Arch. Microbiol. 111, 199–205 (1977)

Archives of
Microbiology

Physiology of a *Methanobacterium* Strain AZ

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Abstract. A methanogenic bacterium using H_2 and CO_2 as sole energy and carbon source has been isolated in pure culture from digested sludge. Its colonies on mineral agar are translucent, convex, circular with entire margins and yellow to brownish in colour. Cells are gram-positive, non motile and appear as straight rods, normally about 3 µm long. A marked pleomorphism depending on the media was observed. The organism is chemolithoheterotrophic, has a pH optimum of 7.0 and an optimal temperature for growth of 33–40° C; no growth occurs above 45° C. The generation time at optimal conditions is less than 5 h. Cysteine must be supplied in the growth medium. It can act as sole sulfur source. The addition of sulfide accelerates the growth at an optimum concentration of 10^{-4} to 10^{-5} molar. A growth factor, not identical with SH-coenzyme M, occurring in anaerobic sewage sludge and yeast extract shows a stimulatory effect. 7.0–8.2% of the total carbon dioxide uptake is assimilated and 11.2% of the energy obtained from the reduction of carbon dioxide to methane is refound in the caloric value of the biomass. 0.01 ppm of dissolved oxygen completely inhibits growth and methane production. However, the bacteria do not loose their viability when exposed to high oxygen concentrations. Further informations are needed before this organism (DSM 744) is specifically identified.

Key words: Methanobacterium strain AZ – New isolate – Oxygen effect – Characterization – Physiology.

In the course of our studies on the ecology of methane producers, we isolated an easy to handle methane bacterium for routine research work. We describe the characteristics of strain AZ which is a *Methanobacterium* sp. which utilizes only H_2 and has features somewhat similar to *Methanobacterium formicicum* and *Methanobacterium* M.O.H.

List of Abbreviations. TOC = total organic carbon; DOC = dissolved organic carbon; POC = particulate organic carbon

MATERIALS AND METHODS

Inocula. Samples of digested sludge from the sewage treatment plant in Opfikon near Zurich were used for enrichment cultures.

Stock Solutions. 1. KH_2PO_4, 27.2 g in 1000 ml H_2O. 2. Na_2HPO_4, 28.4 g in 1000 ml H_2O. 3. Mineral solution: NH_4Cl, 6 g; NaCl, 6 g; $CaCl_2 \cdot 2\,H_2O$, 2.2 g; $MgCl_2 \cdot 6\,H_2O$, 2 g in 1000 ml H_2O. 4. $NaHCO_3$, 80 g in 1000 ml H_2O. 5. Trace metal solution: H_3BO_3 saturated solution, 1 ml; $FeCl_2 \cdot 2\,H_2O$, 2 g; $ZnCl_2$, 0.05 g; $MnCl_2 \cdot 4\,H_2O$, 0.5 g; $CuCl_2 \cdot 2\,H_2O$, 0.03 g; $(NH_4)_6Mo_7O_{24} \cdot 4\,H_2O$, 0.05 g; $AlCl_3$, 0.05 g; $CoCl_2 \cdot 6\,H_2O$, 2 g; HCl conc. 1 ml, in 1000 ml H_2O. 6. Vitamin solution: according to Wolin et al. (1963). 7. Titanium(III) solution $6 \cdot 10^{-2}$ molar (Zehnder, 1976). 8. Sulfide solution: $Na_2S \cdot 9\,H_2O$, 24.02 g in 100 ml H_2O.

The sulfate-free *enrichment culture medium* had the following composition: 20 ml of solution (1) and 47 ml of solution (2) were made up with distilled water to 900 ml and sterilized at 121° C. 1 ml of trace metal solution (5) and 1 ml of vitamin solution (6) in 50 ml of mineral solution (3) were added aseptically to the autoclaved medium by using a micro-syringe filter holder with a 0.2 µm membrane filter (Sartorius GmbH, Göttingen). 0.05 g of cysteine HCl in 50 ml of solution (4) were added in the same way. After gassing the medium with 20% CO_2 and 80% H_2 to remove all oxygen, 30 ml of titanium(III) solution and 0.1 ml of sulfide solution were added. Substrates: H_2 and CO_2.

Growth Medium. Before autoclaving, 2.5 g yeast extract were dissolved in the mixture of the solutions (1) and (2) as described above. For some experiments, we used the supernatant of digested sewage sludge as a medium. The sludge was centrifuged and the supernatant sterilized by membrane filtration (Sartorius, 0.2 µm). No additions were used besides titanium(III) solution.

Gases. All traces of oxygen in the commercial gases were removed by passing them over heated copper (340° C) and through columns of Oxisorb (Messer Griesheim, Düsseldorf).

Culture Techniques. The technique described by Hungate (1950, 1969) and modified by Bryant and Robinson (1961) was adapted for isolation and maintenance of the organism. Cultivation of 100 ml cultures: 1 l serum bottles with serum caps (diameter 35 mm) were used as culture flasks. The enrichment culture media were prepared and sterilized in these bottles as described above.

Gassing. The culture flasks were evacuated and flushed alternately with the gas mixture and brought to the final pressure as desired. We normally worked at approx. 1.8 atmospheres. The pH of the medium depends on the carbonate equilibrium and is, therefore, determined by the partial pressure of CO_2. The gas phase in the bottles was frequently renewed during the growth of the methane bacteria in order to maintain the substrate concentration and pH at a constant level. Bottle cultures were shaken at 115 rpm with an amplitude of 5 cm.

A 10 l anaerobic fermenter (Bioengineering, Rüti, Switzerland) was used for mass cultures at continuous supply of the substrates H_2 and CO_2. A constant pH was achieved by adequate proportioning of the flow of CO_2. In this respect it must be remembered that for calculations of pH in the media from the partial pressure of CO_2, the Henry's constant (K_H) has to be included in the equation

$$\mathrm{pH} = pK_1 + pK_H + \log \frac{[HCO_3^-]}{p_{CO_2}} \quad \text{(Stumm and Morgan, 1970).}$$

For carbon dioxide at 33° C, K_H is 0.03. The equilibrium constant K_1 has to be corrected in solutions with ionic strength of $I > 10^{-2}$ mol/l. Larson and Buswell (1942) recommend the following salinity correction for the first equilibrium constant: $pK_1' = pK_1 - \{0.5\sqrt{I}/(1 + 1.4\sqrt{I})\}$. In our medium pK_1' is equal to 6.03.

Analytics. Methane was detected with a Gow Mac gaschromatograph equipped with a Poropak Q (80/100 mesh) column and a molecular sieve (100/120 mesh) column connected to a thermal conductivity detector. Samples were taken with sterile Gillette gas tight syringes. *Volatile acids* and *alcohols* were identified by a Pye Unicam gas chromatograph equipped with Poropak QS (100/120 mesh) columns joined to a flame ionization detector. Programme: 120° C for 2 min, then heated to 220° C at 4° C per min.

Amino acids were chromatographed with an amino acid analyzer (Biocal). *Total organic carbon* (TOC) and *dissolved organic carbon* (DOC) were determined with the Beckman analyzer. We calculated *particulate organic carbon* (POC) from the difference between TOC and DOC.

Bacterial growth was followed by measuring the optical density of bacterial suspensions at 578 nm with an Eppendorf photometer. Dry weight determinations were performed by using constant weight membrane filters (0.45 µm, Sartorius GmbH, Göttingen). Specific rates of methane production,

Q_{CH_4} are given as $ml_{(CH_4)} \cdot g^{-1}$ (dry wt.) $\cdot$ min^{-1}.

In the present paper, we define the molar growth yield Y_{CH_4} as the mass of cells (dry wt.) per mole of methane released since methane is a very easily detectable product of the energy metabolism of methane bacteria.

Coenzyme M was synthesized according to Taylor and Wolfe (1974). All *chemicals* were obtained from commercial sources.

RESULTS

Enrichment, Isolation and Cultivation of the Methanobacterium Strain AZ

A sample of digested sewage sludge was added to a 1 l serum bottle containing 100 ml of enrichment culture medium, an atmosphere of hydrogen and carbon dioxide in such a proportion as to keep the pH in the medium at 7.0. The flasks were incubated and shaken at 33° C. Within 72 h, the bottles developed a negative pressure, and methane was present. Transfer of the fermenting medium to roll tubes containing 80% H_2 and 20% CO_2 produced colonies, some of which were pure cultures of a methane bacterium. Agar surface colonies of this strain which we provisionally called AZ were translucent, convex, circular with entire margins and yellow to brownish in colour. Cultures of this methane bacterium can be stored in roll tubes at 10° C for more than a year without loss of viability. The purity of our strain is easily checked in a growth medium: any formation of organic acids or alcohols or of bad smells from primary amines immediately indicates a contaminant.

Morphological and Physiological Characteristics of Strain AZ

Morphology. Cells of strain AZ, grown at 33° C in growth media tend to produce aggregates forming rosettes. The straight rods are 0.5–0.8 µm wide and 2–3 µm long. They are non motile and gram-positive. Spores are not formed. A significant pleomorphism depending on the medium was observed (Fig. 1).

Optimum Temperature for Growth. Methane bacteria produce methane from 0° C in temperate glacier ice (Berner et al., 1975) up to 70° C (*Methanobacterium thermoautotrophicum*, Zeikus and Wolfe, 1972). The optimal temperature for our strain is between 33° C and 40° C; no growth and methane formation occur above 45° C (Fig. 2).

Effect of pH. In all of our media, pH is a function of the proportion of CO_2 and bicarbonate. The separation of the influence of the two physiological factors pH and CO_2, however, is easily possible by varying either the proportion of CO_2/HCO_3^- or their absolute concentration at constant proportion. At a pH > 7.5, $CO_{2\,aq}$ (or H_2CO_3) is not present in sufficient quantities for growth in the aqueous medium. This difficulty was avoided by culturing the bacteria under constant gas flow (H_2 + CO_2). All experiments were performed at high and low concentrations of bicarbonate in order to establish its possible influence on growth. The serum bottles used in these experiments were gassed every 90 min with the adequate gas mixture for ensuring a constant pH. The growth rate of strain AZ depends directly on the pH, and the optimal pH is 7.0. Half maximum growth rate is reached at pH 6.6 and 7.4 respectively (Fig. 3).

CO_2 the Source of CH_4. The relationship between CO_2 uptake and CH_4 production was determined by measuring pH, CO_2, CH_4 and POC during growth of strain AZ in a closed system (serum bottle). Although the measurements of CO_2 and CH_4 are subject to an experimental error of about 12% due to the pressure change in the closed system, the results, summarized in Table 1, clearly suggest that CH_4 is formed from CO_2.

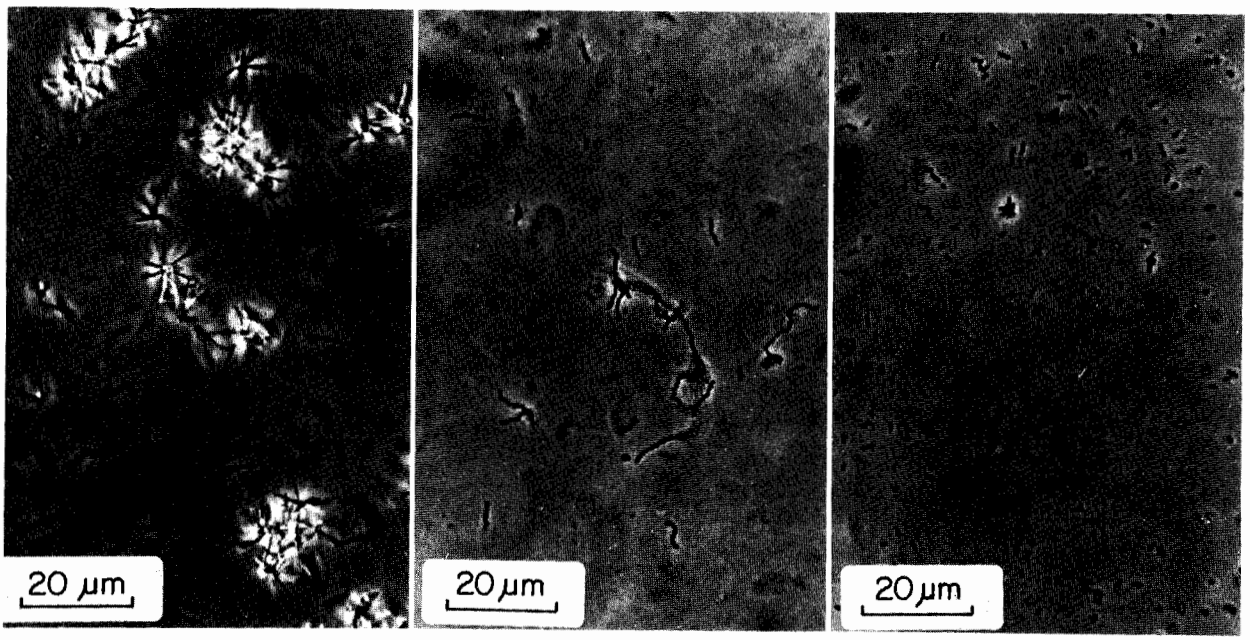

Fig. 1a–c. Pleomorphism of *Methanobacterium* strain AZ, 33° C temperature. (a) Growth medium; growth rate $\mu = 0.110\ h^{-1}$. The cell shape is characteristic for cells grown under optimal conditions. (b) Enrichment medium without vitamins; growth rate $\mu < 0.008\ h^{-1}$. (c) Growth medium without titanium(III) citrate. 0.005 ppm dissolved oxygen were found in the medium. Growth rate $\mu < 0.008\ h^{-1}$. Any of the three forms may be converted into the other ones by transfer to the respective growth conditions

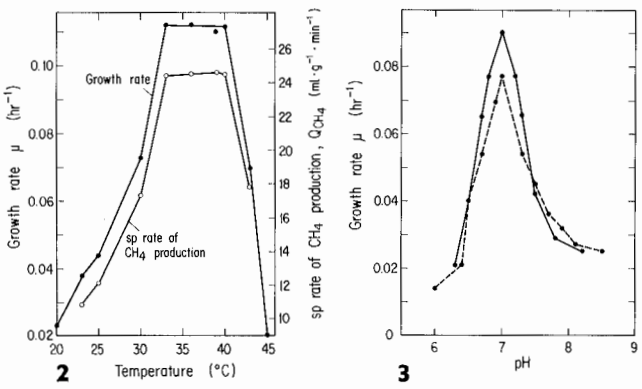

Fig. 2. Optimum temperature for growth and specific methane production of strain AZ. Medium: growth medium. pH: 7.0

Fig. 3. pH dependence of growth of strain AZ. Medium: growth medium. Temperature: 33°C. ●——● growth in serum bottles (closed system); ●——● growth at constant gas flow ($H_2 + CO_2$)

Table 1. Relationship between CO_2 uptake, methane formation and pH of a growing culture of strain AZ (growth medium, temperature = 33°C)

Time (h)	0	2	4	6	7.5	8.5
Total CO_2 (mmol) in culture system	1.83	1.34	0.76	0.19	0.08	0.06
CH_4 formed (mmol)	–	0.44	1.05	1.59	1.79	1.86
Biomass formed (mmol org. C)	–	0.05	0.10	0.13	0.15	0.16
pH (measured)	7.53	7.67	7.91	8.37	8.70	8.82
pH[a]	7.51	7.65	7.87	8.34	8.75	8.88

[a] Calculated according to CO_2/HCO_3 equilibrium in the culture system

Hydrogen Tension. Hydrogen tensions up to 2.3 atm. caused no inhibition of growth and methane production. Higher hydrogen pressures were not investigated for safety reasons.

Effect of Oxygen. Methane bacteria are ubiquitous organisms in anaerobic environments where organic matter is decomposed. Methane is produced even when this environment has originally been aerobic (lake sediments, waste deposits, etc.). The following experiments demonstrated a remarkable oxygen tolerance of our isolate: the atmosphere of $H_2 + CO_2$ in the serum bottles of growing cultures was replaced by helium to which oxygen was added in increasing concentrations of 1, 2, 5, 10, 20 and 100%. Since the volume of the gas phase was 1130 ml and the reducing agents in 100 ml of medium were able to remove only 1.64 ml of oxygen, corresponding to $\frac{1.64 \cdot 100}{1130}$

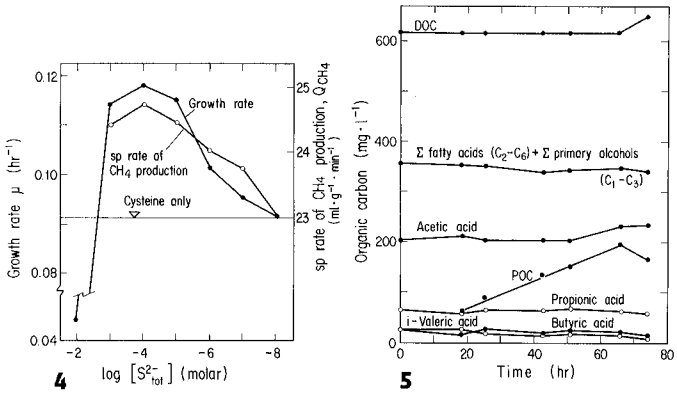

Fig. 4. Effect of various sulfide concentrations on growth and specific methane production of strain AZ. The inoculum was grown in a medium containing cysteine as sole sulfur source. Medium: growth medium. pH: 7.0; temperature: 33° C

Fig. 5. Concentrations of DOC, POC, volatile acids and alcohols (as organic carbon) in the course of growth of strain AZ. Medium: membrane filtered supernatant of digested sewage sludge. pH: 7.0; temperature: 33° C. Substrates: H_2 and CO_2. Variance of acids, alcohols and POC approx. 10%, of DOC approx. 4%. POC in the medium was bacterial biomass. Methanol, ethanol, n-propanol and iso-butyric acid are not stated due to their low concentrations

$= 0.145\%$ of the gas phase, the bacterial environment was strictly aerobic at even the lowest oxygen addition. The culture bottles were then shaken for 4 days at 33° C. Thereafter, the gas phase was replaced by the original atmosphere, and reducing conditions were restored by injection of titanium(III) solution. Growth *and* methane production started again within a lag phase of a few hours in the case of oxygen concentrations of $1-5\%$. It increased to 48 h after exposition to an atmosphere with $10-20\%$ oxygen, and the contact of the cultures with pure oxygen delayed regrowth for more than a month. The length of the lag phase was clearly a function of the preceeding oxygen activity in the medium. When oxygen was injected into the culture flasks still containing the H_2/CO_2 gas phase, stop of growth and methane production recurred as in the previous experimental arrangements, and the resulting lag phases after removal of the oxygen were similar. The composition of the medium showed no effect on the oxygen tolerance or on the period of recovery.

Role of Sulfide and Cysteine. Sulfide and cysteine are reducing agents; at the same time, however, they might be essential physiological factors for growth. Separation of the two activities is only possible with a reductant which is physiologically inert. Titanium(III) citrate meets excellently the requirements for such a redox buffer at $E_H^0 = -480$ mV, pH 7.0 (Zehnder, 1976).

Variation of the sulfide concentration in a medium containing 0.05 g cysteine HCl revealed that sulfide accelerates the growth and the methane production at an optimum concentration of 10^{-4} to 10^{-5} molar (Fig. 4). Methionine, thioglycolic acid, ferredoxin (Sigma) or sulfide as sole sulfur source cannot replace cysteine. It must be considered an irreplaceable, essential amino acid to be supplied in the external medium. In the mineral (enrichment) medium, 40 μg cysteine per mg biomass produced were consumed. Only 3.6 μg cysteine, however, were used during growth in the supernatant of digested sludge. In a later paper (Wellinger et al., in preparation), we shall discuss the role of the sulfur compounds for our strain in more detail.

Utilization of Organic Compounds. Supernatant of digested sludge, sterilized by membrane filtration, was continuously gassed with H_2 and CO_2 at pH 7, 33° C and was inoculated with strain AZ. The composition of the medium was analyzed during growth. Particular parameters were TOC, DOC, short chained volatile acids and alcohols. The results are presented in Figure 5 which indicates no consumption of any organic compound (constant value of DOC during the growth period). In cultures in the growth medium (containing yeast extract as organic component), no decrease of DOC was observed either.

The following compounds (concentration $1-5$ g/l) were assayed for finding other substances which could replace hydrogen for energy supply or carbon dioxide as carbon source and/or as terminal electron acceptor:

1. Fatty acids up to caproate; 2. alcohols up to tertiary butanol; 3. formaldehyde and acetaldehyde; 4. succinic acid and pyruvate.

These compounds were examined in growth medium with and without hydrogen and carbon dioxide, replacing the absent gas by helium. In all experiments, methane production was exclusively observed in the presence of hydrogen *and* carbon dioxide. 50 ppm of formaldehyde and acetaldehyde were already poisonous.

Amino Acid Production. In search of possible amino acid requirements (others than cysteine) of strain AZ, the concentrations of amino acids during growth in sludge supernatant were determined. Table 2 shows that none of the analyzed compounds were consumed. On the contrary, a remarkable excretion of most of them occurred into the external medium. We obtained the same results in the mineral enrichment medium. No distinct increase of amino acids could be detected, however, in the growth medium containing yeast extract due to the already high amino acid content of this substrate.

Table 2. Amino acid production of *Methanobacterium* strain AZ. Medium: supernatant of digested sewage sludge, sterilized by membrane filtration. Substrates: $H_2 + CO_2$, $\mu = 0.058\ h^{-1}$; $Y_{CH_4} = 2.53\ g \cdot mol^{-1}$; pH = 7.0; temperature: 33° C

Amino acid	Sludge supernatant		sp amino acid production µmol/g dry wt. increase
	before growth	after growth of 50 h	
	µmol/l		
Aspartic acid	2.4	6.1	9.8
Threonine	6.0	14.0	21.2
Serine	3.3	16.6	35.4
Glutamic acid	11.7	30.0	48.6
Glycine	11.0	11.0	0
Alanine	43.4	104.6	163.2
Valine	26.0	56.2	80.6
Methionine	4.3	8.1	10.0
Isoleucine	6.6	12.4	15.4
Leucine	8.0	20.5	33.2
Tyrosine	2.4	4.1	4.4
Phenylalanine	2.7	6.4	9.8
β-Alanine	36.0	36.1	0
β-Aminoisobutyric acid	11.0	11.0	0
Lysine	0.7	0.7	0
Arginine	0.8	1.0	in traces

Growth Rate, Specific Methane Production Rate and Molar Growth Yield

Growth rate and specific methane production rate were measured in 1000 ml shaken cultures. The *molar growth yields*, based on methane production (see chapter "Materials and Methods") are contained in Table 3. No significant deviations occurred which could be assigned to the composition of the media. A maximum cell yield of 1.2 g/l (dry wt.) was found in a 10 l fermenter stirred at 1000 rpm, growth medium, pH 7, 33° C, hydrogen and CO_2 as substrates.

Energy Balance

The free enthalpy of the lyophylized biomass of our strain was obtained by calorimetric determination. A heat production of 3.57 kcal per g of biomass was found which permits to establish an overall balance equation for enthalpy changes in the oxidation process as follows:

$$(CH_2O)_{(s)} + O_{2\,(g)} \rightarrow CO_{2\,(g)} + H_2O$$
$$-94.26\ \text{kcal}^a \quad -56.69\ \text{kcal}^a$$
$$-41.75\ \text{kcal}^c \quad -109.2\ \text{kcal}^b$$

[a] Values from Latimer (1956)

[b] Change of free enthalpy, calculated from the heat produced by burning 30 g of biomass (30 · 3.57 kcal) in the calorimeter

[c] "Molar"-free enthalpy of biomass, calculated from [a] and [b]

Table 3. Growth rate (µ), specific methane production rate (Q_{CH_4}) and molar growth yield (Y_{CH_4}) of strain AZ in various media. Substrates: $H_2 + CO_2$, pH: 7.0, temperature: 33° C

Medium	µ (h^{-1})	Q_{CH_4} ($ml \cdot g^{-1} \cdot min^{-1}$)	Y_{CH_4} ($g \cdot mol^{-1}$)
Enrichment medium	0.012	2.9 ± 0.2[a]	2.29 ± 0.21
Supernatant of digested sewage sludge	0.058	12.3 ± 0.7	2.53 ± 0.15
Growth medium	0.144	29.5 ± 2.0	2.68 ± 0.18

[a] Standard deviation of mean of 5–15 assays

According to the experimental findings summarized in Table 3, 2.68 g of biomass with a free enthalpy of 3.73 kcal is bound to the production of 1 mole methane. Assuming its formation from CO_2 and H_2 (− 33.23 kcal), the energy yield based on biomass of strain AZ is then 3.73 kcal/33.23 kcal = 0.112 or 11.2%.

DISCUSSION

The experiments presented show very clearly that the methane bacterium strain AZ is chemolithotrophic. The energy for growth is exclusively obtained by oxidation of hydrogen, and no other energy substrate such as formate, methanol or acetate can be used. The only terminal electron acceptor is carbon dioxide. Strain AZ is most similar to *Methanobacterium formicicum*, *Methanobacterium* strain M.O.H. and *Methanobacterium arbophilicum* (Zeikus and Henning, 1975), it differs however from these in some features (Table 4):

Further comparative studies such as DNA composition and homologies, ultrastructure and chemical composition of cellular components remain to be investigated before a definitive identification and the taxonomic differentiation from the above strains can be made.

Using titanium(III) citrate as a redox buffer, the metabolic role of sulfide and cysteine could be observed independently from their activities as reductants in the medium. Cysteine seems to be the main sulfur source for strain AZ, and furthermore it is an essential amino acid for growth. In inorganic media, cysteine additionally acts as a complexing agent for trace metals preventing their precipitation when sulfide is added. Sulfide demonstrates an accelerating effect on growth and methane production in the presence of cysteine. It is not yet established whether it serves as an additional sulfur source. The dependence on an external supply of cysteine classifies our organism as partially heterotrophic. Consequently, strain AZ should be designated as a chemolithoheterotrophic

Table 4. Comparison of *Methanobacterium* strain characteristics

Strain/Characteristic	*M. formicicum*	*M.* strain M.O.H.	*M. arbophilicum*	*M.* strain AZ
Agar colony	rough	rough	rough	*smooth*
Morphology	rods, single in pairs or chains	rods, single in pairs or chains	rods, single in pairs or chains	rods, single in pairs, *forms rosettes*
Dimension (length)	2–15 µm	2–4 µm	1.8–3.5 µm	2–3 µm
pH optimum			7.5–8.0	6.8–7.2
Electron donor	H_2 + formate	H_2	H_2	H_2
Cysteine	stimulatory	stimulatory	stimulatory	*essential*

bacterium. Partial heterotrophy also exists in regard to some unknown growth stimulating factor(s) occurring in digested sewage sludge or yeast extract.

SH-coenzyme M, even in high concentrations, had no effect on growth and methane formation of strain AZ.

Best growth was observed in a medium containing yeast extract. No stimulation occurred by acetate or other defined organic compounds, as described for *M. ruminantium* and *M.* strain M.O.H. (Bryant et al., 1971).

The generation time of strain AZ of less than 5 h is much lower than that reported for other methane bacteria with the exception of *M. thermoautotrophicum* (Zeikus and Wolfe, 1972) which shows a similar division rate. The molar growth yields of strain AZ in complex synthetical medium vary from $Y_{CH_4} = 2.29$ to 2.68 $g \cdot mol^{-1}$. A value of $Y_{CH_4} = 2.32\ g \cdot mol^{-1}$ can be calculated for *M.* strain M.O.H. on the basis of the results of Roberton and Wolfe (1970) when hydrogen and carbon dioxide are used as substrates. $Y_{CH_4} = 3.3\ g \cdot mol^{-1}$ was found by Stadtman (1967) for *Methanosarcina barkeri* with methanol as sole substrate. It seems that a molar growth yield of about $Y_{CH_4} = 2.5\ g \cdot mol^{-1}$ is a good approximation for methane producers. Assimilated carbon dioxide accounts for 7.0–8.2% of the total of carbon dioxide uptake, and 11.2% of the energy obtained from the overall reaction: $4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$ were refound in the caloric value of the biomass.

Methane bacteria are supposed to be extremely sensitive to oxygen. 0.001 ppm of dissolved oxygen partially inhibits growth of *M. ruminantium*, and 0.01 ppm of dissolved oxygen prevents growth completely (these values were calculated on the basis of Hungate's indications, 1966). Paynter and Hungate (1968) found similar results with *M. mobile*, and so did we with strain AZ. This does not mean, however, that the bacteria are killed by the presence of oxygen. Cultures of strain AZ exposed to high pressures of oxygen (e.g. 20% O_2 in the gas phase, 7 ppm of dissolved oxygen) for several days regrew and produced methane after removal of all oxygen from the culture flasks and the restoration of reducing conditions in the medium with titanium(III) citrate.

The short generation time, the mesophilic temperature optimum, the high oxygen tolerance and the simple nutritional requirements indicate strain AZ as an ideal methane bacterium for routine research work.

Summary of Characteristics of Methanobacterium Strain AZ

Morphology. Straight rods, 0.5–0.8 µm in diameter, 2–3 µm long. Non motile, gram-positive.

Colony Characteristics. Translucent, convex, circular with entire margins and yellow to brownish in colour.

Sensitivity to Oxygen. Growth at strictly anaerobic and highly reducing conditions. Resistant to oxygen contact.

Temperature optimum 33–40°C, tolerated maximum 45°C.

pH optimum for growth at 7.0, half optimum at pH 6.6 and pH 7.4, respectively.

Nutritional Characteristics. Sole energy source is hydrogen, and sole carbon source is carbon dioxide. No utilization of formate, methanol and acetate. Sole sulfur source is cysteine, sulfide at 10^{-4} to 10^{-5} molar concentrations stimulates growth remarkably. Best growth is obtained in a medium containing yeast extract.

Coenzyme M is not required.

Source. Digested sewage sludge.

Type. Strain AZ, isolated from the sewage plant in Opfikon, Zurich, has been deposited in the Deutsche Sammlung von Mikroorganismen (DSM), Göttingen, Germany, under the number DSM 744.

Acknowledgements. We thank Prof. Marvin P. Bryant and Prof. Hans G. Schlegel for the critical review of our paper.

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Received July 12, 1976