110 Thomas, D.J.B., Young, A., Gorsuch, A.N., Bottazzo, G.F., and Cudworth, A.G., Evidence for an association between rheumatoid arthritis and autoimmune endocrine disease. Ann. rheum. Dis. *42* (1983) 297–300.
111 Tosato, G., Steinberg, A.D., and Blaese, R.M., Defective EBV-specific suppressor T-cell function in rheumatoid arthritis. New Engl. J. Med. *305* (1981) 1238–1243.
112 Trentham, D.E., Dynesius, R.A., Rocklin, R.E., and David, J.R., Cellular sensitivity to collagen in rheumatoid arthritis. New Engl. J. Med. *299* (1978) 327–332.
113 Trentham, D.E., Kammer, G.M., McCune, W.J., and David, J.R., Autoimmunity to collagen. A shared feature of psoriatic and rheumatoid arthritis. Arthr. Rheum. *24* (1981) 1363–1369.
114 Trentham, D.E., Townes, A.S., and Kang, A.H., Autoimmunity to type II collagen: an experimental model of arthritis. J. exp. Med. *146* (1977) 857–868.
115 Udén, A.M., Trang, L., Venizelos, N., and Palmblad, J., Neutrophil functions and clinical performance after total fasting in patients with rheumatoid arthritis. Ann. rheum. Dis. *42* (1983) 45–51.
116 Unger, A., Kay, A., Griffin, A.J., and Panayi, G.S., Disease activity and pregnancy associated α_2-glycoprotein in rheumatoid arthritis during pregnancy. Br. med. J. *286* (1983) 750–752.
117 Vandenbroucke, J.P., Valkenburg, H.A., Boersma, J.W., Cats, A., Festen, J.J.M., Huber-Bruning, O., and Rasker, J.J., Oral contraceptives and rheumatoid arthritis: further evidence for a preventive effect. Lancet *2* (1982) 839–842.
118 Vollhardt, B.R., Ackerman, S.H., Grayzel, A.I., and Barland, P., Psychologically distinguishable groups of rheumatoid arthritis patients: a controlled single blind study. Psychosom. Med. *44* (1982) 353–362.
119 Vuorio, E., Rhematoid disease in cultured human synovial cells. Scand. J. clin. Lab. Invest. *37* (1977) suppl. 149, 1–72.
120 Vuorio, E., Einola, S., Hakkarainen, S., and Penttinen, R., Synthesis of underpolymerized hyaluronic acid by fibroblasts cultured from rheumatoid and non-rheumatoid synovitis. Rheumat. Int. *2* (1982) 97–102.
121 Wager, O., Immunopathogenic mechanisms in rheumatoid diseases with special reference to IgM rheumatoid factor. Scand. J. Rheumat. *5* (1975) suppl. 12, 67–76.
122 Waytz, P.H., and Douglas, S.D., Increased antibody-dependent cell-mediated cytotoxicity by monocytes from patients with rheumatoid arthritis. Arthr. Rheum. *22* (1979) 490–494.
123 Willard, K.E., Thorsrud, A.K., Munthe, E., and Jellum, E., Two-dimensional electrophoretic analysis of human leukocyte proteins from patients with rheumatoid arthritis. Clin. Chem. *28* (1982) 1067–1073.
124 Winchester, R.J., Characterization of IgG complexes in patients with rheumatoid arthritis. Ann. N.Y. Acad. Sci. *256* (1975) 73–81.
125 Youssef, A.A.R., and Baron, D.N., Leukocyte superoxide dismutase in rheumatoid arthritis. Ann. rheum. Dis. *42* (1983) 558–562.
126 Ziff, M., Diet in the treatment of rheumatoid arthritis. Arthr. Rheum. *26* (1983) 457–461.
127 Zvaifler, N.J., Etiology and pathogenesis of rheumatoid arthritis, in: Arthritis and allied conditions, 9th ed., pp. 417–428. Ed. D.J. McCarty. Lea & Febiger, Philadelphia 1979.

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Mini-Reviews

Methanogens: a short taxonomic overview

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Key words. Methanogenic bacteria; *Archaebacteria;* classification; physiology; taxonomy.

Introduction

Scientific interest in methanogenic bacteria began in 1776, when the physicist A. Volta described the formation of some sort of 'combustible air' in the sediments of lakes and ponds rich in organic matter. Famous scientists like Béchamp, Popoff, Soehngen and Omelianski studied the microbial origin of methane gas, a development extensively described by another pioneer of the metabolism of the methane formation, Barker[4]. Methanogens live in strongly anaerobic habitats; they are found in two main natural ecosystems, in sediments of lakes and ponds that are rich in organic material, and in the intestines of various animals, among which ruminants and termites may be the most important. In the methane fermentation, unlike other fermentations, no complex substrates are degraded to methane by one single bacterial species, but methane formation needs the cooperation of at least two different bacterial populations. The first one degrades complex organic substrates to low mol.wt. organic acids, CO_2 and hydrogen, and the methanogens use the latter two of these products and eventually acetate to form methane.

All morphological forms found among bacteria, e.g. cocci, rods and spirilla, are also observed among methanogenic bacteria; the unique property of methane formation has been used since the first description of methanogens as the main taxonomic characteristic of this group to distinguish them from the other bacteria.

The findings of novel structures, components and pathways in certain bacterial groups led some years ago to the proposal of the new kingdom of the *Archaebacteria*[42]. *Archaebacteria* have been clearly separated from other procaryotes on the basis of distinct biochemical properties and of differences from all other organisms, e.g. with respect to cell wall composition, structure of lipids, tRNA and RNA-polymerase and the presence of new coenzymes. Besides methanogens, several other organisms have been placed in this new kingdom; the extreme halophiles which form a 1st group together with the methanogens, the *Thermoacidophiles* with *Sulfolobus* as a 2nd one and the genus *Thermoplasma* as a 3rd group[14]. Very recently the latter two groups have been separated from the *Archaebacteria* to form a new kingdom of *Eocyta*[22a].

As mentioned before, the methanogens are strict anaerobes which grow on a narrow range of substrates such as H_2/CO_2, CO, HCOOH, CH_3OH, CH_3COOH and methylamines; they produce methane (and in some cases carbon dioxide) and are best described as obligate anaerobic unicarbonotrophs[44]. Unique properties separate the methanogens from all other bacteria: a) the requirement for an environment of low redox potential as a consequence of strict anaerobiosis; b) the presence of novel coenzymes, e.g. coenzyme M, factors F420 and F430, 7-methylpterin, methanopterin, methenyl-tetrahydromethanopterin, methanofuran; c) their 16S rRNA is distantly related to that of other prokaryotes; d) there are differences in the common arm of the tRNA; e) their cell wall contains neither D-amino acids nor muramic acid; f) their lipids consist of phytanyl ether glycerols (acyclic, cyclic) and squalenes; g) they only have a low genome size (about ⅓ of *E. coli*); h) quinones have not been found, cytochromes are present only in few cases; i) methanophosphagen (cyclic 2,3-diphosphoglycerate) occurs as a possible phosphorous reserve material; j) they possess a novel CO_2-fixation pathway[1].

Some decades ago isolation and identification of methanogens were very difficult. Adequate methods for cultivation and isolation had to be developed first[4]. Until 1967 only six species of methanogenic bacteria had been obtained in pure culture. The development of improved anaerobic culture techniques[5,7,15] made it easier to search for and to isolate new organisms; in the following 10 years at least nine new species or strains were found. The review of Balch et al.[3] induced a dramatic increase in descriptions of new isolates; in only five years more than 24 species of strains have been presented in the literature. How can these organisms be structured systematically in the best way? In 1977 Fox et al.[13] introduced a new system based on the analysis of 16S rRNA oligonucleotide sequence homology. The taxonomic scheme of Balch et al.[3] was based on this sequence homology, and was in strong contrast to the approach evolved by Prévot at about the same time[29,30], which was founded on classical taxonomy.

Table 1. Physiological data on methanogens

Species	DSM No.	Substrate[a]	Morphology	Gram	Temperature	Gen. time (h)	Mol% G+C	pH	Reference
M'bacterium formicium MF	1535	1, 2, 6	Rods	+	43	8.7	40.7	7.0	3
M'bacterium bryantii M.o.H.	863	1	Rods	+	37–39	24	32.7	7.1	3
M'bacterium bryantii M.o.H.G.	862	1	Rods	+	37–39	24	33.2	7.1	3
M'bacterium thermoautotrophicum ΔH	1053	1, 6	Rods	+	65–70	2.2	47.9	7.2–7.6	3
M'bacterium thermoautotrophicum Marburg	2133	1, 6	Rods	+	65–70	1.3	48.5	7.2	6
M'brevibacter ruminantium M1	1093	1, 3	Rods	+	37–42	4	30.6	6.5–7.7	3
M'brevibacter arboriphilus DH1	1125	1, 6	Rods	+	30–37	10	27.5	7.5–8.0	3
M'brevibacter arboriphilus AZ	744	1	Rods	+	33–40	5	31.6	6.6–7.4	3
M'brevibacter arboriphilus DC	1536	1	Rods	+	ND[b]	ND	27.7	ND	3
M'brevibacter arboriphilus A2	2462	1, 2	Rods	+	30–40	14	29.6	7.8	27
M'brevibacter smithii PS	861	1, 2, 6	Cocci	+	37–39	ND	31.0	6.9–7.4	3
M'thermus fervidus V24S	2088	1	Rods	+	83	2.43	33.0	6.5	39
M'coccus vannielii SB	1224	1, 2	Cocci	−	36–40	8	31.1	7.0–9.0	3
M'coccus voltae PS	1537	1, 2	Cocci	−	35–45	1.2	30.7	6.0–7.0	3
M'coccus mazei S-6	–	3, 4, 5	Coccoid	−/+	30–40	7.7	ND	6.1–8.0	23
M'coccus mazei MC_3	–	3, 4, 5	Coccoid	−	25–40	37	ND	6.0–7.7	40
M'coccus deltae ΔRC	–	1, 2	Cocci	ND	37	2	40.5	ND	10
M'coccus maripaludis JJ	2067	1, 2	Coccoid	−	35–39	2.3	33.0	6.8–7.2	19
M'coccus thermolithotrophicus SN1	2095	1, 2	Cocci	−	65	0.92	31.0	6.5–7.5	15
M'coccus jannaschii JAL1	2661	1	Cocci	ND	85	0.43	31.0	6.0	18
M'microbium mobile BP	1539	1, 2	Rods	−	35–42	ND	48.8	6.7–7.4	3
M'microbium paynteri	2545	1	Cocci	−	40	4.8	44.9	7.0	32
M'microbium sp. Ferguson	–	1, 2	Cocci	−/+	60	1.8	59.7	7.2	12
M'genium cariaci JR1	1497	1, 2	Cocci	−	20–25	11	52.0	6.8–7.3	3
M'genium marisnigri JR1	1498	1, 2	Cocci	−	20–25	10	61.0	6.2–6.8	3
M'genium olentangyi RC/ER	–	1	Cocci	ND	37	10.9	54.4	ND	10
M'genium tatii	2702	1, 2	Cocci	−	37–40	12	54.0	7.0	43
M'genium thermophilicum CR1	2373	1, 2	Cocci	−	55	2.5	59.0	7.0	33
M'spirillum hungatei JF1	864	1, 2	Spirillum	−	35–45	17	45.0	6.6–7.4	3
M'spirillum hungatei GP1	1101	1, 2	Spirillum	−	30–40	16	46.5	6.8–7.5	28
M'thrix soehngenii Opfikon	2139	3	Rods	−	37	3.5d	51.9	7.0	17
M'thrix soehngenii VNBF	–	3	Rods	−	40	23	ND	7.0	11
M'coccoides methylutens TMA10	–	4, 5	Cocci	−	30–35	5.2	42.0	7.0–7.5	38
M'sarcina barkeri MS	800	1, 3, 4–6	Sarcina	+	40	12	38.8	7.0	3
M'sarcina barkeri 227	1538	1, 3, 4–6	Sarcina	+	35	24	38.8	7.0	3
M'sarcina barkeri UBS	1311	1, 3, 4–6	Sarcina	+	30	30	43.5	7.4	3
M'sarcina barkeri DM	–	1, 3, 4–6	Sarcina	+	37	28	ND	7.0	3
M'sarcina barkeri FR1	2256	1, 3, 4–6	Sarcina	+	35	33	40.7	ND	2
M'sarcina acetivorans C2A	2834	3–5	Sarcina	−	35–40	5.2	41.0	6.5–7.0	37
M'sarcina vacuolata Z	1232	1, 3, 4–6	Sarcina	+	18–45	ND	51.0	6.0–8.0	47
M'sarcina sp. TM1	–	3–5	Sarcina	ND	45–55	5	ND	5.5–8.0	48
M'planus limicola M3	2279	1–3	Plates	−	40	7	47.5	7.0	41
M'lobus tindarius T3	2278	4, 5	Cocci	−	25	6	45.9	6.5	21
M'plasma elizabethii	–	1, 2	Plasma	ND	37	43	ND	7.2	34

M' = Methano; [a] substrate code: H_2/CO_2 = 1, formate = 2, acetate = 3, methanol = 4, methylamines = 5, carbon monoxide = 6; [b] ND = not determined.

In table 2 the taxonomic scheme of Balch et al.[3] is supplemented with the most recent isolations since 1979. It is difficult to give a complete list of species or strains, since quite a few of the new isolates are not adequately described. Especially the differences from the type strains are often not given in detail. According to Balch's classification we can distinguish at present three orders with 6 families, 14 genera and 30 species with 93 strains; however, only 16 species and 21 strains are documented at present by a 16S rRNA oligonucleotide catalogue (table 2).

Part 1: The diversity

Taxonomic classification of methanogens is not an easy task because of their wide morphological and physiological diversity, as shown in table 1. There is a large variability in cell shape, gram staining, optimal growth temperature, generation time, Mol% G + C and substrate utilization within a single order, family, genus and even species.

According to Balch, methanogens are divided into three orders, the Methanobacteriales, the Methanococcales and the Methanomicrobiales (table 2). In the order Methanobacteriales we find the following properties: 1) Morphology: All members are rods, except for *Methanobrevibacter smithii* PS which is coccoid. The cell wall is formed from pseudomurein. 2) Gram staining is positive. 3) Growth temperature: *Methanobacterium thermoautotrophicum* and *Methanothermus fervidus* are thermophilic, all others are mesophilic species. 4) The generation times range from 1.3 h to 10 h. 5) Substrate: All organisms grow solely on H_2/CO_2, except for *Methanobacterium formicicum* which utilizes formate as 2nd substrate. For *Methanobacterium thermoautotrophicum* at least 12 different strains have been described (ΔH, ΔHR, Marburg, Hveragerdi MT1, R2/3, R1/1, GC1, Z1901, W, YT1, YTA and YTC). Brandis et al.[6] compared the strains ΔH and Marburg more closely and found differencies in the sugar composition of the pseudomurein sacculus, in the structure of subunit O of the DNA-dependent RNA polymerase, in the ATPase activity of the membrane fraction and in the generation time (2.2 h to 1.3 h respectively). Differences are also expected among *Methanobrevibacter arboryphilus* strains DH1, AZ, DC and A2 where Mol% G + C shows a variation of 27.5, 31.6, 27.7 and 29.6, respectively[44].

In the order Methanococcales consisting of only one family, Methanococcaceae, the following variability is observed: 1) Morphology: regular to irregular cocci. The cell wall consists of a protein sacculus. 2) Gram staining: mainly gram negative, with *Methanococcus mazei* S-6

Table 2. Taxonomic classification of methanogens[a]

Order	Family	Genus	Species	Strain	
Methanobacteriales	Methanobacteriaceae	*Methanobacterium*	*M'bacterium formicicum*	MF	*
			M'bacterium bryantii	M.o.H.	*
			M'bacterium bryantii	M.o.H.G.	*
			M'bacterium thermoautotrophicum	ΔH	*
			M'bacterium thermoautotrophicum	Marburg	
		Methanobrevibacter	*M'brevibacter ruminantium*	M1	*
			M'brevibacter arboriphilus	DH1	*
			M'brevibacter arboriphilus	AZ	*
			M'brevibacter arboriphilus	DC	*
			M'brevibacter smithii	PS	*
	Methanothermaceae	*Methanothermus*	*M'thermus fervidus*	V24S	
Methanococcales	Methanococcaceae	*Methanococcus*	*M'coccus vannielli*	SB	*
			M'coccus voltae	PS	*
			M'coccus mazei	S-6	
			M'coccus mazei	MC_3	
			M'coccus deltae	ΔRC	
			M'coccus maripaludis	JJ	*
			M'coccus thermolithotrophicus	SN1	
			M'coccus jannaschii	JAL1	*
Methanomicrobiales	Methanomicrobiaceae	*Methanomicrobium*	*M'microbium mobile*	BP	*
			M'microbium paynteri	–	
		Methanogenium	*M'genium cariaci*	JR1	*
			M'genium marisnigri	JR1	*
			M'genium olentangyi	RC/ER	
			M'genium tatii	–	
			M'genium thermophilicum	CR1	
		Methanospirillum	*M'spirillum hungatei*	JF1	*
			M'spirillum hungatei	GP1	
		Methanothrix	*M'thrix soehngenii*	Opfikon	*
		Methanococcoides	*M'coccoides methylutens*	TMA10	
	Methanosarcinaceae	*Methanosarcina*	*M'sarcina barkeri*	MS	*
			M'sarcina barkeri	227	*
			M'sarcina barkeri	W	*
			M'sarcina barkeri	UBS	
			M'sarcina barkeri	DM	
			M'sarcina barkeri	FR1	
			M'sarcina acetivorans	C2A	
			M'sarcina vacuolata	Z	
			M'sarcina sp.	TM1	
	Methanoplanaceae	*Methanoplanus*	*M'planus limicola*	M3	

M' = Methano; [a]based on classification scheme of Balch et al.[3]; *classified by 16S rRNA oligonucleotide cataloguing.

being gram variable. 3) Growth temperature: *Methanococcus thermophilicus* SN1 and *Methanococcus jannaschii* JAL1 are termophilic, the other species are mesophilic. 4) Generation time ranges from 26 min to 8 h. 5) Mol% G + C is between 30.7 and 40.5. 6) Substrate: *Methanococcus mazei* S-6 utilizes only acetate, *Methanococcus jannaschii* JAL1 only H_2/CO_2, all others utilize H_2/CO_2 or formate as substrates for growth and methanogenesis. 7) Polyamines: *Methanococcus voltae* BP and *Methanococcus vannielii* PS contain only spermidine, *Methanococcus mazei* contains only sym-homospermidine[35]. The order of the Methanococcales exhibits the smallest diversity of all three orders. However, there is some confusion as to whether *Methanococcus mazei* is a *Methanosarcina* and therefore belongs to the order Methanomicrobiales, rather than being an additional species of the Methanococcales[22,35,42]. The most recent attempt at reorganization has been proposed by Mah et al. 1984[24].

The Methanomicrobiales, and in this order especially the family Methanomicrobiaceae, exhibit the largest variability within the methanogenic bacteria. Variations are seen in: 1) Morphology, where rods, cocci and spirillum shape occur. The cell wall consists of one or more protein layers. 2) Gram staining: all gram-negative. 3) Growth temperature: in each genus except for *Methanospirillum* and *Methanococcoides*, there are thermophilic (*Methanomicrobium* sp. Ferguson, *Methanogenium thermophilicum* CR1, *Methanothrix* sp. CAS-1) as well as mesophilic organisms. 4) Generation times range from 1.8 h to 3.5 days. 5) Mol% G + C varies between 42.0 to 61.0. 6) Substrate: in the genus *Methanomicrobium*, *Methanogenium* and *Methanospirillum*, with the exception of *Methanomicrobium paynteri* and of *Methanogenium olentangyi* RC/ER, which only grow on H_2/CO_2, both H_2/CO_2 and formate are substrates for methanogenesis and growth. *Methanothrix* grows only on acetate while *Methanococcoides* uses only methanol and methylamines as substrates. 7) Polyamines: *Methanospirillum hungatei* JF1 and *Methanogenium marisnigri* JR1 contain both spermidine and sym-homospermidine.

In the 2nd family in the order of the Methanomicrobiales, the Methanosarcineae, four species and 20 strains have been described. *Methanosarcina barkeri* strains are MS (the type strain), Fusaro, 3, R1M3, JF, DM, FR1, UBS, 227, W, Wiesmoor, Jülich, Euskirchen, BES1, BES3 and 277BR. This family is unique in morphology (sarcina shape with a cell wall consisting of heteropolysaccharides), positive gram staining and identical substrate requirement (H_2/CO_2, methanol, acetate and methylamines), with the exception of *Methanosarcina acetivorans*, which is gram-negative and is unable to use H_2/CO_2 and formate[37]. All *Methanosarcina* sp. are mesophilic except *Methanosarcina* sp. strains TM1 and CAS-1 which are thermophilic and are unable to use H_2/CO_2 for growth and methanogenesis. The generation times range from 5 to 33 h and the Mol% G + C from 38.8 to 51. Polyamines consist only of sym-homospermidine. The last family, Methanoplanaceae, finally contains the one species *Methanoplanus limicola* M3, which is a plate-shaped, gram-negative, mesophilic bacterium growing on H_2/CO_2, formate and acetate.

Because of their still uncertain taxonomic relationship or existence the following species have been omitted from table 1 and 2: *Methanobacterium propionicum*, *Methanobacterium suboxydans* and *Methanobacillus kuzneceovii* (not available in pure culture according to Mah et al.[26]), *Methanosarcina methanica*[25,45,46], *Methanoplasma elizabethii*[34] and *Methanolobus tindarius* T3[21].

Part 2: The taxonomy

Before 1977 identification and classification of prokaryotes were based on the classical criteria of taxonomy such as cell morphology, gram staining, DNA base content, motility, sporulation and environmental conditions for growth. On these grounds, as late as 1980, Prévot[31] proposed a conservative taxonomic classification which strongly differed not only from the new taxonomy of Balch[3], but also from the classical one[9], in which all the methanogens had been combined on the basis of their ability to produce methane. The taxonomic scheme of Prévot is given in table 3. Several species were renamed, for example (in brackets are the names used by Balch et al.[3] or by the original authors): *Methanopoieticum mobilis (Methanobacterium mobile), Zeikusella thermoautotrophicum (Methanobacterium thermoautotrophicum), Zeikusella ruminantium* and *Z. arboriphilicum (Methanbrevibacter ruminantium* and *M. arboriphilus), Clostridium propionicum (Methanobacterium propionicum), Plectridium omelianskii (Methanobacillus omelianskii), Plectridium suboxydans (Methanobacterium suboxydans).*

Surprising in this scheme is the classification of methanogens in the group of sporulating bacteria, a property not observed within the methanogens. Furthermore, none of the species placed in the order Sporulales have been obtained in pure cultures so far[3,26,30], e.g. *Methanobacillus omelianskii* was found as a coculture of two organisms, *Methanobacterium bryantii* M.o.H. and the 'S-organism'[8]. The unique property of all methanogens, the formation of the specific end product, methane; the presence of unique biochemical markers (e.g. F420, CoM, Methanopterin, Pseudomurein, Methanofuran) and the significant differences of rRNA, DNA or proteins from other bacteria were not accepted by Prévot as a basis of classification. Therefore the division of bacteria into *Eubacteria* and *Archaebacteria* defined by Woese[42] was not accepted.

The modern chemotaxonomy using DNA base-, 16S rRNA- and 5S rRNA composition, lipid and cell wall analysis and serological reactions, has recently become more and more established in bacterial systematics also, and may lead to a phylogenetic foundation for prokaryotic classification. However, there are still some discussions about the weight of each separate criterion, e.g. the minimal difference in Mol% G + C as a prerequisite for classifying strains into species[36].

All the new information available on the methanogens should now be integrated in a natural classification system with the appropriate weight. There are two main chemotaxonomic levels leading to insights in relation to the organisms, the genetic and epigenetic levels. At the epigenetic level, immunological comparative analysis of homologous proteins or of cell surfaces, or functional studies of major metabolic pathways or proteins may give answers about evolutionary relations. On the genetic level the results of DNA-DNA hybridization, DNA-

Table 3. Conservative taxonomic classification[a]

Order	Family	Genus	Species
Asporulales	Micrococcaceae	*Methanococcus*	*M'coccus mazei*
			M'coccus vannieli
		Methanosarcina	*M'sarcina methanica*
			M'sarcina barkerii
			M'sarcina vacuolata
		Methanogenium	*M'genium cariaci*
			M'genium marisnigri
	Ristellaceae	*Methanobacterium*	*M'bacterium soehngenii*
			M'bacterium formicicum
		Methanopoieticum	*M'poieticum mobilis*
	Bacteriaceae	*Zeikusella*	*Zeikusella ruminantium*
			Zeikusella thermoautotrophicum
			Zeikusella ruminantium
	Spirillaceae	*Methanospirillum*	*M'spirillum hungatii*
Sporulales	Clostridiaceae	*Clostridium*	*Clostridium propionicum*
	Plectridiaceae	*Plectridium*	*Plectridium omelianskii*
			Plectridium suboxydans
		Methanobacillus	*M'bacillus kuzneceovii*
	Terminosporaceae	*Terminosporus*	*Therminosporus thermocellulolyticus*

[a] after Prévot[31], for previous names see text.

rRNA hybridization and comparative cataloguing of 16S and 5S rRNA are used. Schleifer and Stackebrandt[36] rated the most important methods for classifying the phylogenetic relations of prokaryotes.

The category from species to urkingdom (defined by Fox et al.[14]) is covered by the sequence of 5S rRNA, from closely related species to urkingdom by the sequence of oligonucleotides of 16S rRNA, from species to orders by sequences of proteins with conserved primary structure, from species to families by comparative immunology of proteins (e.g. ribosomal proteins, DNA-polymerase, adenosinetriphosphatase, etc.), from closely related species to families by DNA 16S/23S rRNA hybridization and finally from strains of one species to closely related species by DNA-DNA hybridization and electropherograms or zymograms of proteins.

Results from such analyses should allow identification and classification of methanogens on a broader basis; besides the traditional description, chemotaxonomic characterization as discussed here is needed to position a new isolate in the taxonomic system. Strict rules and the use of selected specific chemotaxonomic markers for classification, as proposed by Schleifer and Stackebrandt[36] may avoid a flood of species and strains such as that which has arisen on the grounds of only morphological and physiological characterization; a general phenomenon now clearly obvious in the group of the methanogens.

1 Anthony, C., Methanogens and methanogenesis, in: The biochemistry of methylotrophs, chapter 11, pp. 296–327. Academic Press, London 1982.

2 Archer, D. B., and King, N. R., A novel ultrastructural feature of a gas vacuolated *Methanosarcina*. FEMS Microbiol. Lett. *16* (1983) 217–223.

3 Balch, W. E., Fox, G. E., Magrum, L. J., Woese, C. R., and Wolfe, R. S., Methanogens: Reevaluation of a unique biological group. Microb. Rev. *43* (1979) 260–296.

4 Barker, H. A., Studies on methane producing bacteria. Archs Microbiol. *7* (1936) 420–438.

5 Barker, H. A., Bacterial fermentations. John Wiley and Sons, New York 1956.

6 Brandis, A., Thauer, R. K., and Stetter, K. O., Relatedness of Strains ΔH and Marburg of *Methanobacterium thermautotrophicum*. Zbl. Bakt. Hyg., I. Abt. Orig. C2 (1981) 311–317.

7 Bryant, M. P., Commentary on the Hungate technique for culture of anaerobic bacteria. Am. J. clin. Nutr. *25* (1972) 1324–1328.

8 Bryant, M. P., Methane producing bacteria, in: Bergey's manual of determinative bacteriology, part 13, pp. 472–477. Eds. R. E. Buchmann and N. E. Gibbons. The Williams and Wilkinson, Baltimore 1974.

9 Bryant, M. P., Wolin, E. A., Wolin, M. J., and Wolfe, R. S., *Methanobacillus omelianskii*, a new symbiotic association of two species of bacteria. Archs Microbiol. *59* (1967) 20–31.

10 Corder, R. E., Hook, L. A., Larkin, J. M., and Frea, J. I., Isolation and characterization of two new methane-producing cocci: *Methanogenium olentangyi*, sp. nov., and *Methanococcus deltae* sp. nov. Archs Microbiol. *134* (1983) 28–32.

11 Fathepure, B. Z., Isolation and characterization of an aceticlastic methanogen from a biogas digester. FEMS Microbiol. Lett. *19* (1983) 151–156.

12 Ferguson, Th. J., and Mah, R. A., Isolation and characterization of an H_2-oxidizing thermophilic methanogen. Appl. envir. Microbiol. *45* (1983) 265–274.

13 Fox, G. E., Pedman, K. J., and Woese, C. R., Comparative cataloging of 16S ribosomal ribonucleic acid: molecular approach to procaryotic systematics. Int. J. syst. Bact. *27* (1977) 44–57.

14 Fox, G. E., Stackebrandt, E., Hespell, R. B., Gibson, J., Maniloff, J., Dyer, T. A., Wolfe, R. S., Balch, W. E., Tanner, R. S., Magrum, L. J., Zablen, L. B., Blackemore, R., Gupta, R., Bonen, L., Lewis, B. J., Stahl, D. A., Luehrsen, K. R., Chen, K. N., and Woese, C. R., The phylogeny of prokaryotes. Science *209* (1980) 457–463.

15 Huber, H., Thomm, M., König, H., and Stetter, K. O., *Methanococcus thermolithotrophicus*, a novel thermophilic methanogen. Archs Microbiol. *132* (1982) 47–50.

16 Hungate, R. E., A roll tube method for cultivation of strict anaerobes, in: Methods in Microbiology, vol. 3B. Eds J. R. Norris and D. W. Ribbons. Academic Press, New York 1969.

17 Huser, B. A., Wuhrmann, K., and Zehnder, A. J. B., *Methanothrix soehngenii* gen. nov. sp. nov., a new acetotrophic, non-hydrogen oxidizing methane bacterium. Archs Microbiol. *132* (1982) 1–9.

18 Jones, W. J., Leigh, J. A., Mayer, F., Woese, C. R., and Wolfe, R. S., *Methanococcus jannaschii* sp. nov., an extremely thermophilic methanogen from a submarine hydrothermal vent. Archs Microbiol. *136* (1983) 254–261.

19 Jones, W. J., Paynter, M. J. B., and Gupta, R., Characterization of *Methanococcus maripaludis* sp. nov., a new methanogen from salt marsh sediment. Archs Microbiol. *135* (1983) 91–97.

20 Kandler, O., Zellwandstrukturen bei Methanbakterien. Zur Evolution der Prokaryonten. Naturwissenschaften *66* (1979) 95–105.

21 König, H., and Stetter, K. O., Isolation and characterization of *Methanolobus tindarius* sp. nov., a coccoid methanogen growing only on methanol and methylamines. Zbl. Bakt. Hyg., I. Abt. Orig. C3 (1982) 478–490.

22 Kühn, W., Fiebig, K., Hippe, H., Mah, R. A., Huser, B. A., and Gottschalk, G., Distribution of cytochromes in methanogenic bacteria. FEMS Microbiol. Lett. *20* (1983) 407–410.

22a Lake, A.J., Henderson, E., Oakes, M., and Clark, M.W., Eocytes: A new ribosome structure indicates a kingdom with a close relationship to eukaryotes. Proc. natn. Acad. Sci. USA *81* (1984) 3786–3790.
23 Mah, R.A., Isolation and characterization of *Methanococcus mazei*. Curr. Microbiol. *3* (1980) 321–326.
24 Mah, R.A., and Kuhn, D.A., Transfer of the type species of the genus *Methanococcus* to the genus *Methanosarcina*, naming it *Methanosarcina mazei* (Barker 1936) comb. nov. et emend. and conservation of the genus *Methanococcus* (Approved lists 1980) with *Methanococcus vannielii* (Approved lists 1980) as type species. Int. J. syst. Bact. *34* (1984) 263–265.
25 Mah, R.A., and Kuhn, D.A., Rejection of the type species *Methanosarcina methanica* (Approved lists 1980), conservation of the genus *Methanosarcina* with *Methanosarcina barkeri* (Approved lists 1980) as type species and emendation of the genus *Methanosarcina*. Int. J. syst. Bact. *34* (1984) 266–267.
26 Mah, R.A., Ward, D.M., Baresi, L., and Glass, T.N., Biogenesis of methane. A. Rev. Microbiol. *31* (1977) 109–131.
27 Morii, H.N., Nishihara, N., and Koga, Y. Isolation, characterization and physiology of a new formate-assimiable methanogenic strain (A2) of *Methanobrevibacter arboriphilus*. Agric. biol. Chem., Tokyo *47* (1983) 2781–2790.
28 Patel, G.B., Roth, L.A., van den Berg, L., and Clark, D.S., Characterization of a strain of *Methanospirillum hungatii*. Can. J. Microbiol. *22* (1976) 1404–1410.
29 Prévot, A.R., Essai de classification des bactéries méthanogènes. C.r. Acad. Sci., Paris, ser. D *277* (1973) 897–900.
30 Prévot, A.R., Biosynthèse bactérienne du méthane et des pétroles. Pour l'an 2000, in: Le Méthane, pp. 9–47. Maloine S.A. (1977).
31 Prévot, A.R., Nouvel essai de classification des bactéries méthanogènes. C.r. Acad. Sci., Paris, ser. D *290* (1980) 1253–1255.
32 Rivard, Ch.J., Henson, J.M., Thomas, M.V., and Smith, P.H., Isolation and characterization of *Methanomicrobium paynteri* sp. nov. a mesophilic methanogen isolated from marine sediment. Appl. envir. Microbiol. *46* (1983) 484–490.
33 Rivard, C.J., and Smith, P.H., Isolation and characterization of a thermophilic marine methanogenic bacterium, *Methanogenium thermophilicum* sp. nov. Int. J. syst. Bact. *32* (1982) 430–436.
34 Rose, Ch.S., and Pirt, S.J., Conversion of glucose to fatty acids and methane: Roles of two mycoplasmal agents. J. Bact. *147* (1981) 248–254.
35 Scherer, P.H., and Kneifel, H., Distribution of polyamines in methanogenic bacteria. J. Bact. *154* (1983) 1315–1322.
36 Schleifer, K.H., and Stackebrandt, E., Molecular systematics of prokaryotes. A. Rev. Microbiol. *37* (1983) 147–187.
37 Sowers, K.R., Baron, St.F., and Ferry, J.G., *Methanosarcina acetivorans* sp. nov., an anaerobic methane-producing bacterium isolated from marine sediments. Appl. envir. Microbiol. *47* (1984) 971–978.
38 Sowers, K.R., and Ferry, J.G., Isolation and characterization of a methylotrophic marine methanogen, *Methanococcoides methylutens* gen. nov. sp. nov. Appl. envir. Microbiol. *45* (1983) 684–690.
39 Stetter, K.O., Thomm, M., Winter, J., Wildgruber, G., Huber, H., Zillig, W., Janécovic, D., König, H., Palm, P., and Wunderl, S., *Methanothermus fervidus*, sp. nov., a novel extremely thermophilic methanogen isolated from an Iceland hot spring. Zbl. Bakt. Hyg. I. Abt. Orig. C2 (1981) 166–178.
40 Touzel, J.P., and Albagnac, G., Isolation and characterization of *Methanococcus mazei* strain MC_3. FEMS Microbiol. Lett. *16* (1983) 241–245.
41 Wildgruber, G., Thomm, M., König, H., Ober, K., Ricchiuto, Th., and Stetter, K.O., *Methanoplanus limicola*, a plate shaped methanogen representing a novel family, the Methanoplanaceae. Archs Microbiol. *132* (1982) 31–36.
42 Woese, C.R., Archaebacteria and cellular origins: An overview. Zbl. Bakt. Hyg., I. Abt. Orig. C3 (1982) 1–17.
43 Zabel, H.P., König, H., and Winter, J., Isolation and characterization of a new coccoid methanogen, *Methanogenium tatii* spec. nov. from a solfataric field on Mount Tatio. Archs Microbiol. *137* (1984) 308–315.
44 Zeikus, J.G., Metabolism of one-carbon compounds by chemotrophic anaerobes. Adv. Microb. Physiol. *24* (1983) 215–299.
45 Zhilina, T.N., Biotypes of Methanosarcina. Microbiology (USSR) *45* (1976) 481–489.
46 Zhilina, T.N., Chudina, V.I., Ilarianov, S.A., and Bonch-Osmolovskaya, E.A., Thermophilic methane producing bacteria from *Methanobacillus kuzneceovii* methylotrophic associations. Microbiology (USSR) *52* (1983) 268–274.
47 Zhilina, T.N., and Zavarzin, G.A., Comparative cytology of Methanosarcinae and description of *Methanosarcina vacuolata* sp. nova. Microbiology (USSR) *48* (1979) 279–285.
48 Zinder, St.H., and Mah, R.A., Isolation and characterization of a thermophilic strain of *Methanosarcina* unable to use H_2–CO_2 for methanogenesis. Appl. envir. Microbiol. *38* (1979) 996–1008.

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Full Papers

New answers to malaria problems through vector control?

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Key words. Adulticidal pesticides; *Aedes aegypti; Anopheles;* biocontrol; *B.t.i.;* integrated control methodologies; larvivorous fish; methoprene; WHO.

Like so many other of science's latest concepts, integrated anopheline control methodologies were being advocated long before their present name was conceived. Thus almost a century ago, Mrs Carrie B. Aaron of Philadelphia provided a preview[1] of what such an approach to vector suppression would one day comprise. First prizewinner in an essay competition designed to promote mosquito and housefly control via the use of predaceous Odonata, she began by outlining the morphology, life histories and ecology of these pests and their suggested biocontrol agent. Then, proceeding to a consideration of the medical problems posed by Culicidae and Muscidae, she touched (nearly a decade before the *Plasmodium* cycle was elucidated) upon the possibility of the