Effect of temperature stress on structure and function of the methanogenic archaeal community in a rice field soil

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Abstract

The effect of a brief (24 h) temperature shift to 4°C (low-temperature stress) or 50°C (high-temperature stress) was studied in methanogenic slurries of Italian rice field soil incubated at either constant 30°C or 15°C. Low-temperature (low-T) stress showed no effect in either the 30°C or the 15°C incubations. High-temperature (high-T) stress, on the other hand, generally resulted in an increase in the partial pressure of H₂, and the concentrations of acetate and propionate, which accumulated to about 100–200 Pa, 4–6 mM and 0.7–0.9 mM, respectively. The increase in H₂ was transient for 8–15 days. The increases in acetate and propionate were transient for about 20 days only in the 30°C incubations, but persisted in the 15°C incubations until the end of the experiment. The high-T stress did not result in inhibition of CH₄ production in the 30°C incubations, but transiently inhibited the 15°C incubations for about 25–30 days. The archaeal community in the soils was analyzed by terminal restriction fragment length polymorphism of the gene of the SSU rRNA. In the 15°C incubations, the relative gene frequency of members of the Methanosarcinaceae decreased over an incubation period of 54 days, while those of Methanosaetaceae and of methanogenic rice cluster I increased. Temperature stress, high-T stress in particular, tended to reverse this trend. In the 30°C incubations, on the other hand, the relative gene frequency of archaeal members showed the opposite temporal trend or remained constant unlike the 15°C incubations. Again, high-T stress tended to reverse these trends, but the observed effects were much smaller in the 30°C incubations than in the 15°C incubations. In conclusion, a brief high-T stress affected structure and function of the methanogenic archaeal community of rice field soil. © 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Stress is a common factor in nature so that individual microorganisms, Bacteria and Archaea, have developed genes and proteins to cope with stress [1,2]. Heat shock and cold shock regulation systems are found in soil microorganisms, e.g. Bacillus subtilis [2,3]. However, stress adaptation also seems to occur at the community level. Thus, the intermediate disturbance hypothesis predicts maximum diversity at intermediate frequencies of disturbance [4]. This hypothesis has been supported by microbial culture experiments using phytoplankton [5,6] and Pseudomonas fluorescens [7]. More complex microbial systems have received less attention. For example, different soil processes were studied following an experimental change of the biodiversity of a soil microbial community demonstrating that biodiversity can influence ecosystem performance [8,9]. Similarly, a methanogenic bioreactor was shown to exhibit a tremendous variation in the composition of its microbial community, which at least partially also influenced the stability of performance [10–12]. Methanogenic rice field soils exhibited changes in methanogenic pathway and archaeal community structure when the incubation temperature of the soil was changed [13,14]. Methanogenic rice soil adapted to a range of temperatures between 10°C and 37°C also exhibited different methanogenic pathways and archaeal community structures [15] and even resulted in the development of a thermophilic methanogenic community upon incubation at temperatures between 37°C and 50°C [16]. A cellulose-fermenting methanogenic microbial culture originating from rice field soil showed different patterns in function and composition of the methanogenic community upon changes to higher
or lower temperature [17]. This microbial community also exhibited pronounced functional and structural responses following short aeration stress [18].

However, experimental evaluation of the response of the methanogenic community in rice field soil following short-term temperature stress is still missing. Short-term heating has been shown to affect the microbial phospholipid fatty acid patterns in forest humus which may experience occasional fires [19]. In flooded rice fields, it is rather unlikely that a rapid and short-term temperature change takes place. Hence, the functional microbial community may be expected to react sensitively to such a stress. It is interesting to see whether such a stress would affect only the function or also the structure of the community.

Therefore, we have studied the functional and structural response of a rice field soil methanogenic community using brief (for 24 h) heating or cooling as temperature stress. We chose 4°C for low-temperature stress and 50°C for high-temperature stress, since these are temperatures at which in principal methanogenesis in rice field soil can still operate [15,16]. We studied the rice field soil methanogenic community at two incubation temperatures (15°C and 30°C), which had been found previously to select for different methanogenic community structures [14]. We used a model system which mimics the normal situation in situ when the drained, ploughed and harrowed rice fields are flooded in spring, i.e. we prepared anoxic soil slurries from dry Italian rice field soil.

2. Materials and methods

2.1. Incubation of soil slurries

The soil samples were collected from the ploughed but yet unflooded rice field of the Italian Rice Research Institute in Vercelli, Italy in March 1998. Then the soil was air-dried and stored as dry lumps at room temperature and characterized as a sandy-loamy silt [15]. The dry lumps were broken and passed through a stainless steel sieve (1 mm pore size). For preparation of slurries, 5 g (dry weight) soil was suspended in 5 ml distilled anoxic sterile water in 25-ml serum bottle, after which the bottles were flushed with N₂ for 1.5 min.

Soil slurries were pre-incubated for 24 h at 30°C. Then parts of the soil slurries were moved and incubated at 15°C. After incubation for 25 days at both 30 and 15°C, temperature stress was performed: one third of soil slurries at both 30 and 15°C were moved to 50°C, another one third of the soil slurries being moved to 4°C and the remaining one third of the soil slurries was kept at the original 30 and 15°C. After incubation at 4 and 50°C for 24 h, the soil slurries were returned to their previous temperatures, 30 and 15°C respectively. Soil slurries were incubated in sets of many replicates, one for each sampling time point. The soil slurries were classified into six groups, i.e. 15°C and 30°C controls; slurries previously incubated at both 15 and 30°C receiving high-temperature (50°C, high-T) stress; and slurries previously incubated at both 15 and 30°C receiving low-temperature (4°C, low-T) stress.

2.2. Analytical techniques

Gas samples (0.1 ml from three parallel soil slurries of each group) were taken with gas-tight syringes and analyzed for CH₄ and H₂ using a gas chromatograph equipped with a flame ionization detector (GC-8A, Shimadzu, Kyoto, Japan) and a reduced gas detector (RGD2) (Trace Analytical, Menlo Park, CA, USA), respectively [20,21]. After measurement of gas samples, the serum bottles (in triplicate) were sacrificed, soil slurries being transferred into 2-ml Eppendorf cups. The samples were centrifuged for 15 min (12000×g, 4°C) and the supernatant was stored frozen at −20°C until analysis. Before analysis the thawed samples were filtered through 0.2-mm (pore size) membrane filters (Mini-sart SRP 15; Sartorius, Götttingen, Germany). Fatty acids were measured by high-pressure liquid chromatography (Sykam, Gilching, Germany) with a refraction index detector, having a detection limit of 3–5 μM [22].

2.3. Amplification of archaeal 16S rRNA genes

Soil slurries from the serum bottles were also collected in 2-ml Eppendorf cups and stored at −20°C for later DNA extraction. DNA was extracted from duplicate slurries and purified as described in detail by Henckel et al. [23]. Archaeal SSU rRNA genes (SSU rDNA) were amplified from the DNA by PCR with the archaeal group-specific primers described by Grosskopf et al. [24], which amplify the DNA fragment from position 109 to 934 (Escherichia coli 16S rRNA numbering [25]) as described previously [14]. The reverse primer was labeled terminally with FAM (5-carboxyfluorescein). The thermal profile used for amplification included denaturation at 94°C for 3 min, followed by 28 cycles of denaturation (94°C for 45 s), primer annealing at 52°C (45 s), and elongation at 72°C (1.5 min), and a final primer annealing at 52°C for 45 s and elongation at 72°C for 7 min. The 16S rDNA amplicons were purified by use of the QIAquick PCR purification kit (Qiagen, Hilden, Germany) according to the instructions of the manufacturer.

2.4. T-RFLP analysis

Terminal restriction fragment length polymorphism (T-RFLP) analysis is a powerful fingerprinting approach for analysis of microbial community structure [14,15,26]. Aliquots of the purified 16S rDNA were digested by TaqI (Promega, Mannheim, Germany) for 3 h at 65°C. Each 0.5-ml tube contained up to 70 ng of the SSU rDNA amplicons, 1 μl of the incubation buffer, and 1 μl of re-
striction enzyme (10 U) made up to a total volume of 10 μl with deionized water. Aliquots (2.5 μl) of the above mixture containing digested SSU rDNA were mixed with 1.9 μl of formamide and 0.6 μl of an internal lane standard consisting of 17 different 6-carboxy-X-rhodamine (ROX)-labeled fragments ranging in length from 29 to 928 nucleotides (GeneScan-1000 ROX; PE Applied Biosystems). The samples were denatured at 94°C for 3 min and then immediately stored on ice until being loaded onto the gel within 1 h. Electrophoresis and analysis of the resulting bands were performed as described previously [14]. The relative gene frequency of the individual T-RFs is given by the percentage peak areas relative to the total area of all peaks. Differences in the relative gene frequencies of a T-RF among different samples was tested for significance at $P < 0.05$ using a test which compares mean value and range [27].

3. Results

Italian rice field soil incubated at 30°C produced CH$_4$ after a short lag phase with a rate of about 17 nmol h$^{-1}$ g dw$^{-1}$ (Fig. 1). Concentrations of acetate, propionate and H$_2$ were high initially but then decreased to low and relatively constant values of about 20–80 μM, 10–50 μM and 1–5 Pa, respectively (Fig. 1). After 25 days incubation, the soil was exposed for 24 h to either low (4°C) or high

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Fig. 1. Production of CH$_4$ and changes in concentrations of H$_2$, acetate and propionate in slurries of Italian rice field soil incubated anoxically at 30°C. The dashed line indicates the time when the temperature was shifted for 24 h to either 4°C (low-T stress) or 50°C (high-T stress) before incubation at 30°C was resumed. The arrows indicate the time of sampling for later T-RFLP analysis (see Fig. 2); mean±S.E.M. ($n=3$).
temperature, after which incubation at 30°C was resumed. Neither the low-T nor the high-T stress resulted in a change of the CH₄ production compared to the control (Fig. 1). However, the high-T stress, but not the low-T stress, resulted in a transient increase of the H₂ partial pressure for about 8 h, reaching a maximum of almost 90 Pa, and a transient increase of the acetate and propionate concentrations for about 20 h reaching a maximum of about 4.5 mM and 0.8 mM, respectively (Fig. 1). Butyrate and caproate, which were not detectable in the control, also increased transiently to concentrations of about 150-200 μM (data not shown).

Soil samples were taken at time zero, and at another two time points (37 and 54 days) after the temperature stress, and the archaeal community was analyzed by T-RFLP of the SSU rRNA gene (Fig. 2). Seven different T-RFs (83, 91, 185, 284, 380, 392 and 738 bp) could be identified as described before [15,28]. The identification was based on phylogenetic lineages that had previously been characterized by cloning and sequencing of SSU rRNA genes retrieved from Italian rice field soil [14,29], i.e. Methanomicrobiaceae (83 bp), Methanobacteriaceae (91 bp), mainly Methanosarcinaceae (185 bp), mainly Methanosaetaceae (284 bp), rice cluster (RC) III (380 bp), mainly RC I (392 bp) and RC IV (738 bp). The T-RFs representing Methanosarcinaceae and RC I were the most frequent, the others amounted to <20% each (Fig. 2). The relative frequencies of the individual T-RFs stayed relatively constant with time and were also not affected by the temperature stress (Fig. 2). There was a tendency of the T-RFs representing Methanosarcinaceae and Methanosaetaceae to increase and decrease with incubation time, respectively (Fig. 2), but this tendency was not statistically significant.

Analogous experiments were conducted with rice field soil incubated at 15°C. In the non-stressed control, the temporal patterns of change of CH₄, H₂, acetate and propionate were similar to those at 30°C, but the rate of CH₄ production was only about 2 nmol h⁻¹ g dw⁻¹ (Fig. 3). Low-T stress again showed no effect on CH₄ production or substrate concentration. High-T stress, on the other hand, transiently inhibited CH₄ production for about 25 days, resulted in a transient increase of the H₂ partial pressure for about 12 days with maximum values of about 170 Pa, and furthermore resulted in permanently increased concentrations of acetate (4-6 mM) and propionate (0.5-0.7 mM) (Fig. 3). Concentrations of caproate were also permanently increased (about 150 μM) and those of butyrate were transiently increased to about 180 μM (data not shown).

In the 15°C controls, the relative frequencies of several T-RFs systematically changed with incubation time (Fig. 4). The T-RFs representing Methanobacteriaceae, Methanomicrobiaceae and Methanosarcinaceae decreased, and those representing Methanosaetaceae and RC I increased significantly (P<0.05). Low-T stress had no significant
effect on the relative gene frequencies of the different archaeal SSU rRNA genes, but high-T stress decreased those of Methanosaetaceae and increased those of Methanosarcinaceae (Fig. 4).

4. Discussion

Our results show that the microbial community in methanogenic rice field soil reacted to high-T stress but not to low-T stress. The resident methanogenic microbial community was apparently better adapted to resist stress at low than at high temperature. Rice field soil that was kept at 15°C, in particular, reacted markedly to a brief temperature stress of 50°C. High-T stress resulted in transient inhibition of CH₄ production, transient accumulation of H₂ and permanently higher acetate and propionate concentrations. The transient accumulation of H₂ may have been caused by inhibition of H₂-consuming methanogens, but the increase in acetate and propionate must have primarily been caused by stimulated production of these compounds during or immediately after the high-T stress. A decrease in the rate of fatty acid utilization is not an explanation, since the equivalent amount of CH₄ produced in the control was too small. Rice field soil adapted to high temperature by long-term incubation at 50°C also...
exhibited increased production rates of these fatty acids which were not balanced by subsequent methanogenic consumption [16]. This observation indicates that the function of the community of fermenting bacteria was affected by high-T stress. The structure of this community was not studied, but it may well have been affected by the temperature stress.

However, we studied the effect of temperature stress on the composition of the archaeal community. In the non-stressed 15°C control, Methanosarcinaceae decreased while Methanosaetaceae increased with time, as observed before [14]. The opposite trend, though not significant, was seen in the 30°C control. Apparently, low temperatures were selective for acetoclastic Methanosaetaceae versus Methanosarcinaceae confirming earlier studies in soil and methanogenic enrichment culture [14,17,30]. In addition, Methanosaetaceae seem to be selected when acetate concentrations become very low, which usually happens after prolonged incubation [15]. The reason is that Methano- nuseta species exhibit a lower threshold for acetate than Methanosarcina species and thus are selected under these conditions [31]. However, since acetate concentrations were similar in the 15°C and 30°C controls, and Methanosaetaceae specifically increased in the 15°C but decreased in the 30°C incubations, we conclude that the temperature itself was the decisive factor for the change in community structure.

High-T stress apparently reversed the pronounced community change in the 15°C incubations, at least partially, so that the relative gene frequency of Methanosarcinaceae was increased while that of Methanosaetaceae was decreased. One reason may be the high concentrations of acetate that were established permanently upon high-T stress, thus selecting for Methanosarcinaceae. The temperature stress thus could have affected the methanogenic populations indirectly by affecting the bacterial community producing acetate. Another reason, however, may be that the resident Methanosaetaceae were more sensitive to stress by high temperature than the resident Methanosarcinaceae which hence recovered relatively faster from the stress exerted upon the 15°C incubations.

By contrast, high-T stress did not affect the composition of the archaeal community in the 30°C incubations. This result is consistent with the observation that CH4 production was not affected by temperature stress in these incubations. Apparently, the archaeal community that had established at 30°C was more stress-resistant than that at 15°C. It is unknown whether this resistance was due to the better stress response regulation [1] of the resident flora, or a higher diversity with the chance that some of the microbes were better adapted [4]. It should be noted that our analytical techniques resolved the methanogenic community only at the level of families. It is possible that more subtle changes occurred at the level of lower taxa such as genera and species.

In summary, our results show that a rather brief stress...
on a complex soil microbial community may not only change the functional performance immediately after the stress but may also result in a change in the community structure. However, our results also show that the functional response was much stronger than the structural one. This is somewhat opposite to experiments in which the structure of the soil microbial community was changed by experimentally increasing or decreasing biodiversity [8,9]. In these experiments, a direct effect of biodiversity on the variability of, for example, plant organic matter degradation or its stability upon perturbation by heat was not generally obvious. However, in agreement with Griffiths et al. [8] we assume that more specific soil functions, e.g. methanogenesis in anoxic soils, are more sensitive to perturbations than general soil functions. Hence, methanogenic rice field soil may be a useful model to study further the relationship between structure (biodiversity) and function of a soil microbial community.

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References

