ORIGINAL PAPER

Journal of Japanese Society for Extremophiles (2008), Vol. 7, 10-20

Hamana K[a,b,e], Hosoya R[a], Yokota A[c], Niitsu M[d], Hayashi H[e] and Itoh T[b]

Distribution of long linear and branched polyamines in the thermophiles belonging to the domain Bacteria

[a] Gunma University School of Health Sciences, Maebashi, Gunma 371-8514, Japan.
[b] Japan Collection of Microorganisms, RIKEN, BioResource Center, Wako, Saitama 351-0198, Japan.
[c] Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo 113-0032, Japan.
[d] Faculty of Pharmaceutical Sciences, Josai University, Sakado, Saitama 350-0290, Japan.
[e] Faculty of Engineering, Maebashi Institute of Technology, Maebashi, Gunma 371-0816, Japan.

Corresponding author : Koei Hamana, khamana@health.gunma-u.ac.jp
Phone : +81-27-220-8916, FAX : +81-27-220-8999

Received: April 3, 2008/ Reviced:May 26, 2008/ Acepted:June 3, 2008

Abstract Cellular polyamines of 44 newly validated eubacterial thermophiles growing at 45-80℃, belonging to eight orders (six phyla) of the domain Bacteria, were analyzed by HPLC and GC. A quaternary branched penta-amine, N^4-bis(aminopropyl)norspermidine, was found in *Hydrogenivirga* and *Sulfurihydrogenibium* belonging to the order of *Aquificales*. Another quaternary branched penta-amine, N^4-bis(aminopropyl) spermidine, was detected in *Thermovibrio*, *Desulfurobacterium* (*Aquificales*), *Moorella*, *Desulfovirgula* (*Thermoanaerobacteriales*), *Thermaerobacter* (*Clostridiales*), *Caldalkalibacillus*, *Thermobacillus* (*Bacillales*), and *Desulfothermus* (*Desulfovibrionales* of the phylum *Proteobacteria*). The two branched penta-amines as well as linear penta- and hexa-amines were produced by *Thermus* species of the order *Thermales*. *Marinitoga* and *Fervidobacterium* (*Thermotogales*) contained linear penta-amines. The production of these long polyamines was dependent on culture temperature and remarkable at 70-80 ℃ in the extreme/ hyper-thermophiles and to alkaline medium pH 8.5-9.0 in moderately thermophilic bacilli. The long polyamines were not detected in moderately thermophilic *Anaerolinea* (*Anaerolineales* of the phylum *Chloroflexi*). Distribution of either or both long linear polyamines and/or quaternary branched polyamines in the extreme/hyper-thermophiles is also reflected in their phylogenetical evolved locations.

Key words: branched polyamine, eubacteria, long polyamine, thermophiles

Introduction

Various extremely thermophilic eubacteria grown at 65-80℃ and moderately thermophilic eubacteria grown at 45-65℃ distribute as divers phyla or orders of the domain Bacteria [2, 3, 15]. Many hyperthermophiles grown at 80-100℃ have been isolated in archaebacteria (the domain Archaea) and a few of hyperthermophile have been published in eubacteria [15, 16]. However, the degree of thermophily is roughly estimated and not defined exactly. The cellular occurrence of long linear and/or branched polyamines in extremely thermophilic (or hyperthermophilic) eubacteria suggested that the extreme thermophiles (or hyperthermophiles) may have some novel polyamine synthetic abilities possibly associated with their thermophily [8-11, 13-15, 18, 23, 24]. These long and branched polyamines are important to stabilize the cellular nucleic acid structure of extreme thermophiles under extremely high thermal environments [15, 38] and have never been found to our knowledge, in eubacterial moderate thermophiles. Additional distribution catalogues of long-linear and branched polyamines in 44 newly validated thermophiles distributed in the eight eubacterial orders (belonging to six phyla): *Aquificales* (the phylum *Aquifica*), *Thermotogales* (the phylum *Thermotogae*), *Anaerolineales* (the phylum *Chloroflexi*), *Thermales* (the phylum *Deinococcus-Thermus*), *Thermoanaerobacteriales* (the phylum *Firmicutes*), *Bacillales* (the phylum *Firmicutes*), *Clostridiales* (the phylum *Firmicutes*), and *Desulfovibrionales* (the class *Deltaproteobacteria* of the phylum *Proteobacteria*), were presented here to highlight their significance for thermophily and their usefulness for chemotaxonomy within eubacterial thermophiles. Temperature-dependent syntheses of long polyamines were determined in several thermophiles.

Materials and Methods

New eubacteria were supplied by JCM (Japan Collection of Microorganisms, RIKEN, Wako, Saitama, Japan), NBRC (Biological Resource Center, National Institute of Technology and Evaluation, Kisarazu, Chiba, Japan), IAM (Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan) and ATCC (American Type Culture Collection, Manassas, Virginia, USA) and cultivated in the liquid media designated by

the culture collections under aerobic or anaerobic condition. The medium pH and culture temperature used for optimum growth and different maximum culture temperatures in some thermophiles are given in Table 1. The organisms in the stationary phase were harvested by centrifugation. The pellets of these organisms were washed with 0.8% NaCl and then homogenized in equal volumes of cold 1M perchloric acid (PCA) ($HClO_4$). The whole PCA extract was subjected to a Dowex-50W column to concentrate polyamines [7, 18]. The concentrated polyamine fraction was analyzed by high-performance liquid chromatography (HPLC) on a column of cation-exchange resin in a Hitachi L-6000 high speed liquid chromatograph [7, 18]. The polyamine fraction was further purified on a column of Whatman CM23 [7, 21]. Gas chromatography (GC) was performed after heptafluorobutyrization of the purified polyamine samples [19, 33]. Polyamines were identified by gas chromatography-mass spectometry (GC-mass) [33]. High temperature dependent productions of long polyamines were demonstrated in Fig. 3.

Results and discussion

Phylum *Aquificae*
Order *Aquificales*

Hydrogenivirga caldilitoris [31] grown at 70℃, belonging to the family *Aquificaceae* and *Sulfurihydrogenibium yellowstonense* [32] grown at 70℃, belonging to the family *Hydrogenothermaceae*, contained spermidine (and norspermidine) and a quaternary branched penta-amine, N^4-bis(aminopropyl)norspermine (Table 1)

. These polyamine profiles were identical to those found in other extremely thermophilic genera, *Aquifex*, *Thermocrinis*, *Hydrogenobaculum* and *Hydrogenobacter*, belonging to the family *Aquficaceae* and *Persephonella* and *Hydrogenothermus* belonging to the family *Hydrogenothermaceae* [22]. *Sulfurihydrogenibium* sp. and *Hydrogenobacter* sp., newly analyzed, also contained the branched penta-amine (Table 1).

In the third family *Desulfurobacteriaceae* of this order, the occurrence of N^4-bis(aminopropyl)norspermidine has been reported in a *Balnearium* species and two *Thermovibrio* species [23]. Upon reanalyzing the three in the present study, the occurrence of the branched penta-amine should be corrected to the occurrence of another quaternary branched penta-amine, N^4-bis(aminopropyl)spermidine (Fig. 1) (Table 1). The new species of *Thermovibrio*, *T. guarymasencis* [28], and the two species of the new genus *Desulfurobacterium*, *D. pacificum* and *D. crinifex* [1, 28], contained N^4-bis(aminopropyl)spermidine (Fig. 1) (Table 1). The two branched penta-amines were identified completely by HPLC, as shown in Fig. 1. Since N^4-bis(aminopropyl)spermidine is produced from spermidine and N^4-bis(aminopropyl)norspermidine is produced from norspermidine, it is dependent on the synthetic ability of spermidine and/or norspermidine. In fact, the two branched penta-amines were found in some *Hydrogenobacter* and *Hydrogenobaculum* species[23].

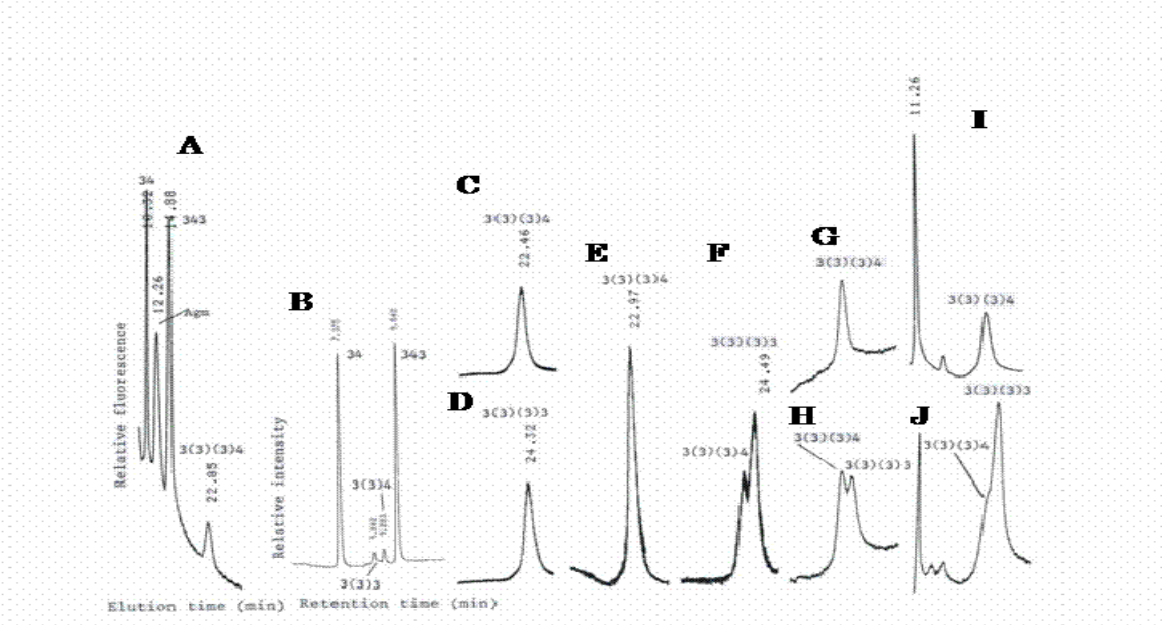

Fig. 1. HPLC analyses (A) and GC analyses (B) of the concentrated polyamine fraction from *Caldoalkalibacillus thermarum* (JCM 13486) grown at 60℃. HPLC analyses of authentic 3(3)(3)4 (C), authentic 3(3)(3)3 (D), the PCA extract from *Desulfurobacterium pacificum* (JCM 12127) grown at 75℃ (E), the PCA extract plus 3(3)(3)3 (F) the PCA extract from *Thermovibrio ammonificans* (JCM 12110) grown at 75℃ (G), the PCA extract plus 3(3)(3)3 (H), the PCA extract from *Desulfothermus okinawensis* (JCM 13304) (I) grown at 55℃ and the PCA extract plus 3(3)(3)3 (J). Abbreviations for polyamines are given in Table 1. During GC analyses, a quaternary penta-amine 3(3)(3)4 was converted to two tertiary tetra-amines 3(3)3 and 3(3)4.

Table 1. Cellular concentrations of polyamines of themophiles belonging to the phylum *Aquificae*.

			Culture	Polyamines (μ mol/g wet wt. cell):												
			℃ (pH)	3	4	5	33	34	333	3(3)3	343/ 334	3(3)4	343/ 334/ 3(3)4	3(3)(3)3	3(3)(3)4
Phylum *Aquificae*															
Order *Aquificales*															
Family *Aquificaceae*															
Hydrogenivirga caldilitoris	JCM 12173		75 (7.0)	–	–	–	0.25	0.20	–	–	–	–	*	1.27	–
Hydrogenobacter sp. (YM752)	NBRC 101523T		75 (7.0)	–	–	–	–	0.15	–	–	–	–	*	1.50	–
Hydrogenobacter hydrogenophilus	JCM 8158^T	(a)	75 (7.0)	–	0.01	–	0.60	0.50	–	–	0.06	0.03	0.09	2.10	–
Hydrogenobacter subterraneus	JCM 10560^T	(b)	75 (7.0)	0.01	–	–	0.06	1.20	–	0.02	0.03	–	0.03	2.40	–
Hydrogenobacter thermophilus (TK-6)	JCM 7687^T	(a)	70 (7.0)	–	–	–	–	1.50	–	0.05	0.03	0.02	0.05	2.60	0.01
Hydrogenobacter thermophilus (TK-G)	The Univ. Tokyo		70 (7.0)	–	–	–	–	1.10	–	0.02	0.30	–	0.30	2.10	–
'*Hydrogenobacter halophilus*' (TH-112)	JCM 7551	(a)	70 (7.0)	–	0.01	–	–	0.05	0.01	0.02	0.01	–	0.01	2.98	–
Hydrogenobaculum acidophilum	JCM 8795^T	(a)	65 (3.0)	–	–	–	–	0.08	–	–	–	–	*	1.12	0.02
Family *Hydrogenothermaceae*															
Sulfurihydrogenibium subterraneum	JCM 11477^T	(b)	62 (7.5)	–	0.40	0.20	–	1.50	–	–	*	*	0.01	1.30	–
Sulfurihydrogenibium yellowstonense	JCM 12773^T		70 (7.5)	–	–	–	–	1.10	–	–	*	*	0.02	1.00	–
Sulfurihydrogenibium sp. (KK701)	NBRC 101520		70 (7.5)	–	–	–	–	0.05	–	–	–	–	–	0.47	–
Sulfurihydrogenibium sp. (BDN702)	NBRC 101521		70 (7.5)	–	–	–	–	0.05	–	–	–	–	–	0.55	–
Sulfurihydrogenibium sp. (Y3c12)	NBRC 101522		70 (7.5)	–	–	–	–	0.05	–	–	–	–	–	0.50	–
Family *Desulfurobacteriaceae*															
Balnearium lithotrophicum	JCM 11970^T	(b*)	75 (5.5)	–	0.04	–	–	0.30	–	–	–	–	–	–	0.60
Thermovibrio ammonificans	JCM 12110^T	(b*)	75 (5.5)	–	–	–	–	0.08	–	–	–	–	–	–	0.80
Thermovibrio ruber	JCM 11468^T	(b*)	75 (6.0)	–	0.02	–	–	0.10	–	–	*	*	0.10	–	1.85
Thermovibrio guaymasensis	JCM 12128^T		75 (6.5)	–	–	–	–	0.08	–	–	–	–	–	–	1.80
Desulfurobacterium pacificum	JCM 12127^T		75 (6.5)	–	–	–	–	0.07	–	–	–	–	–	–	1.50
Desulfurobacterium crinifex	JCM 12825^T		60 (6.5)	–	–	–	–	0.05	–	–	–	–	–	–	1.40

3, diaminopropane; 4, putrescine; 5, cadaverine; 33, norspermidine; 34, spermidine; 44, homospermidine; 333, norspermine; 3(3)3, N^4-aminopropylnorspermidine; 3(3)4, N^4-aminopropylnorspermine; 343, spermine; 334, thermospermine; 344, aminopropylhomospermidine; 3333, caldopentamine; 3334, homocaldopentamine; 3343, thermopentamine; 3(3)(3)3, N^4-bis(aminopropyl)norspermidine; 3(3)(3)4, N^4-bis (aminopropyl)spermidine; 33333, caldohexamine; 33334, homocaldohexamine; 3(3)(3)43, N^4-bis(aminopropyl) spermine; Agm, agmatine; *, not separately determined (the three tetra-amine isomers were separated by GC but not separated HPLC); -, not detected (<0.005); ^{T}, type strain; ℃, culture temperature; ATCC, American Type Culture Collection, Manassas, Virginia, USA; IAM, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan; JCM, Japan Collection of Microorganisms, RIKEN, Wako, Saitama, Japan; NCIMB, the National Collection of Industrial, Food and Marine Bacteria, Aberdeen, Scotland, UK; NBRC, Biological Resource Center, National Institute of Technology and Evaluation, Kisarazu, Chiba, Japan. Quotation mark indicates the scientific name has not been validly published. (a) cited from Hamana et al., 1995 [7]; (b) Hosoya et al., 2004 [20]; (c) Hamana et al., 1999 [13]; (d) Hosoya et al., 2006 [21]; (e) Hamana et al., 1998 [17]; (f) Hamana et al., 1996a [8]; (g) Hamana et al., 1999 [5]; (h) Hamana et al., 1993 [12]; (i) Hamana et al., 2001 [10]; (j) Hamana et al., 1996b[9]. (b*) The reidentification of the branched penta-amine showed that the contents of 3(3)(3)3 should be transferred into 3(3)(3)4 and the previous polyamine data on the three in (b) should be corrected. Recently, the cultures of IFO and IAM were transferred into NBRC and JCM, respectively. The plyamine datum of Hydrogenobacter thermophilus strain TK-G shown in this table was newly calculated from the previous analysis [15].

Phylum *Thermotogae*
Order *Thermotogales*

Caldopentamine was detected in two new moderate thermophiles, *Marinitoga hydrogenitolerans* [36] and *Marinitoga okinawensis* [34], grown at 60℃ (Table 2). In addition to the present result, similar polyamine distribution profiles have been observed in other moderately thermophilic (grown at 55-65℃) *Marinitoga*, *Petrotoga*, and *Thermotoga* species of this order [23]. The cellular concentrations of the penta-amine and the hexa-amine, caldohexamine, in a extremely thermophilic species of *Thermotoga*, *T. maritima*, were demonstrated by the increase of culture temperatures between 60-80℃, as shown in Fig. 3-A, according to our previous data [23]. There was a relative increase of the long polyamines markedly observed at 80℃ (probably at 75℃). A new species of *Fervidobacterium*, *F. changbaicum* [5] grown at 80 ℃ contained caldopentamine. *F. islandicum* grown at 70 ℃ contained two linear penta-amines [10]. Penta-amines were not detected in *F. changbaicum* grown at 70℃ as well as *F. nodosum* grown at 70℃ [10]. Branched penta-amines have never been found in this order.

Phylum *Chloroflexi*
Orders *Anaerolineales* and *Caldilineales*

Two moderate thermophiles, *Anaerolinea thermolimosa* (grown at 50℃) [42] and *Bellilinea caldifistulae* (grown at 55℃) [41] belonging to the order *Anaerolineales* of the phylum *Chloroflexi*, analyzed here, contained spermidine and spermine (Table 2), whereas *Anaerolinea thermophila*, grown at 55℃, contained norspermidine as the major polyamine and lacked triamines [24]. The two *Anaerolinea* species differ in their polyamine profiles. Mesophilic *Longilinea arvoryzae*, grown at 37℃ [41], contained homospermidine. *Caldilinea aerophila*, grown at 55℃, belonging to the order *Caldilineales* of this phylum, contained homospermidine alone as the major polyamine [24], as cited in Table 2. Within the order *Chloroflexi* of this phylum, *Roseiflexus* species grown at 50 ℃ contained putrescine and homospermidine and *Chloroflexus* species grown at 55℃

contained spermidine, homospermidine, and tetra-amines [24]. In this phylum, although the major triamine types and the occurrence of a tetra-amine (spermine) in the thermophiles (grown at 50-55℃) are conflicting, linear penta-amines and a hexa-amine were found in *Thermomicrobium roseum*, grown at 70℃, of the order *Thermomicrobiales*, however, *Sphaerobacter thermophilus*, grown at 55 ℃, of the order *Sphaerobacterales* lacked long polyamines [24].

Table 2. Cellular concentrations of polyamines of themophiles belonging to the phyla *Thermotogae* and *Cloroflexi*.

			Culture		Polyamines (μmol/g wet wt. cell):											
			℃	(pH)	3	4	5	33	34	44	333	343	334	343/334	3333	3343
Phylum *Thermotogae*																
Order *Thermotogales*																
Marinitoga hydrogenitolerans	JCM 12826^T		60	(6.0)	–	–	–	0.07	0.08	–	0.07	*	*	0.07	0.10	–
Marinitoga okinawensis	JCM 13303^T		60	(6.0)	–	–	–	0.14	0.37	–	0.05	*	*	0.05	0.01	–
Marinitoga piezophila	JCM 11233^T	(b)	65	(6.0)	–	0.15	0.02	0.35	0.57	–	0.03	*	*	0.05	–	–
Fervidobacterium changbaicum	JCM 13353^T		70	(7.0)	–	0.02	0.02	0.80	0.40	–	0.20	*	*	0.20	–	–
			80	(7.0)	–	0.02	0.02	0.67	1.02	–	0.40	0.72	0.08	0.80	0.04	–
Fervidobacterium islandicum	ATCC 49647^T	(j)	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	(j)	70	(7.0)	0.04	0.10	0.05	0.60	1.40	–	0.15	*	*	0.45	–	–
Phylum *Chloroflexi*																
Order *Anaerolineales*																
Anaerolinea thermophila	JCM 11388^T	(d)	55	(7.0)	–	–	0.02	0.85	–	–	–	–	–	–	–	–
Anaerolinea thermolimosa	JCM 12577^T		50	(7.0)	–	–	–	–	0.14	–	–	*	*	0.40	–	–
Bellilinea caldifistulae	JCM 13669^T		55	(7.0)	–	–	0.02	–	0.40	–	–	*	*	0.20	–	–
Longilinea arvoryzae	JCM 13670^T		37	(7.0)	–	–	–	–	–	0.42	–	–	–	–	–	–
Order *Caldilineales*																
Caldilinea aerophila	JCM 11387^T	(d)	55	(7.2)	–	–	–	–	–	0.95	–	–	–	–	–	–

Abbreviations are shown in Table 1.

Table 3. Cellular concentrations of polyamines of themophiles belonging to the phylum *Deinococcus –Thermus.*

		Culture		Polyamines (μmol/g wet wt. cell):																		
		℃	(pH)	3	4	5	33	34	44	333	3(3)3	3(3)4	343	334	344	3333	3343	3334	33333	33334	3(3)(3)3	3(3)(3)4	Agm
Phylum *Deinococcus–Thermus*																							
Order *Thermales*																							
Oceanithermus desulfurans	NBRC 100063^T	60	(7.0)	–	–	0.05	–	1.10	–	–	–	–	0.35	–	–	–	–	–	–	–	–	–	–
Oceanithermus profundus	NBRC 100410^T	60	(7.3)	–	–	0.05	–	0.05	–	–	–	–	1.00	–	–	–	–	–	–	–	–	–	–
Thermus kawarayensis	JCM 12314^T	70	(7.2)	0.04	0.04	–	0.40	0.25	0.75	0.98	–	–	0.10	0.48	0.20	0.16	0.06	0.06	–	–	–	–	0.22
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	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	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	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	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	–	–	–	–	–
Thermus scotoductus	ATCC 51532^T	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 (strain HB8	ATCC 27634^T	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 (strain HB2	NBRC 101085	75	(7.2)	0.02	0.10	–	0.15	0.54	0.13	1.20	0.56	0.10	0.55	1.04	0.04	0.77	0.19	0.24	0.01	–	0.32	0.24	0.10
Thermus thermophilus (strain HB1	NBRC 101084^T	75	(7.2)	–	0.10	–	0.10	0.70	0.05	0.90	0.60	0.15	0.20	0.53	0.03	0.26	0.08	0.08	–	–	0.50	0.50	0.10

Abbreviations are shown in Table 1.

Phylum *Deinococcus-Thermus*

Order *Thermales*

In the present study, polyamines of the second species of moderately thermophilic *Oceanithermus*, *O. desulfurans* [29], the nineth species of extremely thermophilic *Thermus*, *T. kawarayensis* [26], and two strains of *Thermus thermophilus* (strain HB8 and HB27) were analyzed. GC analyses of them are shown in Fig. 2. Spermidine and spermine were detected in *O. desulfurans* grown at 60℃ as well as *O. profundus* grown at 60℃ [23]. *T. kawarayensis* grown at 70℃ contained various linear tetra-amines and penta-amines. *T. thermophilus* HB8 and *T. thermophilus* HB27, grown at 75℃ in the present study, were rich in N^4-bis(aminopropyl)norspermidine and N^4-bis(aminopropyl)spermidine (Table 3). The two tertiary branched tetra-amines, N^4-aminopropylnor-spermidine and N^4-aminopropylspermidine, as the precursor of the two quaternary branched penta-amines, were also detected. N^4-bis(aminopropyl)norsperimidine and N^4-bis (aminopropyl)spermidine were found in many other strains of *T. thermophilus*, as previously reported [12, 14, 18, 20, 23]. Temperature dependence of the long-linear or branched numbers of polyamine production in *T. thermophilus* HB8 is shown in Fig. 3-C. The long and branched polyamines markedly increased at 75℃. In *Thermales*, a linear penta-amine was detected in *Marinithermus* species grown at 70 ℃, linear panta-amines and linear hexa-amines were detected in *Vulcanithermus* species grown at 70 ℃: however, branched panta-amines were not found in any of these thermophiles. Long polyamines have never been detected within moderately thermophilic *Meiothermus* species growing at 55-60℃ [18].

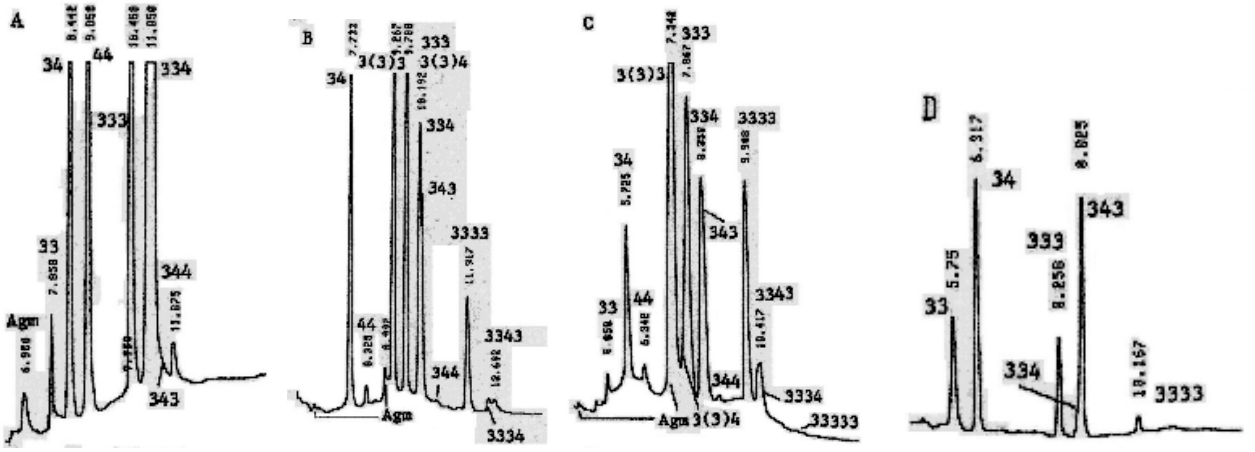

Fig. 2. GC analyses of the concentrated long polyamine fraction from (A) *Thermus kawarayensis* (JCM 12314) grown at 70℃, (B) *Thermus thermophilus* HB8 (NBRC 101084) grown at 75℃, (C) *Thermus thermophilus* HB27 (NBRC 101085) grown at 75℃ and (D) *Fervidobacterium changbaicum* (JCM 13353) grown at 80 ℃. Abbreviations for polyamines are shown in Table 1.

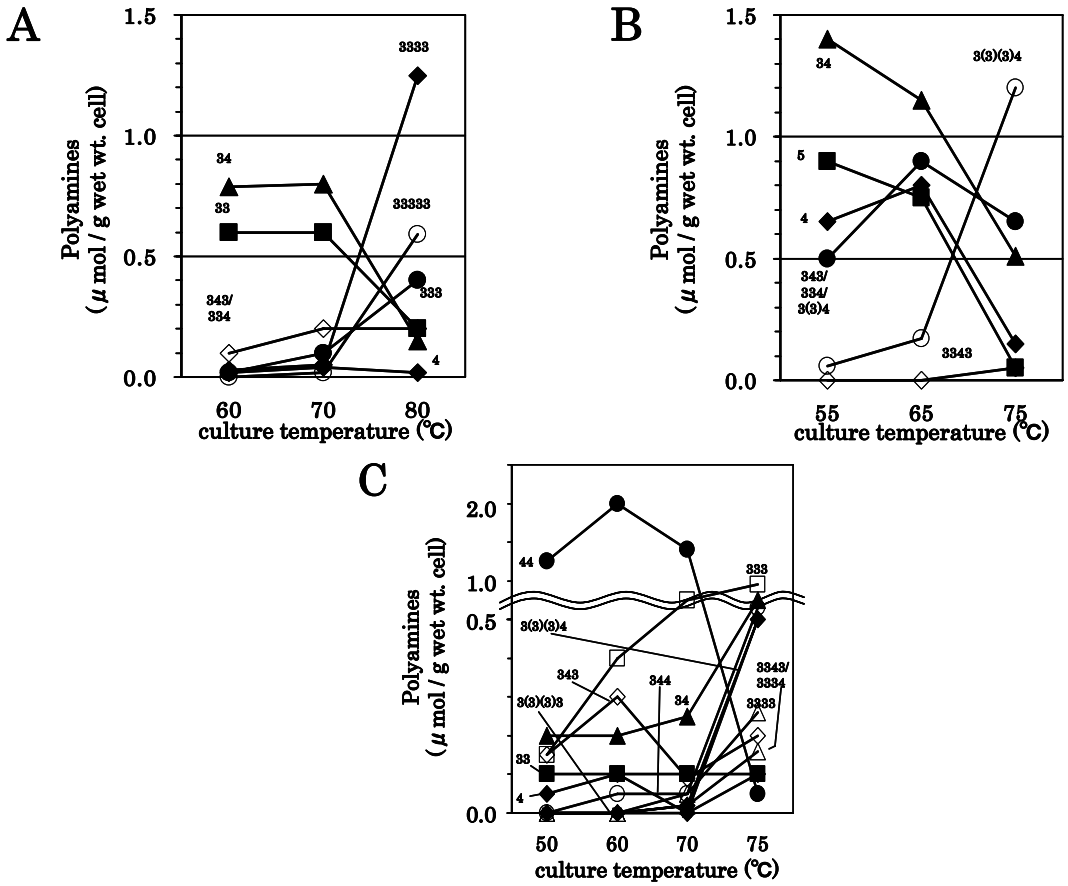

Fig. 3.Production of long linear and branched polyamines depending growth temperature in *Thermotoga maritima* (JCM 10099) (A), *Caldanaerobacter subterraneus* (JCM 11007) (B), *Thermus thermophilus* HB8 (JCM 10941=NBRC 101084) (C). A part of the figures was prepared from our previous data [20] and our review [14]. Abbreviations for polyamines are shown in Table 1.

Phylum *Firmicutes*

Order *Thermoanaerobacteriales*

Thermodesulfobium narugense (grown at 50℃) [30] belonging to the family *Thermodesulfobiaceae* contained spermidine and spermine (Table 4). *Moorella mulderi* (grown at 65℃) [4] and *Desulfovirgula thermocuniculi* (grown at 70℃) [25], belonging to the family *Thermoanaerobacteriaceae*, contained spermidine, spermine, and N^4-bis(aminopropyl)spermidine (Table 4). One of two other *Moorella* species grown at 60℃, contained the branched penta-amine [9]. In the order *Thermoanaerobacteriales*, extremely thermophilic *Thermoanaeromonas*, *Thermoanaerobacter* and *Caldanaerobacter* species grown at 65-75℃ contained the quaternary branched penta-amine. When the thermophiles of the three genera were cultivated at 55-60℃, the branched polyamines were neglectable. The increases of linear and branched penta-amines at 75℃ are shown in Fig. 3-B drawn by the previous data on *Caldanaerobacter subterraneus* [23]. It has been demonstrated that *Thermoanaeromonas toyohensis* grown at 70℃ contained N^4-bis(aminopropyl) norspermidine and a quaternary branched hexa-amine, N^4-bis(aminopropyl) spermine, in addition to N^4-bis(aminopropyl)spermidine [23].

Table4. Cellular concentrations of polyamines of themophiles belonging to the orders *Thermoanaerobacteriales* and *Bacillales* of the phylum *Firmicutes*.

		Culture	Polyamines (μmol/g wet wt. cell):										
		℃ (pH)	4	5	34	343	3(3)4	3343	33343	33433	3(3)(3)4	3(3)(3)43	Agm
Phylum *Firmicutes*													
Order *Thermoanaerobacteriales*													
Family *Thermodesulfobiaceae*													
Themodesulfobium narugense	NBRC 100082^T	55 (5.5)	–	–	0.82	0.20	–	–	–	–	–	–	–
Family *Thermoanaerobacteriaceae*													
Moorella mulderi	ATCC BAA-608^T	65 (6.8)	–	–	0.20	0.47	–	–	–	–	0.05	–	–
Moorella thermoacetica	ATCC 35608^T (f)	60 (6.5)	0.24	0.08	0.72	1.92	–	–	–	–	–	–	–
Moorella thermoautotrophica	ATCC 33924^T (f)	60 (5.6)	1.80	0.05	0.45	2.45	0.13	–	–	–	0.05	–	–
Desulfovirgula thermocuniculi	JCM 13983^T	70 (7.0)	–	–	0.55	1.10	–	–	–	–	0.17	–	–
Order *Bacillales*													
Family *Alicyclobacillaceae*													
Sulfobacillus acidophilus	ATCC 700253^T (g)	45 (2.0)	–	–	1.24	0.50	–	–	–	–	–	–	0.34
Alicyclobacillus disulfidooxidans	ATCC 51911^T (b)	35 (2.5)	–	–	0.75	0.01	–	–	–	–	–	–	0.05
Alicyclobacillus fastidiosus	IAM 15229^T	55 (3.7)	0.02	0.02	1.30	0.04	–	–	–	–	–	–	0.07
Alicyclobacillus fujiensis	IAM 15224^T	55 (3.7)	0.02	0.01	0.70	0.04	–	–	–	–	–	–	0.04
Alicyclobacillus hesperidum	IAM 15232^T	55 (3.7)	0.02	0.01	0.55	0.03	–	–	–	–	–	–	0.02
Alicyclobacillus kakegawaensis	IAM 15227^T	55 (3.7)	0.01	0.01	1.75	0.04	–	–	–	–	–	–	0.06
Alicyclobacillus mali	IAM 14936^T	55 (3.7)	–	–	0.56	–	–	–	–	–	–	–	–
Alicyclobacillus sacchari	IAM 15230^T	55 (3.7)	0.05	0.06	1.55	0.08	–	–	–	–	–	–	0.15
Alicyclobacillus shizuokaensis	IAM 15226^T	55 (3.7)	0.05	0.07	1.65	0.10	–	–	–	–	–	–	0.14
Family *Bacillaceae*													
Bacillus schlegelii	ATCC 43741^T (h)	75 (7.0)	–	–	0.60	1.50	0.15	0.90	0.01	0.01	0.20	–	–
Bacillus smithii	NBRC 15311^T (h)	60 (7.3)	–	–	1.57	0.96	–	–	–	–	–	0.01	0.01
'*Bacillus caldolyticus*'	NBRC 15313 (h)	75 (7.3)	–	–	0.96	2.32	–	–	–	–	0.30	–	0.12
'*Bacillus caldotenax*'	NBRC 15314 (h)	75 (7.3)	–	–	0.45	3.14	–	–	–	–	0.24	–	0.05
'*Bacillus caldovelox*'	NBRC 15315 (h)	65 (7.3)	–	–	0.60	2.20	–	–	–	–	–	–	0.01
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
Caldalkalibacillus thermarum	JCM 13486^T	60 (8.5)	–	–	0.90	0.40	–	–	–	–	0.15	–	0.05
Geobacillus stearothermophilus	IAM 11062^T (h)	65 (7.5)	–	–	0.45	2.50	–	–	–	–	–	0.01	0.05
Geobacillus stearothermophilus	JCM 12894	60 (7.0)	–	–	0.26	0.81	–	–	–	–	–	–	–
Geobacillus kaustophilus	IAM 11001^T (g)	55 (7.0)	–	–	1.50	1.00	–	–	–	–	–	–	0.04
Geobacillus kaustophilus	JCM 12893	60 (7.0)	–	–	0.85	1.65	–	–	–	–	–	–	–
Geobacillus thermocatenulatus	NBRC 15316^T (h)	65 (7.3)	0.04	–	0.14	3.25	–	–	–	–	–	0.02	–
Geobacillus vulcani	JCM 12214^T	60 (7.2)	–	–	0.44	0.50	–	–	–	–	–	–	–
Vulcanibacillus modesticaldus	JCM 12998^T	45 (7.0)	–	–	0.40	0.12	–	–	–	–	–	–	–
Thermalkalibacillus uzonensis	ATCC BAA-1258^T	50 (8.2)	–	–	0.70	0.04	–	–	–	–	–	–	–
Thermobacillus composti	JCM 13945^T	55 (9.0)	–	–	0.97	1.52	0.02	–	–	–	0.04	–	–
Ureibacillus thermosphaericus	NCIMB 13819^T (b)	60 (7.3)	–	–	1.60	0.20	–	–	–	–	–	–	0.04
Ureibacillus terrenus	ATCC BAA-384^T	60 (7.2)	–	–	1.05	0.10	–	–	–	–	–	–	0.15
Family *Paenibacillaceae*													
Aneurinibacillus thermoaerophilus	ATCC 700303^T	60 (7.0)	–	–	0.30	0.70	–	–	–	–	–	–	–
Aneurinibacillus aneurinilyticus	IAM 1077 (g)	30 (7.0)	–	–	1.78	0.40	–	–	–	–	–	–	–
Aneurinibacillus migulans	NBRC 15520 (g)	30 (7.0)	–	–	0.70	0.22	–	–	–	–	–	–	–

Abbreviations are shown in Table 1.

Phylum *Firmicutes*
Order *Bacillales*

In our previous studies, several thermophiles belonging to the genera *Bacillus*, *Alicyclobacillus*, *Geobacillus*, *Anoxybacillus* and *Ureibacillus* grown at 60-65℃ contained spermidine and spermine [6, 23]. *Bacillus schlegelii*, *Saccharococcus thermophilus*, and some *Bacillus* species grown at 65-75℃ contained a few linear and branched panta-amines and hexa-amines [13]. Recently, new moderately thermophilic genera *Vulcanibacillus* grown at 45 ℃ (pH 7.0), *Caldalkalibacillus* grown at 60 ℃ (pH 8.5), *Thermalkalibacillus* grown at 50 ℃ (pH 8.2) and *Thermobacillus* grown at 55℃ (pH 9.0) were published [27, 39, 40, 43]. A quaternary branched penta-amine, N^4-bis(aminopropyl)spermidine was detected in moderately thermophilic, alkaliphilic *Caldalkalibacillus thermarum*, and *Thermobacillus composti* (Fig. 1 and Table 4). The quaternary penta-amine was converted into two tertiary tetra-amines during GC analysis (Fig. 1). *Vulcanibacillus modesticaldus* and *Thermalkalibacillus uzonensis* as well as newly validated, moderately thermophilic *Geobacillus*, *Ureibacillus*, and *Aneurinibacillus* species and moderately-thermophilic, acidophilic *Alicyclobacillus* species, analyzed in the present study, contained spermidine and spermine as the major polyamine. Long-linear and branched polyamines were not detected in other *Geobacillus* species, *G. thermodenitrificans* grown at 65℃, *G. thermoglucosidasius* grown at 60 ℃, and *G. thermoleovorans* grown at 60℃ [23]. Spermine, a tetra-amine, was found in moderately thermophilic *Anoxybacillus* and *Amphibacillus* species grown at 40-60℃, as well as *Bacillus thermocloacae* grown at 60℃, *B. flavothermus* grown at 65℃ and *B. pallidus* grown at 60℃; however, penta-amines were not found in these thermophiles [6]. Spermine was not found in mesophilic *Bacillus*, *Virgibacillus*, *Brevibacillus*, *Paenibacillus*, and *Halobacillus* species [6]. On the other hand, mesophilic *Aneurinibacillus* species as well as moderately thermophilic *Aneurinibacillus* species contained spermine (Table 4). In this order, it is confirmed that the extreme thermophiles grown at 70-75℃ ubiquitously produce some linear and branched panta-amines and/or hexa-amines. Within the moderately thermophilic genus of *Bacillales* growing at 55-65℃, the branched penta-amine was selectively found in the alkaliphilic *Caldalkalibacillus* and *Thermobacillus* species cultivated at a pH of 8.5-9.0 but not detected in acidophilic (grown at pH 2.0-3.7) and neutrophilic (grown at pH 7.0-8.2) genera. These findings suggest that the production of branched panta-amines is dependent on the alkalinic conditions of their growth media.

When *Alicyclobacillus acidiphilus*, *A. herbarius*, and *A. pomorum* were cultured at 45℃, 55℃, and 65℃, the cellular concentrations of spermidine and spermine did not changed among the three cultures [23]. When *Alicyclobacillus acidocaldarius* was cultured at 55℃ and 65℃, spermine level was remarkably greater at 65℃ [23]. It is suggested that the occurrence of spermine is essential for the thermophily at 65℃ within the order *Bacillales*. Spermine level of *Sulfobacillus acidophilus* grown at 45℃ was higher than that of *Alicyclobacillus disulfidooxidans* (formerly *Sulfobacillus disulfido-oxidans*) grown at 35℃, as cited in Table 4.

Phylum *Firmicutes*
Order *Clostridiales*

Within the order *Clostridiales*, novel thermoaerobic *Thermaerobacter* species grown at 70℃, belonging to the family *Syntrophomonadaceae*, contained N^4-bis(aminopropyl)spermidine as a major polyamine [23]. A new *Thermaerobacter* species [37] grown at 70℃ contained the quaternary branched penta-amine and agmatine, as shown in the present study (Table 5). *Caldicellulosiruptor* species, grown at 70℃, contained the quaternary branched panta-amine [9, 11]. Since this branched panta-amine has not been detected in the moderately thermophilic *Desulfotomaculum* and *Pelotomaculum* species belonging to the family *Peptococcaceae* or *Thermobrachium*, belonging to the family *Clostridiaceae*, or *Thermosyntropha* and *Thermoanaerovibrio* belonging to the family *Syntrophomonadaceae* (grown at 55-60℃) [23], the occurrence of this branched penta-amine seems to correlate to a growth temperature of 70℃.

Phylum *Proteobacteria* (*Class Deltaproteobacteria*)
Order *Desulfovibrionales*

In the phylum *Proteobacteria*, spermine, a tetra-amine, has been found only in some moderate thermophiles grown at 50-60 ℃, belonging to the classes *Betaproteobacteria*, *Deltaproteobacteria*, or *Epsilonproteobacteria*, suggesting that the occurrence of spermine correlates to their thermophily [22]. In the new two moderately thermophilic deltaproteobacteria, *Desulfothermus naphtha* and *Desulfothermus okinawensis* [35] grown at 55-60 ℃, contained N^4-bis(aminopropyl)spermidine (Fig. 1) (Table 5). This is the first report on the occurrence of branched penta-amine in the phlum *Proteobacteria*. Mesophilic nine *Desulfovibrio* species belonging to this order did not containe branched penta-amines [22].

Table 5. Cellular concentrations of polyamines of themophiles belonging to the order *Clostridiales* of the phylum *Firmicutes* and the phylum *Proteobacteria*.

			Culture		Polyamines (μmol/g wet wt. cell):									
			℃	(pH)	4	5	33	34	3(3)4	343	334	343/334/3(3)4	3(3)(3)4	Agm
Phylum *Firmicutes*														
Order *Clostridiales*														
Family *Peptococcaceae*														
Pelotomaculum thermopropionicus	JCM 10971^T	(b)	55	(7.0)	0.05	−	−	0.05	−	0.50	−	0.50	−	−
Pelotomaculum terephthalicum	JCM 11824^T		37	(7.0)	−	−	−	0.20	−	−	−	*	−	−
Desulfotomaculum acetoxidans	ATCC 49208^T	(g)	37	(7.2)	−	−	−	0.35	−	0.77	−	0.77	−	−
Desulfotomaculum nigrificans	NBRC 13698^T	(g)	55	(7.5)	0.02	0.04	−	1.20	−	0.20	−	0.20	−	0.45
Desulfotomaculum ruminis	ATCC 23193^T	(g)	37	(7.5)	0.10	−	−	0.05	−	−	−	*	−	−
Desulfotomaculum thermobenzoicum	ATCC 46756^T	(g)	60	(7.0)	−	0.25	−	2.05	−	0.45	−	0.45	−	0.02
Desulfotomaculum thermosapovorans	NCIMB 13375^T	(b)	50	(7.4)	−	−	−	0.22	−	0.18	−	0.18	−	−
Desulfotomaculum arcticum	JCM 12923^T		44	(7.0)	−	−	−	0.20	−	0.15	−	0.15	−	−
Desulfotomaculum thermosubtrraneum	JCM 13837^T		60	(7.0)	−	−	−	0.18	−	0.30	−	0.30	−	−
Family *Syntrophomonadaceae*														
Thermaerobacter litoralis	JCM 13210^T		70	(7.2)	−	0.05	−	0.95	*	*	*	0.35	0.26	0.30
Thermaerobacter marianensis	JCM 10246^T	(i)	70	(7.6)	−	0.04	0.16	0.60	*	*	*	0.75	0.60	0.70
Thermaerobacter nagasakiensis	JCM 11223^T	(b)	70	(7.0)	−	0.05	0.02	0.55	*	*	*	0.35	0.55	1.40
Thermaerobacter subterraneus	ATCC BAA−137^T	(b)	70	(7.3)	−	−	−	0.50	*	*	*	0.85	0.12	0.30
Phylum *Proteobacteria*														
Class *Deltaproteobacteria*														
Order *Desulfovibrionales*														
Desulfothermus naphthae	JCM 12298^T		60	(6.9)	−	−	−	0.10	*	*	*	−	0.60	−
Desulfothermus okinawensis	JCM 13304^T		55	(7.0)	−	−	−	0.55	*	*	*	0.10	0.40	−

Abbreviations are shown in Table 1.

Long polyamines in six other eubacterial phyla including thermophiles:

Actinobacteria

In the phylum, quaternary branched polyamines were detected in *Thermoleophylum* species grown at 70℃, whereas long and branched polyamines were not found in *Acidothermus* species grown at 60℃ [6, 20]. When 1 mM norspermidine was supplemented into the culture of *Thermoleophylum minutum* synthesizing cellular spermidine and N^4-bis(aminopropyl)spermidine, another quaternary branched penta-amine, N^4-bis(aminopropyl) norspermidine, was newly produced [17, 18].

Nitrospirae

A small amount of a linear penta-amine, homocaldo-pentamine, was found in *Thermodesulfovibrio yellowstonii* grown at 60℃ [18, 24].

Fibrobacter

Penta-amines were not detected in *Defferribacter abyssi* grown at 60℃ [24].

Bacteroidetes

Extremely thermophilic *Rhodothermus marinus* grown at 70℃, as well as moderately thermophilic *Thermonema lapsum* grown at 65℃, and *Acetomicrobium flavidum* grown at 60℃, phylogenetically belong to the phylum. *R. marinus* contained two penta-amines, thermo-pentamine and N^4-bis(aminopropyl)spermidine and two linear hexa-amines [12]. *A. flavidum* contained the linear penta-amine, thermopentamine [6]. *T. lapsum* was absent in the long polyamines [12].

Dictyoglomi

Dictyogromus thermophilum grown at 73 ℃ was proposed as a single genus of the phylum and it has been reported that *D. thermophilum* contained the two long linear polyamines, caldopentamine and thermopentamine [10].

Thermodesulfobacteria

Extreme thermophiles belonging to the phylum contained linear penta-amines as a major polyamine in addition to a branched penta-amine as a major polyamine [23].

Long polyamine distributions within eubacterial thermophiles

Polyamine profiles of the eubacterial themophiles distributing within total 12 phyla, presented here and previously reported by us, are sunmmarized. At least one of the long polyamines were widespread ubiquitously within eubacterial extreme and hyper thermophiles. The distribution of linear panta-amines and hexa-amines, without branched polyamines, was found in the phylogeneticaly early branched phyla *Thermotogae*, *Dictyoglomi*, and *Nitrospirae*. Occurrences of quaternary branched penta-amines as the major polyamine, without long linear polyamines, were limited to the phyla *Aquificae*, as another phylogeneticaly early diverged group. Linear and branched penta-amines were distributed in the extreme thermophiles belonging to the phylum *Thermodesulfobacteria*. The linear and branched long polyamine types were mixed, as a minor polyamine, in various extreme thermophiles belonging to the phyla *Bacteroides*, *Actinobacteria* and *Deinococcus-Thermus*, and the orders *Thermoanaerobacteriales*, *Bacillales* and *Clostridiales* of the phylum *Firmicutes*, located in delayed branching lineages. A branched penta-amine alone was found in the aerobic, extremely thermophilic genus, *Thermaerobacter* of the order *Clostridiales* and

moderately thermophilic genus, *Desulfothermus* of the phylum *Proteobacteria*. Although enzymatic synthetic process of long linear and/or branched polyamines remains unknown, their distribution profiles seems to be correlated to phylogenetic evolution of eubacterial thermophiles under high temperature environments on the Earth.

Acknowledgments
We would like to thank JCM, NBRC (Dr. K. Mori and Dr. Y. Uchino), and ATCC for their generous donation of bacterial strains. Furthermore, we would also like to thank Dr. Yanfem Xue from the Chinese Academy of Sciences for the strain, *Caldalkalibacillus thermarum* JCM13486.

References

1) Alain, K., Rolland, S., Crassous, P., Lesongeur, F., Zbinden, M., Gall, C., Godfroy, A., Page, A., Juiper, S.K., Cambon-Bonavita, M.-A., Duchiron, F., and Querellou, J. 2003. *Desulfurobacterium crinifex* sp. nov., a novel thermophilic, pinkish-streamer forming, chemolithoautotrophic bacterium isolated from a Juan de Fuca Ridge hydrothermal vent and amendment of the genus *Desulfurobacterium*. Extremophiles 7: 361-370.
2) Bacteria, The NCBI Entrez Taxonomy website 2007. National Center for Biotechnology Information. http://www.ncbi.nlm.nih.gov/Taxonomy/
3) Bacteria, Bergey's Manual of Systematic Bacteriology 2nd ed. Vol. 1. D. R. Boone and R. W. Castenholz eds. Springer, 2001.
4) Balk, M., Weijma, J., Friedrich, M.W., and Stams, A.J. 2003. Methanol utilization by a novel thermophilic homoacetogenic bacterium, *Moorella mulderi* sp. nov., isolated from a bioreactor. Arch. Microbiol. 179: 315-320.
5) Cai, J., Wang, Y., Liu, D., Zeng, Y., Xue, Y., Ma, Y., and Feng, Y. 2007. *Fervidobacterium changbaicum* sp. nov., a novel thermophilic anaerobic bacterium isolated from a hot spring of the Changbai Mountains, China. Int. J. Syst. Evol. Microbiol. 57: 2333-2336.
6) Hamana, K. 1999. Polyamine distribution catalogues of *clostridia*, acetogenic anaerobes, *actinobacteria*, *bacilli*, *heliobacteria* and haloanaerobes within gram-positive eubacteria—Distribution of spermine and agmatine in thermophiles and halophiles—. Microbiol. Cult. Coll. 15: 9-28.
7) Hamana, K. 2002. Extraction and HPLC analysis of bacterial polyamines. Ann. Gunma Health Sci. 23: 149-153 (in Japanese).
8)Hamana, K., Hamana, H., and Itoh, T. 1995. Polyamines in the hydrogen-oxidizing eubacteria *Hydrogenobacter*, *Calderobacterium* and *Hydrogenovibrio* and sulfur-redusing archaebacteria *Stygiolobus* and *Desulfurolobus*. Microbios 81: 223-229.
9) Hamana, K., Hamana, H., Niitsu, M., and Samejima, K. 1996a. Polyamines of thermophilic Gram-positive anaerobes belonging to the genera *Caldicellulosiruptor*, *Caloramator*, *Clostridium*, *Coprothermobacter*, *Moorella*, *Thermoanaerobacter* and *Thermoanaerobacterium*. Microbios 85: 213-222.
10) Hamana, K., Hamana, H., Niitsu, M., Samejima, K., and Itoh, T. 1996b. Distribution of long and branched polyamines in thermophilic eubacteria and hyperthermophilic archaebacteria. Microbios 85: 19-33.
11) Hamana, K., Hamana, H., Niitsu, M., Samejima, K., and Itoh, T. 2001. Polyamines of the thermophilic eubacteria belonging to the genera *Thermosipho*, *Thermaerobacter*, *Caldicellulosiruptor*. Microbios 104: 177-185.
12) Hamana, K., Hamana, H., Niitsu, M., Samejima, K., and Mtsuzaki, S. 1992. Distriution of unusual long and branched polyamines in thermophilic eubacteria belonging to *Rhodothermus*, *Thermus* and *Thermonema*. J. Gen. Appl. Microbiol. 38: 575-584.
13) Hamana, K., Hamana, H., Niitsu, M., Samejima, K., Sakane, T., and Yokota, A. 1993. Tertiary and quaternary branched polyamines distribution in thermophilic *Saccharococcus* and *Bacillus*. Microbios 75: 23-32.
14) Hamana, K., Hamana, H., Shinozawa, T., Niitsu, M., Samejima, K., and Itoh, T. 1999. Polyamines of the thermophilic eubacteria belonging to the genera *Apuifex*, *Thermodesulfobacterium*, *Thermus* and *Meiothermus*, and the thermophilic archaebacteria belonging to the genera *Sulfurisphaera*, *Sulfophobococcus*, *Stetteria*, *Thermocladium*, *Pyrococcus*, *Thermococcus*, *Methanopyrus* and *Methanothermus*. Microbios 97: 117-130.
15) Hamana, K. and Hosoya, R. 2006. Polyamines of thermophilic *Eubacteria* and *Archaebacteria*. KASEAA (Chemistry and Biology) 44: 320-330 (in Japanese).
16) Hamana, K., Hosoya, R., and Itoh, T. 2007. Polyamine analysis of methanogens, thermophiles and extreme halophiles belonging to the domain Archaea. J. Jpn. Soc. Extremophiles 6: 25-31.
17) Hamana, K., Niitsu, M., Matsuzaki, S., Samejima, K., Igarashi, Y., and Kodama, T. 1992. Novel linear and branched polyamines in the extremely thermophilic eubacteria *Thermoleophilum*, *Bacillus* and *Hydrogenobacter*. Biochem. J. 284: 741-747.
18) Hamana, K., Niitsu, M., Samejima, K., Itoh, T., Hamana, H., and Shinozawa, T. 1998. Polyamines of the thermophilic eubacteria belonging to the genera *Thermotoga*, *Thermodesulfovibrio*, *Thermoleophilum*, *Thermus*, *Rhodothermus* and *Meiothermus*, and the thermophilic archaebacteria belonging to the genera *Aeropyrum*, *Picrophilus*, *Methanobacterium* and *Methanococcus*. Microbios 94: 7-21.
19) Hamana, K., Niitsu, M., Matsuzaki, S., Samejima, K., Igarashi, Y., and Kodama, T. 1992. Novel linear and branched polyamines in the extremely thermophilic eubacteria *Thermoleophilum*, *Bacillus* and *Hydrogenobacter*. Biochem. J. 284: 741-747.
20) Hamana, K., Niitsu, M., Samejima, K., and Matsuzaki, S. 1991. Polyamine distributions in thermophilic eubacteria belonging to *Thermus* and *Acidothermus*. J. Biochem. 109: 444-449.

21) Hamana, K., Otsuka, E., Eguchi, F., and Niitsu, M. 2005. Occurrence of homospermidine and canavalmine as a major polyamine in mushrooms. Mushroom Sci. Biotech. 13: 95-102.
22) Hamana, K., Sato, W., Gouma, K., Yu, J., Ino, Y., Umemura, Y., Mochizuki, C., Takatsuka, K., Kigure, Y., Tanaka, N., Itoh,T., and Yokota, A. 2006. Cellular polyamine catalogues of the five classes of the phylum proteobacteria : distributions of homospermidine within the class *Alpha-proteobacteria*, hydroxyputrescine within the class *Betaproteobacteria*, norspermidine within the class *Gammaproteobacteria*, and spermine within the classes *Deltaproteobacteria* and *Epsilon-proteobacteria*. Ann. Gunma Health Sci. 27: 1-16.
23) Hosoya, R., Hamana, K., Niitsu, M., and Itoh, T. 2004. Polyamine analysis for chemotaxonomy of thermophilic eubacteria: Polyamine distribution profiles within the orders *Aquificales*, *Thermotogales*, *Thermodesulfobacteriales*, *Thermales*, *Thermo-anaerobacteriales*, *Clostridiales* and *Bacillales*. J. Gen. Apll. Microbiol. 50: 271-287.
24) Hosoya, R., Yokoyama, Y., Hamana K., and Itoh, T. 2006. Polyamine analysis within the eubacterial thirteen phyla *Acidobacteria*, *Actinobacteria*, *Chlorobi*, *Chloroflexi*, *Chrysiogenetes*, *Deferri-bacteres*, *Fibrobacteres*, *Firmicutes*, *Fuso- bacteria*, *Gemmatimonadetes*, *Nitrospirae*, *Plancto- mycetes* and *Verrucomicrobia*. Microbiol. Cult. Coll. 22: 21-33.
25) Kaksonen, A.H., Spring, S., Schumann, P., Kroppenstedt, R.M., and Puhakka, J.A. 2007. *Desulfovirgula thermocuniculi* gen. nov., sp. nov., a thermophilic sulfate-reducer isolated from a geothermal underground mine in Japan. Int. J. Syst. Evol. Microbiol. 57: 98-102.
26) Kurokawa, N., Itoh, Y.H., and Itoh, T. 2005. *Thermus kawaraensis* sp. nov., a new member of the genus *Thermus,* isolated from Japanese hot spring. Extremophiles 9: 81-84.
27) L'Haridon, S., Miroshnichenko, M.L., Kostrikina, N.A., Tindall, B.J., Spring, S., Schumann, P., Stackebrandt, E., Bonch-Osmolovskaya, E.A., and Jeanthon, C. 2006. *Vulcanibacillus modesticaldus* gen. nov., sp. nov., a strictly anaerobic, nitrate-reducing bacterium from deep-sea hydro-thermal vents. Int. J. Syst. Evol. Microbiol. 56: 1047-1053.
28) L'Haridon, S., Reysenbach, A.-L., Tindall, B.J., Schonheit, P., Banta, A., Johnsen, U., Schumann, P., Gambacorta, A., Stackebrandt, E., and Jeanthon, C. 2006. *Desulfobacterium atlanticum* sp. nov., *Desulfurobacterium pacificum* sp. nov. and *Thermovibrio guaymasensis* sp. nov., three thermophilic members of the *Desulfurobacteriaceae* fam. nov., a deep branching lineage within the *Bacteria*. Int. J. Syst. Evol. Microbiol. 56: 2843-2852.
29) Mori, K., Kakegawa, T., Higashi, Y., Nakamura, K., Maruyama, A., and Hanada, S. 2004. *Oceanithermus desulfurans* sp. nov., a novel thermophilic, sulfur-reducing bacterium isolated from a sulfide chimney in Suiyo Seamount. Int. J. Syst. Evol. Microbiol. 54: 1561-1566.
30) Mori, K., Kim, H., Kakegawa, T., and Hanada, S. 2003. A novel lineage of sulfate-reducing microorganisms: *Thermodesulfobiaceae* fam. nov., *Thermodesulfobium narugense*, gen. nov., sp. nov., a new thermophilic isolated from a hot spring. Entremophiles 7: 283-290.
31) Nakagawa, S., Nakamura, S., Inagaki, F., Takai, K., Shirai, N., and Sako, Y. 2004. *Hydrogenivirga caldilitoris* gen. nov., sp. nov., a novel extremely thermophilic, hydrogen- and sulfur-oxidizing bacterium from a coastal hydrothermal field. Int. J. Syst. Evol. Microbiol. 54: 2079-2084.
32) Nakagawa, S., Shtaih, Z., Banta, A., Beveridge, T.J., Sako, Y., and Reysenbach, A.-L. 2005. *Sulfurihydrogenibium yellowstonense* sp. nov., an extremely thermophilic, facultatively heterotrophic, sulfuroxidizing bacterium from Yellowstone National Park, and emended descriptions of the genus *Sulfurihydrogenibium*, *Sulfurihydrogenibium subterraneum* and *Sulfurihydrogenibium azorense*. Int. J. Syst. Evol. Microbiol. 55: 2263-2268.
33) Niitsu, M., Samejima, K., Matsuzaki, S., and Hamana, K. 1993. Systematic analysis of naturally occurring linear and branched polyamines by gas chromatogaraphy and gas chromatography-mass spectrometry. J. Chromatogr. 641: 115-123.
34) Nunoura, T., Oida, H., Miyazaki, M., Suzuki, Y., Takai, K., and Horikoshi, K. 2007. *Marinitoga okinawensis* sp. nov., a novel thermophilic and anaerobic heterotroph isolated from a deep-sea hydrothermal field, Southern Okinawa Trough. Int. J. Syst. Evol. Microbiol. 57: 467-471.
35) Nunoura, T., Oida, H., Miyazaki, M., Suzuki, Y., Takai, K., and Horikoshi, K. 2007. *Desulfothermus okinawensis* sp. nov., a thermophilic and hetero-trophic sulfate-reducing bacterium isolated from a deep-sea hydrothermal field. Int. J. Syst. Evol. Microbiol. 57: 2360-2364.
36) Postec, A., Le Breton, C., Fardeau, M.-L., Lesongeur, F., Pignet, P., Querellou, J., Ollivier, B., and Godfroy, A. 2005. *Marinitoga hydrogenitolerans* sp. nov., a novel member of the order *Thermotogales* isolated from a black smoker chimney on the Mid-Atlantic Ridge. Int. J. Syst. Evol. Microbiol. 55: 1217-1221.
37) Tanaka, R., Kawaichi, S., Nishimura, H., and Sako, Y. 2006. *Thermaerobacter litoralis* sp. nov., a strictly aerobic and thermophilic bacterium isolated from a coastal hydrothermal field. Int. J. Syst. Evol. Microbiol. 56: 1531-1534.
38) Terui, Y., Ohnuma, M., Hiraga, K., Kawashima, E., and Oshima, T. 2005. Stabilization of nucleic acids by unusual polyamines produced by an extreme thermophile, *Thermus thermophilus*. Biochem. J. 388: 427-433.
39) Watanabe, K., Nagao, N., Yamamoto, S., Toda, T., and Kurosawa, N. 2007. *Thermobacillus composti* sp. nov., a moderately thermophilic bacterium isolated from a composting reactor. Int. J. Syst. Evol. Microbiol. 57: 1473-1477.
40) Xue, Y., Zhang. X., Zhou, C., Zhao, Y., Cowan, D.A.,

Heaphy, S., Grant, W.D., Jones, B.E., Ventosa, A., and Ma, Y. 2006. *Caldalkalibacillus thermarum* gen. nov., sp. nov., a novel alkalithermophilic bacterium from a hot spring in China. Int. J. Syst. Evol. Microbiol. 56: 1217-1221.

41) Yamada, T., Imachi, H., Ohashi, A., Harada, H., Hanada, S., Kamagata, Y., and Sekiguchi, Y. 2007. *Bellilinea caldifistulae* gen. nov., sp. nov. and *Longilinea arvoryzae* gen. nov., sp. nov., strictly anaerobic, filamentous bacteria of the phylum *Chloroflexi* isolated from methanogenic propionate-degrading consortia. Int. J. Syst. Evol. Microbiol. 57: 2299-2306.

42) Yamada, T., Sekiguchi, Y., Hanada, S., Imachi, H., Ohashi, A., Harada, H., and Kamagata, Y. 2006. *Anaerolinea thermolimosa* sp. nov., *Levilinea saccharolytica* gen. nov., sp. nov. and *Leptolinea tardivitalis* gen. nov., sp. nov., novel filamentous anaerobes, and description of the new classes *Anaerolineae* classis nov. and *Caldilineae* classis nov. in the bacterial phylum *Chloroflexi*. Int. J. Syst. Evol. Microbiol. 56: 1331-1340.

43) Zhao, W., Weber, C., Zhang, C.L., Romanek, C.S., King, G.M., Mills, G., Sokolova, T., and Wiegel, J. 2006. *Thermalkalibacillus uzonensis* gen. nov., sp. nov, a novel aerobic alkali-tolerant thermophilic bacterium isolated from a hot spring in Uzon Caldera Kamchatka. Extremophiles 10: 337-345.