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Novel linear and branched polyamines in the extremely thermophilic eubacteria *Thermoleophilum*, *Bacillus* and *Hydrogenobacter*

Koei HAMANA,*† Masaru NIITSU,‡ Shigeru MATSUZAKI,§ Keijiro SAMEJIMA,‡ Yasuo IGARASHI‖ and Tohru KODAMA‖

*College of Medical Care and Technology, Gunma University, Maebashi, Gunma 371, Japan,
‡Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Josai University, Sakado, Saitama 350-02, Japan,
§Department of Physiology, Institute of Endocrinology, Gunma University, Maebashi 371, Japan,
and ‖Department of Agricultural Chemistry, The University of Tokyo, Tokyo 113, Japan

Novel tertiary branched tetra-amines, quaternary branched penta-amines, linear penta-amines and linear hexa-amines were distributed as the major polyamines in six obligately extremely thermophilic eubacteria belonging to *Thermoleophilum*, *Bacillus* or *Hydrogenobacter*. The major polyamine of *Thermoleophilum album* and *Thermoleophilum minutum* was identified as a quaternary branched penta-amine, 4,4-bis(3-aminopropyl)-1,8-diamino-4-azaoctane $\{NH_2[CH_2]_3N^+([CH_2]_3NH_2)_2[CH_2]_4NH_2\}$ by h.p.l.c., t.l.c. and g.c.–m.s. *Hydrogenobacter thermophilus* and *Hydrogenobacter halophilus* contained another quaternary branched penta-amine, 4,4-bis(3-aminopropyl)-1,7-diamino-4-azaheptane $\{NH_2[CH_2]_3N^+([CH_2]_3NH_2)_2[CH_2]_3NH_2\}$ as the major polyamine, and tertiary branched tetra-amines (4-(3-aminopropyl)-1,7-diamino-4-azaheptane $\{NH_2[CH_2]_3N([CH_2]_3NH_2)[CH_2]_3NH_2\}$, 4-(3-aminopropyl)-1,8-diamino-4-azaoctane $\{NH_2[CH_2]_3N([CH_2]_3NH_2)[CH_2]_4NH_2\}$) and 4,4-bis(3-aminopropyl)-1,8-diamino-4-azaoctane were confirmed as minor components. *Bacillus schlegelii* contained a branched tetra-amine, 4-(3-aminopropyl)-1,8-diamino-4-azaoctane, a branched penta-amine, 4,4-bis(3-aminopropyl)-1,8-diamino-4-azaoctane, a linear penta-amine, 1,16-diamino-4,8,13-triazahexadecane ($NH_2[CH_2]_3NH[CH_2]_3NH[CH_2]_4NH[CH_2]_3NH_2$) and linear hexa-amine(s), 1,20-diamino-4,8,12,17-tetra-azaeicosane ($NH_2[CH_2]_3NH[CH_2]_3NH[CH_2]_3NH[CH_2]_4NH[CH_2]_3NH_2$) and/or 1,20-diamino-4,8,13,17-tetra-azaeicosane ($NH_2[CH_2]_3NH[CH_2]_3NH[CH_2]_4NH[CH_2]_3NH[CH_2]_3NH_2$).

INTRODUCTION

Linear penta-amines and hexa-amines were found in extremely thermophilic aerobic Gram-negative non-sporulating eubacteria, various species of *Thermus* (Oshima, 1982; Oshima & Kawahata, 1983; Oshima *et al.*, 1987; Hamana *et al.*, 1990*b*, 1991) and *Thermomicrobium roseum* (Hamana *et al.*, 1990*c*), growing at 70–80 °C. *Thermus thermophilus* also contains a tertiary branched tetra-amine, XXI, and a quaternary branched penta-amine, XXIV, as minor polyamines (Oshima *et al.*, 1987; Humana *et al.*, 1991). We have previously reported the occurrence of two tertiary branched tetra-amines, XXI and XXII, in extremely thermophilic Gram-negative eubacteria, *Thermoleophilum album* and *Thermoleophilum minutum* (Hamana *et al.*, 1990*a*). We re-analysed polyamines of *Thermoleophilum* to detect quaternary branched penta-amines. Some thermophiles have been isolated in Gram-positive aerobic sporulating rods belonging to *Bacillus* (Claus & Berkely, 1986). The occurrence of polyamine IX is limited to the moderate thermophiles such as *Bacillus stearothermophilus*, *Bacillus thermodenitrificans* and *Bacillus acidocaldarius* (Stevens & Morrison, 1968; Hamana *et al.*, 1989) and is associated with their moderate thermophily. The moderately thermophilic bacilli growing at 50–65 °C lack long and branched polyamines. Since the occurrence of long linear or branched polyamines is limited to the extremely thermophilic eubacteria and these polyamines are not found in archaebacteria including extreme thermophiles (Kneifel *et al.*, 1986), the presence of these polyamines is apparently associated with their extreme thermophily and relevant to chemotaxonomy within thermophilic eubacteria.

In order to survey the distribution of polyamines in thermophilic bacilli, we analysed polyamines in an extreme thermophile, *Bacillus schlegelii* (Schenk & Aragno, 1979). Previously, we isolated extremely thermophilic aerobic Gram-negative non-sporulating hydrogen-oxidizing eubacteria, *Hydrogenobacter thermophilus* (Kawasumi *et al.*, 1984) and *Hydrogenobacter halophilus* (Nishihara *et al.*, 1990), growing at 70–75 °C. It seemed to us of interest to investigate the possible relationship between their thermophily and polyamine-distribution patterns. A list of all polyamines described in this report is shown in Table 1.

MATERIALS AND METHODS

Culture

Culture of *Thermoleophilum album* YS-3 (A.T.C.C. 35264) and *Thermoleophilum minutum* PTA-1 (A.T.C.C. 35268) was carried out as described previously (Hamana *et al.*, 1990*a*).

B. schlegelii MA-48 (A.T.C.C. 43741 = DSM 2000), an obligately thermophilic facultatively chemolithoautotrophic bacterium which oxidizes molecular hydrogen, was grown heterotrophically in the polyamine-free basal mineral medium containing NH_4^+/pyruvate medium, pH 7.0 at 60 or 75 °C (Schenk & Aragno, 1979). For autotrophic growth, $NaHCO_3$ was added to the basal mineral medium, pH 7.1, and cultures were then incubated under $O_2/CO_2/H_2$ (HCO_3^-/H_2 medium) at 60 °C (Schenk & Aragno, 1979).

H. thermophilus strains TK-6 and TK-G were autotrophically cultivated in the basal medium, pH 7.2, at 70 °C under a gas mixture consisting of H_2, O_2 and CO_2 (15:3:2, by vol.) (Kawasumi *et al.*, 1984). Cultivation of a halophile, *H. halophilus* TH-112, was performed autotrophically in the basal medium, pH 7.0, containing 0.5 M-NaCl at 70 °C under the gas phase, $H_2/O_2/CO_2 = 7:1:1$, by vol. (Nishihara *et al.*, 1990).

† To whom all correspondence should be addressed.

Table 1. Trivial names, systematic names, chemical formulae and abbreviation numerals of polyamines described in the present paper

Group	Trivial name	Systematic name	Chemical formula	Abbreviation numeral
Diamines	Diaminopropane	1,3-Diaminopropane	$NH_2[CH_2]_3NH_2$	I
	Putrescine	1,4-Diaminobutane	$NH_2[CH_2]_4NH_2$	II
	Cadaverine	1,5-Diaminopentane	$NH_2[CH_2]_5NH_2$	III
Triamines	Norspermidine	1,7-Diamino-4-azaheptane	$NH_2[CH_2]_3NH[CH_2]_3NH_2$	IV
	Spermidine	1,8-Diamino-4-azaoctane	$NH_2[CH_2]_3NH[CH_2]_4NH_2$	V
	Homospermidine	1,9-Diamino-5-azanonane	$NH_2[CH_2]_4NH[CH_2]_4NH_2$	VI
	Aminopropylcadaverine	1,9-Diamino-4-azanonane	$NH_2[CH_2]_3NH[CH_2]_5NH_2$	VII
Linear tetra-amines	Norspermine	1,11-Diamino-4,8-diazaundecane	$NH_2[CH_2]_3NH[CH_2]_3NH[CH_2]_3NH_2$	VIII
	Spermine	1,12-Diamino-4,9-diazadodecane	$NH_2[CH_2]_3NH[CH_2]_4NH[CH_2]_3NH_2$	IX
	Thermospermine	1,12-Diamino-4,8-diazadodecane	$NH_2[CH_2]_3NH[CH_2]_3NH[CH_2]_4NH_2$	X
	Aminopropylhomospermidine	1,13-Diamino-4,9-diazatridecane	$NH_2[CH_2]_3NH[CH_2]_4NH[CH_2]_4NH_2$	XI
	Canavalmine	1,13-Diamino-5,9-diazatridecane	$NH_2[CH_2]_4NH[CH_2]_3NH[CH_2]_4NH_2$	XII
	Homospermine	1,14-Diamino-5,10-diazatetradecane	$NH_2[CH_2]_4NH[CH_2]_4NH[CH_2]_4NH_2$	XIII
Linear penta-amines	Caldopentamine	1,15-Diamino-4,8,12-triazapentadecane	$NH_2[CH_2]_3NH[CH_2]_3NH[CH_2]_3NH[CH_2]_3NH_2$	XIV
	Homocaldopentamine	1,16-Diamino-4,8,12-triazahexadecane	$NH_2[CH_2]_3NH[CH_2]_3NH[CH_2]_3NH[CH_2]_4NH_2$	XV
	Thermopentamine	1,16-Diamino-4,8,13-triazahexadecane	$NH_2[CH_2]_3NH[CH_2]_3NH[CH_2]_4NH[CH_2]_3NH_2$	XVI
Linear hexa-amines	Caldohexamine	1,19-Diamino-4,8,12,16-tetra-azanonadecane	$NH_2[CH_2]_3NH[CH_2]_3NH[CH_2]_3NH[CH_2]_3NH[CH_2]_3NH_2$	XVII
	Homocaldohexamine	1,20-Diamino-4,8,12,16-tetra-azaeicosane	$NH_2[CH_2]_3NH[CH_2]_3NH[CH_2]_3NH[CH_2]_3NH[CH_2]_4NH_2$	XVIII
	Thermohexamine	1,20-Diamino-4,8,12,17-tetra-azaeicosane	$NH_2[CH_2]_3NH[CH_2]_3NH[CH_2]_3NH[CH_2]_4NH[CH_2]_3NH_2$	XIX
	Homothermohexamine	1,20-Diamino-4,8,13,17-tetra-azaeicosane	$NH_2[CH_2]_3NH[CH_2]_3NH[CH_2]_4NH[CH_2]_3NH[CH_2]_3NH_2$	XX
Tertiary branched tetra-amines	N^4-Aminopropylnorspermidine	4-(3-Aminopropyl)-1,7-diamino-4-azaheptane	$NH_2[CH_2]_3N([CH_2]_3NH_2)[CH_2]_3NH_2$	XXI
	N^4-Aminopropylspermidine	4-(3-Aminopropyl)-1,8-diamino-4-azaoctane	$NH_2[CH_2]_3N([CH_2]_3NH_2)[CH_2]_4NH_2$	XXII
	N^5-Aminopropylhomospermidine	5-(3-Aminopropyl)-1,9-diamino-5-azanonane	$NH_2[CH_2]_4N([CH_2]_3NH_2)[CH_2]_4NH_2$	XXIII
Quaternary branched penta-amines	N^4-Bis(aminopropyl)norspermidine	4,4-Bis(3-aminopropyl)-1,7-diamino-4-azaheptane	$NH_2[CH_2]_3N^+([CH_2]_3NH_2)_2[CH_2]_3NH_2$	XXIV
	N^4-Bis(aminopropyl)spermidine	4,4-Bis(3-aminopropyl)-1,8-diamino-4-azaoctane	$NH_2[CH_2]_3N^+([CH_2]_3NH_2)_2[CH_2]_4NH_2$	XXV
	N^5-Bis(aminopropyl)homospermidine	5,5-Bis(3-aminopropyl)-1,9-diamino-5-azanonane	$NH_2[CH_2]_4N^+([CH_2]_3NH_2)_2[CH_2]_4NH_2$	XXVI

Polyamine analysis

The organisms were harvested and homogenized in equal volumes of cold 1 M-$HClO_4$. The $HClO_4$ extracts were analysed for their polyamine contents by h.p.l.c. on a column of cation-exchange resin as described previously (Matsuzaki *et al.*, 1982). G.c. of polyamines was performed on a GC-9A gas chromatograph (Shimadzu Co. Ltd., Tokyo, Japan) after treatment of the polyamine samples with heptafluorobutyrate (Matsuzaki *et al.*, 1989). The Pyrex glass column (2.1 m, 3 mm internal diameter) was packed with 3% SE-30 on 100–120-mesh Chromosorb WHP (Gasukuro Kogyo Inc., Tokyo, Japan). Helium was used as the carrier gas with a flow rate of 40 ml/min. The column oven temperature was raised from 120 to 280 °C (16 °C/min) or 200–280 °C (8 °C/min) and the injector temperature was 300 °C. The identity of polyamines was confirmed by g.c.–m.s. on a JMS-DX 300 (JEOL Co. Ltd., Tokyo, Japan) which was operated in the electron-impact mode at an ionization energy of 70 eV. The identity of major polyamines was also confirmed by t.l.c. on cellulose (Avicel SF, Funakoshi, Tokyo, Japan) using the solvent system propan-2-ol/ammonia (7:3, v/v) (Hamana & Matsuzaki, 1984). Various linear tetra-amines, penta-amines and hexa-amines, and tertiary branched tetra-amines (XXI, XXII and XXIII) and quaternary branched penta-amines (XXIV, XXV and XXVI) were synthesized according to our previous report (Niitsu & Samejima, 1986) or Oshima *et al.* (1987). *NN*-Bis(3-aminopropyl)methylamine was purchased from Tokyo Kasei (Tokyo, Japan).

RESULTS

Thermoleophilum

H.p.l.c. analysis of the polyamines extracted from the normal culture of the two species of *Thermoleophilum* showed that the major unknown peak was XXV (Fig. 1*a* and 1*f*). Minor peaks corresponding to polyamines I, II, IV, V, XXI, XXII and XXIV were also detected (Fig. 1*f*). When the polyamine fraction was analysed by g.c., two major peaks corresponding to tertiary branched tetra-amines, XXI and XXII, were found (Fig. 2*a*). The identity of these tetra-amines was confirmed by m.s. On the other hand, the g.c. analysis of synthesized authentic polyamine XXV also gave the two tertiary tetra-amines and two peaks corresponding to the degradation products ($NH_2CH_2CH{=}CH_2$ and $NH_2CH_2CH_2CH{=}CH_2$), as shown in Fig. 3. The products were also detected during g.c. of the polyamine sample from *Thermoleophilum* on g.c. were identified as IV and V by g.c.–m.s. degraded to XXI during g.c. analysis (Fig. 3*a*). Polyamine XXVI produced XXII and XXIII (Fig. 3*c*). The conversion of quaternary penta-amines into tertiary tetra-amines was observed during g.c. analysis after the treatment of polyamines with heptafluorobutyrate. Ratios between the two products and two tertiary tetra-amines produced suggest the preferential elimination of the aminobutyl moiety of the quaternary penta-amines.

The two minor peaks detected in the polyamine fractions of *Thermoleophilum* on g.c. were identified as IV and V by g.c.–m.s. analysis. The major polyamine of *Thermoleophilum* behaved identically with authentic XXV on t.l.c. (results not shown). Although separation of the three quaternary branched penta-amines was incomplete, they were clearly separated from linear penta-amines and hexa-amines, and tertiary branched tetra-amines by t.l.c. on cellulose. It is concluded that intact XXV detected on h.p.l.c. and t.l.c. as the major polyamine of the two species of *Thermoleophilum* was detected as the two tertiary tetra-amines which had been converted from the quaternary penta-amines on g.c.

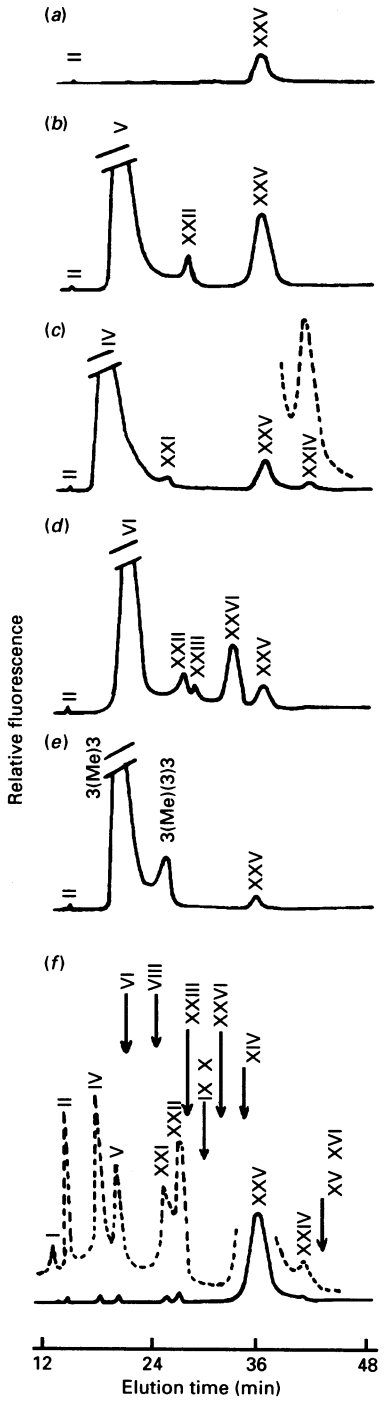

Fig. 1. H.p.l.c. separation of polyamines of *Thermoleophilum minutum* PTA-1 grown in the absence (*a*) or presence of 1 mM-spermidine (*b*), -norspermidine (*c*), -homospermidine (*d*) or -*NN*-bis(3-aminopropyl)methylamine (*e*)

Polyamines were extracted from the same amounts of the cells. Analysis of the concentrated polyamine fraction obtained from the normal culture is shown in (*f*). Broken lines indicate the curves at 25 times standard sensitivity. Polyamines corresponding to each peak are shown. Arrows indicate elution peaks of other authentic polyamine standards. Abbreviations: 3(Me)3, *NN*-bis(3-aminopropyl)methylamine; 3(Me)(3)3, *NNN*-tris(3-aminopropyl)methylamine.

Addition of polyamine V to the culture medium resulted in an increase in concentrations of XXV in the organism (Fig. 1*b*). A small peak corresponding to polyamine XXIV appeared after

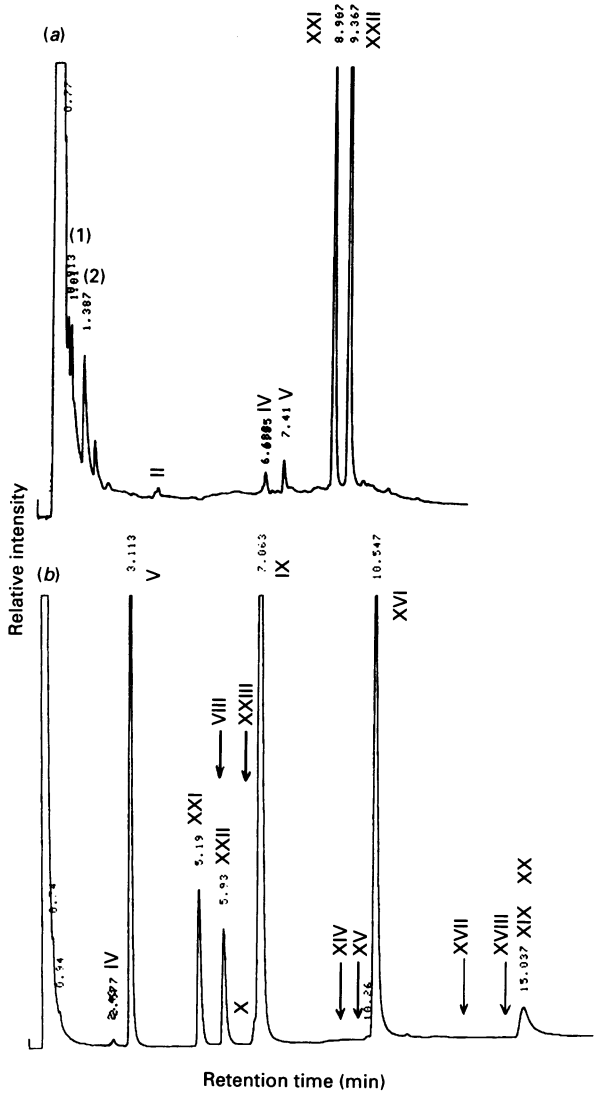

Fig. 2. G.c. analysis of polyamines of *Thermoleophilum minutum* PTA-1 (*a*) and *B. schlegelii* (*b*) under the g.c. column temperature at 120–200 °C (*a*) or 200–280 °C (*b*)

Peak (1) and (2) in (*a*) correspond to peak (1) and peak (2) of Fig. 3(*b*) respectively.

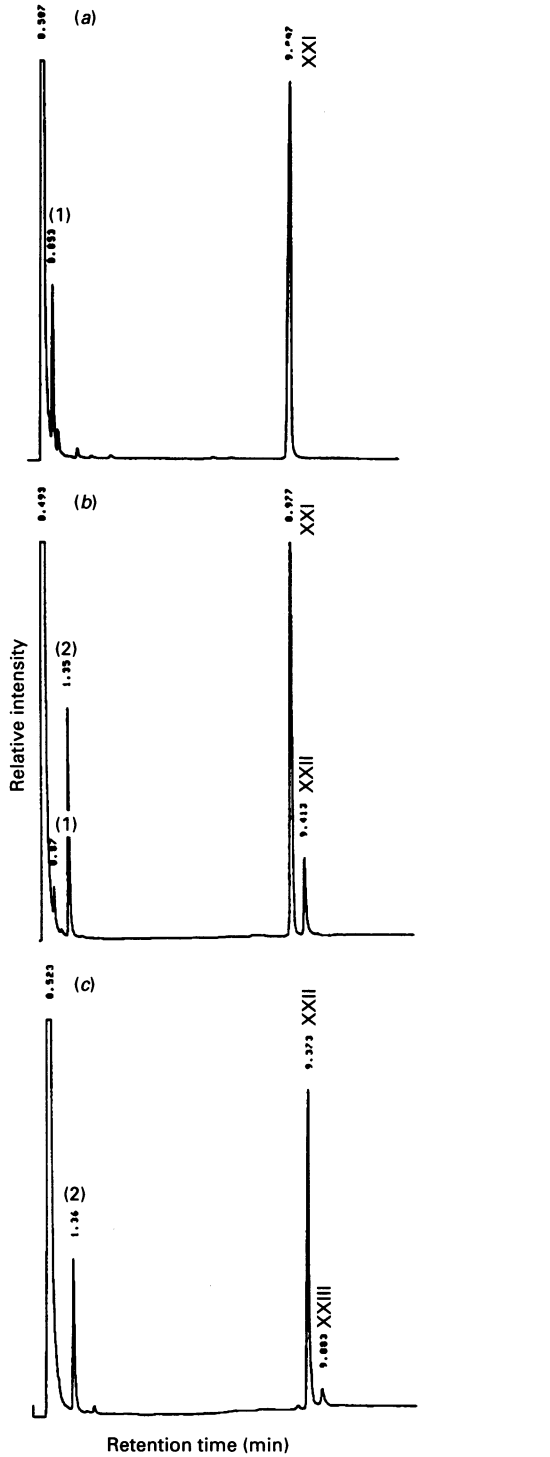

Fig. 3. G.c. analysis of authentic quaternary branched penta-amines, 4,4-bis(3-aminopropyl)-1,7-diamino-4-azaheptane (*a*), 4,4-bis(3-aminopropyl)-1,8-diamino-4-azaoctane (*b*) and 5,5-bis(3-aminopropyl)-1,9-diamino-5-azanonane (*c*) under the g.c. column temperature at 120–280 °C

Peak (1) and peak (2) correspond to $NH_2CH_2CH{=}CH_2$ (a degradation product from the aminopropyl group of quaternary penta-amines) and $NH_2CH_2CH_2CH{=}CH_2$ (a product from the aminobutyl group of quaternary penta-amines).

supplementation with 1 mM-polyamine IV (Fig. 1*c*). Two new peaks corresponding to polyamines XXIII and XXVI were formed from the added polyamine VI at 1 mM (Fig. 1*d*). These findings suggest that aminopropyl transfer to N^4 of triamines occurs significantly in polyamines V and VI but only slightly in polyamine IV in *Thermoleophilum*. Neither XXIII nor XXVI, however, would be produced naturally in the organisms which lack polyamine VI. When a tertiary amine, *NN*-bis(3-aminopropyl)methylamine, was added to the medium at 1 mM, a new peak appeared, which probably corresponds to a quaternary amine, *NNN*-tris(3-aminopropyl)methylamine (Fig. 1*e*).

Bacillus

Typical h.p.l.c. profiles of the polyamines in *B. schlegelii* grown under different growth conditions are shown in Fig. 4. Peaks 1, 2 and 3 correspond to polyamines I, IV and V respectively. Peak 4 corresponds to polyamine XXII. Peak 5 corresponds to polyamines IX and/or X. Peak 6 corresponds to polyamine XXV. The elution time of authentic polyamines XVI and XV was identical with that of peak 7. Peak 8 corresponds to polyamines XVIII, XIX and/or XX. The latter two hexa-amines were named by us in this report. The polyamine-distribution

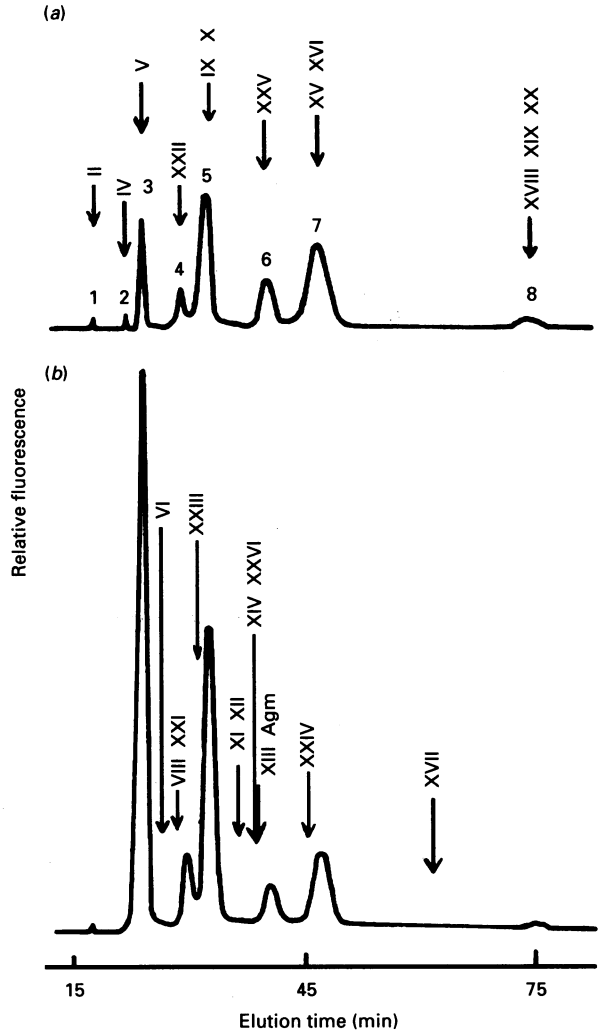

Fig. 4. H.p.l.c. separation of polyamines of *B. schlegelii* grown in the synthetic NH_4^+/pyruvate medium at 75 °C (*a*) and in the synthetic HCO_3^-/H_2 medium at 60 °C (*b*)

Arrows indicate the elution positions of the polyamines. Abbreviation: Agm, agmatine.

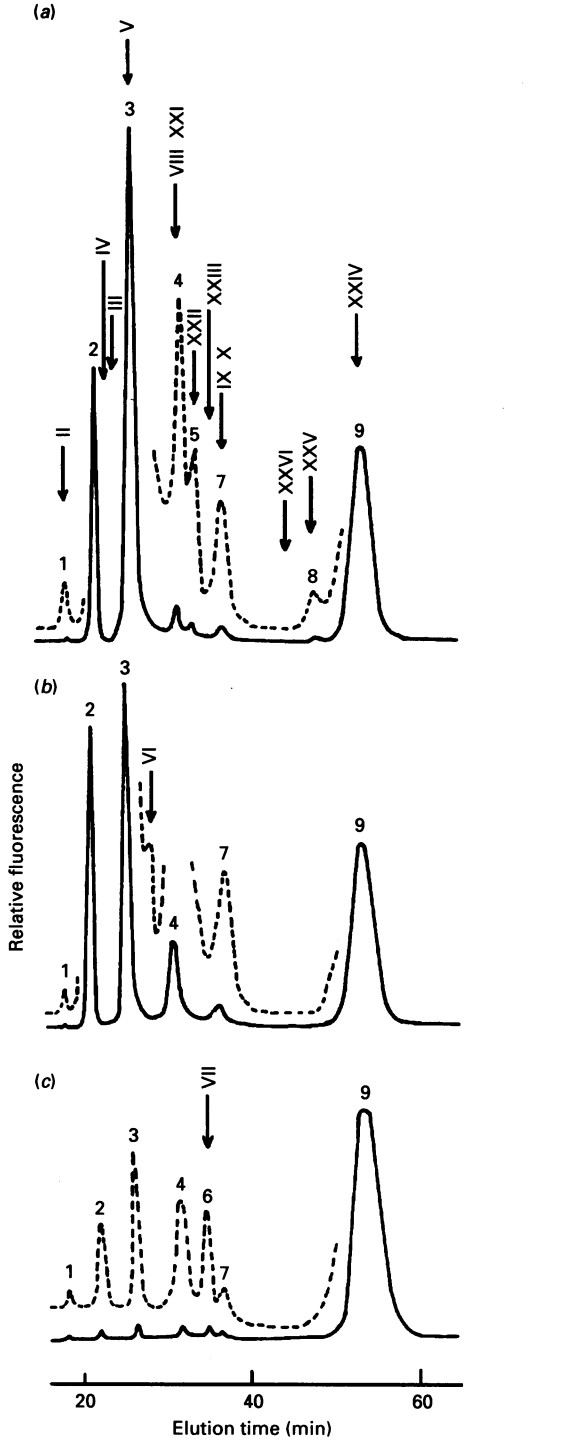

Fig. 5. H.p.l.c. separation of polyamines of *H. thermophilus* TK-6 (*a*), *H. thermophilus* TK-G (*b*) and *H. halophilus* TH-112 (*c*)

Broken lines indicate the curves at 10 times the standard sensitivity. Arrows indicate the elution peaks of authentic polyamines.

patterns were not much affected by heterotrophic and chemolithoautotrophic growth conditions (Fig. 4). Essentially the same patterns were obtained after the heterotrophic culture in Medium 199 (results not shown). In contrast, the hexa-amine fraction was relatively increased at higher growth temperatures ranging from 60 to 75 °C. The polyamine IV peak slightly increased in height when polyamine I was added to the medium. Neither triamine nor tetra-amine was produced from the supplemented polyamine III.

Two tertiary branched tetra-amines, XXI and XXII, were identified by g.c.–m.s. (Fig. 2*b*). X was detected as a minor tetra-amine by g.c. analysis. The ratio of polyamine X to IX was estimated to be roughly 1:100. Polyamine XVI, first found in *Thermus thermophilus* (Hamana *et al.*, 1990*a*), was identified by g.c. analysis (Fig. 2*b*) and by m.s. It was concluded that polyamine XXI detected by g.c. was derived from polyamine XXV (peak 6 on h.p.l.c.) and that polyamine XXII on g.c. included the product from polyamine XXV. The occurrence of the quaternary branched penta-amine, XXIV, and tertiary branched tetra-amine, XXI, in *B. schlegelii* was excluded. Peak 7 on h.p.l.c. profiles corresponds to polyamine XVI. The last peak on the gas chromatogram corresponds to two hexa-amine isomers, XIX and XX, but these two were not separated from each other by g.c. analysis. On the mass spectrum of the hexa-amine fraction treated with heptafluorobutyrate, the fragment ion at m/z 1323 for $(M^+ - C_3F_7)$, exhibiting the same fragment ion for three authentic isomeric hexa-amines (XVIII, XIX and XX), was observed in the fraction. However, the spectra of the sample lacked the typical fragment ion at m/z 521 for polyamine XX. Although hexa-amines XIX and XX were not distinguished from

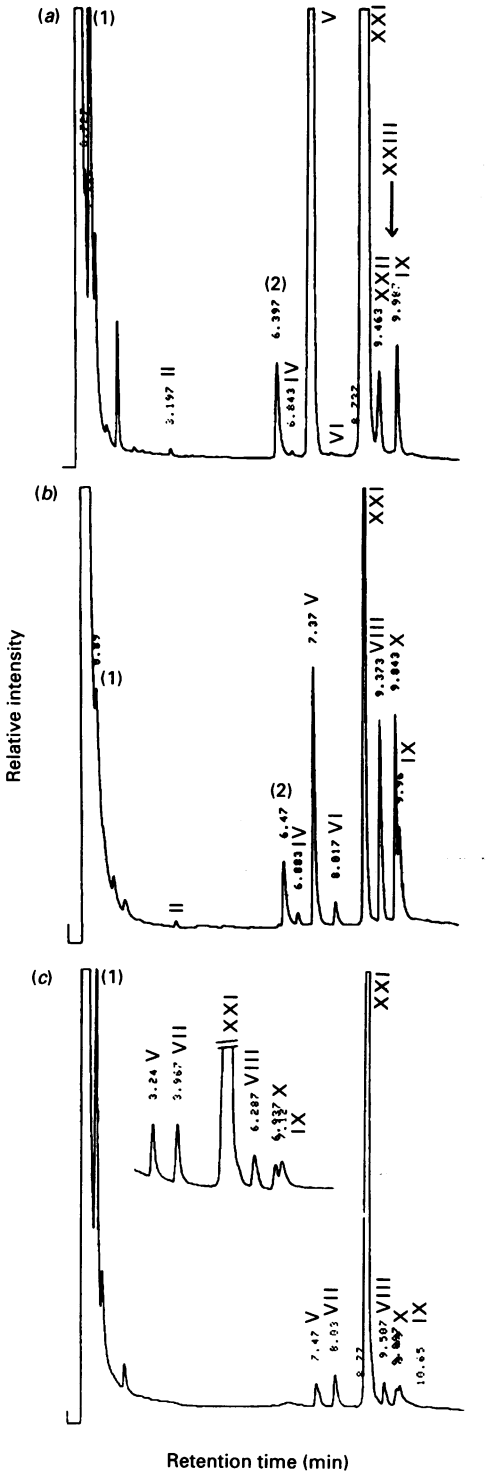

Fig. 6. G.c. analysis of polyamines extracted from *H. thermophilus* TK-6 (*a*), *H. thermophilus* TK-G (*b*) and *H. halophilus* TH-112 (*c*)

The chromatogram inserted in (*c*) shows the results of a column temperature change from 200 to 280 °C. In all the other profiles, it was changed from 120 to 280 °C. Polyamines corresponding to respective peaks are shown in abbreviations. The position of polyamine XXIII is indicated with an arrow. Quaternary penta-amines in polyamine samples were detected as tertiary tetra-amines by g.c. Peak (1) corresponds to the degradation product ($NH_2CH_2CH{=}CH_2$) from polyamine XXIV. Peak (2) is an unidentified component.

each other on the mass spectrum, one or both may be present in *B. schlegelii*.

Hydrogenobacter

Nine peaks were detected on h.p.l.c. profiles when polyamine fractions of the hydrogen bacteria *Hydrogenobacter* were analysed (Fig. 5). The major peak (peak 3) in TK-6 and TK-G corresponds to polyamine V. Another major peak, peak 9, corresponding to polyamine XXIV, was found in the three strains. Among minor peaks, peak 1 corresponds to polyamine II, peak 4 to polyamine VIII and/or XXI, and peak 7 to polyamine IX and/or X. Peaks 5 and 8 found in TK-6 correspond to polyamines XXII and XXV respectively. An elution shoulder corresponding to polyamine VI and a minor peak corresponding to polyamine IV were detected in TK-G. Peak 6 which was identical with polyamine VII was observed only in TH-112. An acid- and alkaline-stable component, peak 2, was not identified.

When the polyamine fractions of these three strains were analysed by t.l.c., a major ninhydrin-positive spot corresponding to polyamine XXIV was found (results not shown).

Polyamine XXI was detected as a major component during g.c. of the three samples (Fig. 6), suggesting that most of the tertiary tetra-amine was derived from polyamine XXIV corresponding to peak 9 in h.p.l.c. Peak (1) in g.c. corresponds to another degradation product from authentic polyamine XXIV. Polyamine XXII found in TK-6 included the intact one (peak 4 in h.p.l.c. profiles) and a product from polyamine XXV corresponding to peak 8 found by h.p.l.c. The occurrence of polyamine VI in TK-G was confirmed by g.c. analysis. Polyamine VII in TH-112 was also confirmed by g.c. and identified by g.c.–m.s. Among the tetra-amines, only IX was detected in TK-6, whereas three linear tetra-amines, such as VIII, IX and X, were found in TK-G and TH-112 (Fig. 6). An unidentified peak (2) was detected in the g.c. profiles of TK-6 and TK-G. The peak probably corresponds to the unknown peak 2 in h.p.l.c. profiles.

DISCUSSION

Polyamine XXV was first found in *Thermoleophilum* as the major polyamine, as shown in this report. Another quaternary penta-amine, XXIV, was first found in extremely thermophilic eubacteria belonging to *Thermus* species as a minor polyamine (Oshima *et al.*, 1987; Hamana *et al.*, 1991). The latter penta-amine was also detected as a trace polyamine in *Thermoleophilum album* and *Thermoleophilum minutum*, and the former penta-amine was found as a minor polyamine in *Thermus* species (Hamana *et al.*, 1991). Production of linear tetra-amines, penta-amines and hexa-amines may be the main pathway of polyamine synthesis in *Thermus thermus*, but it was not found in *Thermoleophilum*.

XXIV was also found to be the major polyamine in *H. thermophilus* and *H. halophilus*. Although a variety of other minor triamines and linear and branched tetra-amines were observed, it is suggested that the main pathway of quaternary penta-amine synthesis is common in the two strains, TK-6 and TK-G, of *H. thermophilus* and a halophile, *H. halophilus* TH-112. These extremely thermophilic hydrogen bacteria lack linear penta-amines and hexa-amines as does the extreme thermophile, *Thermoleophilum*. However, the major polyamine of *Thermoleophilum* is another quaternary penta-amine. The extremely thermophilic eubacterial *Thermus* species including *Thermus thermophilus*, *Thermus caldophilus*, *Thermus filiformis* and *Thermus flavus* (Oshima, 1982; Oshima & Kawahata, 1983; Oshima *et al.*, 1987; Hamana *et al.*, 1990*b*, 1991), and *B. schlegelii* contain linear penta-amines and hexa-amines in addition to some of the tertiary tetra-amines and quaternary penta-amines. XXV was detected in *B. schlegelii* as a minor polyamine.

An extremely thermophilic bacterium, *Thermomicrobium roseum*, contains linear penta-amines and hexa-amine but not branched tetra-amines and penta-amines (Hamana *et al.*, 1990*c*).

Novel hexa-amines, XIX and XX, were first found in *B. schlegelii*. XVII (and XVIII) have been found in *Thermus* (Oshima *et al.*, 1987; Hamana *et al.*, 1991) and *Thermomicrobium roseum* (Hamana *et al.*, 1990*c*) but not in *B. schlegelii*, as shown in this study. XVI has been detected in *Thermus* and *Thermomicrobium roseum* (Hamana *et al.*, 1990*b*,*c*, 1991) as well as in *B. schlegelii*. The polyamine-synthetic pattern of the obligately extremely thermophilic eubacteria belonging to five groups, *Thermus*, *Thermomicrobium*, *Thermoleophilum*, *Hydrogenobacter* and *Bacillus*, is similar but different among them. The polyamine-distribution pattern found in each extreme thermophile is unique but would be one of the types found in extremely thermophilic eubacteria.

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