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Polyamine Distribution Profiles among Some Members within Delta-and Epsilon-Subclasses of *Proteobacteria*

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Cellular polyamines of 18 species（13 genera）belonging to the delta and epsilon subclasses of the class *Proteobacteria* were analyzed by HPLC and GC. In the delta subclass, the four marine myxobacteria（the order *Myxococcales*）, *Enhygromyxa salina*, *Haliangium ochroceum*, *Haliangium tepidum* and *Plesiocystis pacifica* contained spermidine. Fe(Ⅲ)-reducing two *Geobacter* species and two *Pelobacter* species belonging to the order *Desulfuromonadales* contained spermidine. *Bdellovibrio bacteriovorus* was absent in cellular polyamines. *Bacteriovorax starrii* contained putrescine and spermidine. *Bacteriovorax stolpii* contained spermidine and homospermidine. Spermidine was the major polyamine in the sulfate-reducing delta proteobacteria belonging to the genera *Desulfovibrio*, *Desulfacinum*, *Desulfobulbus*, *Desulfococcus* and *Desulfurella*, and some species of them contained cadaverine. Within the epsilon subclass, three *Sulfurospirillum* species ubiquitously contained spermidine and one of the three contained spermidine and cadaverine. *Thiomicrospora denitrificans* contained cadaverine and spermidine as the major polyamine. These data show that cellular polyamine profiles can be used as a chemotaxonomic marker within delta and epsilon subclasses.

Key words: polyamine, spermidine, homospermidine, *Proteobacteria*

The class *Proteobacteria* is a major taxon of the domain *Bacteria* and is phylogenetically divided into the alpha, beta, gamma, delta and epsilon subclasses. We have reported the cellular polyamine distribution profiles within proteobacteria, as a chemotaxonomic marker（8-15）. Within the delta subclass, homospermidine was found in some myxobacteria（the order *Myxococcales*）（10）comprising the two suborders, *Sorangineae* and *Cystobacterineae*, and the four families, *Myxococcaceae*, *Cystobacteraceae*, *Polyangiaceae* and *Archangiaceae*（2, 18, 26）. Fe(Ⅲ)-reducing members belonging to the genera *Pelobacter*, *Geobacter*, *Desulfuromonas* and *Desulfuromusa*, form a monophyletic group（the order *Desulfuromonadales*）(2, 3, 4, 20). Two *Pelobacter* species, previously analyzed, contained spermidine as the major polyamine（10）. Within the order *Campylobacterales* of the epsilon subclass, various species belonging to the four genera *Helicobacter*, *Wolinella*, *Campylobacter* and *Arcobacter*（2, 6）scarcely contained any polyamines but *Sulfurospirillum deleyianum* contained spermidine（10）. In the present study, polyamines of some new members of the delta and epsilon subclasses were analyzed.

Newly validated 20 delta and epsilon proteobacteria

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Table 1. Polyamines of delta and epsilon subclasses of the class *Proteobacteria*

Organism		Medium−Temp. (℃)	Polyamine (μ mol/g wet cell)						
			Dap	Put	Cad	Spd	HSpd	Spm	Agm
Delta subclass									
Myxococcales									
Enhygromyxa salina	JCM 11769^T	JCM 341−30	−	−	−	0.54	−	0.10	−
Haliangium ochraceum	JCM 11303^T	JCM 341−30	−	−	−	1.15	−	0.31	0.03
Haliangium tepidum	JCM 11304^T	JCM 341−37	−	−	−	0.70	−	0.15	0.02
Plesiocystis pacifica	JCM 11591^T	JCM 341−30	−	0.10	−	0.65	−	0.02	−
Desulfovibrionales									
Desulfovibrio africanus	NCIMB 8401^T	NCIMB17−37	0.01	0.12	0.02	0.95	−	0.02	0.03
Desulfovibrio desulfuricans subsp. *desulfuricans*	IFO 13699	(a)	−	−	−	1.16	−	0.01	0.02
Desulfovibrio desulfuricans subsp. *desulfuricans*	NCIMB 8307^T	NCIMB17−30	−	0.24	1.98	0.40	−	−	−
Desulfovibrio desulfuricans subsp. *aestuarii*	NCIMB 9335^T	NCIMB104−30	−	0.02	0.01	1.11	−	0.05	0.03
Desulfovibrio salexigens	NCIMB 8403^T	NCIMB104−30	−	0.05	0.04	0.65	−	−	0.20
Desulfovibrio vulgaris subsp. *oxamicus*	NCIMB 9442^T	NCIMB17−30	−	0.35	0.10	0.50	−	−	0.10
Desulfovibrio vulgaris subsp. *vulgaris*	NCIMB 8303^T	NCIMB17−30	−	0.02	0.01	1.50	−	0.10	0.03
Syntrophobacterales									
Desulfacinum infernum	NCIMB 13416^T	NCIMB109−60	−	0.02	−	0.68	−	0.06	−
Desulfobacterales									
Desulfobulbus propionicus	NCIMB 12907^T	NCIMB17−37	−	0.02	−	0.88	−	0.04	−
Desulfococcus multivorans	NCIMB 12965^T	NCIMB295−30	−	0.02	−	0.70	−	0.04	−
Desulfuromonadales									
Pelobacter acidigallici	ATCC 49970^T	(a)	−	−	−	1.45	−	−	0.24
Pelobacter massiliensis	ATCC 49973^T	(a)	−	−	−	1.20	−	−	0.20
Geobacter sulfurreducens	ATCC 51573^T	ATCC1957−30	−	−	−	1.10	−	−	0.03
Geobacter hydrogenophilus	ATCC 51590^T	ATCC1957−30	−	−	−	0.92	−	−	0.10
Desulfurellales									
Desulfurella acetivorans	ATCC 51451^T	ATCC1920−55	−	−	−	0.97	−	0.02	−
Bdellovibrionales									
Bdellovibrio bacteriovorus	NCIMB 9529^T	ATCC137−30	−	−	−	0.05	−	0.10	−
		199−30	−	−	−	−	−	−	−
Bdellovibrio bacteriovorus	IFO 14261	(a)	−	2.10	−	−	−	−	−
Incertae sedis									
Bacteriovorax starrii (*Bdellovibrio starrii*)	ATCC 15145^T	(a)	−	1.52	0.10	1.77	−	−	−
Bacteriovorax stolpii (*Bdellovibrio stolpii*)	ATCC 27052^T	(a)	−	−	−	0.70	0.90	0.07	−
Epsilon subclass									
Campylobacterales									
Sulfurospirillum barnesii (“*Geospirillum barnesii*”)	ATCC 700032^T	ATCC2034−30	−	−	−	1.10	−	0.01	−
Sulfurospirillum arsenophilum (“*Geospirillum arsenophillus*”)	ATCC 700056^T	ATCC2018−30	0.02	−	0.85	0.10	−	0.03	−
Sulfurospirillum deleyianum	ATCC 51133^T	(a)	−	−	−	1.25	−	−	−
Incertae sedis									
Thiomicrospira denitrificans	ATCC 33889	ATCC1255−26	−	−	0.40	2.10	−	−	−

Note: Dap, 1, 3−diaminopropane; Put, putrescine; Cad, cadaverine; Spd, spermidine; HSpd, homospermidine; Spm, spermine; Agm, agmatine; NCIMB, The National Collections of Industrial, Food and Marine Bacteria, Aberdeen, Scotland, UK; ATCC, American Type Culture Collection, Manassas, Virginia, USA; IFO, Institute for Fermentation, Osaka, Japan; JCM, Japan Collection of Microorganisms, RIKEN, Saitama, Japan; ^{T}, Type strain; −, not detectable (<0.005). Quotation marks indicate that the scientific name has not been validly published. Former names are shown in parentheses. (a) was cited from Hamana and Takeuchi (10).

were grown in the media designated by the culture collections (JCM Catalogue of Strains, 2002 and The Catalogue of Strains of NCIMB, 1994 and The Catalogue of Strains of ATCC, 1996) and in the polyamine-free 199 medium (Nissui Pharmaceutical Co., Tokyo, Japan), at the optimum growth temperature listed in Table 1. The cells in the stationary phase were harvested. The whole polyamine extract in 0.5 M perchloric acid was analyzed by high-performance liquid chromatography (HPLC) (10). Gas chromatography (GC) was performed after heptafluorobutyrization of the concentrated polyamine sample (21).

Cellular concentrations of 1, 3-diaminopropane, putrescine, cadaverine, agmatine, spermidine, homospermidine and spermine are shown in Table 1. Spermine detected as a minor component was judged as a contaminant from the media containing spermine.

The delta subclass

Our previous study showed that several myxobacteria belonging to the genera *Myxococcus*, *Cystobacter*, *Stigmatella*, *Melittangium*, *Polyangium* and *Archangium* ubiquitously contained homospermidine as the major polyamine (10). The new marine myxobacteria, *Enhygromyxa salina*, *Haliangium ochroceum*, *Haliangium tepidum* and *Plesiocystis pacifica* phylogenetically located in *Sorangineae* of *Myxococcales* (7, 17-19) contained spermidine as the major polyamine and spermine as a minor polyamine, as shown in Table 1. Although widespread occurrence of homospermidine within terrestrial myxobacteria serves as a chemotaxonomic marker for *Myxococcales*, homospermidine was not found in the four marine slightly halophilic myxobacteria.

The two *Geobacter* species, *G. sulfurreducens* and *G. hydrogenophilus* (3, 4, 20), as well as the two *Pelobacter* species (24), contained spermidine as the major polyamine (Table 1).

Polyamines of three *Bdellovibrio* species, including a reference strain of *Bdellovibrio bacteriovorus* (IFO 14261) isolated in Japan (28), have been analyzed (10). Recently, *Bdellovibrio starrii* and *Bdellovibrio stolpii* were reclassified into the new genus *Bacteriovorax* (1). Polyamines of the type strain of *Bdellovibrio bacteriovorus* (NCIMB 9529) analyzed in the present work, were lacking when this organism was grown in 199 medium. A little amount of spermidine and spermine was incorporated into this organism from the ATCC 137 medium containing these amines (Table 1). The strain IFO 14261 contained putrescine but not triamines (10). The polyamine profile of *Bdellovibrio bacteriovorus* is unique within this subclass. The two *Bacteriovorax species* differ in their polyamine profiles, however, *Bacteriovorax starrii* contained putrescine and spermidine and *Bacteriovorax stolpii* contained spermidine and homospermidine (10).

The sulfate reducers such as *Desulfovibrio*, *Desulfobulbus*, *Desulfococcus*, *Desulfacinum*, *Desulfobacter*, *Desulfobacterium*, *Desulfomicrobium*, *Desulfomonas*, *Desulfomonile*, *Desulfonema*, *Desulfosarcina*, *Desulforhopalus*, *Desulfohalobium*, *Desulfotulus*, *Desulforhobdus*, *Desulfuromonas*, *Syntrophus*, and *Syntrophobacter* phylogenetically spread within the delta subclass (5, 16, 19, 23). Polyamine analysis of a strain of *Desulfovibrio desulfuricans* has been reported previously (10). In the present study, polyamines of *Desulfobulbus propionicus*, *Desulfococcus multivorans*, *Desulfacinum infernum*, and seven species of the genus *Desulfovibrio*, were analyzed. Cadaverine level differed among the three strains of *Desulfovibrio desulfuricans* (Table 1), correlating to their phylogenetic heterogeneity (5). Spermidine was the major polyamine in all four *Desulfovibrio* species and all three species belonging to the genera *Desulfobulbus* (the order *Desulfobacterales*), *Desulfococcus* (the order *Desulfobacterales*) and *Desulfacinum* (*Syntrophobacterales*), which are located in other clusters divergent from the *Desulfovibrio* clade (the order *Desulfovibrionales*). *Desulfurella acetivorans*, a thermophilic, acetate-oxidizing and sulfur-reducing proteobacterium, contained spermidine as the major polyamine (Table 1). This organism represents a distinct lineage (the order *Desulfurellales*) within the delta subclass (2) but was formerly located in the epsilon subclass (22).

The epsilon subclass

A species of the genus *Sulfurospirillum*, *S. deleyianum* (25), contained an appreciable amount of spermidine, indicating high spermidine synthetic ability (10). New two species of *Sulfurospirillum*, *S. barnesii* and *S. arsenophilum*, formerly classified as "*Geospirillum*" (27), also contained spermidine (Table 1). This result

indicates the ubiquitous occurrence of spermidine in the *Sulfurospirillum* clade. Cadaverine was found in *S. arsenophilum*. The polyamine profile of this clade was distingushed from the poor polyamine levels found in *Helicobacter*, *Wolinella*, *Campylobacter* and *Arcobacter* species within *Campylobacterales* (10). Authentic *Thiomicrospira* species such as *T. crunogena*, *T. pelophila* and *T. thyasira* were phylogenetically located in the gamma subclass and contained putrescine and spermidine (11). *Thiomicrospira denitrificans* ATCC 33889 phylogenetically belongs to a clade of the epsilon subclass (6, 27). Spermidine and cadaverine were the major polyamines of this organism (Table 1).

These polyamine catalogues added by this study on 18 species (13 genera) display the whole polyamine distribution profiles in the delta and epsilon subclasses. The distribution of putrescine, cadaverine and homospermidine can be used as a chemotaxonomic marker for genus and species levels within the two subclasses. Homospermidine has not been observed within the epsilon subclass, however, it was mostly limited in *Bacteriovorax* and terrestrial myxobacteria within the delta subclass. This unusual triamine was found also in alpha and gamma proteobacteria and its distribution was closely related to their phylogenetic positions (10, 11, 13-15). 2-Hydroxyputrescine was widely distributed within beta subclass (10, 12) and norspermidine was selectively distributed in the gamma subclass (10, 11). They have not yet been detected in the delta or epsilon subclasses.

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数種のδおよびεプロテオバクテリアにおけるポリアミン構成

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δおよびεプロテオバクテリアに属する18種（13属）についてHPLCとGCによるポリアミン分析を行なった。δサブクラスでは，海水産ミキソバクテリア（*Myxococcales* 目）の *Enhygromyxa* 属1種と *Haliangium* 属2種と *Plesiocystis* 属1種ではスペルミジンが主ポリアミンであり，*Desulfuromonadales* 目の鉄（Ⅲ）還元性 *Geobacter* 属2種と *Pelobacter* 属2種でもスペルミジンが主ポリアミンであった。*Bdellovibrio* 属の *B. bacteriovorus* はポリアミンを欠き，*Bacteriovorax* 属の *B. starrii* はプトレスシンと スペルミジンを，*B. stolpii* はスペルミジンおよびホモスペルミジンを含有していた。*Desulfovibrio* 属，*Desulfacinum* 属，*Desulfobulbus* 属，*Desulfococcus* 属，*Desulfurella* 属 の硫酸塩還元菌ではスペルミジンが共通する主ポリアミンであったが，カダベリンを有する種も存在した。εサブクラスでは，*Sulfurospirillum* 属の3種では主ポリアミン はスペルミジンであったが，1種のみカダベリンも有していた。*Thiomicrospira denitrificans* はεサブクラスの1系統に位置していて，主ポリアミンとしてカダベリン とスペルミジンを含有していた。これらのポリアミン分析データはδおよびεサブクラスにおける属または種レベルでの化学分類マーカーとして有用と思われた。