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Isolation and Characterization of Methanogenic Bacteria from Landfills

ELIZABETH R. FIELDING,[1]† DAVID B. ARCHER,[1]* EVERLY CONWAY DE MACARIO,[2] AND ALBERTO J. L. MACARIO[2]

Institute of Food Research, Agricultural and Food Research Council, Colney Lane, Norwich NR4 7UA, United Kingdom,[1] and Wadsworth Center for Laboratories and Research, New York State Department of Health, and School of Public Health Sciences, State University of New York, Albany, New York 12201[2]

Received 22 October 1987/Accepted 12 December 1987

Methanogenic bacteria were isolated from landfill sites in the United Kingdom. Strains of *Methanobacterium formicicum*, *Methanosarcina barkeri*, several different immunotypes of *Methanobacterium bryantii*, and a coccoid methanogen distinct from the reference immunotypes were identified.

In both the United Kingdom and the United States, a high proportion of domestic refuse is disposed of in landfills (1, 11). The methane generated by anaerobic digestion of the refuse in United Kingdom landfills is a potential energy resource of approximately 35 GJ per year (11). To realize this potential, landfill management practices should be adapted to optimize conditions for methanogenesis. Hitherto, however, there has been very little information published on landfill microbiology apart from studies of public health aspects (2). Attempts have been made to enumerate the predominant species of methanogenic bacteria from samples taken from different depths within landfills (3, 5), and in this paper, we report the first isolation and characterization of methanogenic species from landfills.

Landfill samples were kindly supplied by the Landfill Research and Management Section, Harwell Laboratory, United Kingdom, and the sources are listed in Table 1. Samples were taken aerobically and packed tightly into screw-top jars. The numbers of viable methanogens was not increased by filling the jars under an atmosphere of nitrogen (unpublished results). The landfill samples were incubated at 37°C in a defined mineral salts medium (BM3) (6) containing cefoxitin (Merck Sharp & Dohme, United Kingdom) with either acetate (50 mM) or H_2-CO_2 (4:1, vol/vol) at 203 kPa as the substrate. Methane was assayed by gas chromatography (7). Methanogenic bacteria were isolated by subculturing in liquid BM3 and by picking colonies from plates of BM3 solidified with agar without cefoxitin. The purity of the isolates was assessed microscopically after growth of methanogens in a yeast extract-containing medium (Met3) lacking cefoxitin (4). The isolates were grown in Met3 for characterization, except for the *Methanosarcina* sp., which grew better in BM3 without cefoxitin. Microscopic examinations were performed with a Leitz Ortholux II microscope. Optimum temperatures for methane production were assessed by measuring the rates of methane production in triplicate over the temperature range of 11.7 to 48.4°C at intervals of approximately 1.5°C. The specific rate of methanogenesis of each isolate was assessed in Met3 medium buffered to pH 6, 6.5, 7, 7.5, or 8. The buffers used were sodium phosphate (50 mM) at all pH values, TES (*N*-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid) and HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (both 50 mM; Sigma Chemical Co.) at pH 7, 7.5, and 8; each buffer was used separately. Isolation of DNA and estimation of base ratios were carried out as previously described (6).

Antigenic fingerprinting was done as described (8) by indirect immunofluorescence and quantitative slide immunoenzymatic assay (9). The antibody probes used, taken from a numbered collection of 29 probes for reference methanogens, were as follows: 1, *Methanobrevibacter smithii* PS; 2, *Methanobacterium formicicum* MF; 3, *Methanosarcina barkeri* MS; 4, *Methanobacterium bryantii* MoH; 5, *M. bryantii* MoHG; 6, *M. barkeri* R1M3; 8, *Methanobrevibacter ruminantium* M1; 10, *M. smithii* AL1; 11, *Methanobacterium thermoautotrophicum* GC1; 12, *M. thermoautotrophicum* ΔH; 13, *Methanococcus vannielii* (DSM1224); 14, *Methanococcus voltae* PS; 15, *Methanogenium marisnigri JR1*; 16, *M. barkeri* 227; 17, *Methanogenium cariaci* JR1; 18, *Methanosarcina mazei* S-6; 19, *M. barkeri* W; 20, *Methanosarcina thermophila* TM1; 24, *Methanothermus fervidus* V24S; 26, *Methanococcus maripaludis* JJ; and 29, *Methanococcus thermolithotrophicus* SN1. Antigenic relatedness among the isolates and between the isolates and reference methanogens was determined by comparative analysis of antigenic fingerprints with a reference table and by procedures previously described (9).

Physiological properties of the seven isolates obtained are given in Table 1. The specific rate of methanogenesis of each isolate was highest at pH 7. Antigenic fingerprinting data were obtained at positions of interest for each isolate, considering its morphological and physiological properties. A comparative analysis of the fingerprints of the isolates with those of the reference methanogens showed that some isolates were immunologically close to a reference organism, whereas others were distant. Illustrative data obtained by indirect immunofluorescence and quantitative slide immunoenzymatic assay are shown in Table 2.

Isolates EF1 and EF5 were closely related in their physiological and antigenic properties to the reference methanogens *M. formicicum* MF and *M. barkeri* MS, respectively. The coccoid isolate EF2, on the other hand, was unrelated to any of the reference methanogens. The G+C content of DNA extracted from EF2 was 52 mol%, outside the range of

* Corresponding author.
† Present address: Sittingbourne Research Centre, Sittingbourne, Kent, ME9 8AG, United Kingdom.

TABLE 1. Characterization of methanogenic isolates

Isolate	Source	Depth of sample (m)	Morphology (diam [μm])	Substrate(s) for methanogenesis	Optimum temp (°C)
EF1	Aveley, Essex	4	Bent rod (0.5)	H_2–CO_2, formate	40.4
EF2	Aveley, Essex	4	Irregular coccus (1–2)	H_2–CO_2, formate	40.4
EF3	Stangate, Kent	7	Bent rod (0.8–1.0)	H_2–CO_2	37.7
EF4	RF3	0[a]	Bent rod (0.8–1.0)	H_2–CO_2	37.7
EF5	Aveley, Essex	4	Sarcina	H_2–CO_2, acetate, methanol, trimethylamine	37.7
EF6	Enderby, Leicestershire	7.3	Straight rod (0.5)	H_2–CO_2	40.4
EF7	Blue Circle, Kent	3	Straight rod (0.5)	H_2–CO_2, formate	37.7

[a] Fraction from manually sorted refuse.

values reported for *Methanococcus* spp. (10). EF2 did not require the addition of sodium chloride to Met3 for optimal growth. The specific growth rate of EF2 was reduced at salt concentrations greater than 1% (wt/vol), and growth did not occur at salt concentrations of 3% (wt/vol) and above. Acetate stimulated, but was not required for, growth in Met3 medium. EF2 lysed in the presence of 1% (wt/vol) sodium dodecyl sulfate and stained gram negative. Although the motility of EF2 was not demonstrated, electron micrographs revealed the presence of cells with flagella. Further characterization of EF2 is required before it can be assigned to a genus, but it is probably a *Methanogenium* sp. (10) or a *Methanocorpusculum* sp. (12).

Isolates EF3, EF4, EF6, and EF7 showed various degrees of antigenic relatedness to the reference methanogens *M. bryantii* MoH and *M. bryantii* MoHG. They were, however, different from these two reference strains and from each other. Isolate EF7 was a thin rod capable of utilizing H_2-CO_2 and formate as *M. formicicum* was but was antigenically related to *M. bryantii*. The data presented in this study show that a diversity exists within the genus *Methanobacterium* and that landfills harbor a variety of methanogens.

TABLE 2. Antigenic fingerprints of methanogens isolated from landfills

Reference methanogen and no.	Reference methanogen or isolate	Reaction with S probe no.[a]								
		1	2	3	4	5...	10...	12...	24...	29
M. formicicum MF, 2	MF		4						2	
	EF1		3						3	
M. barkeri MS, 3	MS			4						
	EF5			4						
M. bryantii MoH, 4	MoH	1			4	3	1			
M. bryantii MoHG, 5	MoHG	1			3	4	1			
	EF3				2	3				
	EF4				1	2				
	EF6					1		1		
	EF7				1	1				

[a] Each probe defines a position of the antigenic fingerprint (8, 9). The position and probe numbers are the same as the numbers assigned to the reference methanogens used to generate the probes (see text for a list of these reference organisms). Positions determined for the fingerprint of each isolate were as follows: EF1, EF3, EF6, and EF7, positions 2, 4, 5, 11, 12, and 24; EF5, positions 3, 6, 16, 18, 19, and 20; and EF2, positions 1, 3, 6, 8, 10, 13–20, 26, and 29. Only values ≥1 (positive reactions) are shown for clarity. None of the antigenic fingerprints of the reference methanogens was similar to that of EF2.

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