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Methanothermus fervidus, sp. nov., a Novel Extremely Thermophilic Methanogen Isolated from an Icelandic Hot Spring

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Summary

A rod-shaped extremely thermophilic methanogen is described, growing between 65 and 97 °C with an optimal temperature around 83 °C and a doubling time of 170 min. The GC-content of its DNA is 33 mol %. The isolated cell wall sacculus contains pseudomurein. The complex cell envelope exhibits two layers, each about 12 nm thick; the inner represents the pseudomurein sacculus and the outer a protein envelope.

An enriched fraction of RNA polymerase does not react with antiserum against RNA polymerase from *Methanobacterium thermoautotrophicum,* indicating that the isolate belongs to a new family, the *Methanothermaceae,* within the order *Methanobacteriales.* The new organism is named *Methanothermus fervidus.*

Key words: Thermophilic – Archaebacteria – Methanogens – RNA polymerase – Methane – Bacterial Gas – Volcanism

Introduction

Thermophilic methanogens known to date were isolated from sewage sludge (*Zeikus* and *Wolfe,* 1972) and from thermal volcanic environments (*Zeikus* et al., 1980). They all belong to the species *Methanobacterium thermoautotrophicum,* which is characterized by morphology, GC-content (*Zeikus* and *Wolfe,* 1972), cell wall composition (*Kandler* and *König,* 1978), 16S r-RNA oligonucleotide pattern (*Balch* et al., 1979), and RNA polymerase (*Stetter* et al., 1980).

The maximal growth temperature of *Mb. thermoautotrophicum* is around 75 °C. Therefore, it was assumed (*Zeikus* et al., 1980), that the upper temperature limit

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of methanogenesis is below 80 °C, possibly because of thermal desintegration of an inner membrane system necessary for methane production (*Zeikus* et al., 1980).

Here we report the isolation and first characterization of a new highly thermophilic methanogen which can grow at temperatures of up to 97 °C, belonging to the order *Methanobacteriales* but differing strongly from *Mb. thermoautotrophicum*.

Materials and Methods

Strains

Methanobacterium thermoautotrophicum, strain ΔH, *Methanobacterium bryantii*, strains M.O.H. and M.O.H.G., *Methanobacterium formicicum*, strain MF, *Methanobrevibacter arboriphilus*, strain AZ, *Methanobrevibacter smithii*, strain PS, *Methanococcus vannielii*, strain SB, and *Methanosarcina barkeri*, strain MS, were kindly supplied by R.S. Wolfe, Urbana. *Methanobacterium thermoautotrophicum*, strain JW 510, was obtained from *J. Wiegel*, Göttingen. *Methanobacterium thermoautotrophicum*, strain Marb., was obtained from *R. Thauer*. *Methanobacterium thermoautotrophicum*, strain W, was isolated by *J. Winter* as an aberrant derivative of *Methanobacterium thermoautotrophicum*, strain ΔH. *Methanosarcina fusaro*, DSM 805, and *Sulfolobus acidocaldarius*, DSM 639, were obtained from the Deutsche Sammlung für Mikroorganismen, Göttingen. *Thermoplasma acidophilum* 122-1B2 was obtained from *E.A. Freundt*, Aarhus. *Halobacterium halobium*, strain R, was obtained from *D. Oesterhelt*, Martinsried.

Culture conditions

All methanogens were cultivated by using the culture technique described by *Balch* and *Wolfe* (1976).

MM-medium was used to grow the isolate V24S. It consists of medium 1 of *Balch* et al. (1979), modified by the omission of formate and the addition of 2-mercapto-ethanesulfonic acid (0.1 mg/l). The pH is adjusted to 6.5 with acetic acid. The other isolates were grown in medium 1 of *Balch* et al. (1979).

20 ml cultures were grown in stoppered pressurized 100 ml serum bottles (Bormioli, Italy) made of "type III"-glass by incubation in shakers (New Brunswick), employing a glycerol bath.

The strains of *Mb. thermoautotrophicum*, *Mb. bryantii*, *Mb. formicicum*, *Mbr. arboriphilus*, *Mbr. smithii*, *Ms. barkeri* and *Ms. fusaro* were grown in medium 1, *Mc. vannielii* in medium 3 as described by *Balch* et al. (1979). *Sulfolobus acidocaldarius* was cultivated in a medium described by *Brock* et al. (1972). *Thermoplasma acidophilum* was grown in a medium recommended by *E.A. Freundt* (*Sturm* et al., 1980). *Halobacterium halobium* was cultivated as described by *Oesterhelt* and *Stoeckenius* (1974).

Preparation of polysilicate plates

In an anaerobic chamber (Coy, Ann Arbor, Mich.) at 4 °C, trace amounts of solid sodium dithionite are added to a mixture containing 20 ml sodium silicate solution (DAB6; 1.37 kg/l; Merck, Darmstadt, FRG), 20 µl of a resazurin solution in water (1 g/l), and 180 ml MM-medium, until resazurin becomes colorless. Then, under vigorous stirring, the pH is quickly adjusted to 7.0 with H_2SO_4 (about 3.4 ml diluted 1:1 in water) and 40 ml portions are poured immediately into glass petri dishes. After 2 to 5 min at room temperature the plates become solid. Then, the plates are equilibrated with MM-medium by superposing them with 20 ml of MM-medium, containing vancomycin (20 mg/l) in order to avoid infections. The equilibration medium was changed 5 times within 24 h. During equilibration, the plates are gently rotary-shaken at room temperature in the anaerobic

chamber. The equilibrated plates are sterilized for 2 h at 120 °C in a pressure cylinder described by *Balch* et al. (1979), containing 100 kPa of an 80 % H_2 and 20% CO_2 gas atmosphere. The covers of the petri dishes contain paper discs (Selecta, 11 cm ⌀; Schleicher & Schüll, FRG) absorbing the residual medium during sterilisation.

The inoculated plates are incubated at 85 °C and 200 kPa H_2:CO_2 (80:20) in the pressure cylinder described by *Balch* et al. (1979).

Electron microscopy

For thin sectioning, cell sediments were fixed in MM-medium not containing yeast extract, with 25 g/l glutaraldehyde for 2 hours and postfixed with 10 g/l OsO_4 (2 h). Epon 812 (Serva, Heidelberg) epoxy resin was used for embedding and thin sections were contrasted with lead citrate (5 min), uranyl acetate (5 min) and again with lead citrate (3 min). The sections were examined in a Siemens Elmiskop 102 (80 KV).

Preparation of antibodies

A rabbit was immunized with a total of 170 µg of purified RNA polymerase from *Methanobacterium thermoautotrophicum*, strain W (*Stetter* et al., 1980) using a micro method described previously (*Stetter*, 1977).

The γ-globulines were enriched from the serum by the method of *Linn* et al. (1973).

Enrichment of RNA polymerase

RNA polymerase was enriched as described previously (*Stetter* et al., 1980), but protein was eluted from the polymine precipitate in one step by extraction with 1.5 mol/l NH_4Cl in TAG buffer, followed by an ammonium sulfate precipitation (95% final concentration). After centrifugation, the precipitate was resuspended and dialyzed and was used for antibody precipitation.

Isolation of DNA

2 g cells were suspended in 5 ml buffer (10 mmol/l Tris-HCl, 10 mmol/l $MgCl_2$, pH 7.7) and disrupted in the French press (Aminco) at 130000 kPa. After the debris was removed (21000 rpm; 5 min; Beckman J21-2), 100 µg RNAase (Ribonuclease A from bovine pancreas, heated to 100 °C for 10 min; Boehringer, Mannheim) and, 15 min later, 50 µg proteinase K were added to the supernatant. After 30 min incubation at 25 °C, SDS was added to a final concentration of 5 g/l and the mixture was heated to 65 °C for 15 min. Then, KCl was added to a final concentration of 0.5 mol/l. After 30 min at 4 °C, the K-SDS was removed (21000 rpm; 10 min; Beckman J2-21) and the supernatant was extracted 3 times with an equal volume of buffer-saturated freshly distilled phenol, containing 0.8 g/l hydroxychinolin. The DNA-containing upper (lighter) phase was then again extracted 3 times with an equal volume of chloroform and n-octanol (9:1) and 3 times with chloroform, followed by dialysis of the upper phase against a buffer containing 10 mmol/l Tris-HCl, pH 7.7, 10 mmol/l NaCl and 10 mmol/l EDTA. In the next step, ethidium bromide (250 ng/l) and solid CsCl (0.95 g/ml; ϱ = 1.64 g/cm³, η = 1.3947) were added to the dialyzed solution and then centrifuged 24 h at 47000 rpm (rotor 50 Ti, 20 °C, Beckman L5-50). The DNA band was removed with a syringe and again centrifuged for 48 h under the same conditions. Finally, ethidium bromide was removed by shaking with n-butanol and the solution was dialyzed against 1 × SSC (0.15 mol/l NaCl; 0.015 mol/l Na_3-citrate). Yield: 0.4 mg DNA.

Cell wall analysis

Cell walls were prepared by suspending whole cells (yield of a 4 × 20 ml serum bottle culture) in 1 ml sodium dodecylsulfate (20 g/l) and treating the suspension in a Bransonic 220 bath until no intact cells were detectable microscopically (about 30 min). Then, the suspension was incubated at 100 °C for 30 min, washed (4 ×) with 1 ml distilled water,

spun off in an Eppendorf centrifuge and freeze-dried (16 h, WKF-Gefrieranlage L05-60). Yield: 5 mg dried cell walls.

Amino acids, amino sugars and neutral sugars were determined as described by *Kandler* and *König* (1978). Talosaminuronic acid was detected by thin layer chromatography after spraying with ninhydrin-reagent, using α-picoline: 25% ammonia:water = 70:2:28 as a solvent (*König* and *Kandler*, 1979).

Removal of the outer envelope

Pronase treatment: Cells (18 mg) were suspended in 1 ml buffer (phosphate 0.07 mol/l; pH 7.4). After addition of 1 mg pronase (Merck), the suspension was incubated for 8 h at 37 °C.

Sodium dodecylsulfate treatment: Cells (18 mg) were suspended in 1 ml sodium dodecylsulfate solution (20 g/l) and heated for 10 min in a boiling water bath.

After pronase or sodium dodecylsulfate treatment, the cells were washed (4 ×) with 1 ml buffer (phosphate 0.07 mol/l; pH 7.4) and spun off in an Eppendorf centrifuge. As a reference, untreated cells were washed 2 times in the same buffer.

Results

Collecting of samples

Samples of water from 131 Icelandic volcanic spring and mud holes with temperatures between 37 °C and 100 °C and pH-values ranging from 1 to 9.5 were taken. They were drawn from the bottom of the source, either with a 50 ml beaker mounted on a long stick, or, if possible, directly with a 20 ml-syringe. At pH-values below 4, the pH was raised to around 6 by the addition of $CaCO_3$. Then, 20 ml samples were injected with a syringe into stoppered gassed (95 % N_2; 5 % H_2; 100 kPa (≙ 1 bar)) 25 ml serum tubes (Schott, Germany) sealed with an aluminium cap (*Balch* et al., 1979). Samples of mud and coarse-grained sediments were filled directly into open tubes which were then stoppered. The samples were transported to the laboratory without temperature control and then stored at 4 °C. Resazurin (0.0001 %) was added as an oxygen indicator. During storage, traces of sodium dithionite were added until the resazurin became colorless again.

Enrichment cultures

Serum tubes (25 ml, Schott, Germany), containing either 6 ml of medium 2 (*Balch* et al., 1979) or medium 2, supplemented with 0.2 % yeast extract were pressurized (200 kPa $H_2 : CO_2 = 80 : 20$; *Balch* and *Wolfe*, 1976) and then inoculated with 1 ml original source water. The pH was roughly adjusted (± 0.5) with sulfuric acid or NaOH, as required, to that of the source of the sample. The samples were then incubated by shaking at 37, 45, 60 or 72 °C, depending on the temperature of the source.

After 3, 7 and 14 days methane production was determined by gas chromatography (Hewlett Packard 7620 A).

No methanogens could be enriched in pure *M*H-medium. When supplemented with yeast extract, however, after 3 days, three samples, R1 a, R2 a and V24 a had yielded methane positive enrichment cultures containing rods strongly fluorescent at 420 nm. No additional isolates of methanogens could be obtained after prolonged incubation.

Methanogens isolated from a field of slightly alkaline springs (pH 8.7) at Reykir, not far from Varmahlith (samples R1 a and R2 a), turned out to be closely related to *Mb. thermoautotrophicum* based on GC-content, RNA polymerase structure, cell wall composition, and upper temperature limit of growth. Therefore, they are not further described in this paper.

The sample V24 a, taken from a tiny spring of 85 °C and pH 6.5 in the Hveradalir solfataric field in Kerlingarfjöll yielded methanogens of different characters. They are described in more detail.

Isolation procedure

The enrichment culture of V24 a was plated on MM nutrient agar and polysilicate plates equilibrated with MM-medium (pH 6.5). The plates were incubated anaerobically in an $H_2 + CO_2$ atmosphere at 200 kPa at 85 °C. After 3 days, the pressure decreased and round, smooth, opaque, slightly greyish colonies, 1 to 3 mm in diameter appeared on the polysilicate plates, while no growth occured on agar. All colonies had the same macroscopic and microscopic appearance. The isolate was designated as V24 S. It consists of rods occuring singly and in pairs, but never in long chains. A second strain, V24 K, could be isolated from the same enrichment culture on plates of pH 8.0 incubated at 72 °C. V24 K grows in long chains. We found it to be related to *Mb. thermoautotrophicum* and will not further describe it in this paper.

Cultivation

V24 S grows well in MM-medium at pH 6.5 under an atmosphere of 200 kPa of $H_2 : CO_2$ (80 : 20). No growth occurs above pH 7. The addition of 2-mercaptoethanesulfonic acid (coenzyme M) enhances growth, especially when small inoculates have been used. $H_2 + CO_2$ can not be substituted by formate or acetate. Oxygen is highly toxic. Even very short exposure leads to complete inactivation of V24 S which is not the case with *Mb. thermoautotrophicum* (*J. Wiegel,* personal communication).

Influence of the glass brand

Growth occurs in serum bottles (Bormioli, Italy), made of alkali-rich soda lime glass ("type III"-glass), but not in serum tubes (Schott, Germany) made of borosilicate glass ("type I"-glass) with a high hydrolytic resistance. Addition of glass powder from ground bottles of "type III"-glass to the culture medium in "type I"-glass tubes did not, however, yield significant growth. The isolates resembling *Mb. thermoautotrophicum* do not show this dependence.

Storage

The cells of V24S die within a few hours after the H_2 and CO_2 supply is exhausted. Therefore, the gas atmosphere must be renewed or the culture be transferred before the gas mixture is used up.

Stock cultures, stable at least 4 months without transfer, were obtained by 6 hours growth (5 % inoculation), followed by renewing the gas phase of 200 kPa of $H_2 + CO_2$ and storage at 4 °C. They serve as an excellent inoculum. This storage technique was also found to be useful for other methanogens.

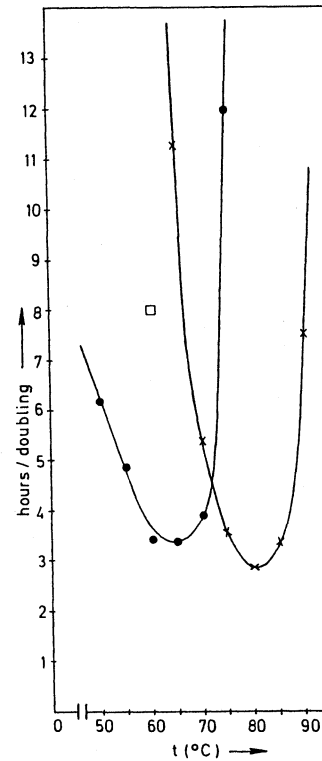

Fig. 1. Optimal growth temperature.
×---× *Methanothermus fervidus* (grown in MM-medium); ●---● *Methanobacterium thermoautotrophicum* ΔH (grown in Medium 1 of *Balch* et al., 1979); □ *Methanobacterium thermoautotrophicum* ΔH at its temperature optimum (grown in medium 2 of *Balch* et al., 1979). Growth was determined several times during the exponential phase by $O.D._{578}$-measurement. The hrs/doubling were calculated from the slopes of the growth curves.

Growth temperature

The optimal growth temperature is around 83 °C (Fig. 1). The new organism does not grow at 60 °C or below, nor above 97 °C. Above 97 °C, the rods turn into "large bodies". Thus, cell wall synthesis is apparently less heat-stable than growth.

The shortest doubling time was 170 min (Fig. 1). The *Mb. thermoautotrophicum* strain ΔH, grown as a control, had its temperature optimum at 65 °C (Fig. 1).

Morphology

The cells are immotile rods, about 1–3 μm long and 0.3–0.4 μm in width. They are sometimes slightly curved, especially at the end of the growth phase. They are gram positive. By electron microscopy of thin sections a bilayered cell envelope can be seen (Fig. 2a), consisting of an inner darker and an outer light layer, each about 12 nm thick. During cell division, only the inner wall forms diaphragm-like insertions (Fig. 2, a, b, c), whereas the outer envelope does not participate in the formation of septa. The outer envelope appears to consist of subunits (Fig. 2, d). A double-layered envelope has already been seen in *Mb. thermoautotrophicum* by *Doddema* et al. (1979), who considered the outer layer as a sheath embracing several cells. The outer layer of V24S, however, appears more regular and shows more contrast in electron micrographs than that of *Mb. thermoautotrophicum*.

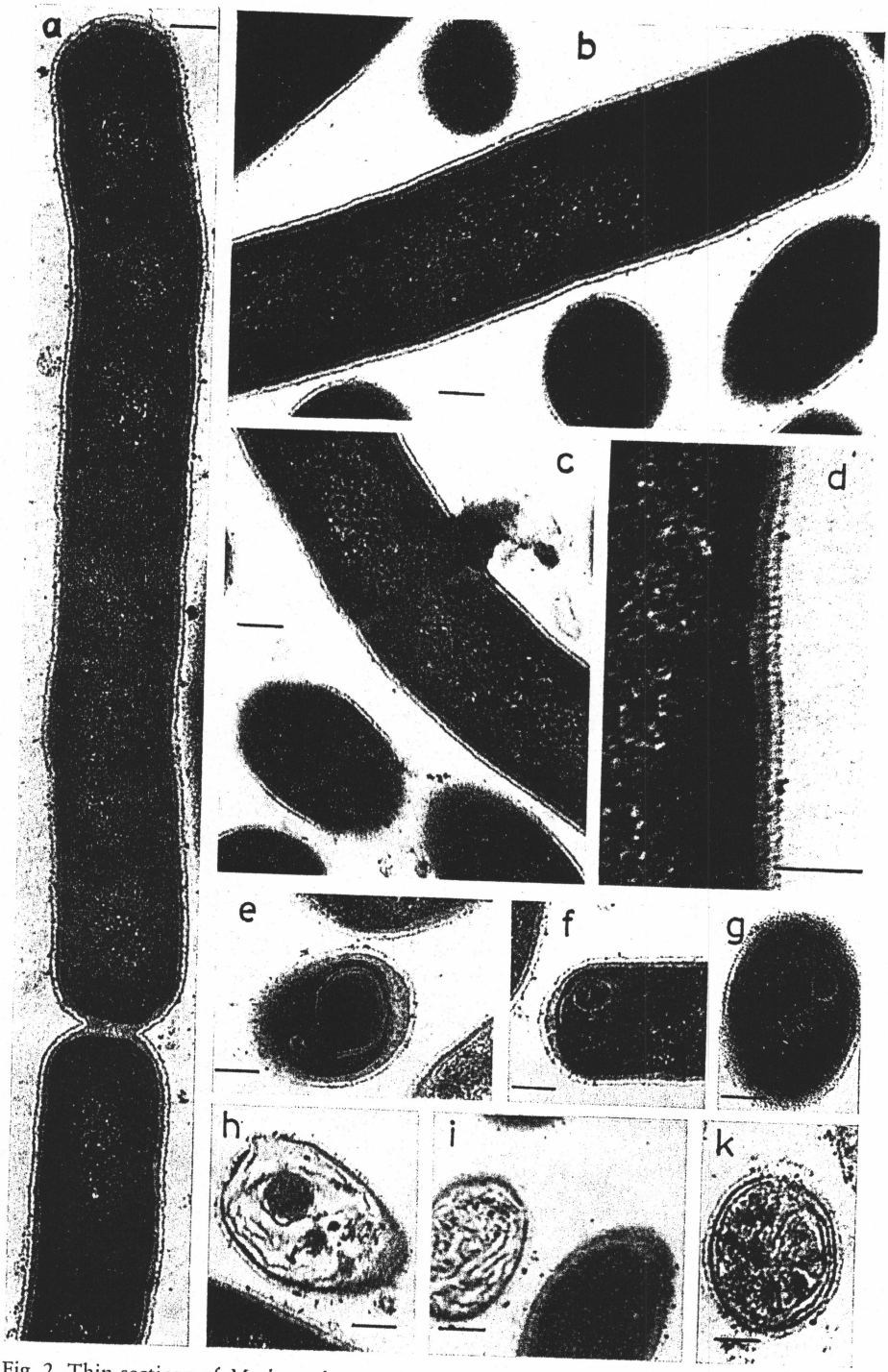

Fig. 2. Thin sections of *Methanothermus fervidus*.
Negative contrast with uranyl acetate and lead citrate. Bar indicates 0.1 μm.

Therefore, the compositions may be different. Membranous structures are often visible within the cells (Fig. 2, e, f, g), particularly when these have lost their cytoplasm (Fig. 2, h, i, k).

Cell wall composition

The composition of the hydrolysate of isolated cell wall sacculi (Table 1) indicates the presence of pseudomurein (*König* and *Kandler*, 1978) with N-acetyl-

Table 1. Components of the hydrolysate of isolated cell wall sacculi of V24S

Component	μmol/mg cell wall	molar ratio
Lysine	0.330	1.00
Glutamic acid	0.736	2.23
Alanine	0.488	1.47
NH_3	0.542	1.64
N-acetyl-glucosamine	0.163	0.49
N-acetyl-galactosamine	0.116	0.35
N-talosaminuronic acid	present in partial hydrolysates	

glucosamine, N-acetyl-galactosamine and N-talosaminuronic acid in the carbohydrate backbone. N-talosaminuronic acid was positively identified by thin layer chromatography of a partial hydrolysate (4 N HCl, 2 h, 100 °C). In addition to the components of pseudomurein 1 g cell wall preparation contained $5.8 \cdot 10^{-3}$ g glucose. Significant amounts of other neutral sugars could not be detected. On account of its high contrast in thin sections in electron micrographs and its participation in septum formation (Fig. 2) the inner layer is assumed to be the pseudomurein sacculus. The fact that the pseudomurein sacculus is much thicker in Fig. 3 than in Fig. 2 may be explained as an effect of the very high temperature of 95 °C at which this batch of cells was grown. The outer layer consists of protein, as evidenced by the result of the SDS-treatment (Fig. 3 b) and the digestion with pronase (Fig. 3 c), both treatments removing the outer layer.

DNA base composition

The DNA contains 33 mol % GC as determined by the melting point in 1 × SSC (*Marmur* and *Doty*, 1962), using calf thymus DNA (42 mol % GC) as a reference. For the *Mb. thermoautotrophicum*, strain ΔH, DNA 51 mol % GC was determined in good agreement with the literature (*Balch* et al., 1979).

A second analysis of the DNA base composition was performed by HPLC chromatography of a nuclease P1 hydrolysate (*Zillig* et al., 1980), yielding a GC-content of 33 mol % for V24S and 50 % for *Mb. thermoautotrophicum*. No unusual bases could be detected. In spite of the new organisms' high growth temperature, its DNA denatures in vitro at 83 °C (1 × SSC). Although the GC-content in *Mb. thermoautotrophicum* is relatively high, this does not seem to be essential for growth at high temperatures. Our results, together wih the GC values of *Sulfolobus* (*Zillig* et al., 1980), show that even extreme thermophiles may have DNA with very low GC-contents. Hence, the mechanism of protection of the DNA structure remains unclear.

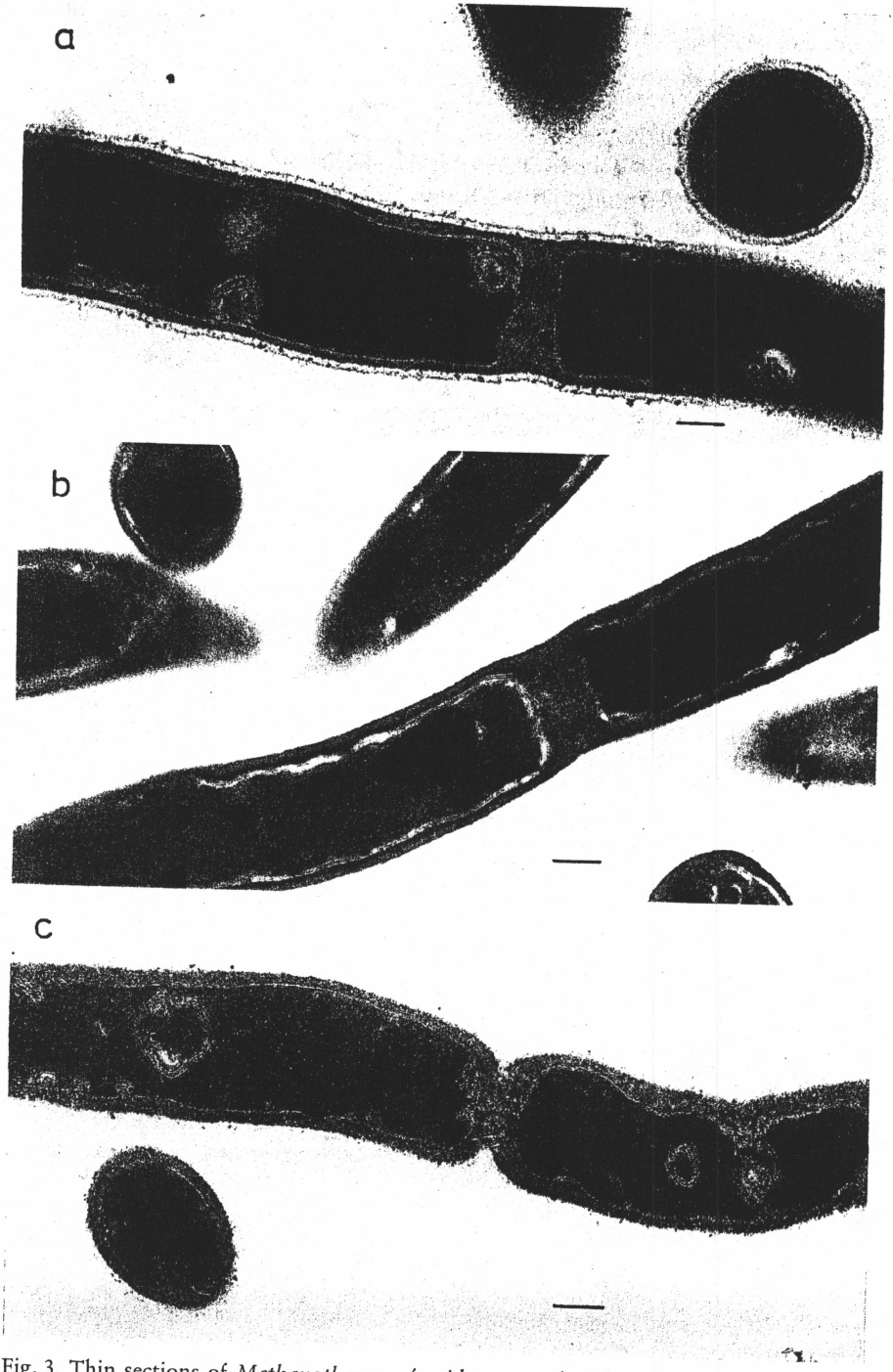

Fig. 3. Thin sections of *Methanothermus fervidus*, treated with sodium dodecylsulfate or pronase.
Negative contrast with uranyl acetate and lead citrate. Bar indicates 0.1 μm.
(a) untreated cells; (b) cells treated with SDS; (c) cells treated with pronase.

RNA polymerase

An enriched RNA polymerase fraction from V24S does not show a precipitation line with an antibody against *Mb. thermoautotrophicum* RNA polymerase in the *Ouchterlony* immunodiffusion test (*Ouchterlony*, 1962), while polymerase fractions of other methanogens of hot springs and of other members of the *Methanobacteriaceae* do (Fig. 4). As expected, members of other orders of methanogens, and other Archaebacteria show no precipitation with this antibody (Table 2).

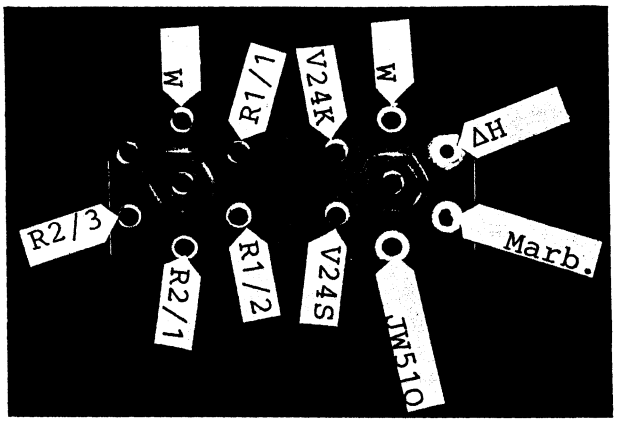

Fig. 4. Immunodiffusion of purified anti-*Methanobacterium thermoautotrophicum* (strain W) RNA polymerase – γ-globulin against RNA polymerase from different thermophilic methanogens.

Table 2. Ouchterlony immunodiffusion test of antibodies against *Mb. thermoautotrophicum* RNA polymerase with extracts from different methanogens and other Archaebacteria

Species	Strain	precipitation line
Methanobacterium thermoautotrophicum	ΔH, V24K, JW510, W, Marb., R-strains[a]	+
Methanobacterium bryantii	M.O.H., M.O.H.G.	+
Methanobacterium formicicum	MF	+
Methanobrevibacter arboriphilus	AZ	+
Methanobrevibacter smithii	PS	+
Methanothermus fervidus	V24S	−
Methanococcus vannielii	SB	−
Methanosarcina barkeri	MS	−
Methanosarcina fusaro	DSM 805	−
Sulfolobus acidocaldarius	DSM 639	−
Thermoplasma acidophilum	122-1B2	−
Halobacterium halobium	R	−

[a] Isolated from samples R1a and R2a.

Discussion

The new methanogen V24 S is clearly different from *Mb. thermoautotrophicum* according to its growth temperature, nutritional requirements, DNA composition and RNA polymerase. The existence of pseudomurein in the cell wall classifies it as a member of the order *Methanobacteriales* (*Kandler* and *König,* 1978; *Balch* et al., 1979).

The RNA polymerase of V24 S does not precipitate with antibodies against RNA polymerase from *Mb. thermoautotrophicum* in the Ouchterlony immunodiffusion test, whereas the members of the two genera of the *Methanobacteriaceae, Methanobacterium* and *Methanobrevibacter* show distinct precipitation lines. The multiplicity of the lines may be caused by fragmentation of the enzymes. With RNA polymerase from other orders of methanogens and other Archaebacteria, however, no precipitation could be observed with antiserum against RNA polymerase of *Mb. thermoautotrophicum*. Therefore, we consider the new isolate to be a member of a new family of the *Methanobacteriales,* which we name *Methanothermaceae.* Because of its ability to grow close to the boiling point, the isolate V24 S is named *Methanothermus fervidus.*

The ecological niche of *Methanothermus fervidus* may be different from that of *Mb. thermoautotrophicum:* the latter seems to be mainly involved in the thermophilic decomposition of organic matter in sewage digestors and waste piles, and of decaying blue green algal mats in hot springs (*Zeikus* et al., 1980). Since no algal mats can grow at temperatures around 90 °C, *Methanothermus* appears to depend on geothermal hydrogen and CO_2. As inferred from our survey of Icelandic solfataras, where *Methanothermus* could be found only once, and from a survey of *Zeikus* et al. (1980) in the Yellowstone National Park, U.S.A., where it was not detected it appears to be very rare in volcanic spring holes. As indicated by its extreme oxygen sensitivity it may, however, occur more frequently in the depth of volcanic areas.

One could speculate that *Methanothermus fervidus* and other so far unknown members of the *Methanothermaceae,* may also be responsible for bacterial methane production in geothermally heated deep sediments, e. g. in the area of Porto Corsini in northern Italy, where methane is found in pliocene sediments situated in 3,000 m depth at temperatures around 90 °C (*Schoell,* 1980). The $\delta\ ^{13}C$ value of -72.7, indicates that this methane is of bacterial origin (*Schoell,* 1980).

Description and Classification of the Methanothermaceae

Order *Methanobacteriales, Balch* and *Wolfe* 1979

Family I, *Methanobacteriaceae, Barker* 1956

Family II, *Methanothermaceae, Stetter* (fam. nov.)

Methanothermaceae, Me.tha.no.ther.ma.ce'ae. M.L.neut.n.*Methanothermus* type genus of the family; – aceae ending to denote a family; M.L.fem.pl.n. *Methanothermaceae* the Methanothermus family. The *Methanothermaceae* belong to the order *Methanobacteriales, Balch* and *Wolfe* 1979. The family *Methanothermaceae* contains one genus.

Gram-positive rods, occuring singly and in pairs. In ultra thin sections, the cell envelope appears as a distinct double-layer. The inner layer is a sacculus contain-

ing pseudomurein. The outer layer consists of protein. RNA polymerase does not show serological relationship with members of *Methanobacteriacaeae* in the immunodiffusion test. Cells are extremely thermophilic, not growing at 60 °C or below. Methan is formed from $H_2 + CO_2$. Habitat: anaerobic environments within solfataric fields.

Genus 1 *Methanothermus, Stetter* (gen. nov.)

Me.tha.no.ther'mus. M.L.n. *methanum* methane; Gr.fem.n.*therme* heat; M.L.-masc.n. *Methanothermus* the methane (-producing) thermophile. The description of the genus is the same as that of the family.

Methanothermus fervidus, Stetter (sp. nov.)

fer.vid.us M.L. *fervidus*, L.masc.adj.fervent; on account of its growth in almost boiling water.

Straight to slightly curved rods, 1 to 3 μm long and 0.3–0.4 μm in width, occurring singly and in pairs, but never in long filaments. They are non-motile and gram-positive. No growth can be detected at 60 °C or below. Optimal growth is at 83 °C at pH 6.5. The upper temperature limit is 97 °C. Methane is formed from H_2 and CO_2. Yeast extract is required for growth in artificial medium. Formate and acetate do not serve as substrates. Colonies are formed on polysilicate plates 1 to 3 mm in diameter, and are round, smooth, opaque, and slightly greyish. No growth can be observed on agar. The cell wall contains pseudomurein consisting of N-acetyl-glucosamine, N-acetyl-galactosamine, N-talosaminuronic acid, glutamic acid, alanine, and lysine. The cell envelope consists of a double-layer of pseudomurein and protein. The DNA base composition is 33 mol % G + C. The RNA polymerase does not precipitate with antibodies against RNA polymerase from *Methanobacterium thermoautotrophicum*. The type strain is DSM 2088 (isolated from an Icelandic hot spring).

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