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Full Paper

Polyamine analysis for chemotaxonomy of thermophilic eubacteria: Polyamine distribution profiles within the orders *Aquificales*, *Thermotogales*, *Thermodesulfobacteriales*, *Thermales*, *Thermoanaerobacteriales*, *Clostridiales* and *Bacillales*

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Cellular polyamines of 45 thermophilic and 8 related mesophilic eubacteria were investigated by HPLC and GC analyses for the thermophilic and chemotaxonomic significance of polyamine distribution profiles. Spermidine and a quaternary branched penta-amine, N^4-bis(aminopropyl)norspermidine, were the major polyamine in *Thermocrinis*, *Hydrogenobacter*, *Hydrogenobaculum*, *Aquifex*, *Persephonella*, *Sulfurihydrogenibium*, *Hydrogenothermus*, *Balnearium* and *Thermovibrio*, located in the order *Aquificales*. *Thermodesulfobacterium* and *Thermodesulfatator* belonging to the order *Thermodesulfobacteriales* contained another quaternary penta-amine, N^4-bis(aminopropyl)spermidine. In the order *Thermotogales*, *Thermotoga* contained spermidine, norspermidine, caldopentamine and homocaldopentamine. The latter two linear penta-amines were not found in *Marinitoga* and *Petrotoga*. In the order *Thermales*, *Thermus* and *Marinithermus* contained homospermidine, norspermine and the linear penta-amines. *Meiothermus* lacked penta-amines. *Vulcanithermus* contained linear penta-amines and hexa-amines but not homospermidine. *Oceanithermus* contained spermine alone. Within the order *Thermoanaerobacteriales*, the two quaternary branched penta-amines were found in *Thermanaeromonas* and *Thermoanaerobacter*. *Caldanaerobacter* contained N^4-bis(aminopropyl)spermidine. *Thermoanaerobacterium* lacked penta-amines. *Thermaerobacter* of the order *Clostridiales* contained N^4-bis(aminopropyl)spermidine and agmatine. *Thermosyntropha*, *Thermanaerovibrio*, *Thermobrachium* (the order *Clostridiales*), *Sulfobacillus*, *Alicyclobacillus*, *Anoxybacillus*, *Ureibacillus*, *Thermicanus* (the order *Bacillales*), *Desulfotomaculum*, *Desulfitobacterium* and *Pelotomaculum* (the family *Peptococcaceae*) ubiquitously contained spermine. Some thermophiles of *Bacillales* added linear and branched penta-amines.

Key Words——*Alicyclobacillaceae*; *Aquificales*; *polyamine*; *Thermales*; *Thermoanaerobacteriales*; *Thermodesulfobacteriales*; thermophile; *Thermotogales*

Introduction

Aliphatic cellular polyamines, linear diamines, triamines, tetra-amines, penta-amines and hexa-amines, and quaternary branched penta-amines, were found in eubacteria (domain Bacteria) and archaebacteria (do-

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main Archaea), and are important to stabilize cellular nucleic acid structure as a major function (Hamana, 2002; Hamana and Matsuzaki, 1992). The chemotaxonomic and phylogenetic significance of the distribution of long and branched polyamines, possibly associated with their thermophily, has been proposed (Hamana and Itoh, 2001; Hamana and Kato, 2000; Hamana et al., 1994, 1995, 1996a, b, 1998, 1999, 2001, 2003).

The three deeply branching lineages of eubacteria, the orders *Aquificales*, *Thermotogales* and *Thermodesulfobacteriales*, are phylogenetically located in early eubacterial evolution and comprise entirely thermophiles. We have attempted to analyze the polyamines of hydrogen-oxidizing *Hydrogenothermus*, *Hydrogenobacter* (Stohr et al., 2001; Takai et al., 2001a), *Persephonella*, *Sulfurihydrogenibium*, *Thermocrinis* and *Balnearium*, and nitrate-reducing *Thermovibrio* (Eder and Huber, 2002; Huber et al., 1998, 2002; Nakagawa et al., 2003; Takai et al., 2003a, b), belonging to *Aquificales*. Polyamines of new members of *Thermotoga*, *Petrotoga* and *Marinitoga* (Alain et al., 2002; Balk et al., 2002; L'Haridon et al., 2002; Takahata et al., 2001) of *Thermotogales*, and thermophilic sulfate-reducing, *Thermodesulfatator* belonging to the order *Thermodesulfobacteriales* (Moussard et al., 2004), were analyzed.

The thermophilic order *Thermales* currently comprises *Thermus*, *Meiothermus*, *Marinithermus* and *Oceanithermus* (Miroshnichenko et al., 2003a; Sako et al., 2003). A novel member, *Vulcanithermus*, was isolated as the fifth genus (Miroshnichenko et al., 2003b). Thus, polyamine profiles of new species of the five genera were determined, in relation to their growth temperature and their phylogenetic distance.

Thermophiles are distributed also in the Gram-positives of the orders *Thermoanaerobacteriales*, *Clostridiales* and *Bacillales*. Polyamines of new species of *Thermaerobacter* (Nunoura et al., 2002; Spanevello et al., 2002), *Caldanaerobacter* (Fardeau et al., 2000, 2004; Xue et al., 2001), *Thermanaeromonas* (Mori et al., 2002) and *Thermoanaerobacterium* (Cann et al., 2001) were determined. The thermophilic anaerobes, *Gelria* (Plugge et al., 2002), *Thermanaerovibrio* (Baena et al., 1999b), *Thermosyntropha* (Svetlitshnyi et al., 1996), *Thermicanus* (Gossner et al., 1999) and *Thermobrachium* (Engle et al., 1996), and the mesophiles, *Aminobacterium* and *Aminomonas* (Baena et al., 1999a, 2000), and several anaerobes located in the *Desulfotomaculum* lineage I (Brauman et al., 1998; Fardeau et al., 1995; Imachi et al., 2002; Qiu et al., 2003; Robertson et al., 2001), and the alkaliphilic clostridia, *Alkaliphilus* (Cao et al., 2003; Takai et al., 2001b) were available in the present study. Polyamines of thermophilic *Anoxybacillus* and *Ureibacillus* (Belduz et al., 2003; Fortina et al., 2001; Yumoto et al., 2004), as well as thermoacidophilic *Sulfobacillus* and *Alicyclobacillus* (Dufresne et al., 1996; Goto et al., 2002a, b, 2003; Matsubara et al., 2002; Tsuruoka et al., 2003), were also determined.

Distribution catalogues of long and branched polyamines in the 53 newly validated species were presented here to elucidate their chemotaxonomic significance. Their distribution profiles, at different culture temperatures under acidic, neutral and alkaline conditions, were also described.

Materials and Methods

The culture media designated by the culture collections were used according to the Catalogue of Strains (2004) of ATCC, JCM, NBRC and NCIMB. The medium pH and culture temperature used for optimum growth are given in Table 1. *Thermocrinis*, *Persephonella*, *Sulfurihydrogenibium*, *Balnearium* and *Thermovibrio* were grown under N_2-O_2-H_2 or H_2-CO_2. *Hydrogenothermus* was aerobically grown. *Marinitoga*, *Petrotoga* and *Thermotoga* were cultivated under N_2 or N_2-CO_2. *Thermodesulfatator* was grown under H_2-CO_2. *Thermus*, *Marinithermus* and *Meiothermus* were aerobically grown whereas *Vulcanithermus* and *Oceanithermus* were grown under a N_2 atmosphere. *Thermanaeromonas*, *Thermoanaerobacterium*, *Caldanaerobacter*, *Gelria*, *Thermosyntropha*, *Thermanaerovibrio*, *Thermobrachium*, *Aminobacterium* and *Aminomonas* were grown anaerobically. *Themaerobacter*, *Alkaliphilus*, *Desulfotomaculum*, *Desulfosporosinus*, *Pelotomaculum* and *Sporotomaculum* were grown under N_2-CO_2. *Sulfobacillus*, *Alicyclobacillus*, *Anoxybacillus* and *Ureibacillus* were grown aerobically and *Thermicanus* anaerobically.

The organisms at stationary phase were harvested and homogenized in equal volumes of cold 1 M $HClO_4$. The whole $HClO_4$ extract was analyzed by high-performance liquid chromatography (HPLC) on a column of cation-exchange resin in a Hitachi L6000 high-speed liquid chromatograph (Hamana et al., 1998). Polyamines were detected with the *o*-phthalaldehyde reagent. The whole extract was subjected to a Dowex

Table 1. Cellular concentration of polyamines of thermophilic eubacteria.

					Polyamines (μmol/g wet wt. cell)												
			°C	pH	3	4	5	33	34	333	333/ 3(3)3	343/ 334	3(3)3	3(3)4	343/ 334/ 3(3)4	3(3)(3)3	3(3)(3)4
Order *Aquificales*																	
Family *Aquificaceae*																	
Thermocrinis albus	JCM 11386^T		80	7.0	—	0.02	—	0.10	0.56	*	0.02	*	*	*	0.02	0.30	—
Hydrogenobacter hydrogenophilus	JCM 8158^T	(c)	75	7.0	—	0.01	—	0.60	0.50	—	*	0.06	—	0.03	*	2.10	—
Hydrogenobacter subterraneus	JCM 10560^T		75	7.0	0.01	—	—	0.06	1.20	—	*	0.03	0.02	—	*	2.40	—
Hydrogenobacter thermophilus	JCM 7687^T	(c)	70	7.0	—	—	—	—	1.50	—	*	0.03	0.05	0.02	*	2.60	0.01
Hydrogenobaculum acidophilum	JCM 8795^T	(c)	65	3.0	—	—	—	—	0.08	—	*	—	—	—	*	1.12	0.02
Aquifex pyrophilus	JCM 9492^T	(b)	85	6.5	—	—	—	0.02	—	—	—	—	0.02	—	—	1.60	—
Family *Hydrogenothermaceae*																	
Persephonella hydrogeniphila	JCM 11663^T		70	7.0	—	0.02	0.10	—	0.03	*	0.02	—	*	—	—	1.60	—
Sulfurihydrogenibium subterraneum	JCM 11477^T		62	7.5	—	0.40	0.20	—	1.50	—	—	*	—	*	0.01	1.30	—
Hydrogenothermus marinus	JCM 10974^T		65	7.0	0.01	—	—	0.18	0.40	—	*	—	0.08	—	—	3.10	—
Family *Desulfurobacteriaceae*																	
Balnearium lithotrophicum	JCM 11970^T		75	5.5	—	0.04	—	—	0.30	—	—	—	—	—	—	0.60	—
Thermovibrio ammonificans	JCM 12110^T		75	5.5	—	—	—	—	0.08	—	—	—	—	—	—	0.80	—
Thermovibrio ruber	JCM 11468^T		75	6.0	—	0.02	—	—	0.10	—	—	*	—	*	0.10	1.85	—
			°C	pH	3	4	5	33	34	44	333	343/ 334	3333	3343	3334	33333	
Order *Thermotogales*																	
Family *Thermotogaceae*																	
Marinitoga piezophila	JCM 11233^T		65	6.0	—	0.15	0.02	0.35	0.57	—	0.03	0.05	—	—	—	—	
Petrotoga miotherma	ATCC 51224^T	(d)	55	7.2	—	0.57	0.16	0.02	0.55	—	0.02	0.17	—	—	—	—	
Petrotoga olearia	JCM 11234^T		55	7.0	—	0.90	0.15	—	1.20	—	—	0.30	—	—	—	—	
Petrotoga sibirica	JCM 11235^T		55	7.0	—	0.25	—	—	0.80	—	—	0.25	—	—	—	—	
Thermotoga elfii	ATCC 51869^T	(a)	65	8.0	—	0.08	—	—	0.36	—	—	0.66	0.04	—	—	—	
Thermotoga lettingae	ATCC BAA-301^T		65	6.9	—	0.10	0.05	1.15	0.40	0.04	—	0.90	—	—	—	—	
Thermotoga maritima	JCM 10099^T		60	6.5	—	0.02	—	0.60	0.79	—	0.02	0.10	0.03	—	—	—	
			70	6.5	—	0.04	—	0.60	0.80	—	0.10	0.20	0.05	—	—	0.02	
		(a)	80	6.5	0.02	0.02	—	0.20	0.15	—	0.40	0.20	1.25	—	—	0.59	

Table 1. (Continued.)

			°C	pH	Polyamines (μmol/g wet wt. cell) 3	4	5	33	34	44	333	343/334	3333	3343	3334	33333
Thermotoga naphthophila	JCM 10882[T]		80	6.5	—	0.25	0.05	0.80	1.20	—	1.30	0.02	0.01	0.01	—	0.01
Thermotoga neapolitana	ATCC 49049[T]	(a)	80	6.5	0.20	0.15	0.02	1.35	1.15	—	0.15	0.14	0.16	0.05	0.02	0.02
Thermotoga petrophila	JCM 10881[T]		80	6.5	—	0.19	0.10	0.65	1.40	—	1.52	0.02	0.04	0.02	—	0.02
Thermosipho japonicus	JCM 10495[T]	(f)	70	7.0	—	0.27	—	1.05	1.45	—	0.02	0.40	—	—	—	—
Fervidobacterium islandicum	ATCC 4964[T]	(d)	70	7.0	0.02	0.14	0.02	0.85	1.42	—	0.12	0.22	0.10	0.12	—	—
Fervidobacterium nodosum	ATCC 35602[T]	(d)	70	7.0	0.04	0.10	0.05	0.60	1.40	—	0.15	0.45	—	—	—	—

			°C	pH	3	4	33	34	333	343	334	3333	3343	3334	3(3)4	3(3)(3)4
Order *Thermodesulfobacteriales*																
Family *Thermodesulfobacteriaceae*																
Thermodesulfobacterium commune	ATCC 33708[T]	(b)	70	7.5	—	0.02	0.02	0.18	0.05	0.08	—	0.04	—	—	0.03	1.00
Thermodesulfobacterium thermophilum	NCIMB 12254[T]	(b)	65	7.4	—	0.02	0.02	0.77	0.05	1.05	0.20	0.50	—	0.02	0.08	1.50
Thermodesulfatator indicus	JCM 11887[T]		70	6.7	—	0.10	—	0.90	—	0.01	—	—	—	—	—	0.80

			°C	pH	3	4	33	34	44	333	3(3)3	3(3)3/333	343	334	343/334	344	3333	3343	3334	3343/3334	33333	33334	3(3)(3)3
Order *Thermales*																							
Family *Thermaceae*																							
Thermus antranikianii	ATCC 700961[T]		70	7.6	—	0.10	0.01	0.25	1.00	0.32	—	*	0.10	0.30	*	0.05	0.02	0.01	0.02	*	—	—	—
Thermus aquaticus	ATCC 25104[T]	(a)	70	7.6	—	0.26	0.14	0.68	1.38	1.06	—	*	0.21	0.80	*	0.05	0.16	0.10	0.10	*	—	—	—
Thermus brockianus	NCIMB 12676[T]	(b)	70	7.6	—	0.10	0.10	0.75	1.30	0.80	—	*	0.20	2.50	*	0.20	0.02	0.02	0.01	*	—	—	—
Thermus filiformis	ATCC 43280[T]	(a)	70	7.6	0.04	0.20	0.09	0.88	1.04	0.16	0.02	*	0.10	0.41	*	0.09	0.21	0.05	0.05	*	0.05	—	0.05
Thermus igniterrae	ATCC 700962[T]		70	7.6	—	0.15	0.01	0.10	1.00	0.38	—	*	0.05	0.10	*	0.05	0.02	0.02	0.02	*	—	—	—
Thermus oshimai	NCIMB 13400[T]	(b)	70	8.2	0.01	0.02	0.05	0.18	0.42	0.60	—	*	0.20	1.40	*	0.06	0.06	0.04	0.05	*	—	—	—

Table 1. (Continued.)

					Polyamines (µmol/g wet wt. cell)																		
			°C	pH	3	4	33	34	44	333	3(3)3	3(3)3/333	343	334	343/334	344	3333	3343	3334	3343/3334	33333	33334	3(3)(3)3
Thermus scotoductus	ATCC 51532^T	(a)	65	7.2	—	0.15	—	0.24	1.70	0.30	—	*	0.22	1.50	*	0.45	0.10	0.02	0.03	*	—	—	—
Thermus thermophilus	ATCC 27634^T	(a)	70	7.2	—	0.51	0.14	0.14	0.95	0.53	0.04	*	0.19	1.00	*	0.05	0.11	0.06	0.04	*	0.04	0.04	0.02
Thermus thermophilus	JCM 10941^T		50	7.2	—	0.05	0.10	0.20	1.25	*	*	0.15	*	*	0.15	—	—	*	*	—	—	—	—
			60	7.2	—	0.10	0.10	0.30	2.10	*	*	0.40	*	*	0.30	0.05	—	*	*	—	—	—	—
			70	7.2	—	—	0.10	0.25	1.40	*	*	0.80	*	*	0.09	0.05	0.05	*	*	0.02	—	—	0.02

			°C	pH	3	4	5	33	34	44	333	343	334	3(3)4/343/334	344	444	3333	3334	3334/3343	3343	33333	33334
Meiothermus chiarophilus	NCIMB 13439^T	(b)	55	8.2	0.02	0.10	—	—	1.54	0.80	0.02	0.15	0.02	*	0.02	—	—	—	*	—	—	—
Meiothermus ruber	ATCC 34958^T	(a)	60	7.2	0.01	0.04	—	0.01	0.12	1.81	—	0.02	0.01	*	0.05	—	—	—	*	—	—	—
Meiothermus silvanus	NCIMB 13440^T	(b)	55	8.2	0.01	0.15	—	—	0.72	1.14	—	0.07	0.01	*	0.01	0.01	—	—	*	—	—	—
Meiothermus taiwanensis	ATCC BAA-399^T		60	7.6	—	0.12	—	0.02	0.85	0.95	—	0.23	0.02	*	0.02	—	—	—	*	—	—	—
Marinithermus hydrothermalis	JCM 11576^T		70	7.0	—	0.10	0.15	0.10	0.75	0.90	0.12	*	*	0.15	—	—	—	*	0.10	*	—	—
Oceanithermus profundus	NBRC 100410^T		60	7.3	—	—	0.05	—	0.05	—	—	1.00	—	*	—	—	—	—	*	—	—	—
			68	7.3	—	—	0.24	—	0.05	—	—	1.50	—	*	—	—	—	—	*	—	—	—
Vulcanithermus mediatlanticus	JCM 11956^T		70	6.5	—	0.02	—	0.02	0.50	—	0.03	0.10	0.40	*	—	—	0.10	0.20	*	0.02	0.04	0.01

Table 1. (Continued.)

			°C	pH	Polyamines (μmol/g wet wt. cell) 4	5	33	34	44	3(3)3	343/334/3(3)4	343	334	344	3(3)4	3343	3(3)(3)4	3(3)(3)3	3(3)(3)43
Order *Thermoanaerobacteriales*																			
Family *Thermoanaerobacteriaceae*																			
Thermanaeromonas toyohensis	JCM 11376^T		70	6.5	—	0.02	0.01	0.10	—	0.15	*	0.85	0.10	—	—	—	0.20	0.16	0.05
Thermoanaerobacter acetoethylicus	ATCC 33265^T	(e)	60	7.2	0.95	—	—	2.80	—	—	*	0.45	—	—	—	—	—	—	—
Thermoanaerobacter brockii	ATCC 53556^T	(e)	60	6.5	0.35	0.10	—	0.45	—	—	*	0.80	—	—	—	—	—	—	—
Thermoanaerobacter ethanolicus	ATCC 31550^T	(e)	60	6.5	0.68	0.14	—	1.30	—	—	*	0.50	—	—	—	—	—	—	—
Thermoanaerobacter kivui	ATCC 33488^T	(d)	65	7.2	0.01	0.03	—	0.50	—	—	*	2.15	—	—	0.04	—	0.40	—	—
Thermoanaerobacter thermocopriae	IAM 13577^T	(d)	60	7.1	0.61	0.02	—	1.25	—	—	*	2.75	—	—	0.01	—	0.16	—	—
Thermoanaerobacter thermohydrosulfuricus	ATCC 35045^T	(e)	60	7.2	0.12	0.08	—	1.48	—	—	*	2.15	—	—	—	—	—	—	—
Caldanaerobacter subterraneus subsp. *subterraneus*	ATCC BAA-225^T		65	7.5	0.15	0.25	—	0.69	—	—	1.62	*	*	—	*	—	0.24	—	—
Caldanaerobacter subterraneus subsp. *tengcongensis*	JCM 11007^T		55	7.5	0.65	0.90	—	1.40	—	—	0.50	*	*	—	*	—	0.06	—	—
			65	7.5	0.80	0.75	—	1.15	—	—	0.90	*	*	—	*	—	0.17	—	—
			75	7.5	0.15	0.05	0.01	0.51	0.01	—	*	0.55	0.10	0.01	—	0.05	1.20	—	—
Thermoanaerobacterium polysaccharolyticum	ATCC BAA-17^T		65	6.8	—	0.10	—	0.56	—	—	0.46	*	*	—	*	—	—	—	—
Thermoanaerobacterium saccharolyticum	ATCC 49915^T	(e)	60	7.0	0.40	0.10	—	1.64	—	—	*	0.20	—	—	—	—	—	—	—
Thermoanaerobacterium thermosaccharolyticum	ATCC 7956^T	(e)	50	7.0	1.35	0.02	—	1.49	—	—	*	0.35	—	—	—	—	—	—	—
Thermoanaerobacterium thermosulfurigenes	ATCC 33743^T	(e)	60	7.3	0.64	0.05	—	1.22	—	—	*	2.45	—	—	—	—	—	—	—
Thermoanaerobacterium zeae	ATCC BAA-16^T		65	6.8	—	0.10	—	0.45	—	—	0.35	*	*	—	*	—	—	—	—
Gelria glutamica	ATCC BAA-262^T		55	7.0	—	—	—	0.15	—	—	*	0.85	—	—	—	—	—	—	—

Table 1. (Continued.)

					Polyamines (µmol/g wet wt. cell)									
			°C	pH	4	5	33	34	343	334	3(3)4	343/ 334/ 3(3)4	3(3)(3)4	Agm
Order *Clostridiales*														
Family *Syntrophomonadaceae*														
Thermaerobacter marianensis	JCM 10246^{T}	(f)	70	7.6	—	0.04	0.16	0.60	*	*	*	0.75	0.60	0.70
Thermaerobacter nagasakiensis	JCM 11223^{T}		70	7.0	—	0.05	0.02	0.55	*	*	*	0.35	0.55	1.40
Thermaerobacter subterraneus	ATCC BAA-137^{T}		70	7.3	—	—	—	0.50	*	*	*	0.85	0.12	0.30
Thermosyntropha lipolytica	ATCC 700317^{T}		60	9.0	0.01	—	—	0.70	0.90	—	—	*	—	—
Thermanaerovibrio acidaminovorans	ATCC 49978^{T}		55	7.1	0.02	0.10	—	9.50	0.40	—	—	*	—	—
Aminobacterium mobile	ATCC BAA-7^{T}		37	7.2	0.50	—	—	0.60	0.15	—	—	*	—	—
Aminomonas paucivorans	ATCC BAA-6^{T}		37	7.2	0.45	—	—	1.10	0.30	—	—	*	—	—
Family *Clostridiaceae*														
Thermobrachium celere	ATCC 700318^{T}		60	8.0	0.12	0.10	—	0.45	0.45	—	—	*	—	—
Alkaliphilus transvaalensis	JCM 10712^{T}		37	11.5	—	—	—	1.15	0.09	—	—	*	—	0.03
Alkaliphilus crotonatoxidans	JCM 11672^{T}		37	7.5	—	—	—	1.15	0.15	—	—	*	—	0.02
			°C	pH	4	34	343	3343	33343	33433	3(3)4	3(3)(3)4	3(3)(3)43	Agm
Order *Bacillales*														
Family *Alicyclobacillaceae*														
Sulfobacillus acidophilus	ATCC 700253^{T}	(g)	45	2.0	—	1.24	0.50	—	—	—	—	—	—	0.34
Sulfobacillus disulfidooxidans	ATCC 51911^{T}		35	2.5	—	0.75	0.01	—	—	—	—	—	—	0.05
Alicyclobacillus acidoterretris	ATCC 49025^{T}	(g)	50	4.0	0.03	1.00	0.04	—	—	—	—	—	—	0.05
Alicyclobacillus acidocaldarius	JCM 5260^{T}	(g)	65	4.0	—	0.31	1.40	—	—	—	—	—	—	0.02
	IAM 14934		55	4.5	—	0.09	0.70	—	—	—	—	—	—	0.02
Alicyclobacillus cycloheptanicus	IFO 15310^{T}	(g)	50	4.0	—	1.60	0.56	—	—	—	—	—	—	0.40
Alicyclobacillus herbarius	IAM 14883^{T}		55	4.5	—	0.50	0.50	—	—	—	—	—	—	0.15
			45	4.0	—	0.30	0.50	—	—	—	—	—	—	—
			55	4.0	—	0.45	0.70	—	—	—	—	—	—	—
			60	4.0	—	0.40	0.60	—	—	—	—	—	—	—
Alicyclobacillus acidiphilus	IAM 14935^{T}		50	3.0	—	1.04	0.04	—	—	—	—	—	—	0.03
			35	4.0	—	1.20	0.22	—	—	—	—	—	—	—
			45	4.0	—	1.20	0.18	—	—	—	—	—	—	—
			55	4.0	—	1.00	0.24	—	—	—	—	—	—	0.02

Table 1. (Continued.)

					Polyamines (μmol/g wet wt. cell)									
			°C	pH	4	34	343	3343	33343	33433	3(3)4	3(3)(3)4	3(3)(3)43	Agm
Alicyclobacillus pomorum	IAM 14988^{T}		55	4.5	—	0.83	0.06	—	—	—	—	—	—	—
			45	4.0	—	0.75	0.15	—	—	—	—	—	—	0.09
			60	4.0	—	0.80	0.15	—	—	—	—	—	—	0.08
Alicyclobacillus sendaiensis	JCM 11817^{T}		55	4.5	—	1.04	0.75	—	—	—	—	—	—	0.20
Bacillus schlegelii	ATCC 43741^{T}	(h)	75	7.0	—	0.60	1.50	0.90	0.01	0.01	0.15	0.20	—	—
Bacillus tusciae	IFO 15132^{T}	(h)	50	5.0	0.06	1.90	1.78	—	—	—	—	—	—	0.01
Family *Paenibacillaceae*														
Thermicanus aegyptius	ATCC 700890^{T}		55	7.0	0.10	1.20	0.10	—	—	—	—	—	—	—
Family *Bacillaceae*														
Bacillus smithii	IFO 15311^{T}	(h)	60	7.3	—	1.57	0.96	—	—	—	—	—	0.01	0.01
Bacillus thermocloacae	ATCC 49805^{T}	(g)	60	7.0	—	0.74	1.60	—	—	—	—	—	—	—
Saccharococcus thermophilus	ATCC 43125^{T}	(h)	70	7.0	0.10	0.76	0.93	0.02	—	—	0.02	0.02	0.03	0.04
Geobacillus stearothermophilus	IAM 11062^{T}	(h)	65	7.5	—	0.45	2.50	—	—	—	—	—	0.01	0.05
Geobacillus thermocatenulatus	IFO 15316^{T}	(h)	65	7.3	0.04	0.14	3.25	—	—	—	—	—	0.02	0.04
Anoxybacillus flavithermus	IFO 15317	(h)	65	7.3	—	1.15	0.50	—	—	—	—	—	—	0.02
Anoxybacillus gonensis (Aero)	NCIMB 13933^{T}		60	7.3	—	0.35	0.90	—	—	—	—	—	—	—
(Anaero)			60	7.3	—	0.50	1.05	—	—	—	—	—	—	—
Anoxybacillus (Aero)	JCM 12111^{T}		50	7.5	0.03	0.90	0.70	0.04	—	—	—	—	—	0.05
voinovskiensis (Anaero)			50	7.5	—	0.72	0.60	0.04	—	—	—	—	—	0.10
Ureibacillus thermosphaericus	NCIMB 13819^{T}		60	7.3	—	1.60	0.20	—	—	—	—	—	—	0.04

			°C	pH	4	5	34	343	Agm
Desulfotomaculum lineage I									
Family *Peptococcaceae*									
Desulfotomaculum acetoxidans	ATCC 49208^{T}	(g)	37	7.2	—	—	0.35	0.77	—
Desulfotomaculum nigrificans	ATCC 7946	(g)	55	7.5	0.04	0.04	0.74	0.10	0.52
	IFO 13698^{T}	(g)	55	7.5	0.02	0.04	1.20	0.20	0.45
Desulfotomaculum ruminis	ATCC 23193^{T}	(g)	37	7.5	0.10	—	0.05	—	—
Desulfotomaculum thermobenzoicum	ATCC 49756^{T}	(g)	60	7.0	—	0.25	2.05	0.45	0.02
Desulfotomaculum thermosapovorans	NCIMB 13375^{T}		50	7.4	—	—	0.22	0.18	—
Desulfitobacterium dehalogenans	ATCC 51507^{T}	(g)	35	6.5	—	—	0.57	0.02	—
	ATCC 700041	(g)	35	7.3	—	—	0.74	0.14	—

Table 1. (Continued.)

			°C	pH	Polyamines (μmol/g wet wt. cell) 4	5	34	343	Agm
Desulfosporosinus meridiei	NCIMB 13706^T		30	7.4	—	—	0.40	0.10	—
Desulfosporosinus orientis	ATCC 23598	(g)	30	7.5	—	—	0.97	0.30	—
Pelotomaculum thermopropionicus	JCM 10971^T		55	7.0	0.05	—	0.05	0.50	—
Family *Thermoanaerobacteriaceae*									
Sporotomaculum hydroxybenzoicum	ATCC 700645^T		30	7.6	—	—	0.65	0.15	—
Sporotomaculum syntrophicum	JCM 11495^T		37	6.5	—	—	0.35	0.45	—
Family *Lachnospiraceae*									
Acetitomaculum ruminis	ATCC 43876^T	(g)	37	7.5	0.20	—	0.24	0.06	0.02

3, diaminopropane; 4, putrescine; 5, cadaverine; 33, norspermidine; 34, spermidine; 44, homospermidine; 333, norspermine; 343, spermine; 334, thermospermine; 344, aminopropylhomospermidine; 3333, caldopentamine; 3334, homocaldopentamine; 3343, thermopentamine; 33333, caldohexamine; 33334, homocaldohexamine; 33343, thermohexamine; 33433, homothermohexamine; 3(3)3, N^4-aminopropylnorspermidine; 3(3)4, N^4-aminopropylspermidine; 3(3)(3)3, N^4-bis(aminopropyl)norspermidine; 3(3)(3)4, N^4-bis(aminopropyl)spermidine; 3(3)43, N^4-aminopropylspermine; 3(3)(3)43, N^4-bis(aminopropyl)spermine; Agm, agmatine; (Aero), aerobically grown; (Anaero), anaerobically grown; —, not detected (<0.005); *, data not separated; ^{T}, type strain; ATCC, American Type Culture Collection, Manassas, Virginia, USA; IAM, IAM Culture Collection, Institute of Molecular and Cellular Biosciences, the University of Tokyo, Tokyo, Japan; JCM, Japan Collection of Microorganisms, RIKEN, Wako, Saitama, Japan; NBRC, Biological Resourse Center, National Institute of Technology and Evaluation, Kisarazu, Chiba, Japan; NCIMB, the National Collections of Industrial, Food and Marine Bacteria, Aberdeen, Scotland, UK. (a) Cited from Hamana et al.(1998); (b) Hamana et al.(1999); (c) Hamana et al.(1995); (d) Hamana et al.(1996a); (e) Hamana et al. (1996c); (f) Hamana et al. (2001); (g) Hamana (1999); (h) Hamana et al. (1993). °C, culture temperature.

50W column to concentrate long and branched polyamines (Hamana et al., 1998). Gas chromatography (GC) was performed after heptafluorobutyrization of the concentrated polyamine samples (Niitsu et al., 1993). Polyamines were identified by gas chromatography-mass spectrometry (GC-mass) (Niitsu et al., 1993).

Results and Discussion

HPLC analysis of polyamines has been developed in our laboratory and resolution of many polyamines was improved (Hamana and Itoh, 2001; Hamana et al., 2001). Norspermine [333] and N^4-aminopropylnorspermidine [3(3)3] were co-eluted in the HPLC. Separation of thermospermine [334], spermine [343] and N^4-aminopropylspermidine [3(3)4] was difficult by HPLC. When a concentrated polyamine sample is subjected to GC analysis, they are identified. Thermopentamine [3343] and homocaldopentamine [3334] were separately eluted in the HPLC and GC. Typical HPLC chromatograms of the acid extracted polyamines are shown in Fig. 1. Some GC chromatograms of the concentrated polyamine samples are shown in Fig. 2. Polyamine peaks were further identified by GC-mass. During GC analysis, a quaternary penta-amine, N^4-bis(aminopropyl)norspermidine [3(3)(3)3], was converted to a tertiary tetra-amine, [3(3)3], and another quaternary penta-amine, N^4-bis(aminopropyl) spermidine [3(3)(3)4] was converted to two tertiary tetra-amines, [3(3)3] and [3(3)4] (Hamana et al. 1992). Cellular concentrations of polyamines estimated from the HPLC and GC analyses are shown in Table 1. A list of trivial names, systematic names and chemical formulae of polyamines has been recorded elsewhere (Hamana, 2002; Hamana and Matsuzaki, 1992; Hamana et al., 1994). Polyamine profiles within each order and lineage are followed.

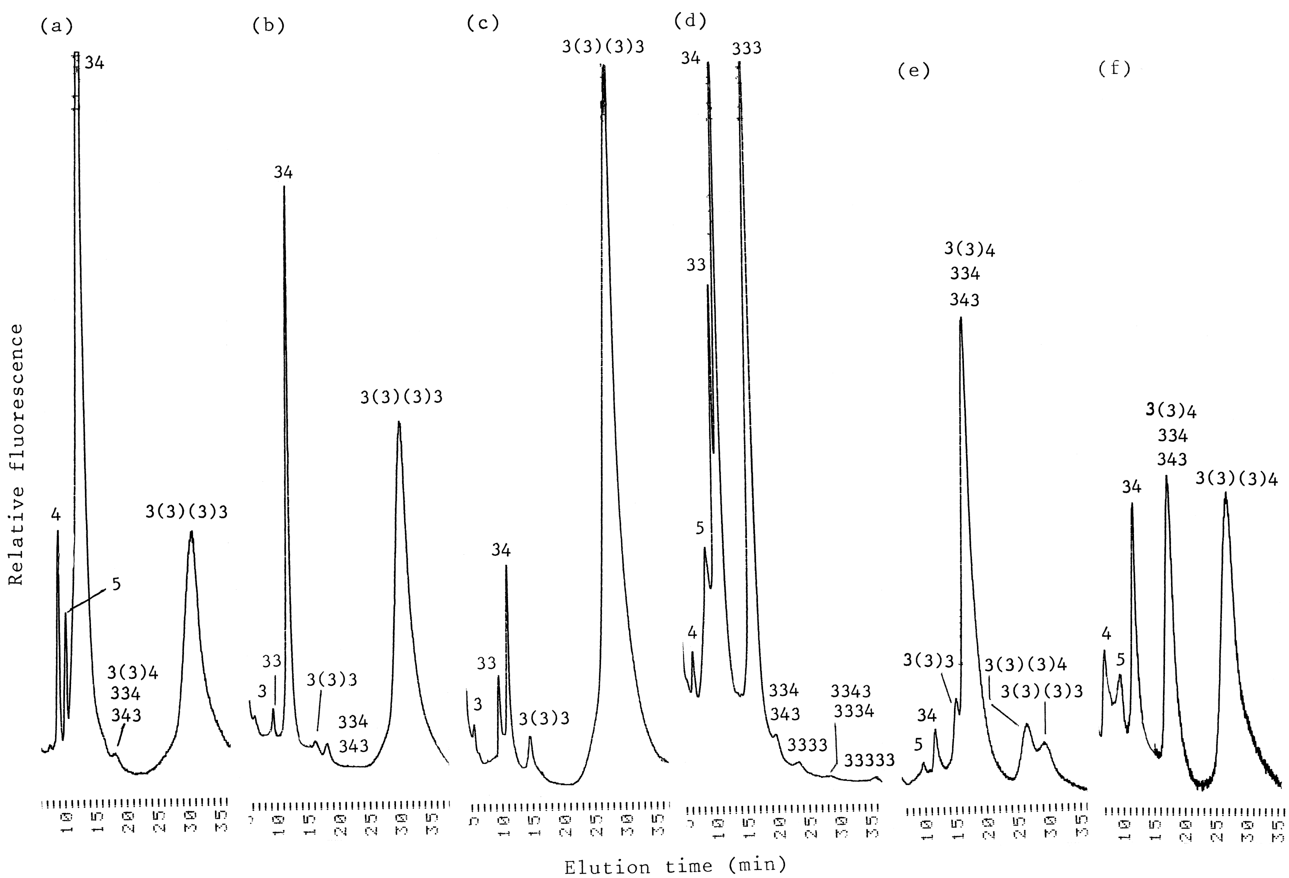

Fig. 1. HPLC analysis of polyamines extracted from (a) *Sulfurihydrogenibium subterraneum* JCM 11477; (b) *Hydrogenobacter subterraneus* JCM 10560; (c) *Hydrogenothermus marinus* JCM 10974; (d) *Thermotoga petrophila* JCM 10881; (e) *Thermanaeromonas toyohensis* JCM 11376; (f) *Caldanaerobacter subterraneus* subsp. *tengcongensis* JCM 11007 (grown at 75°C).

Abbreviations for polyamines are given in Table 1.

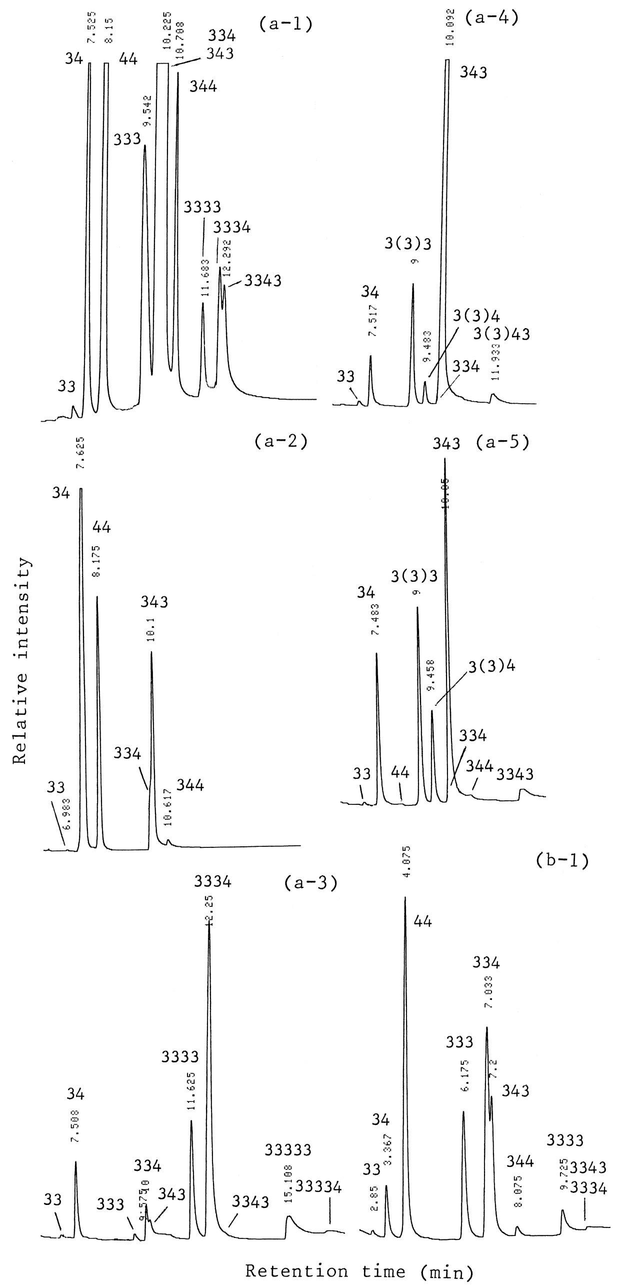

Fig. 2. GC analysis of the concentrated long polyamine fractions from (a-1) *Thermus igniterrae* ATCC 700962; (a-2) *Meiothermus taiwanensis* ATCC BAA-399; (a-3) *Vulcanithermus mediatlanticus* JCM 11956; (a-4) *Thermanaeromonas toyohensis* JCM 11376; (a-5) *Caldanaerobacter subterraneus* subsp. *tengcongensis* JCM 11007 (grown at 75°C); (b-1) *Thermus antranikianii* ATCC 700961.

GC-column oven temperature was raised from 120 to 280°C in (a) and from 200 to 280°C in (b) (Niitsu et al., 1993). Abbreviations for polyamines are given in Table 1. Retention times (min) are printed on each peak.

Aquificales

The genera *Aquifex*, *Thermocrinis*, *Hydrogenobacter* and *Hydrogenobaculum* phylogenetically belong to the family *Aquificaceae* of the order *Aquificales*. *Persephonella*, *Hydrogenothermus* and *Sulfurihydrogenibium* are located in the family *Hydrogenothermaceae* of the order (Eder and Huder, 2002; Takai et al., 2003a). *Thermovibrio* and *Balnearium* together with *Desulfurobacterium*, represent a tentative family *Desulfurobacteriaceae* of the order (Huber et al., 2002; Takai et al., 2003b). We reported the occurrence of a quaternary branched penta-amine, N^4-bis(aminopropyl)norspermidine, as the main polyamine of a species of *Aquifex*, a species of *Hydrogenobaculum* and two *Hydrogenobacter* species (Hamana et al., 1995, 1999). The eight new members of *Aquificales* ubiquitously contained N^4-bis(aminopropyl)norspermidine as the major polyamine.

The ubiquitous and abundant occurrence of N^4-bis(aminopropyl)norspermidine within the moderate-, extreme- and hyper-thermophiles, ranging from 62 to 85°C as the optimum growth temperature, characterizes the order *Aquificales*.

Thermotogales

Several thermophiles belonging to *Thermotogales*, previously analyzed, contained liniar triamines, tetra-amines, penta-amines and a hexa-amine (Hamana et al., 1998, 2001). Among the *Thermotoga* species examined, *Thermotoga naphthophila* and *Thermotoga petrophila* growing at 80°C contained norspermidine, norspermine, caldopentamine and thermopentamine, in addition to spermidine and spermine (and/or thermospermine). On the other hand, *Thermotoga littingae* growing at 65°C was devoid of norspermine, caldopentamine and thermopentamine. Existence of homospermidine in *T. littingae* was confirmed by the GC analysis. Such difference in the polyamine profile of *T. littingae*, as well as that of *Thermotoga elfii*, might reflect their distinct phylogenetic position and growth temperature from other *Thermotoga* species. When *Thermotoga maritina* was cultivated at lower temperatures, penta-amine and hexa-amine were decreased. Therefore, the difference in the long polyamine composition may be attributed to the difference of growth temperature. Likewise, *Marinitoga piezophila*, *Petrotoga olearia* and *Petrotoga sibirica* growing at 55–65°C showed polyamine profiles lacking panta-amines and hexa-amines. The level of norspermidine was low in all the *Petrotoga* species and not significantly affected by the growth temperatures in *Thermotoga maritina*. The low norspermidine level in the genus *Petrotoga* and *T. elfii* may represent a chemotaxonomic feature.

Within other members of the order *Thermotogales* (the family *Thermotogaceae*), linear penta-amines have been detected in *Fervidobacterium islandicum* growing at 70°C, but not in *Fervidobacterium nodosum* and *Thermosipho japonicus* growing at 70°C (Hamana et al., 1996a, 2001).

Thermodesulfobacteriales

The occurrence of a quaternary branched panta-amine, N^4-bis(aminopropyl)spermidine, in the two species of *Thermodesulfobacterium* growing at 65–70°C, have been reported in our previous reports (Hamana et al., 1996a, 1999). A new member of the order *Thermodesulfobacteriales* (the family *Thermodesulfobacteriaceae*), *Thermodesulfatator indicus*, growing at 70°C, contained the branched penta-amine indicating the ubiquitous occurrence of the branched panta-amine in the two extremely-thermophilic, sulfate-reducing genera of this order.

Thermales

Six *Thermus* species previously analyzed contained many triamines, tetra-amines and penta-amines (Hamana et al., 1991, 1998, 1999), as listed in Table 1. A branched tetra-amine, N^4-aminopropylnorspermidine, and a branched penta-amine, N^4-bis(aminopropyl)norspermidine, have been detected in two of the six species. In the two new members of extremely thermophilic *Thermus* growing at 70°C, almost the same polyamine components were identified; however, hexa-amines and branched polyamines were not detected. Penta-amines increased at higher growth temperature of *T. thermophilus*. A new species of the genus *Meiothermus*, *M. taiwanensis* had a polyamine pattern similar to that of the three *Meiothermus* species previously reported (Hamana et al., 1991, 1998, 1999). Spermidine, homospermidine and spermine were found as the major polyamine and norspermidine and norspermine were minor in the moderately thermophilic genus *Meiothermus* growing at 55–60°C. Although levels of norspermine were affected by growth temperature in *T. thermophilus*, a significant amount of norspermine as well as norspermidine was found in the cells grown at the lower temperature 50°C.

A marine species, *Marinithermus hydrothermalis* growing at 70°C, contained norspermidine, spermidine, homospermidine, spermine (and/or thermospermine) and homocaldopentamine (and/or thermopentamine). Two hexa-amines were detected but not homospermidine in *Vulcanithermus mediatlanticus* growing at 70°C. *Oceanithermus profundus* growing at 60–68°C contained spermine alone as the major polyamine and lacked norspermidine and homospermidine as well as long and branched polyamines. Therefore, some genus-specific polyamine profiles among the five genera of the *Thermacea* were observed.

The family *Thermaceae* (the order *Thermales*) belongs to the phylum *Deinococcus-Thermus*, however, mesophilic *Deinococcus* species belonging to another family *Deinococcaceae* (the order *Deinococcales*) lacked norspermidine, homospermidine, and long and branched polyamines (Hamana, 1994).

Thermoanaerobacteriales

Two quaternary penta-amines, N^4-bis(aminopropyl)spermidine and N^4-bis(aminopropyl)norspermidine, were found as major polyamines in extremely thermophilic *Thermanaeromonas toyohensis* growing at 70°C. In addition, a novel tertiary branched penta-amine, N^4-aminopropylspermine, was detected by GC analysis of the concentrated polyamine fraction from this organism. This tertiary penta-amine is produced from a quaternary hexa-amine, N^4-bis(aminopropyl)spermine, during GC analysis (Hanama et al., 1992, 1993). It is strongly suggested that the quaternary branched hexa-amine exists in *T. toyohensis*.

Recently, *Caldanaerobacter subterraneus* subsp. *subterraneus* (formerly *Thermoanaerobacter subterraneus*) and *Caldanaerobacter subterraneus* subsp. *tengcongensis* (formerly *Thermoanaerobacter tengcongensis*) were phylogenetically separated from other *Thermoanaerobacter* species (Fardeau et al., 2004). N^4-bis(aminopropyl)spermidine was found in the two *Caldanaerobacter* species, as a major polyamine, as well as *Thermoanaerobacter kivui* and *Thermoanaerobacter thermocopriae*, previously reported (1996c). Extremely thermophilic *C. subterraneus* subsp. *tengcongensis* growing at 75°C contained norspermidine, homospermidine, aminopropylhomospermidine and thermopentamine as a minor polyamine component. A minor occurrence of thermospermine was found by GC-mass analysis. When *C. subterraneus* subsp.

tengcongensis was cultivated at lower temperatures, 55°C and 65°C, it still had the branched penta-amine in decreased amounts. Moderate thermophilic *Thermoanaerobacterium* species and *Gelria glutamica*, growing at 55–65°C, contained putrescine, spermidine and spermine and lacked long and branched polyamines (Table 1). In *Caprothermobacter* and *Moorella* species growing at 60°C, N^4-bis(aminopropyl)spermidine was found in some species (Hamana et al., 1996c). Thus, the distribution profiles of long and branched polyamines were variable within the order *Thermoanaerobacteriales*.

Clostridiales

Strictly-aerobic, thermophilic *Thermaerobacter marianensis* contained a quaternary branched penta-amine, N^4-bis(aminopropyl)spermidine, and agmatine (Hamana et al., 2001). Two new species of *Thermaerobacter*, *T. nagasakiensis* and *T. subterraneus*, also contained N^4-bis(aminopropyl)spermidine and agmatine. These extreme thermophiles, growing at 70°C, show same polyamine profiles.

Moderately thermophilic anaerobes, belonging to the genera *Thermosyntropha* and *Thermanaerovibrio*, growing at 55–60°C, contained spermidine and spermine, but lacked long and branched polyamines. Two mesophiles, *Aminobacterium* and *Aminomonas*, were rich in putrescine and spermidine and poor in spermine. These findings suggest that the occurrence of long and branched polyamines are coupled with extreme thermophily within *Syntrophomonadaceae*, a family of the order *Clostridiales*. Thermophilic *Caldicellulosiruptor* species (growing at 70°C) of this family contained also linear and branched penta-amines (Hamana et al., 2001).

Moderately thermophilic *Clostridium* species contained spermine as a major polyamine and mesophilic *Clostridium* species lacked a significant amount of spermine, as reported in our previous studies (Hamana, 1999; Hamana et al., 1996c). Two mesophilic anaerobes, *Alkaliphilus transvaalensis* and *Alkaliphilus crotonatoxidans*, growing at 37°C, and a moderately thermophilic anaerobe, *Thermobrachium celere*, growing at 60°C, are located in the family *Clostridiaceae*. The former two strains contained spermidine as the major polyamine and the latter strain contained spermine as a major polyamine. *Caloramator fervidus* of this family, growing at 70°C, also contained spermine but not long branched polyamine (Hamana et al., 1996c). The genus *Alkaliphilus* contained alkaliphilic and non-alkaliphilic species, and alkaliphily did not affect the polyamine composition in the two species.

Bacillales

The family *Alicyclobacillaceae* of the order *Bacillales* includes mesophilic, acidophiles and moderately (or slightly) thermophilic acidophiles (Dufresne et al., 1996; Goto et al., 2002a, b, 2003; Matsubara et al., 2002; Tsuruoka et al., 2003). When two *Sulfobacillus* species were cultivated at optimum growth temperature, spermine level was higher at higher temperatures. In the three *Alicyclobacillus* species, spermine level did not change significantly in temperature ranges between the optimal and 15°C below. Nevertheless, the levels of spermine among the different species seemed to be variable in accordance with their optimal growth temperatures. A acidic pH range from 3.0 to 4.0 in *Alicyclobacillus* species did not change significantly in their polyamine profiles. Considering that neutrophilic, moderate thermophiles of the families *Bacillaceae* and *Thermoactinomycetaceae* also contained spermine (Hamana, 1999; Hamana et al., 1993), it seems that acidophily does not affect the polyamine composition and the occurrence of spermine correlates to moderate thermophily. This family also contains thermoacidophilic *Bacillus tusciae* (growing at 50°C) containing spermidine and spermine and extremely thermophilic *Bacillus schlegelii* (growing at 75°C) containing linear penta- and hexa-amines and a branched penta-amine (Hamana et al., 1993) (Table 1).

In the family *Bacillaceae*, the occurrence of a quaternary branched hexa-amine, N^4-bis(aminopropyl)-spermine (detected as N^4-aminopropylspermine in GC analysis), in addition to spermidine and spermine, was reported in thermophilic *Saccharococcus*, *Geobacillus* (Nazina et al., 2001) and *Bacillus* species growing at 60–75°C (Hamana et al., 1993). New thermophilic *Anoxybacillus* and *Ureibacillus* species (growing at 50–65°C) (Belduz et al., 2003; Fortina et al., 2001; Yumoto et al., 2004), analyzed here, contained spermidine and spermine as the major polyamine. Thermopentamine and agmatine were detected in *Anoxybacillus voinovskiensis*. Some polyamine profiles were observed in the both aerobically and anaerobically grown *Anoxybacillus* species (Table 1). The branched hexa-amine was abundant in the extreme thermophiles containing high spermine levels within *Bacillaceae* and *Thermoanaerobacteriaceae*.

A moderately thermophilic acetogen growing at 55°C, *Thermicanus aegyptius*, belongs to the family *Paenibacillaceae* and is related to the other three families of this order (Gossner et al., 1999). This organism contained spermidine and spermine. In *Paenibacillaceae*, it is known that mesophilic *Paenibacillus* and *Brevibacillus* species lack spermine whereas mesophilic *Aneurinibacillus* species contain a small amount of spermine (Hamana, 1999).

Desulfotomaculum lineage I

Sulfate-reducing *Desulfotomaculum* and *Desulfosporosinus*, sulfite-reducing *Desulfitobacterium*, propionate-oxidizing *Pelotomaculum* and benzoate-degrading *Sporotomaculum*, located in the family *Peptococcaceae* of the order *Clostridiales* or the family *Thermoanaerobacteriaceae* of the order *Thermoanaerobacteriales*, are moderate thermophiles growing at 50–60°C or mesophiles growing at 37–30°C (Brauman et al., 1998; Fardeau et al., 1995; Imachi et al., 2002; Qiu et al., 2003; Robertson et al., 2001). The polyamine pattern of an acetogenic anaerobe, *Acetitomaculum ruminis*, belonging to the family *Lachnospiraceae* of the order *Clostridiales* (Hamana, 1999), was cited in Table 1. Although putrescine, spermidine and/or spermine were found as the major polyamines in the *Desulfotomaculum* lineage I, spermine was relatively abundant in the moderate thermophiles as suggested in our previous report (Hamana, 1999). Some of the thermophiles contained agmatine, suggesting that the cellular occurrence of spermine and agmatine may correlate to their moderate thermophily, as found in the thermophiles of *Clostridiales* and *Bacillales*.

Polyamine profiles found in thermophilic eubacteria

The thermophiles belonging to *Aquificales*, lacking long linear polyamines, ubiquitously contained a quaternary branched penta-amine. In *Thermodesulfobacteriales*, the thermophiles ubiquitously contained another quaternary branched penta-amine. The occurrence of two different quaternary branched penta-amines is chemotaxonomically significant and was not strictly affected by growth temperature in the two orders. The thermophiles of *Thermotogales* contained long linear polyamines. In the three orders phylogenetically located in early eubacterial evolution, the occurrence of the branched polyamines or the linear long polyamines seems to be coupled with their thermophily, and serve for a chemotaxonomy.

Extremely thermophilic *Thermus* contained long linear and branched polyamines whereas other extreme thermophiles, *Marinithermus* and *Vulcanithermus*, contained long linear polyamines. Moderately-slightly thermophilic *Meiothermus* and *Oceanithermus* lacked them. The presence or absence of homospermidine, norspermidine and its derivatives differed in the five genera; however, they serve as a chemotaxonomic marker within the *Thermales*. Linear long polyamines and branched polyamines were simultaneously found in some extreme thermophiles belonging to *Thermoanaerobacteriales*, *Clostridiales* and *Bacillales* located in Gram-positives. Various moderate thermophiles of this taxa contained spermidine and spermine alone.

It is suggested that the synthetic abilities of long linear polyamines and branched polyamines were separately developed in early evolved thermophilic eubacteria and the two polyamine synthetic pathways were mixed during later eubacterial evolution. One of them seems to be essential for extremely thermophilic eubacteria. Norspermidine- and homospermidine-syntheses might be phylogenetically developed or elimitated in eubacteria independently of their thermophily.

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