

Pathways for Methanogenesis and Diversity of Methanogenic Archaea in Three Boreal Peatland Ecosystems

P. E. Galand,^{1*} H. Fritze,² R. Conrad,³ and K. Yrjälä¹

Department of Biological and Environmental Sciences, General Microbiology, University of Helsinki, Helsinki,¹ and Finnish Forest Research Institute, Vantaa Research Centre, Vantaa,² Finland, and Max-Planck-Institut für Terrestrische Mikrobiologie, Marburg, Germany³

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The main objectives of this study were to uncover the pathways used for methanogenesis in three different boreal peatland ecosystems and to describe the methanogenic populations involved. The mesotrophic fen had the lowest proportion of CH₄ produced from H₂-CO₂. The oligotrophic fen was the most hydrogenotrophic, followed by the ombrotrophic bog. Each site was characterized by a specific group of methanogenic sequences belonging to *Methanosaeta* spp. (mesotrophic fen), rice cluster-I (oligotrophic fen), and fen cluster (ombrotrophic bog).

Northern peatlands are important emitters of the green house gas methane (CH₄) produced by methanogenic archaea (3, 18, 30). Methanogens utilize a limited number of substrates, the most important of which are acetate and H₂-CO₂ (38). In peatlands, H₂-CO₂-dependent methanogenesis is thought to be the main pathway for CH₄ production (20, 21, 26, 37), but in some minerotrophic peatlands (fens), acetoclastic methanogenesis is often predominant in upper peat layers (4, 23, 31). The diversity of methanogenic communities of fen (15, 17) and bog (2, 16, 35) ecosystems has recently been described, but data on the combined investigation of methanogenic pathways and methanogen populations are scarce. To the best of our knowledge, community studies have never been associated with the detection of methanogenic pathways in fen ecosystems, and only one study of an acidic bog ecosystem has been published (20). The aim of our study was to determine the precursors used for methanogenesis in three peatland ecosystems (a mesotrophic fen, an oligotrophic fen, and an ombrotrophic bog) and to describe the diversity of methane-producing archaea by using molecular methods that target the functional methyl coenzyme M reductase gene (*mcrA*).

Replicate samples from depths with the highest potential CH₄ production rates were taken from a mesotrophic fen, an oligotrophic fen, and an ombrotrophic bog at the Lakkasuo mire complex in central Finland (61°48'N, 24°19'E) in August 2003. The fraction of CH₄ produced from H₂-CO₂ was estimated at each site by a tracer experiment (8, 11) and by the inhibition of acetoclastic methanogenesis with CH₃F (methyl fluoride) (6, 14, 22). The Gibbs free energy (ΔG) of H₂-dependent methanogenesis was calculated (10, 12), and potential CH₄ production was measured as described earlier (17). Fatty acids and alcohols dissolved in the pore water were analyzed by high-pressure liquid chromatography (25). All experiments were conducted on triplicate samples from each peat ecosys-

tem. DNA was extracted directly from peat samples (17), and a portion of the methanogen-specific *mcrA* was amplified with the primer pair ML (28). Clone libraries were subjected to restriction fragment length polymorphism (RFLP) analysis with the restriction enzymes MspI and TaqI, and representatives of the biggest RFLP groups were sequenced for phylogenetic analysis.

The contributions of H₂-CO₂-dependent methanogenesis to total CH₄ production varied clearly among the three peatland ecosystems (Fig. 1). The mesotrophic fen had the lowest proportion of CH₄ formed from H₂-CO₂. Since H₂-CO₂ and acetate are the main precursors for methanogenesis in wetlands (7, 38), the fraction of CH₄ which is not produced from H₂-CO₂ predominantly results from acetoclastic methanogenesis. Acetotrophy has been described previously as an important pathway for CH₄ formation in nutrient-rich fens (23) and in fens covered with *Carex* sedges (4, 31). Vascular plants, primarily sedges such as *Carex* spp. and *Eriophorum* spp., were the dominant field layer vegetation at the Lakkasuo mesotrophic fen site. Vascular plant species, as an adaptation to wetland conditions, develop a cortical oxygen-transporting gas space (aerenchyma), which transports oxygen (O₂) to the roots situated in O₂-depleted layers of soil (1, 24). The root systems of vascular plants tend to be less shallow than those of non-aerenchymatous peatland plants. Thus, the roots of vascular plants penetrate into anoxic layers and allow the entrance of potential carbon substrates, like acetate, into the deeper peat layers (29). The presence of vascular plants in peatland ecosystems has been suggested to favor acetoclastic methanogenesis by providing acetate to the anaerobic peat layers. Strom et al. (34), for example, pointed out that acetate is the main labile carbon exudation from the roots of the sedge *Eriophorum scheuchzeri* in an arctic wetland; consequently, acetoclastic methanogenesis is the dominant reaction in the vicinity of that sedge. Interestingly, our phylogenetic analysis showed that the *mcrA* clone libraries of the mesotrophic fen were dominated by RFLP group 2 (Fig. 2), which had the closest similarity to obligate acetotrophic methanogens of the family *Methanosaetaceae*, order *Methanosarcinales* (Fig. 3). Sequences from

* Corresponding author. Present address: Département de Biologie, Université Laval, Québec, Québec G1K 7P4, Canada. Phone: (418) 656-2131, ext. 12004. Fax: (418) 656-2043. E-mail: pierre.galand@bio.ulaval.ca.

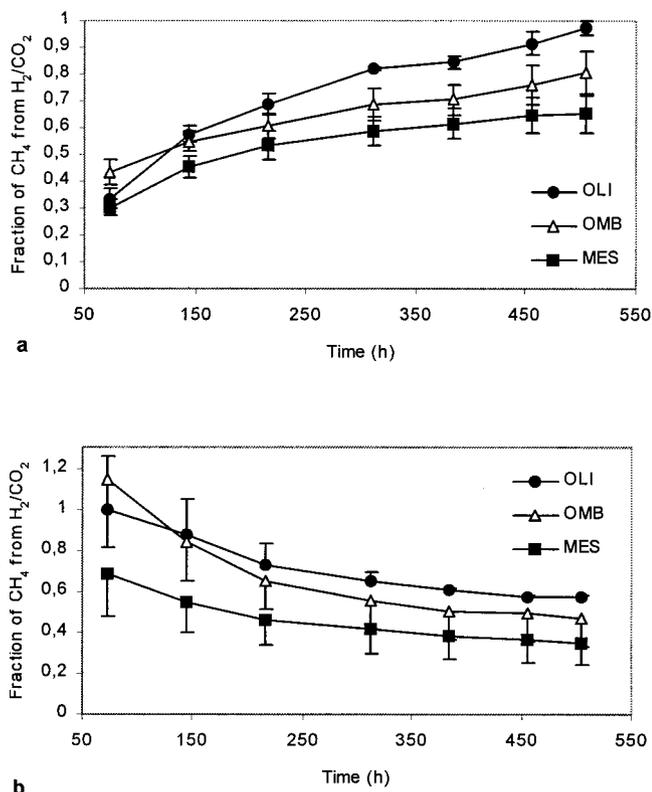


FIG. 1. Fraction of CH₄ produced from H₂-CO₂ in mesotrophic (MES), oligotrophic (OLI), and ombrotrophic (OMB) peatlands as determined by tracer (a) and inhibition (b) experiment. Data are means of results of triplicate experiments \pm standard errors.

the family *Methanosarcinaceae* were not detected in the mesotrophic fen; rather, the acetotrophic methanogen sequences belonged exclusively to *Methanosaeta* spp. The low concentration of acetate in the mesotrophic peat (≤ 0.65 mM) probably

favors *Methanosaeta* spp., which have a lower threshold for acetate than other acetotrophs belonging to the family *Methanosarcinaceae*. In ecosystems where acetate concentrations are high, *Methanosaeta* spp. are outcompeted by *Methanosarcina* spp. (5, 13, 36). The mesotrophic site had, by far, the highest rates of CH₄ production (Table 1). Nutrient-rich peat at the site promotes vegetation growth; consequently, decomposition of organic matter and exudates from plant roots increase the quantity of carbon substrates available for methanogenesis.

In contrast, the oligotrophic fen had low potential CH₄ production (Table 1), and a high fraction of CH₄ was produced from H₂-CO₂ (Fig. 1). The site is nutrient poor, the concentration of fatty acids in peat pore water is low (<0.07 [± 0.02] mM), and few vascular plants are present. Little labile carbon from plant exudates is introduced to the anaerobic layers of the peat, and recalcitrant old peat is the main carbon source for methanogenesis. In natural environments, most of the H₂ used by hydrogenotrophs for CO₂ reduction is obtained directly from syntrophic bacteria by interspecies transfer (8, 9, 19). This process results in a low partial pressure of H₂, which is necessary for the metabolism of syntrophs (32). The partial pressure of H₂ in the gas phase of the incubated oligotrophic peat samples was low (result not shown). This result provides additional evidence of the predominance of H₂-CO₂-dependent methanogenesis at the site. RFLP groups 3, 4, and 6 were found exclusively in the oligotrophic clone libraries (Fig. 2) and the corresponding sequences grouped with rice cluster-I (Fig. 3). Members of rice cluster-I were selectively enriched earlier with H₂-CO₂ as the energy source (27, 33), indicating that the group includes hydrogenotrophs. One of the sequences (Lak1) dominating the oligotrophic clone libraries belonged, however, to fen cluster-III; the function of that subcluster remains unknown (17).

The ombrotrophic bog also had a high proportion of CH₄ production that originated from H₂-CO₂ (Fig. 1). This result is

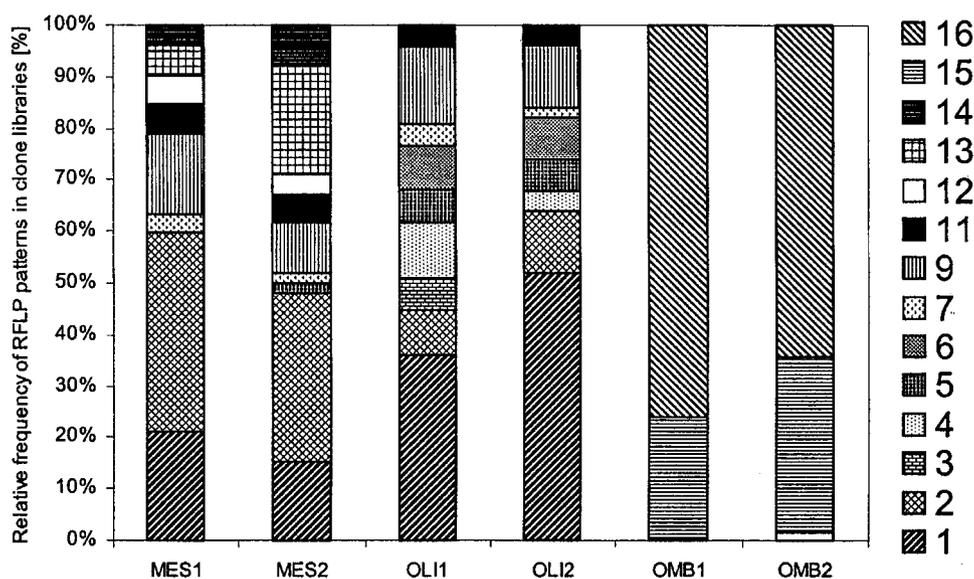


FIG. 2. Methanogen community structure in upper layers of mesotrophic (MES), oligotrophic (OLI), and ombrotrophic (OMB) peatland soils. Communities are represented by the relative abundances of RFLP patterns in *mcrA* clone libraries. Two replicate peat samples (1 and 2) were analyzed at each site.

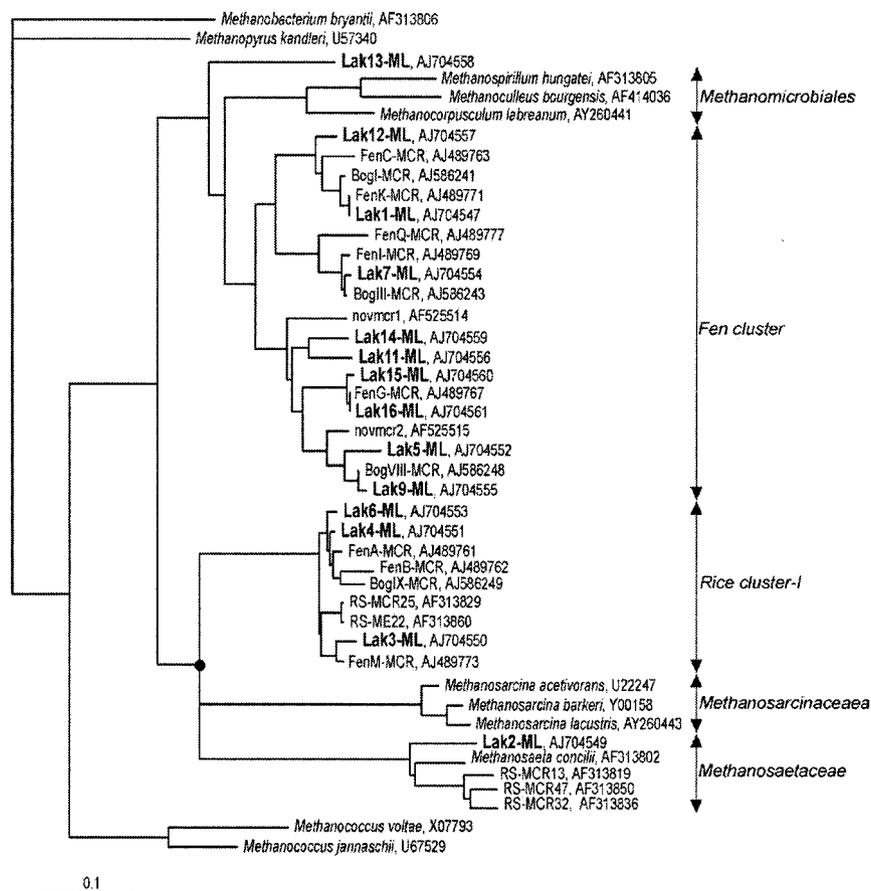


FIG. 3. Phylogenetic dendrogram representing 137-amino-acid-long *mcrA* sequences retrieved from three different boreal peatlands (boldface) in relation to other sequences of methanogenic archaea. The number in the sequence name corresponds to the RFLP pattern. The tree was constructed using FITCH distance matrix analysis. The full circle indicates a manually adjusted consensus trifurcation. GenBank accession numbers are indicated for all sequences. *Methanopyrus kandleri* was used as outgroup. The scale bar represents 10% sequence divergence.

consistent with earlier findings, which showed hydrogenotrophy to be the dominant reaction in bogs (4, 20, 26). The characteristic vegetation cover of the ombrotrophic bog can explain the small proportion of acetotrophic methanogenesis at the site. The plant species covering the Lakkasuo bog are almost exclusively *Sphagnum* mosses. *Sphagnum*-dominated bogs have been identified previously as peatlands where CO₂ reduction is an important pathway (23). Mosses have a shallow rhizoid system and lack aerenchyma; as a result, they do not introduce any labile carbon substrates to anaerobic peat layers. The methanogenic community at the bog site differed significantly from the communities characteristic of fen ecosystems. The methanogen diversity at the bog site was extremely low;

only two RFLP groups dominated all libraries (RFLP groups 15 and 16) (Fig. 2), and their corresponding sequences belonged to a cluster named fen cluster-IV (17) (Fig. 3). Previous studies of bog methanogen communities have revealed higher methanogen diversity and have detected sequences belonging to the orders *Methanomicrobiales*, *Methanobacteriales*, and *Methanosarcinales* (2, 20, 35). The low diversity observed at the Lakkasuo bog may be induced by the specific characteristics of the peat at the site. The peat pH, for example, was much lower at Lakkasuo (pH <4) (Table 1) than at other bogs studied (2, 20), which may be a factor in the selection of populations that are dominated by acid-tolerant methanogens. Further experiments should be conducted with ombrotrophic bogs in order to

TABLE 1. Water table depths, peat pHs, average potential CH₄ production, and ΔG values for H₂-dependent methanogenesis calculated for oligotrophic, mesotrophic, and ombrotrophic peatland sites

Peatland type	Water table depth (cm)	Pore water pH (SE)	Potential CH ₄ production ^a (nmol g [dry wt] ⁻¹ h ⁻¹) (SE)	ΔG (kJ mol ⁻¹) (SE) (nmol g [dry wt] ⁻¹ h ⁻¹)
Mesotrophic	0	5.27 (0.13)	209.73 (76.65)	-26.7 (1.9)
Oligotrophic	33	5.16 (0.05)	14.88 (3.63)	-15.7 (1)
Ombrotrophic	25	3.89 (0.19)	39.93 (13.37)	-17.2 (1.4)

^a Three parallel peat profiles were taken at each site; CH₄ production was calculated from control samples from inhibition experiment and from tracer experiment samples incubated at 10°C.

confirm the observed low diversity and to define possible selection factors.

Nucleotide sequence accession numbers. The *mcrA* sequences obtained in this study were deposited in the EMBL database under accession no. AJ704547 to AJ704561.

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