotrophs, are a diverse group of Gram-negative proteobacteria which obligately or facultatively oxidize methane, methanol and methylamine as sole sources of carbon and energy. The class *Proteobacteria* is phylogenetically comprised of alpha, beta, gamma, delta and epsilon subclasses; furthermore, each subclass is divided into several clusters corresponding to orders or families (19). *Methylobacterium*, *Methylosinus*, and *Methylocystis* belong to the alpha-2 subclass (12,21). *Methylobacillus*, *Methylophilus*, and *Methylovorus* are located in the beta subclass (12). *Methylococcus*, *Methylobacter*, *Methylomicrobium*, and *Methylomonas* are the members of the family Methylococcaceae of the gamma subclass (1,12). The phylogenetic position of *Methylophaga* within the *Proteobacteria*, however, is still not clear. A methanol oxidizer, *Ancylobacter aquaticus* was confirmed as belonging to the alpha subclass (22). Non-methane-, non-methanol-, and methylamine-utilizing proteobacteria; a genus, *Aminobacter*, and three species, *A. aminovorans*, *A. aganoensis*, and *A. niigataensis*, probably belong to the alpha subclass (20). A study of a non-methane-, methanol-, and methylamine-utilizing, budding proteobacterium, *Hyphomicrobium*, belonging to the alpha subclass, was published recently (23).

New members of acidophilic chemo-organotrophic proteobacteria belonging to the genus *Acidiphilium* (14,16,25) and the genus *Acidocella* transferred from

*Address reprint requests to: Dr. Koei Hamana, College of Medical Care and Technology, Gunma University, 3-39-15 Showa-machi, Maebashi 371, Japan.

Abbreviations: IAM, IAM Culture Collection, Center for Cellular and Molecular Research, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan; IFO, Institute for Fermentation, Osaka, Osaka, Japan; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research (RIKEN), Wako, Japan; ATCC, American Type Culture Collection, Rockville, MD, U.S.A.

Acidiphilium (17), and a new acidophilic, facultatively methanol-oxidizing *Acidomonas methanolica* (24) were isolated. These acidophiles belong to the alpha-1 subclass and are distinguished from acidophilic chemolithotrophically sulfur-oxidizing thiobacilli. However, an acidophilic, facultatively heterotrophic thiobacillus, *Thiobacillus acidophilus*, was phylogenetically located on the same cluster as *Acidiphilium* species (18). The slightly acidophilic bacteria, *Acetobacter* and *Gluconobacter*, belong to the alpha subclass (19). Acidophilic chemo-organotrophic, Gram-negative *Acidobacterium capsulatum* was phylogenetically different from these acidophilic proteobacteria (13, 15).

Studies on cellular polyamines have already provided some valuable chemotaxonomic information within the class *Proteobacteria*. The polyamine distribution profiles were characteristic for higher taxonomic rank; the beta subclass which features 2-hydroxyputrescine, or the alpha subclass which features homospermidine, or the gamma subclass which features spermidine (2-9, 11). Polyamines of C₁ compound oxidizers belonging to 14 genera were analyzed in the present study chemotaxonomically. These genera are not an evolutionary coherent grouping but rather connect into the three subclasses of the *Proteobacteria*. Polyamines of acidophiles belonging to seven different genera of Gram-negative eubacteria were also analyzed.

Facultatively methanol-oxidizing *Methylobacterium*, *Methylophaga*, and *Methylophilus* were grown heterotrophically in synthetic 199 medium (polyamine-free) (Nissui Pharmaceutical Co., Tokyo, Japan) or in JCM 79 medium containing 1% methanol, at pH 7.0 and 30°C. *Methylophaga marina* was cultivated in the presence of 3% NaCl (199-NaCl). Of the obligate methanol- and methane-oxidizers, *Methylobacillus* was cultivated in JCM 79 medium and *Methylomonas* and *Methylococcus* in ATCC 167 medium (L medium), in 50% methane-50% air, at 30°C. Facultatively methanol- and methane-oxidizing *Methylosinus*, *Methylovorus*, *Methylocystis*, *Methylomicrobium*, and *Methylobacter* were cultivated in ATCC 1306 medium (nitrate mineral salts medium; NMS medium) in the presence of 0.5% methanol or 0.3% glucose, or in 50% methane-50% air, at 30°C. *Aminobacter* and *Ancylobacter* species were grown heterotrophically in 199 medium and IAM B-44 medium. *Hyphomicrobium* was grown in IFO 233 medium containing 1% methanol or JCM 79 medium, at 25°C. *Acidiphilium*, *Acidocella*, *Acidomonas*, and *Acidobacterium* were heterotrophically cultivated in ATCC 1421 medium, pH 3.0, JCM 122 medium, pH 4.0 and JCM 142 medium, pH 3.5, respectively, at 30°C. The latter two organisms were also grown in 199 medium, pH 4.0 (199-pH 4). *Acetobacter* and *Gluconobacter* were grown in the 199 medium supplemented with 0.5% glucose (199G), pH 6.8 at 30°C. The polyamines extracted from the cells harvested at the stationary phase with cold 0.5 M perchloric acid were analyzed by high-performance liquid chromatography on a column of cation-exchange resin, as described previously (6, 11).

The same polyamine profiles were obtained in the cultures grown heterotrophically and in cultures grown in the presence of methanol or methane in facultative

methanol and/or methane oxidizers (Table 1). These nutritional growth conditions did not affect their polyamine distribution patterns. Among all the species of *Methylobacterium*, *Methylosinus*, and *Methylocystis* tested and belonging to the alpha-2 subclass, the major polyamines were putrescine and homospermidine (Table 1). It has been demonstrated that homospermidine is synthesized from 2 mol of putrescine mediated by Schiff-base complex formation; distribution of the triamine as a major polyamine is specific for the alpha-2 subclass within the *Proteobacteria* (2,6). The C₁ oxidizers belonging to the alpha-2 subclass were of the homospermidine-dominant type, as supporting by previous findings. The limited occurrence of the hydroxylated putrescine, 2-hydroxyputrescine, in the beta subclass has been proposed (2,6). The present study shows that the C₁ oxidizers belonging to the beta subclass, *Methylobacillus*, *Methylophilus*, and *Methylovorus*, also contain 2-hydroxyputrescine and putrescine (Table 1). *Methylophaga* as well as the three methanol oxidizers *Methylobacillus*, *Methylophilus*, and *Methylovorus* belonging to the beta subclass have the same quinone system and fatty acid composition (22), however, putrescine and spermidine are the major polyamine in *Methylophaga* species. This result suggests that the location of *Methylophaga* is outside the beta and alpha-2 subclasses.

Methylococcus, *Methylobacter*, *Methylomicrobium* and *Methylomonas* species belonging to the gamma subclass as the members of the family Methylococcaceae ubiquitously contained spermidine as the major polyamine (Table 1). The occurrence of cadaverine was found in the latter three of the above four methane oxidizers. *Methylococcus capsulatus* was devoid of lysine decarboxylase activity to produce cadaverine. This chemotaxonomic difference in polyamine pattern supports the phylogenetic finding that the genus *Methylococcus* is located in a cluster divergent from other three genera within the family (1). Although similar polyamine profiles have also been found in *Alteromonas*, *Halomonas*, *Deleya*, *Azomonas*, *Azotobacter*, and *Chromatium* of the gamma subclass (5,6), members of this subclass varied in their polyamine distribution profiles i.e.; norspermidine was found in Vibrionaceae (26,27); diaminopropane and acetylspermidine were widely spread in Enterobacteriaceae (3); Aeromonadaceae lacked in spermidine (27); and *Acinetobacter* contained diaminopropane only (8).

The occurrence of putrescine and homospermidine in *Ancylobacter* and *Aminobacter* species suggest that their correct location is in the alpha-2 subclass. Incorporation of cadaverine and spermidine from the medium was observed in the cultures of *Ancylobacter* grown in B-44 medium containing these amines (Table 1). Putrescine and spermidine were detected in *Hyphomicrobium* species. It is suggested that these homospermidine-lacking organisms are located in a cluster divergent from the alpha-2 subclass within the alpha subclass. The C₁-oxidizing alpha proteobacterium, *Xanthobacter*, contained homospermidine as the major polyamine (9). The C₁ oxidizers, five *Paracoccus* species belonging to the alpha subclass, contained spermidine as the major polyamine (7). A C₁-oxidizing neutrophilic thiobacilli, *Thiobacillus novellus* belonging to the alpha-2 subclass, contained homo-

Table 1. Polyamines in C₁ compound-utilizing eubacteria.

	Medium	Polyamines ($\mu\text{mol/g}$ wet cell)				
		H-Put	Put	Cad	Spd	HSpd
<i>Methylobacterium aminovorans</i> JCM 8240 ^T	199	—	0.9	—	—	20.1
<i>Methylobacterium extorquens</i> JCM 2802 ^T	199	—	0.2	—	—	7.2
IAM 12631	199	—	0.5	—	—	10.5
<i>Methylobacterium fujisawaense</i> ATCC 43884 ^T	199	—	0.7	—	—	25.4
<i>Methylobacterium mesophilicum</i> JCM 2829 ^T	199	—	0.6	—	—	16.5
<i>Methylobacterium organophilum</i> JCM 2833 ^T	199	—	0.2	—	—	11.0
	79	—	0.5	—	—	6.7
<i>Methylobacterium radiotolerans</i> IAM 12098 ^T	199	—	3.5	—	—	14.5
	79	—	1.1	—	—	6.7
<i>Methylobacterium rhodesianum</i> JCM 2810	199	—	0.2	—	—	16.0
	79	—	1.3	—	—	8.6
<i>Methylobacterium rhodinum</i> JCM 2811 ^T	199	—	0.5	—	—	14.0
<i>Methylobacterium zatmanii</i> JCM 2819	199	—	0.5	—	—	15.0
<i>Methylosinus sporium</i> ATCC 35069 ^T	NMS + Methane	—	0.05	—	—	2.37
<i>Methylosinus trichosporium</i> ATCC 35070 ^T	NMS + Methane	—	0.20	—	—	3.54
<i>Methylocystis parvus</i> ATCC 35066 ^T	NMS + Methane	—	0.10	—	—	3.10
<i>Methylophaga marina</i> JCM 6886 ^T	199-NaCl	—	0.2	—	3.7	—
<i>Methylophaga thalassica</i> JCM 6887 ^T	199	—	0.2	—	2.0	—
	79	—	0.3	—	6.5	—
<i>Methylobacillus glycogenes</i> JCM 2850 ^T	79	0.30	1.50	—	—	—
JCM 2851	79	0.60	2.74	—	—	—
("Methanomonas methylovora") JCM 2840	79	0.75	4.50	—	—	—
("Achromobacter methanophila") JCM 2841	79	0.10	1.30	—	—	—
("Methylomonas methylovora") JCM 2844	79	0.20	1.44	—	—	—
<i>Methylophilus methylotrophus</i> ATCC 53528 ^T	199	0.41	2.40	—	—	—
<i>Methylovorus glucosotrophus</i> ATCC 49758 ^T	NMS + Methanol	0.20	0.27	—	—	—
	NMS + Glucose	1.50	2.50	—	—	—
<i>Methylococcus capsulatus</i> ATCC 19069 ^T	L + Methane	—	0.01	—	0.50	—
<i>Methylobacter luteus</i> ATCC 49878 ^T	NMS + Methane	—	0.01	0.26	0.90	—
<i>Methylomicrobium agile</i> ATCC 35068 ^T	NMS + Methanol	—	0.02	0.85	2.40	—
("Methanomonas agilis")	NMS + Methane	—	0.01	0.12	0.30	—
<i>Methylomonas methanica</i> ATCC 35067 ^T	L + Methane	—	0.01	0.19	0.70	—
<i>Ancylobacter aquaticus</i> IAM 12361	199	—	0.21	—	—	1.95
	B-44	—	0.56	0.11	0.02	1.49
IAM 12362	199	—	0.01	—	—	0.78
	B-44	—	0.38	0.08	0.05	1.15
IAM 12363	199	—	0.25	—	—	1.45
	B-44	—	0.45	0.30	0.10	1.88
IAM 12364 ^T	199	—	0.03	—	—	0.75
<i>Aminobacter aminovorans</i> JCM 7852 ^T	199	—	0.20	—	—	1.50
(<i>Pseudomonas aminovorans</i>)						
<i>Aminobacter aganoensis</i> JCM 7854 ^T	199	—	0.32	—	—	2.10
<i>Aminobacter niigataensis</i> JCM 7853 ^T	199	—	0.27	—	—	2.25
<i>Hyphomicrobium aestuarii</i> JCM 6889 (NCIMB 9698)	79	—	0.10	—	2.75	—
<i>Hyphomicrobium methylovorum</i> IFO 14180 ^T	233	—	0.62	—	0.40	—

Abbreviations: H-Put, 2-hydroxyputrescine; Put, putrescine; Cad, cadaverine; Spd, spermidine; HSpd, homospermidine; ^T, type strain; —, not detectable (<0.005 or $<0.05 \mu\text{mol/g}$ wet cell). Former names are shown in parentheses. Names which do not appear on the Approved Lists of Bacterial Names are enclosed in quotation marks.

spermidine whereas *Thiobacillus versutus* belonging to the alpha-1 subclass contained spermidine (4).

Although *Acidiphilium* species, which produce bacteriochlorophyll *a*, should be classified as a quasi-photosynthetic bacterium (14,17,25), all the species of *Acidiphilium* and *Acidocella* contained putrescine and spermidine (Table 2). The major polyamines of *Acidomonas methanolica* were putrescine and spermidine (Table 2). Homospermidine was not detected in these proteobacteria classified into alpha-1 subclass as well as *Thiobacillus acidophilus* located on a cluster including *Acidiphilium*. Thus, these acidophiles can not be distinguished by their polyamine types. The cellular concentrations of polyamines detected in the acidophilic proteobacteria were similar to those of neutrophilic alpha-1 proteobacteria, suggesting that acidophily of the acidophiles is not connected to changes in cellular polyamine levels. All the species of *Acetobacter* and *Gluconobacter* tested in our

Table 2. Polyamines in acidophilic eubacteria.

	Medium	Polyamines ($\mu\text{mol/g}$ wet cell)		
		Put	Spd	HSpd
<i>Acidiphilium angustum</i> ATCC 35903 ^T	1421	0.01	1.80	—
<i>Acidiphilium cryptum</i> IFO 14242 ^T (a)	1421	0.01	0.63	—
<i>Acidiphilium multivorum</i> JCM 8867 ^T	129	0.01	0.89	—
<i>Acidiphilium organovorum</i> ATCC 43141 ^T	1421	0.06	1.80	—
<i>Acidiphilium rubrum</i> ATCC 35905 ^T	1421	0.04	1.90	—
<i>Acidocella aminolytica</i> JCM 8796 ^T (<i>Acidiphilium aminolytica</i>)	1421	0.04	1.56	—
<i>Acidocella facilis</i> ATCC 35904 ^T (<i>Acidiphilium facilis</i>)	1421	0.06	1.98	—
<i>Thiobacillus acidophilus</i> ATCC 27809 ^T (b)	199-pH 3.5	0.02	0.64	—
<i>Acidomonas methanolica</i> JCM 6891 ^T (<i>Acetobacter methanolicus</i>)	199-pH 4 122	0.10 0.13	1.75 1.64	— —
<i>Acetobacter aceti</i> IFO 14818 ^T (c)	199G	0.08	0.50	—
<i>Acetobacter hansenii</i> IFO 14820 ^T (c)	199G	0.05	1.10	—
<i>Acetobacter liquefaciens</i> IFO 12388 ^T (c)	199G	0.08	0.74	—
<i>Acetobacter pasteurianus</i> IFO 14814 (c)	199G	0.08	1.35	—
<i>Acetobacter xylinum</i> IFO 15237 ^T	199G	0.02	0.50	—
<i>Gluconobacter asaii</i> IFO 3276 ^T	199G	0.05	0.55	—
<i>Gluconobacter cerinus</i> IFO 3276 ^T (c)	199G	0.21	1.25	—
<i>Gluconobacter frateurii</i> IFO 3264 ^T	199G	0.05	1.55	—
<i>Gluconobacter oxydans</i> subsp. <i>oxydans</i> (c) IFO 14819 ^T	199G	0.03	0.74	—
<i>Gluconobacter oxydans</i> subsp. <i>sphaericus</i> IFO 12467 ^T	199G	0.04	0.80	—
<i>Gluconobacter oxydans</i> subsp. <i>suboxydans</i> IFO 3172	199G	0.01	1.20	—
<i>Acidobacterium capsulatum</i> JCM 7670 ^T	199-pH 4 142	— —	— —	1.93 1.30

Abbreviations are shown in Table 1. a, cited from Hamana et al. (9); b, cited from Hamana and Matsuzaki (4); c, cited from Hamana and Matsuzaki (6).

previous (6) and present studies contained putrescine and spermidine (Table 2). It is known that these two genera belong to the alpha subclass (19). There are five types of polyamine distribution patterns in the genus *Thiobacillus*, including other acidophilic thiobacilli (4). Thus, thiobacilli could be rearranged into different taxonomic positions within the *Proteobacteria* on the basis of their polyamine profiles (4). The present results demonstrate that the polyamine profiles serve in assisting the division of proteobacteria into the subclasses. Spermidine-dominant type and homospermidine-dominant type organisms have been observed among the alpha subclass which is divided into 4 clusters, alpha-1, alpha-2, alpha-3 and alpha-4 (2,6). However more polyamine analyses are expected to recognize the chemotaxonomic usefulness of the cellular polyamine composition within the alpha subclass.

A Gram-negative eubacterium, *Acidobacterium capsulatum*, which has a weak relationship to all of the previously known phylogenetic groups of Gram-negative proteobacteria and Gram-positive eubacteria (13,15), contained homospermidine but not spermidine (Table 2). Homospermidine has been found in the organisms belonging to the alpha-2 subclass of the class *Proteobacteria* and *Flavobacterium-Cytophaga-Flexibacter* complex (10), although it has not been detected in Gram-positive eubacteria. The occurrence of homospermidine in Gram-negative *A. capsulatum* located outside the class *Proteobacteria* is phylogenetically significant when considering the evolution of the class *Proteobacteria*.

We are grateful to Drs. K. Suzuki and Y. Kosako of JCM, and Dr. T. Urakami of Mitsubishi Gas Chemical Co. for their valuable suggestions and to JCM, IFO, IAM, and ATCC for supplying bacterial strains.

REFERENCES

- 1) Bowman, J. P., Sly, L. I., and Stackebrandt, E., The phylogenetic position of the family Methylococcaceae. *Int. J. Syst. Bacteriol.*, **45**, 182-185 (1995).
- 2) Busse, J. and Auling, G., Polyamine pattern as a chemotaxonomic marker within the *Proteobacteria*. *Syst. Appl. Microbiol.*, **11**, 1-8 (1988).
- 3) Hamana, K., Distribution of diaminopropane and acetylspermidine in Enterobacteriaceae. *Can. J. Microbiol.*, **42**, 107-114 (1996).
- 4) Hamana, K. and Matsuzaki, S., Five types of polyamine distribution patterns in thiobacilli. *FEMS Microbiol. Lett.*, **70**, 347-352 (1990).
- 5) Hamana, K. and Matsuzaki, S., Polyamines as a chemotaxonomic marker in bacterial systematics. *Crit. Rev. Microbiol.*, **18**, 261-283 (1992).
- 6) 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 (1992).
- 7) Hamana, K. and Matsuzaki, S., Taxonomic significance of polyamine synthesis in *Paracoccus*. *J. Gen. Appl. Microbiol.*, **38**, 98-103 (1992).
- 8) Hamana, K. and Matsuzaki, S., Diaminopropane occurs ubiquitously in *Acinetobacter* as the major polyamine. *J. Gen. Appl. Microbiol.*, **38**, 191-194 (1992).
- 9) Hamana, K., Matsuzaki, S., and Sakakibara, M., Distribution of sym-homospermidine in eubacteria, cyanobacteria, algae and ferns. *FEMS Microbiol. Lett.*, **90**, 11-16 (1992).

- 10) Hamana, K., Nakagawa, Y., and Yamasato, K., Chemotaxonomic significance of polyamine distribution patterns in the *Flavobacterium-Cytophaga* complex and related genera. *Microbios*, **81**, 135–145 (1995).
- 11) Hamana, K., Sakane, T., and Yokota, A., Polyamine analysis of the genera *Aquaspirillum*, *Magnetospirillum*, *Oceanospirillum* and *Spirillum*. *J. Gen. Appl. Microbiol.*, **40**, 75–82 (1994).
- 12) Hanson, R. S. and Hanson, T. E., Methanotrophic bacteria. *Microbiol. Rev.*, **60**, 439–471 (1996).
- 13) Hiraishi, A., Kishimoto, N., Kosako, Y., Wakao, N., and Tano, T., Phylogenetic position of the menaquinone-containing acidophilic chemo-organotroph *Acidobacterium capsulatum*. *FEMS Microbiol. Lett.*, **132**, 91–94 (1995).
- 14) Kishimoto, N., Fukaya, F., Inagaki, K., Sugio, T., Tanaka, H., and Tano, T., Distribution of bacteriochlorophyll *a* among aerobic and acidophilic bacteria and light-enhanced CO₂-incorporation in *Acidiphilium rubrum*. *FEMS Microbiol. Ecol.*, **16**, 291–296 (1995).
- 15) Kishimoto, N., Kosako, Y., and Tano, T., *Acidobacterium capsulatum* gen. nov., sp. nov.: An acidophilic chemoorganotrophic bacterium containing menaquinone from acidic mineral environment. *Curr. Microbiol.*, **22**, 1–7 (1991).
- 16) Kishimoto, N., Kosako, Y., and Tano, T., *Acidiphilium aminolytica* sp. nov.: An acidophilic chemoorganotrophic bacterium isolated from acidic mineral environment. *Curr. Microbiol.*, **27**, 131–136 (1993).
- 17) Kishimoto, N., Kosako, Y., Wakao, N., Tano, T., and Hiraishi, A., Transfer of *Acidiphilium aminolytica* to the genus *Acidocella* gen. nov., and emendation of the genus *Acidiphilium*. *Syst. Appl. Microbiol.*, **18**, 85–91 (1995).
- 18) Lane, D. J., Harrison, A. P., Jr., Stahl, D., Pace, B., Giovannoni, S. J., Olson, G. J., and Pace, N. R., Evolutionary relationships among sulfur- and iron-oxidizing eubacteria. *J. Bacteriol.*, **174**, 269–278 (1992).
- 19) Stackebrandt, E., Murray, R. G. E., and Truper, H. G., *Proteobacteria* class nov., a name for the phylogenetic taxon that includes the “purple bacteria and their relatives.” *Int. J. Syst. Bacteriol.*, **38**, 321–325 (1988).
- 20) Urakami, T., Araki, H., Oyaizu, H., Suzuki, K., and Komagata, K., Transfer of *Pseudomonas aminovorans* (den Dooren de Jong 1926) to *Aminobacter* gen. nov. as *Aminobacter aminovorans* com. nov. and description of *Aminobacter aganoensis* sp. nov. and *Aminobacter niigataensis* sp. nov. *Int. J. Syst. Bacteriol.*, **42**, 84–92 (1992).
- 21) Urakami, T., Araki, H., Suzuki, K., and Komagata, K., Further studies of the genus *Methylobacterium* and description of *Methylobacterium aminovorans* sp. nov. *Int. J. Syst. Bacteriol.*, **43**, 504–513 (1993).
- 22) Urakami, T. and Komagata, K., Occurrence of isoprenoid compounds in gram-negative methanol-, methane-, and methylamine-utilizing bacteria. *J. Gen. Appl. Microbiol.*, **32**, 317–341 (1986).
- 23) Urakami, T., Sasaki, J., Suzuki, K., and Komagata, K., Characterization and description of *Hyphomicrobium denitrificans* sp. nov. *Int. J. Syst. Bacteriol.*, **45**, 528–532 (1995).
- 24) Urakami, T., Tamaoka, T., Suzuki, K., and Komagata, K., *Acidomonas* gen. nov., incorporating *Acetobacter methanolicus* as *Acidomonas methanolica* comb. nov. *Int. J. Syst. Bacteriol.*, **39**, 50–55 (1989).
- 25) Wakao, N., Nagasawa, N., Matsuura, T., Matsukura, H., Matsumoto, T., Hiraishi, A., Sakurai, Y., and Shiota, H., *Acidiphilium multivorum* sp. nov., an acidophilic chemoorganotrophic bacterium from pyritic acid mine drainage. *J. Gen. Appl. Microbiol.*, **40**, 143–159 (1994).
- 26) Yamamoto, S., Chowdhury, M. A. R., Kuroda, M., Nakao, T., Koumoto, Y., and Shinoda, S., Further study on polyamine composition in Vibrionaceae. *Can. J. Microbiol.*, **37**, 148–153 (1991).
- 27) Yamamoto, S., Shinoda, S., Kawaguchi, M., Wakamatsu, K., and Makita, M., Polyamine distribution in Vibrionaceae: Norspermidine as a general constituent of *Vibrio* species. *Can. J. Microbiol.*, **29**, 724–728 (1983).