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Effect of temperature on the structure and activity of a methanogenic archaeal community during rice straw decomposition

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ABSTRACT

In recent years, the rice fields in Sanjiang Plain of Northeast China have drawn more and more attention because of the unique high-latitude location and the important contributions to rice production and methane emission. In the present study, a rice field soil in Sanjiang Plain was anaerobically incubated in presence and absence of rice straw at three temperatures (10 °C, 30 °C and 45 °C). The community structure and activity dynamics of methanogenic archaea were investigated by the terminal restriction fragment length polymorphism analyses in combination with cloning and sequencing of *mcrA* genes and transcripts and archaeal 16S rRNA genes. We found rice straw addition significantly shortened the lag phase of methanogenesis and enhanced the production of CH_4 and the accumulation of methanogenic precursors (CO_2, H_2 and acetate). The T-RFLP analysis of *mcrA* gene revealed that structure of methanogenic archaeal community was relatively stable during the anoxic incubation. By contrast, analysis at the transcript level showed a highly dynamic composition of the active methanogens. In the beginning and early stages of incubation, *Methanosarcinaceae* actively utilized the accumulated acetate and H_2/CO_2 for CH_4 production. Then the different methanogenic community developed along with the incubation time, and each representative methanogen group became predominant in different temperatures and treatments. At 10 °C, *Methanobacteriales* became more abundant in the soil without straw, while *Methanosarcinaceae* dominated in the soil with straw. The acetoclastic *Methanosaetaceae*, in particular, appeared to be active at 30 °C, probably due to the low concentration of acetate. High temperature of 45 °C significantly favored the hydrogenotrophic methanogens, with the increasing abundance of *Methanobacteriales* early and then the *Methanocellales* in the later stages.

1. Introduction

Methane (CH_4) is the second most important greenhouse gas after carbon dioxide (CO_2) and rice fields have been identified as a major source of atmospheric CH_4 emission (IPCC, 2007). In submerged rice field soil, CH_4 is exclusively produced by methanogenic archaea in the final step of the anaerobic degradation of organic matters (Liesack et al., 2000). Returning rice straw to soil, as a common practice to maintain the soil fertility in Asia, has been proved to significantly enhance CH_4 production and emission (Yagi and Minami, 1990; Zou et al., 2005). Several slurry incubation experiments showed that addition of rice straw also changed the structure of methanogenic community. It is generally found that the relative abundances of *Methanosarcinaceae* and/or *Methanobacteriales* increased after straw addition (Weber et al., 2001; Conrad and Klose, 2006; Peng et al., 2008; Conrad et al., 2012; Bao et al., 2014). The concentrations of acetate and H_2 were considered as the key factors to regulate the shift of methanogen composition during the degradation of rice straw (Conrad, 2007). However, all these results were obtained from the analyses at the gene level, which might have showed the existence of total methanogens or their growth but not their activity (Ma et al., 2012).

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To our knowledge, the structure and dynamics of metabolically active methanogens and their responses to the substrate concentration during rice straw decomposition has not been studied yet.

Temperature is another key factor controlling the CH_4 production in rice field soil (Fey and Conrad, 2000; Fey et al., 2001; Peng et al., 2008; Conrad et al., 2009). Although *in situ* soil temperature in field is generally lower than 30 °C (Schütz et al., 1990), the optimal temperature for CH_4 production is 35–40 °C as revealed by the previous slurry incubation experiments (Yao and Conrad, 2000; Fey et al., 2001; Conrad, 2007). The pathway of CH_4 production as well as the structure of methanogenic community change with the temperature shift. At low temperature, about 85% CH_4 was produced from acetate rather than H_2/CO_2 with acetoclastic *Methanosaetaceae* as the predominant methanogen (Conrad, 2002). At moderate temperatures, members of both hydrogenotrophic and acetoclastic methanogens including *Methanosarcinaceae*, *Methanosaetaceae*, *Methanocellales*, *Methanobacteriales* and *Methanomicrobiales* were all responsible for CH_4 production (Conrad, 2009). However, at high temperatures, CH_4 was exclusively produced from H_2/CO_2 rather than acetate. *Methanocellales* was generally found to be exclusively selected by high temperature (Fey et al., 2001; Conrad, 2009), as well as other thermophilic methanogens including *Methanosarcinaceae*, *Methanobacteriales* or *Methanomicrobiales* (Wu et al., 2006). Whether similar effects of temperatures could be observed for the composition of the active methanogenic community is unclear.

The Sanjiang Plain, located in northeastern China, formerly had the largest concentrated area of freshwater wetlands in China. During the past half century, incredible land-use changes happened. The considerable areas of natural marsh (more than 3 million ha) had been reclaimed for agricultural purpose (Huang et al., 2010). The area of rice fields especially increased after 1990s and now accounts for one third of total cultivated area (Li et al., 2012). Total CH_4 emission from rice fields in Sanjiang Plain has already increased to 0.203 Tg CH_4–C in 2010, compared with 0.025 Tg CH_4–C in 1990 (Zhang et al., 2012). The emission amount is predicted to increase much faster in the next several decades, due to the continuous expansion of rice field areas and remarkable global warming in high latitudes there (Zhang et al., 2011). However, the microbial methanogenesis process and the methanogenic community structure of rice field soil in Sanjiang Plain are poorly understood (Wang et al., 2010). As far as we know, the effects of straw incorporation on microbial CH_4 production under different temperatures have never been studied for the rice field soils in the Sanjiang Plain.

Therefore, one rice field soil which is representative for the soils in Sanjiang Plain was tested in the present study. The soil in presence and absence of rice straw was anaerobically incubated under low (10 °C), moderate (30 °C) and high (45 °C) temperatures. The objectives were: (1) to investigate the structure and dynamics of methanogenic community during the decomposition of rice straw; (2) to compare the composition of existing and active methanogens based on analyses of functional *mcrA* gene and transcript levels respectively; (3) to determine the responses of different methanogenic groups to different temperatures.

2. Materials and methods

2.1. Soil sampling and incubation

Soil from the plow layer (0–20 cm) was collected in October, 2010 from a rice field in Qixing farmland (47.3′N, 132.6′ E), which locates in Sanjiang Plain in northeastern China. Soil was dried, crushed, sieved (2-mm mesh size), mixed and stored at room temperature. Standard methods (Page et al., 1982) were used to measure soil characteristics (per kg soil): pH 6.58, organic C of 38 g kg^{-1}, total N of 1.65 g kg^{-1}, available K of 112.8 mg kg^{-1} and available P of 26.3 mg kg^{-1}. Rice straw was collected at the maturity of rice plants (*Oriza sativa*, Kongyu 131), dried to constant weight at 60 °C, and cut into 3–5 mm pieces.

About 10 g of dried soil and 20 ml of N_2-flushed demineralized water were placed into serum bottles (50 ml). The rice straw (0.1 g) was added into half of the bottles which were referred to as straw treatment. The other half of the bottles without straw addition were referred to as no-straw treatment. The no-straw and straw treatment bottles were set up in replicates each (81 bottles for each treatment). Then the serum bottles were capped with butyl stoppers and aluminum caps, flushed with N_2 for 6 min, and incubated without shaking at 10 °C, 30 °C or 45 °C, respectively. On 9 different sampling dates, 18 bottles (2 straw treatments * 3 temperatures * 3 replicates) were sacrificed. The bottles after incubating for 2 h were sampled as day 0.

2.2. Chemical measurements

Gas samples (0.1 ml) were collected from the headspace of serum bottles using a pressure-lock syringe for various time points. The concentrations of H_2, CH_4 and CO_2 were analyzed using a gas chromatograph (GC, 7890A, Agilent Technologies, USA) equipped with a thermal conductivity detector (TCD) and a flame ionization detector (FID). The water content, pH and extractable ferrous iron of fresh slurry were measured as described before (Ma et al., 2012). Short chain fatty acids of the centrifuged supernatant were analyzed using high-performance liquid chromatography (HPLC, 1200series, Agilent Technologies, USA) (Rui et al., 2009), and NO_3^-, NO_2^-, Cl^- and SO_4^{2-} of the supernatant were analyzed using ion chromatography (C14508, Metrohm, Switzerland) (Yuan and Lu, 2009).

2.3. Nucleic acid extraction and cDNA synthesis

At different time points, triplicate bottles from each treatment were destructively sampled and the fresh slurry was stored at −80 °C within 15 min. The total DNA and RNA contents of soil samples were coextracted by a modified phenol extraction protocol (Ma et al., 2012). In brief, 0.5 g of slurry sample was extracted with 0.7 g of glass beads (0.1 mm) and 700 μl of TPM buffer (50 mM Tris–HCl [pH 7.5], 1.7% [wt/vol] polyvinylpyrrolidone K25, 20 mM $MgCl_2$) and then extracted with 900 μl of phenol-based lysis buffer (50 mM Tris–HCl [pH 7.5], 10 mM Na_2EDTA, 1% [wt/vol] sodium dodecyl sulfate, and 6% [vol/vol] water-saturated phenol). The supernatant was collected and further extracted with 900 μl of water-saturated phenol, 800 μl of phenol-chloroform-isoamyl alcohol (25:24:1 [vol/vol/vol]) and 650 μl of chloroform-isoamyl alcohol (24:1[vol/vol]). The total nucleic acids were precipitated with 550 μl of cold isopropyl alcohol and 55 μl of 3 M sodium acetate (pH 5.2), washed with 700 μl of cold 70% ethanol, dried for around 10 min, and finally dissolved in 80 μl of DNase/RNase-free water.

For the analysis of DNA, no further purification steps were carried out and nucleic acids were stored at −20 °C. For the analysis of RNA, 40 μl of nucleic acids were incubated at 37 °C for 30 min with an RNase-Free DNase (TaKaRa, China) to hydrolyze the DNA, and RNA was further purified with an RNeasy Mini Kit (Qiagen, Germany). The removal of DNA was confirmed by PCR amplification as described previously (Ma et al., 2012). The quality and purity of DNA and RNA were checked by agarose gel electrophoresis and NanoDrop1000 spectrophotometry (NanoDrop Technologies, Wilmington, DE). The RNA was synthesized into cDNA using a PrimeScript cDNA Synthesis Kit (TaKaRa, China) according to the manufacturer's instructions. cDNA samples were stored at −20 °C.