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Evidence for the Existence of Psychrophilic Methanogenic Communities in Anoxic Sediments of Deep Lakes

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In order to obtain evidence for the existence of psychrophilic methanogenic communities in sediments of deep lakes that are low-temperature environments (4 to 5°C), slurries were first incubated at temperatures between 4 and 60°C for several weeks, at which time they were amended, or not, with an additional substrate, such as cellulose, butyrate, propionate, acetate, or hydrogen, and further incubated at 6°C. Initial methane production rates were highest in slurries preincubated at temperatures between 4 and 15°C, with maximal rates in slurries kept at 6°C. Hydrogen-amended cultures were the only exceptions, with the highest methane production rates at 6°C after preincubation at 30°C.

Sediments of deep lakes where carbon dioxide is the major electron acceptor for biological oxidation of organic matter leading to methane production (3) are, with the exception of the littoral portion, constantly exposed to low temperatures (28). Methanogenesis has been repeatedly shown to occur in low-temperature lake sediments (2, 3, 7, 8, 17, 20, 30, 31), and the activities of these methanogenic microbial communities are apparently dependent on the availability of organic matter (8, 22).

In studies in which sediments have been incubated at different temperatures, the highest methane production rates have always occurred in the mesophilic range (3, 31), leading to the assumption that methanogenesis is carried out by mesophilic communities still active in the low in situ temperature range of 4 to 6°C. Evidence of specific psychrophilic methanogenic communities in acid peat and soil of a river marshland has been obtained, with maximal methane production at 20 and 10°C, respectively (27, 29). In recent years, a number of anaerobic bacteria and methanogenic archaea have been isolated from cold environments; these organisms have optimal growth temperatures of 15 to 25°C and are unable to grow at temperatures above 30 to 35°C (5, 12, 18, 21, 25, 26).

In order to obtain evidence of the existence of specific psychrophilic methanogenic communities in sediments of deep lakes, various sediment incubation experiments were performed. Sediment samples were collected from Lake Baldegg and Lake Soppen, two central Switzerland lakes with histories of anoxic hypolimnia and sediments (16, 19), at maximum depth (66 and 27 m, respectively) with a gravity corer (9). Inside a 6°C cold room, in a glovebox under a nitrogen atmosphere, the upper 50 cm of each sediment core was diluted twofold with anaerobic mineral medium (in milligrams per liter: NH_4Cl, 330; $MgCl_2 \cdot 6H_2O$, 500; $CaCl_2$, 168; KCl, 330; KH_2PO_4, 330; $NaHCO_3$, 500; Na_2S, 500). The final pH was 6.8, the absolute dry mass was between 161.1 and 179.5 mg/ml, and the organic carbon content was between 18.3 and 20.2 mg/g of absolute dry mass (determined as described in reference 6).

Aliquots of 25 ml of sediment slurry were transferred into 50-ml serum bottles. The gas phase was N_2. Where indicated, substrates were added from sterile stock solutions (microcrystalline cellulose, 5 g/liter; acetate, propionate, butyrate, and methanol, 10 mM) or by replacing the gas phase with H_2-CO_2 (4:1, vol/vol; 1.5×10^5 Pa). The sediment samples were incubated at temperatures ranging from 2 to 70°C. All experiments were done in triplicate. Methane production was measured every 2 to 5 days during the first month of incubation and once or twice per month in long-term experiments. Methane was measured with a gas chromatograph (model HRGC 51 60; Carlo Erba, Milan, Italy) equipped with a flame ionization detector (FID-40; Carlo Erba) and a megabore GS-Q column (30 m length). Volatile fatty acids were measured by ion-exchange chromatography on a low-capacity column, using a step gradient procedure (1).

Methane was produced from indigenous substrates in slurries of sediment from Lake Baldegg at all temperatures tested. After 3 days of incubation, methanogenesis was highest at 30°C, but after 7 additional days, another peak was also observed at around 50°C (Fig. 1). After 6 weeks, only one peak, at 50°C, was present (data not shown). Addition of cellulose, butyrate, propionate, or a direct methane precursor such as acetate, methanol, or H_2-CO_2 to sediment slurries from Lake Baldegg and Lake Soppen resulted in increased methane production at all temperatures tested. With cellulose, the peak in methane production in the thermophilic temperature range shifted to 63°C (Fig. 1). A significant accumulation of acetate and other volatile fatty acids was found only at 70°C, a temperature at which methane production was very low. At 30 to 60°C, small amounts of volatile fatty acids (no more than 2 to 3 mM) were detected during the first 2 to 5 days of incubation.

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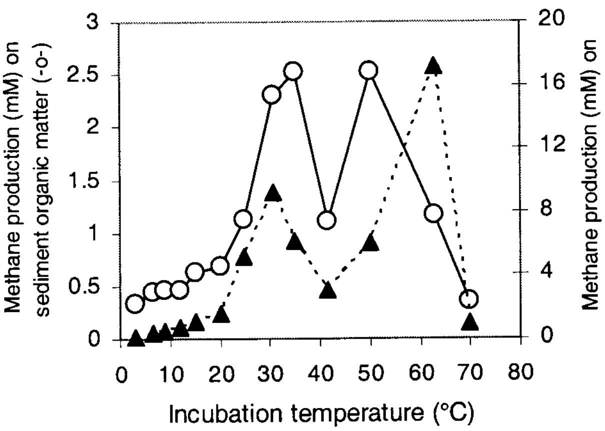

FIG. 1. Methane production in sediment slurries from Lake Baldegg at 3 to 70°C after 10 days of incubation. ○, sediment organic matter; ▲, cellulose-amended slurry.

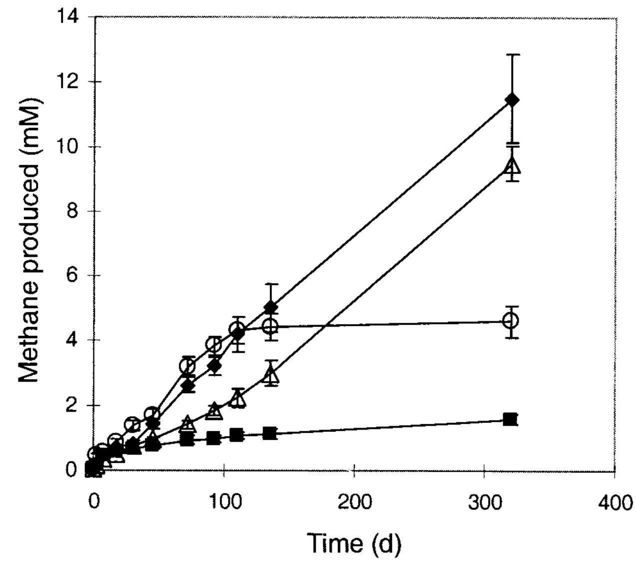

FIG. 2. Methane production in sediment slurries from Lake Baldegg at 2°C without substrate amendment (■) and after amendment with acetate (△), H_2-CO_2 (○), or cellulose (◆).

These results confirmed that methanogenesis in lake sediments exhibited a temperature optimum that was much higher than the in situ temperatures, as has been shown with other cold profundal lake sediments (24, 31). Although the temperature of the sediments of Lake Baldegg and Lake Soppen is about 5°C year round, increasing incubation temperatures triggered a sharp increase of methane production from both indigenous sources and cellulose, with maxima at around 35 and 50°C, suggesting the presence of mesophilic and thermophilic methanogenic populations. This is in accordance with data obtained by Zeikus and Winfrey (31) but in contrast to the study of Schulz et al. (24), in which no methane production was found for sediments from Lake Constance at 50°C. Whether thermophilic bacteria are active, although at very low rates, at in situ temperatures is not yet clear.

Unlike the studies involving soil of a river marshland (29) or acid peat (27), in which peaks in methane production have been found at 10 and 20°C, no clear peaks were found with profundal lake sediments at low temperatures. A closer look at the temperature range of 2 to 20°C showed, however, that the methane production curve had slight shoulders at 6 and 15°C, a possible result of activity peaks of psychrophilic communities overlapping with those of mesophilic communities. A similar activity shoulder was found at 25°C for sediments of Lake Constance (24), suggesting the presence of psychrophilic, or at least psychrotrophic, communities in these environmental niches.

With Lake Baldegg sediment slurries, quite constant rates of methane production from indigenous substrates, of 35.1 and 73.4 μmol kg of dry sediment^{-1} day^{-1}, were found at 2 and 6°C, respectively, over a period of at least 18 months. Similar rates were found with sediment from Lake Soppen. Addition of cellulose or a direct methane precursor such as acetate or H_2-CO_2 enhanced methane production in slurries incubated at 2°C (Fig. 2). When H_2-CO_2 was used as a substrate, mainly acetate was produced.

To obtain more evidence for the existence of specific psychrophilic methanogenic communities, sediment slurries preincubated at up to 12 different temperatures ranging between 4 and 60°C for 4 or 6 weeks were amended with additional substrate, or not, and then transferred to 6°C. During the first month of incubation, methane production rates were highest in cellulose-amended slurries that had been preincubated at low temperatures of between 4 and 15°C, with maximal rates in slurries kept at 6°C (Fig. 3). With prolonged incubation, methane production at 6°C resumed in some of the slurries preincubated at temperatures above 15°C, but the time needed to regain the ability to produce methane at low temperatures increased with increasing temperature of preincubation. After 8 months of incubation, slurries preincubated at 35 or 43°C produced only 35 and 10%, respectively, of the methane found in slurries preincubated at 6°C. Just traces of methane were produced in slurries preincubated at 50°C, and no methane at all was produced in slurries preincubated at 60°C after 1 year

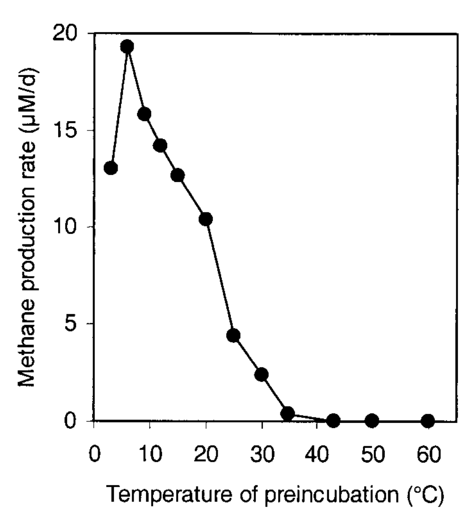

FIG. 3. Rates of methane production from cellulose at 6°C during the first 3 weeks of incubation of sediment slurries from Lake Baldegg that were preincubated for 1 month at different temperatures.

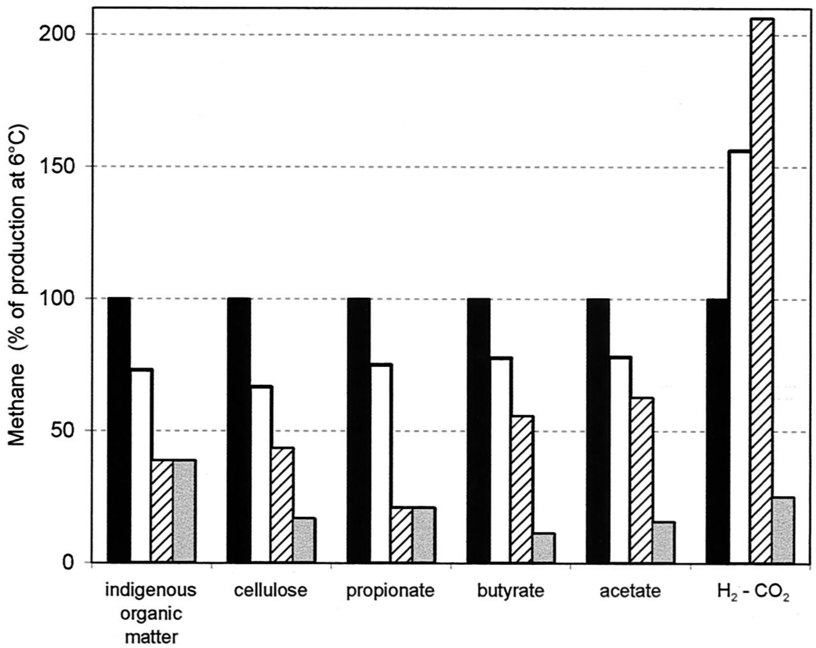

FIG. 4. Methane production, normalized to the production at 6°C, in Lake Soppen sediment slurries preincubated at 6°C (black bars), 15°C (white bars), 30°C (hatched bars), or 50°C (grey bars), then amended with different substrates and incubated at 6°C for 11 days. A methane production value of 100% corresponds to 0.13 mM methane produced on indigenous organic matter, 0.3 mM on cellulose, 0.24 mM on propionate, 0.45 mM on butyrate, 0.32 mM on acetate, and 0.8 mM on H_2-CO_2.

of incubation at 6°C. These results provide strong evidence that specific psychrophilic methanogenic communities are present in profundal lake sediments.

The same pattern of methane production at 6°C was observed in slurries that were amended with butyrate, propionate, or acetate after preincubation (Fig. 4). The highest methane production rates were found in slurries preincubated at 6°C, gradually decreasing rates were evident after preincubation at 15 or 30°C, and no methane production occurred after preincubation at 50°C.

This indicated that different physiological groups involved in methanogenic degradation of organic matter had psychrophilic representatives. Psychrophilic fermentative bacteria have been isolated (4, 11, 18), but no syntrophic fatty acid- or alcohol-oxidizing bacteria or acetoclastic methanogens have been isolated so far. It had been proposed earlier that the degradation of the carbohydrate monomers of polymers such as cellulose mainly occurs through fermentation via homoacetogenesis at low temperatures, thus eliminating the need for low-temperature-adapted syntrophic bacteria (23, 24). It is not known whether this process occurred in the lake sediments studied here, but the results obtained with Lake Soppen sediment amended with syntrophically degraded substrates such as propionate and butyrate indicated that psychrophilic syntrophs also exist. Rapid butyrate degradation has also been observed in anaerobic bioreactors operated at low temperatures (15). In Lake Constance sediments, on the other hand, syntrophic bacteria seemed to become active only at temperatures above 20°C (24) and degradation of artificial algal deposition in sediment cores incubated at 4°C caused an accumulation of these syntrophically degraded substrates (22).

Acetate has been proposed as the predominant methane precursor in lake sediments under low-temperature conditions (20, 24, 32), and a detailed analysis of the archaeal population of a low-temperature lake sediment showed the predominant presence of methanogens hybridizing with a molecular probe targeting members of the genus *Methanosaeta* and having also the corresponding morphology (32). The fact that the highest rates of methane production from acetate were observed at 6°C after preincubation of the microbial population at 6°C indicated that the acetoclastic methanogens of Lake Soppen sediment producing methane at low temperatures were psychrophilic organisms.

Only slurries amended with H_2-CO_2 showed a different pattern (Fig. 4). More methane was formed in slurries preincubated at 15 or 30°C than in slurries preincubated at 6°C. This indicates that methane formation from H_2-CO_2 in this sediment at low temperatures was catalyzed by mesophilic or psychrotolerant hydrogenophilic methanogens and not psychrophilic ones. The isolation of the psychrotolerant methanogen *Methanosarcina lacustris,* which is able to utilize H_2-CO_2 for methane production, from sediments of Lake Soppen (26) corroborates this deduction. This methanogen grew at temperatures ranging from 1 to 35°C, with the highest rate of growth occurring at 20 to 30°C.

However, methane production from H_2-CO_2 at low temperatures has been proposed to occur as a two-step process: first acetate is produced, and then acetoclastic methane formation occurs (10, 13, 20). There are no thermodynamic or kinetic arguments for such a process. Recent investigations have shown that a predominantly homoacetogenic hydrogen consumption in a balanced anaerobic community under substrate-limiting conditions and at a low temperature can be explained by the metabolic versatility of homoacetogens and the better ability of these bacteria to adapt to low-temperature conditions (14). Two strains of the psychrophilic acetogen *Acetobacterium paludosum* have actually been isolated from Lakes Baldegg and Soppen (21).

Measurements of production of methane from ^{14}C-labeled substrates indicated that hydrogenophilic methanogenesis occurs at low temperatures under certain conditions. In the organic-material-enriched upper sediment layers, ^{14}C-labeled methane was formed from [^{14}C]bicarbonate (32), in agreement with other work indicating that this group of methanogens is mainly substrate limited (23, 24).

In conclusion, the results presented here provide strong evidence that low-temperature-adapted psychrophilic methanogenic communities exist in profundal lake sediments, although classic incubation experiments with samples obtained from such constant-low-temperature environments always reveal that the highest levels of methane production occur in the mesophilic or even thermophilic temperature range.

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