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Distribution of Polyamines in Methanogenic Bacteria

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Members of all four families of methanogenic bacteria were analyzed for polyamine concentrations. High-performance liquid chromatography analysis of dansylated cell extracts revealed typical polyamine patterns for each family. Members of *Methanobacteriaceae* (family I) were characterized by very low polyamine concentrations; members of *Methanococcaceae* (family II) were characterized by putrescine and high spermidine concentrations; members of *Methanomicrobiaceae* (family III) were characterized by the presence of putrescine, spermidine, and sym-homospermidine; and members of *Methanosarcinaceae* (family IV) contained only high concentrations of sym-homospermidine in addition to putrescine. The highest polyamine concentration was found in *Methanosarcina barkeri* Jülich, with 0.35% putrescine in the dry cell material. The polyamine distribution found coincides with the dendrogram based on comparative cataloguing of 16S rRNA and offers a new, rapid chemotaxonomic method for characterizing methanogenic bacteria. Variation of the growth substrates (H_2–CO_2, methanol, acetate, and trimethylamine) for *M. barkeri* resulted in quantitative but not qualitative differences in polyamine composition.

Methanogenic bacteria are strict anaerobes, growing usually on hydrogen and carbon dioxide and thereby producing methane (3). Together with the halobacteria and certain other thermoacidophilic bacteria, they are considered to represent their own kingdom of organisms, called archaebacteria (39) or metabacteria (19), which was suggested to be equivalent to the remaining kingdoms of eucaryotes and eubacteria.

Systematic studies on methanogens show that they possess some unique biochemical characteristics. They contain deazaflavine as part of the electron carrier factor F_{420} (12), 2-mercaptoethanesulfonic acid as terminal methyl carrier (4), a pteridine called methanopterine (37), and factor F_{430}, a compound containing a nickel tetrapyrrol complex (9). They synthesize biphytanylglycerol ethers—instead of fatty acid ethers—as well as large amounts of squalene derivatives (26, 29). Instead of a murein sacculus, the cells consist of components like a pseudomurein sacculus, a protein sheath, or a heteropolysaccharide envelope (22). Typical for all archaebacteria is a reaction with diphtheria toxin (23).

Classifications of methanogenic bacteria can be based on the comparative cataloguing of 16S rRNA sequences (3, 14) as well as on the abovementioned biochemical properties. The scheme resulting from these studies is in good agreement with a classification based on immunological analyses (7). So far, however, no other cell constituents have been shown to be useful for chemosystematics.

Polyamines (Fig. 1), which occur as polycations under physiological conditions, have been detected in practically all organisms analyzed (2, 6, 24, 35, 36). Their function is mostly related to the biosynthesis of protein and nucleic acids and, therefore, they are involved in all processes connected with growth and differentiation. They can also stabilize membranes and nucleic acids and influence a multitude of enzymatic reactions (1, 2, 6, 36). Whereas putrescine, spermidine, and spermine are classical representatives of the polyamines, 1,3-diaminopropane, sym-norspermidine, sym-homospermidine, and sym-norspermine have also been identified in many bacteria and algae (8, 16, 24).

The starting point of the biosynthesis of polyamines is putrescine, usually derived from ornithine by decarboxylation (see Fig 1). Addition of one or two aminopropyl groups yields spermidine and spermine, respectively (36). 1,3-Diaminopropane, the precursor of sym-norspermidine and sym-norspermine, is thought to be derived from spermidine (8). Of the other natural polyamines, cadaverine does not seem to have a

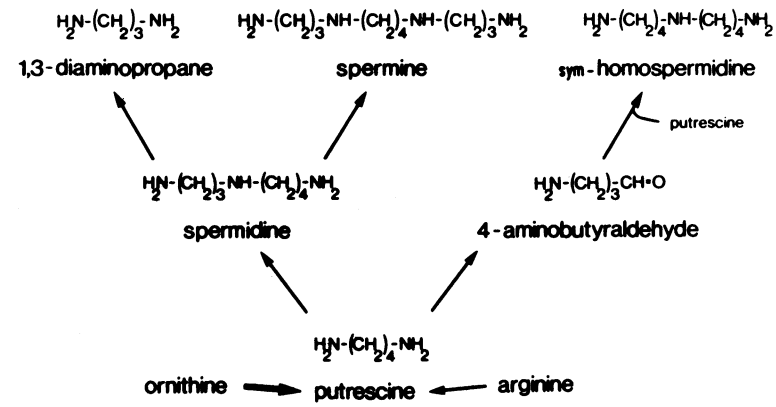

FIG. 1. Biosynthesis of polyamines. The scheme is a modification of that of De Rosa et al. (8).

typical polyamine function, and its aminopropyl derivative, asym-homospermidine, has been detected in a mutant of *Escherichia coli* (10).

The distribution of polyamines was shown to be a valuable tool in the chemosystematics of green algae (17). As the occurrence of polyamines in methanogenic bacteria has not been described in the literature, the following investigation was carried out to determine whether methanogens contain polyamines. In the case of different polyamine patterns occurring in different families, the value of polyamine analysis for chemosystematics should be evaluated in comparison with more elaborate methods like 16S rRNA analysis (3, 14).

MATERIALS AND METHODS

Organisms. All organisms with a DSM number can be obtained from the Deutsche Sammlung von Mikroorganismen (DSM; Göttingen, Federal Republic of Germany). The new list of this group of the DSM, available on request from H. Hippe, contains all isolation details or refers to the original literature about these strains. The strains were obtained from different donor sources (Table 1).

TABLE 1. Sources of strains used

Species	Strain designation	DSM no.	Donor source
Methanosarcina barkeri	MS	800	J. Winter, Müchen/Regensburg
Methanosarcina barkeri	Fusaro	804	DSM (H. Hippe)
Methanosarcina barkeri	227	1538	R. A. Mah, Los Angeles
Methanosarcina sp. (50°C)	TM-1	1825	S. Zinder, Los Angeles/Ithaca, N.Y.
Methanosarcina vacuolata		1232	DSM (H. Hippe)
Methanococcus mazei	S-6[a]	2053	R. A. Mah, Los Angeles
Methanococcus vannielli	SB	1224	DSM (H. Hippe)
Methanococcus voltae	PS	1537	DSM (H. Hippe)
Methanobrevibacter arboriphilus	AZ	744	DSM (H. Hippe)
Methanobacterium bryantii	M.o.H.G.	862	DSM (H. Hippe)
Methanobrevibacter smithii	PS	861	G. Diekert, Marburg
Methanobacterium thermoautotrophicum (65°C)	Marburg[b]	2133	R. K. Thauer, Marburg
Methanospirillum hungatei	JF 1[c]	864	H. König, Regensburg

[a] This strain, which manifests itself as a sarcina or as a coccus morphotype, was subsequently grown three times in the coccoid stage.

[b] This strain is somewhat different from the originally described ΔH, DSM 1053 (3, 5).

[c] Cells were harvested in the log phase.

New strains. *Methanosarcina barkeri* Wiesmoor was isolated without yeast extract via a single roll tube colony (20) from a drainage creek of the peat bog Wiesmoor, in the northern part of West Germany. The pellet-forming (up to 3 mm or larger) *M. barkeri* Jülich was also isolated without yeast extract, but from a small single pellet (<1 mm) of a homogeneous methanogenic culture obtained from a sewage digester near Jülich. *M. barkeri* Euskirchen was isolated from an Anamet plant in Euskirchen (anaerobic treatment of washing waters of sugar beets), as described by Godsy (15), with cefotaxim as antibiotic and without the addition of yeast extract. By the same method, a *Methanococcus* sp. Jülich was isolated from a sewage digester near Jülich. This strain grew always as a coccus (mainly in pairs of two or four regularly shaped cells morphologically distinct from *Methanococcus mazei*) and only with methanol as substrate (acetate and H_2–CO_2 were tested). From the homogeneous methanogenic enrichment culture of this coccus on methanol, a rod-shaped (about 0.6 by 4 μm) methanogenic bacterium could be separated, growing on H_2–CO_2 but not on methanol.

All new strains were controlled by epifluorescence microscopy (11) and by using a glucose-peptone broth, for the elimination of non-methanogenic contaminants.

Culture methods. A modified Hungate technique (20) was used throughout the investigation, as previously described (31). To obtain strictly anaerobic conditions, the media were alternatively evacuated and gassed with argon. Transfers were made by using syringes (31). The culture vessels were rounded glass cylinders (0.5 liter of medium to 2.3 liters total volume; diameter 15 cm) equipped with two serum bottlenecks as filling holes (KFA workshops, Jülich).

Cells were generally harvested in the early stationary phase.

Culture media. The basal medium was an optimized mineral medium (31, 32): imidazole-hydrochloride buffer, 40 mM (adjusted to the given pH with HCl); NaH_2PO_4, 0.5 mM; Na_2HPO_4, 0.5 mM; $MgCl_2$, 2 mM; $CaCl_2$, 2 mM; KCl, 5 mM; NaCl, 34 mM; NH_4Cl, 10 mM; $NiCl_2$, 5 μM; $CoCl_2$, 1 μM; Na_2MoO_4, 0.5 μM; Na_2SeO_3, 0.1 μM; riboflavin, 3 μM; folic acid, 1 μM; resazurin, 4.4 μM; titanium-III-citrate, made from $TiCl_3$, trisodium citrate, and NaOH (modified [41]), 0.17 mM; L-cysteine-hydrochloride, 0.85 mM. The Ni^{2+} concentration was increased to 5 μM (formerly 1 μM [32]) because in 500-ml vessels the Ni^{2+} concentration caused by contamination from syringe canules (chromium-nickel-steel) was smaller than in previously used 5-ml tubes.

The Na_2S concentration of the medium solutions was ≤50 μM (S^{2-} + HS^-), according to Scherer and Sahm (31). To sustain such a concentration in the described 0.5-liter vessels at the given pH (Table 2), only 0.1 to 0.3 mmol of Na_2S had to be added per liter in 1:4 portions after sterilization. The addition of Na_2S after autoclaving drastically reduces the total quantity of Na_2S needed compared with addition before sterilization (31) and averts precipitation of sulfides. For growth under H_2–CO_2 (300 kPa pressure; 100 rpm on a rotary shaker), a low but sufficient concentration of Na_2S seemed to be essential for good growth (31). The growth temperature was generally 37°C. Slight variations of the basal medium are listed in Tables 1 and 2.

Analysis of amines. The method for analysis of amines was a modification of the procedure originally developed by Seiler and Wiechmann (33), using dansyl chloride (5-dimethylamino naphthalene-1-sulfochloride). Amines were extracted from approximately 40-mg freeze-dried samples with 1 ml of 0.2 M $HClO_4$ at 100°C (30 min) with shaking once after 15 min. Internal standard (200 nmol; 43.44 μg of 1,8-diaminooctane hydrochloride in 50 μl of water) was added before the heating. After extraction, the samples were centrifuged; then 0.2 ml of the supernatant was incubated with 300 μl of Na_2CO_3 solution (100 mg/ml in water) and 800 μl of dansyl chloride solution (7.5 mg/ml in dry acetone) in 0.5-dram vials with Teflon-lined caps (20 min at 60°C). Thereafter, 100 μl of a proline solution (50 mg/ml in water) was added to bind excessive dansyl chloride (10 min at 60°C). After cooling in a refrigerator (to 5°C), the mixture was shaken with 100 μl of toluene. Then 10 μl of the upper layer was injected for analysis.

High-performance liquid chromatography conditions. Separation of the dansyl derivatives was effected by using a Waters (GmbH) system equipped with M 6000 and M 6000A pumps, U6K injector, M 660 programmer, and a Micro-Bondapak C18 reverse-phase column (30 by 0.4 cm; 10-μm particles). Detection was achieved with a fluorescence detector (Du Pont de Nemours 836) equipped with a 16-μl flow cell. The excitation wavelength was λ = 250 to 390 nm (filter: Corning CS-7-54), and emitted light was passed through a cutoff filter of λ = 451 nm (Corning CS-3-72). A linear gradient of 40 to 80% acetonitrile-water at 40°C with a flow rate of 1 ml/min was used to elute the dansylated products. Amine concentrations were calculated from peak areas by means of an integrator (Pye Unicam DP 101), using the method of internal standardization. The maximum deviation found was <10%. The identity of the separated polyamines was confirmed by mass spectrometry and comparison with authentic samples.

HCl hydrolysis of samples. To 20 mg of sample, 200 nmol of internal standard and 1 ml of 6 M HCl were added. Hydrolysis was achieved by heating this mixture for 6 h at 105°C in screw-capped vials with Teflon-lined caps. Solvent was removed in an evaporator, and the residues were dissolved in 500 μl of water. An aliquot of 200 μl from this aqueous solution was derivatized and analyzed as described above.

Mass spectrometry. A Varian Mat mass spectrometer CH 7 with a direct inlet system and data processing by Spectrosystem 100 MS was used with the electron energy of 70 eV, trap current 600 μA, and ion source temperature 250°C.

Chemicals. All chemicals were of the highest purity available, generally of analytical grade, and were purchased from Merck (Darmstadt, West Germany) and Fluka (Neu-Ulm, West Germany). sym-Homospermidine was synthesized as described before (25). The gases obtained from Messer Griesheim (Duisburg, West Germany) had a purity of greater than 99.9995% (vol/vol). The resulting oxygen impurity of 1.4 to 1.7 ppm (μl/ml [vol/vol]) was not corrected by a gas-purifying apparatus as done by other scientists (3).

RESULTS

Figure 2 and Tables 3 and 4 summarize the results of polyamine analysis of methanogenic

TABLE 2. Cultivation of methanogenic bacteria on a defined medium: slight variations of the basal defined medium

Organism	Carbon source						Carbon source supplement			Additional vitamins[a]	Additional trace elements[a]	Iron source (μM)	$CaCl_2$ (0.1 mM, not 2 mM)	pH at t_0
	H_2–CO_2 (80:20)	Methanol (170–190 mM)	Sodium acetate (mM)	Sodium formate (mM)	$NaHCO_3$ (mM)	$N(CH_3)_3 \cdot HCl$ (mM)	Sodium citrate 2 (mM)	Malate 1 (mM)	L-Methionine 1 (mM)					
DSM 744	+	–	5	5	10	–	–	+	–	+	+	$(NH_4)_2Fe(SO_4)_2$ (50); FeII-citrate (50)	+	6.8
DSM 1224	–	–	–	100	5[b]	–	–	–	–	+	+	$FeCl_2$ (50)[c]	–	8.0
DSM 800	–	+	–	–	5[b]	–	–	–	–	–	–	$(NH_4)_2Fe(SO_4)_2$ (100); FeII-citrate (100)	–	6.3
DSM 804	–	+	–	–	–	–	–	–	–	–	–	$(NH_4)_2Fe(SO_4)_2$ (100); FeII-citrate (100)	–	6.3
DSM804	–	–	115	–	–	–	–	–	–	–	–	$(NH_4)_2Fe(SO_4)_2$ (100); FeII-citrate (100)	–	6.2
DSM 804	+	–	–	–	–	–	–	–	–	–	–	$FeSO_4$ (100)	–	6.4
DSM 804	–	–	–	–	–	100	–	–	–	–	–	$(NH_4)_2Fe(SO_4)_2$ (100); FeII-citrate (100)	–	6.5
DSM 1232	–	+	–	–	5[b]	–	–	–	–	–	+	FeII-citrate (100)	–	6.3
DSM 1538	–	+	–	–	5[b]	–	–	–	–	+	+	$(NH_4)_2Fe(SO_4)_2$ (100); FeII-citrate (100)	–	6.3
DSM 1825	–	+	–	–	5[b]	–	+	–	+	+	+	$(NH_4)_2Fe(SO_4)_2$ (100); FeII-citrate (100)	–	6.4
DSM 2053	–	+	–	–	5[b]	–	–	–	–	–	+	$FeCl_2$ (50)[c]	–	6.3
M. barkeri Juelich	–	+	–	–	5[b]	–	–	–	–	–	+	$FeCl_2$ (50)[c]	–	6.3
M. barkeri Wiesmoor	–	+	–	–	5[b]	–	–	–	–	–	–	FeII-citrate (100)	–	6.3
M. barkeri Euskirchen	–	+	10		10[b]	–	–	–	5[d]	–	–	$FeCl_2$ (1,500)[d]	–	6.2

[a] The trace element solution was according to reference 18, and vitamins were according to reference 40. The organism *M. bryantii* (DSM 862, not listed) and *Methanobacterium* sp. were cultivated like *M. arboriphilus* (DSM 744). The strain *Methanococcus* sp. Jülich had the culture medium of *M. barkeri* (DSM 1825; see above). *M. smithii* (DSM 861) and *M. hungatei* (DSM 864) were grown according to reference 3, *M. thermoautotrophicum* (DSM 2133) was grown according to reference 5, and *M. voltae* was grown according to reference 38, all on H_2-CO_2.

[b] $NaHCO_3$ was used preventively, but its necessity for good growth was not checked (21).

[c] A 0.1 M $FeCl_2$ solution was complexed by 0.1 M sodium citrate and served as stock solution.

[d] Special medium: the common sulfur sources Na_2S and L-cysteine HCl were completely omitted and were replaced by 5 mM L-methionine. To sustain a sufficiently reduced medium, the FeII concentration and the TiIII-citrate concentration were increased (1.5 mM and 1.35 mM, respectively). Fe^{2+} was added as $FeCl_2$ and was complexed by an equivalent amount of methylaminodiacetic acid. The Ni^{2+} concentration was increased to 50 μM according to the higher content of complexing agents. Furthermore, the medium contained 125 mM NaCl instead of 34 mM and, preventively, 0.2 mM cefotaxim as an antibiotic.

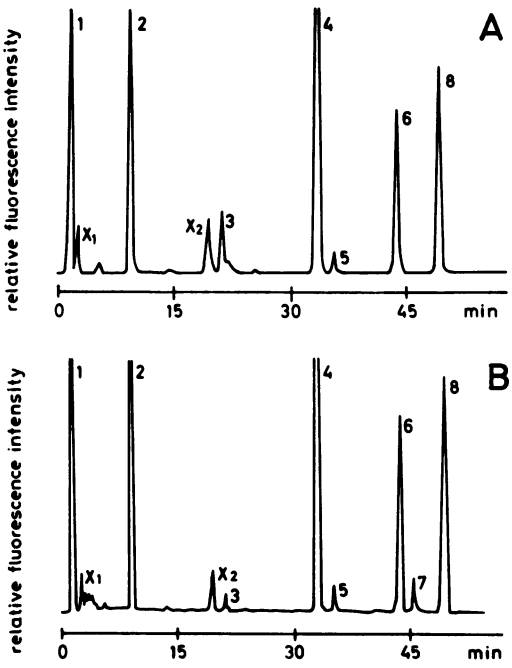

FIG. 2. High-performance liquid chromatogram of cell material from *M. barkeri* 227 (DSM 1538). (A) $HClO_4$ extracted. (B) HCl treated. Peaks represent dansyl derivatives of the following compounds: 1, H_2O plus proline; 2, ammonia; 3, dimethylamine (artifact, derived from dansylation); 4, putrescine; 5, cadaverine; 6, 1,8-diamino-octane (internal standard); 7, tyramine; 8, sym-homospermidine; x_1 and x_2, unknown components.

bacteria. With the bacteria being grouped in four families according to the relationship of their 16S rRNA (S_{AB} values [3]), typical polyamine patterns could be observed for each family. Whereas norspermidine, spermine, and norspermine were not detectable in methanogenic bacteria, and diaminopropane was found only in a few cases (Table 3), putrescine was present in all four families. Members of *Methanobacteriaceae* (family I) contained putrescine only in small amounts ($\ll$1 μmol/g dry weight) in contrast to families II, III, and IV (>1 μmol of putrescine per g dry weight). Upon HCl hydrolysis of the cells, distinctly higher putrescine concentrations were found in bacteria of family I, but these concentrations were still always low (<1 μmol of putrescine per g dry weight). On the other hand, the concentration of 40 μmole of putrescine per g in *M. barkeri* Jülich corresponded to more than 0.35% of the dry weight.

The most characteristic differences could be seen between spermidine and sym-homospermidine. Spermidine (>25 μmol/g dry weight) was detected in family II (*Methanococcaceae*) with *M. vannielii* and *M. voltae*, and in family III (*Methanomicrobiaceae*) with *M. hungatei* as representatives. The largest amounts of sym-homospermidine (up to 9 μmol/g dry weight) could be found in family IV (*Methanosarcinaceae*), where it was the only triamine detected. Family III, which follows on family IV in the scheme of Balch et al. (3), was characterized also by the presence of smaller amounts of sym-homospermidine (0.3 μmol/g dry weight) and,

TABLE 3. Quantitative determinations of polyamines in methanogenic bacteria including *Methanobacteriaceae* (family I), *Methanococcaceae* (family II) and *Methanomicrobiaceae* (family III)

Family	Species	Polyamine concn (μmol/g dry wt)[a]		
		Putrescine	Spermidine	sym-Homospermidine
I	*Methanobacterium bryantii*	0.04 (0.14)	<0.03	<0.03
I	*Methanobacterium thermoautotrophicum*	0.10 (0.3)	<0.03	<0.03
I	*Methanobrevibacter arboriphilus*	0.03 (0.3)	<0.03	<0.03
I	*Methanobrevibacter smithii*	0.03 (0.7)	<0.03	<0.03
II	*Methanococcus vannielii*[b]	3.4 (3.4)	28.5 (27.4)	<0.03
II	*Methanococcus voltae*	0.48	40.0	<0.03
III	*Methanospirillum*[b] *hungatei*	3.1	4.0	0.30
I(?)	*Methanobacterium* sp.	0.07	<0.03	<0.03

[a] Norspermidine, norspermine, and spermine were not detectable (<0.03 μmol/g dry weight). Analyses were made from $HClO_4$-extracted cells. Numbers in parentheses refer to HCl-treated cells of the same organism. HCl treatment revealed tyramine as an additional amine (not listed). For names of strains and other details, see the text.

[b] *M. vannielli* and *M. hungatei* also contained small amounts of 1,3-diamino-propane: 0.27 and 0.37 μmol/g dry weight, respectively ($HClO_4$ extract).

TABLE 4. Quantitative determinations of polyamines in *Methanosarcinaceae* (family IV)

Species	Strain	Substrate	Polyamine concn (μmol/g dry wt)[a]		
			Putrescine	Spermidine	sym-Homospermidine
M. barkeri	MS	Methanol	18.7	<0.03	4.1
M. barkeri	227	Methanol	31.9 (34.2)[b]	<0.03	8.7 (9.2)[b]
M. barkeri	TM-1	Methanol	1.2	<0.03	6.5
M. barkeri	Euskirchen	Methanol	6.5	<0.03	4.7
M. barkeri	Wiesmoor	Methanol	22.9	<0.03	5.4
M. barkeri	Jülich	Methanol	40.8	<0.03	6.9
M. vacuolata	(DSM 1232)	Methanol	11.5	<0.03	5.4
Methanococcus mazei	S-6	Methanol	19.2	<0.03	6.9
Methanococcus sp.	Jülich	Methanol	17.7	<0.03	3.4
M. barkeri	Fusaro		26.2	<0.03	4.7
M. barkeri	Fusaro 14-day stationary		0.8	<0.03	0.5
M. barkeri	Fusaro	Trimethylamine	4.4	<0.03	3.6
M. barkeri	Fusaro	H_2–CO_2	12.5	<0.03	2.8
M. barkeri	Fusaro	Acetate[c]	1.1	<0.03	0.9

[a] Norspermidine, norspermine, and spermine were not detectable (<0.03 μmol/g dry weight). Cells of the early stationary growth phase were used (see the text) unless otherwise indicated.

[b] Values in parentheses refer to HCl-treated cells. HCl treatment revealed tyramine as an additional amine (not listed).

[c] Cells grown more than 20 times subsequently on acetate with a gassing rate about one-fifth that of methanol-grown cells were used (an adaptation time was not observed [31]).

therefore, contained two triamines. In bacteria of family I, no tri- or tetraamines could be detected, even after HCl hydrolysis of bacterial cells.

Analytical results of an unidentified new strain of a methanogenic bacterium growing on H_2–CO_2 were included in Table 3. According to its polyamine pattern, which was in excellent agreement with family I, it could be assigned to the *Methanobacteriaceae*.

As *M. barkeri* is the most versatile methanogen with respect to catabolism, the possible influence of different energy and carbon sources (H_2–CO_2, methanol, acetate, trimethylamine) on the polyamine concentrations was studied. The results (Table 4) show that only the quantitative values varied, whereas the qualitative polyamine pattern remained unchanged. It is noteworthy, however, that in the case of acetate, the content of polyamines was obviously decreased and that trimethylamine did not increase the polyamine quantities. From other organisms, it is known that rapidly growing cells have a higher content of polyamines than do non-proliferating cells (1). This influence was found to be true also for *M. barkeri* as the content of polyamines fell off if the cells were kept without substrate in the stationary phase for 5 to 14 days (Table 4). For *M. barkeri* Fusaro grown on methanol, it was also determined that polyamines (putrescine and sym-homospermidine) could be detected almost exclusively in the supernatant of disintegrated cells (centrifuged for 10 min at 25,000 × *g*).

DISCUSSION

The systematic investigation of polyamine concentrations in methanogenic archaebacteria revealed different types of polyamine patterns. A classification according to these patterns is in full agreement with the scheme based on the analysis of 16S rRNA (3, 14). A combination of the polyamine analyses with this scheme based on S_{AB} values is shown in Fig. 3. The analysis of polyamines offers an additional convenient method for the chemosystematics of methanogenic bacteria and can give preliminary hints for the classification of new strains and species. As already shown with algae, triamines (spermidine and its homologs) seem to be the most relevant polyamines for use in chemotaxonomy (17). Whereas polyamine patterns in families I, II, and IV were obtained from at least two different members of these families, of family III only *M. hungatei* was investigated. Therefore, although with respect to family III the basis for taxonomic generalizations is limited, the simultaneous presence of spermidine and sym-homospermidine in *M. hungatei* indicates an intermediate position between families II and IV.

Putrescine is certainly the key substance in polyamine biochemistry (Fig. 1). Ornithine decarboxylase, the main enzyme for putrescine biosynthesis, has an activity which is highly dependent on the state of growth. On the other hand, putrescine concentration is reduced by the formation of spermidine and sym-homospermidine and, therefore, shows the greatest variation

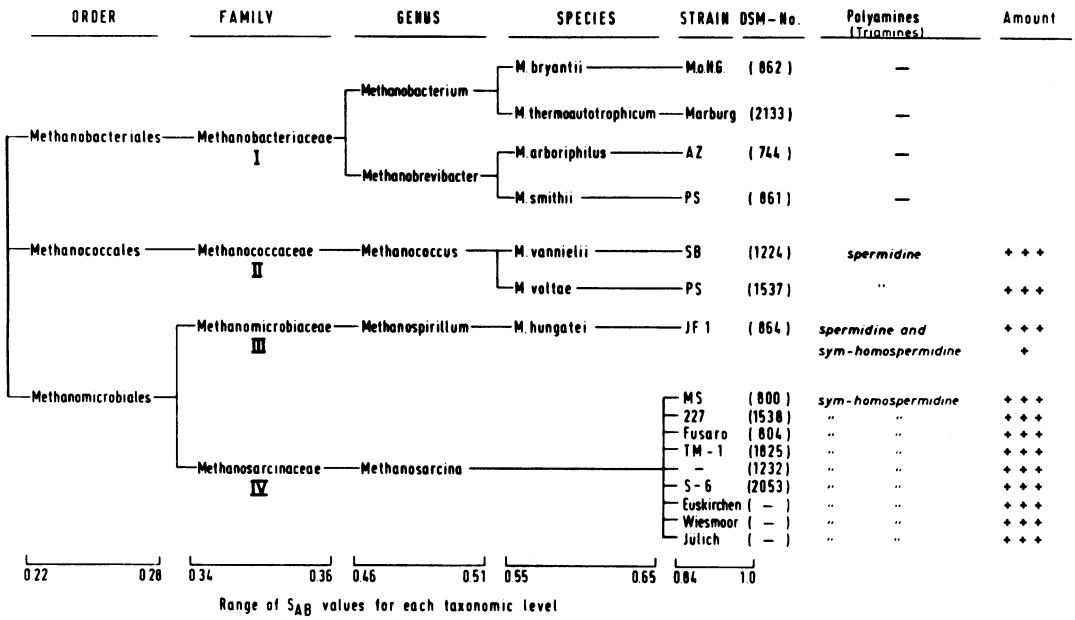

FIG. 3. Dendrogram of methanogenic bacteria based on comparative cataloguing of 16S rRNA values (S_{AB} values, varied from references 3 and 14) and completed by the found distribution of polyamines. The *Methanosarcinaceae* (family IV) were extended in the scheme (3) by the species *M. vacuolata* (formerly designated as *M. barkeri* [42]), by *M. mazei* S-6 (27), and by *Methanococcus* sp. Jülich (new isolate). The following additional strains of *M. barkeri* were used: *M. barkeri* TM-1 (43), Fusaro (18), Euskirchen, Wiesmoor, and Jülich (new isolates). The *M. thermoautotrophicum* strain ΔH was replaced by the faster-growing strain Marburg (5).

of all polyamine concentrations. For this reason, putrescine concentrations are more an indication of metabolic activity and possess less value in chemotaxonomy.

Spermidine, which is one of the frequently found polyamines (24, 35, 36) was detected in two families of methanogenic bacteria (families II and III). One of the functions of spermidine could be the participation at the level of protein translation (1, 2, 36). Interestingly, the in vivo translation system of *M. vannielii* SB (family II) needs 1 mM spermidine for optimal activity (13), suggesting that spermidine is involved in the protein translation system in vivo as well.

The occurrence of sym-homospermidine in methanogenic bacteria is another example of the abundance of symmetrical polyamines in microorganisms, as demonstrated earlier with algae and with thermophilic and other bacteria (24, 30, 34). But there is still not much known about the relative functions of symmetrical polyamines in comparison with the "classical" polyamines spermidine and spermine. First results with *Chlorella emersonii* show a close relationship between concentrations of sym-norspermidine and RNA (28).

In family I, the almost total lack of polyamines is striking. Hydrolysis experiments with HCl showed that polyamines also did not occur in the form of acid-labile derivatives. Taking into account that polyamines are essential for optimal growth, this apparent deficiency in the family *Methanobacteriaceae* should deserve special attention. As the function of polyamines in methanogenic bacteria has not yet been studied, and in view of an unusual biochemistry related to methanogenesis, a situation different from other organisms cannot be fully excluded.

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ADDENDUM IN PROOF

A second species and genus of family III can be cultivated according to Romesser et al. (J. A. Romesser, R. S. Wolfe, F. Mayer, E. Spiess, and A. Walther-Mauruschat, Arch. Microbiol. **121**:147–153, 1978). This member, *Methanogenium marisnigri* JR1 (DSM 1498), was grown with H_2-CO_2 as the substrate. The analyses revealed 41.3 μmol of putrescine, 6.76

μmol of sym-homospermidine, 0.23 μmol of spermidine, and 0.21 μmol of spermine per g (dry weight). Therefore, the analyses fit with the scheme of Fig. 3.

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