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Dynamics of methanogenic archaeal communities based on rRNA analysis and their relation to methanogenic activity in Japanese paddy field soils

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Abstract

We investigated the relationships between dynamics of methanogenic archaeal communities and methanogenic activities in Japanese paddy field soils by reverse transcription-PCR-denaturing gradient gel electrophoresis (RT-PCR-DGGE) and real-time PCR targeting 16S rRNA gene (16S rDNA) and 16S rRNA. The investigated paddy fields were managed on a double-cropping system, where rice was cultivated under flooded conditions in summer and wheat was cropped after drainage in winter under drained conditions. Methanogenic archaeal 16S rRNAs were retrieved from all collected soil samples even under drained conditions by RT-PCR. DGGE band patterns from methanogenic archaeal 16S rRNAs were stable throughout the year and were similar to the patterns from their 16S rDNAs. Sequences of main DGGE bands were closely related to Methanomicrobiales, Methanosarcinales and Rice cluster I, indicating those methanogenic archaea predominantly maintained basal metabolism in the paddy fields. Numbers of methanogenic archaeal 16S rDNAs and 16S rRNAs ranged from 5.9×10^7 to 1.3×10^9 and from 5.3×10^9 to 2.9×10^{11} g^{-1} dry soil, respectively. The ratio of 16S rRNAs per one 16S rDNA fragment fluctuated between 9.4 and 1.2×10^3 and increased during rice cultivations although contrary results were also obtained in some investigated plots. Methanogenic activities under flooded conditions were higher than those under drained conditions. These results suggested that activities of methanogenic archaeal communities fluctuated in a year depending on soil conditions. Surprisingly, 6.7×10^7 g^{-1} dry soil of methanogenic archaeal 16S rDNAs and certain number of their 16S rRNAs were detected even in a long-term air-dried paddy soil (15 years). When the air-dried soil was incubated under anoxic flooded condition, methane was vigorously produced after 6 days and the number of methanogenic archaeal 16S rRNA gradually increased during the incubation. These results indicated that methanogenic archaea were dormant or starved, but survived in oxic paddy soils, and their activity increased under the conditions suitable for growth.
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Keywords: Methanogenic archaea; Denaturing gradient gel electrophoresis (DGGE); Real-time PCR; Paddy field soil; 16S rRNA

1. Introduction

Methane is one of greenhouse gases and greatly contributes to global warming (Prather and Ehhalt, 2001). Major part of methane on earth is biologically produced as a consequence of anaerobic decomposition of organic materials and emitted from various anoxic environments. Flooded paddy field is one of the representative anaerobic sites and of the major anthropogenic sources for methane emission. Methanogenic archaea, which belong to the Euryarchaeota in Archaea, play the unique role for biological methane production in those anoxic environments. Therefore, it is important to understand the ecology of methanogenic archaea in paddy field soil.

In the previous studies, methanogenic archaeal communities were investigated in Japanese paddy fields under double cropping (rice and wheat) by the most probable number (MPN) method (Asakawa and Hayano, 1995; Asakawa et al., 1998), analysis of specific ether lipids for archaea (Asakawa et al., 1998) and PCR-denaturing

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gradient gel electrophoresis (PCR-DGGE) method targeting 16S rRNA gene (16S rDNA) (Watanabe et al., 2006), and it was shown that the methanogenic archaeal populations and their community structure did not change conspicuously between flooded and drained conditions. These results suggested that methanogenic archaea, which are obligate anaerobe and need low redox potential (less than −0.33 V) for their growth, survived in paddy field soil even under drained conditions and also indicated that the community structure did not reflect the methane flux from paddy fields, i.e. methane was actively emitted under flooded conditions (e.g. Yagi and Minami, 1990). The communities of methanogenic archaea determined by the methods as mentioned above may have not corresponded to active populations of methanogenic archaea in paddy field soil.

Recently, many studies have been carried out to estimate metabolically active bacterial members in various environments, targeting bacterial 16S rRNA (e.g. Poulsen et al., 1993; Felske and Akkermans, 1998; Lüdemann et al., 2000; Duineveld et al., 2001; Tanahashi et al., 2005) since ribosome- and rRNA-contents in bacterial cells were correlated with the activity and the growth (Nomura et al., 1984; Poulsen et al., 1993). Some studies also targeted methanogenic archaeal 16S rRNA in incubated paddy soil (Lueders and Friedrich, 2002), on decomposing rice straw (Glissmann et al., 2001; Weber et al., 2001) and on rice root (Lu and Conrad, 2005), using terminal restriction fragment length polymorphism (T-RFLP), slot blot hybridization or stable isotope probing (SIP) methods. However, no work has been reported so far, which has targeted 16S rRNA of methanogenic archaea in paddy soil under field conditions and has estimated the relationships between their 16S rRNA numbers and the methanogenic activities in soil. Since irrigated paddy fields, which are common in the temperate areas of Asia, are flooded during rice cultivation period in summer and drained under fallow condition or cropped with wheat or barley under upland (drained) condition in winter. Therefore, soils in paddy fields undergo seasonal changes in redox potentials between +0.6 to +0.5 V and −0.2 to −0.3 V (Takai and Kamura, 1966). Thus, the study under field conditions is requisite to understand ecology of methanogenic archaea in paddy soil.

In the present study, therefore, we targeted methanogenic archaeal 16S rRNA and investigated the relationships between the metabolically active communities of methanogenic archaea by reverse transcription-PCR-DGGE (RT-PCR-DGGE) and real-time PCR methods and the methanogenic activities in paddy soil under field conditions. We hypothesized and expected that the methods targeting 16S rRNA allowed to detect drastic structural changes in metabolically active communities of methanogenic archaea reflecting soil conditions (flooded or drained) and seasonality. We also investigated 16S rRNA of the methanogenic archaea in air-dried paddy soils to show their survival under extremely oxic and dry conditions.

2. Materials and methods

2.1. Soil samples

The study fields were two paddy fields (Anjo and Chikugo) located in central and southwestern Japan, which were also investigated in the previous study (Watanabe et al., 2006). Both Anjo and Chikugo fields were under double cropping (rice [*Oryza sativa* L.] as a summer crop and wheat [*Triticum aestivum* L.] as a winter crop). In Anjo field (total C content, 13 g kg^{-1}; total N content, 1.1 g kg^{-1}; pH [H_2O], 6.3), wheat straw residues were incorporated into the field after wheat harvest, but rice straw residues were taken out. Chikugo field consisted of four plots (total C content, 22–30 g kg^{-1}; total N content, 2.1–2.9 g kg^{-1}; pH [H_2O], 5.9–6.2 (Yoo et al., 1992)), which were treated with chemical fertilizer (CF) that consisted of urea, ammonium sulfate, ammonium phosphate and potassium sulfate, rice straw plus chemical fertilizer (RS), rice straw compost plus chemical fertilizer (RSC) and wheat straw plus chemical fertilizer (WS). Soil samples were collected during wheat cultivation (11 April and 11 December in Anjo and 11 March and 9 December in Chikugo), flooded period (26 June and 28 July in Anjo and 11 July in Chikugo), midseason drainage (11 August in Anjo and 4 August in Chikugo), re-flooded period (4 September in Anjo and 10 September in Chikugo) and drainage (14 October in Anjo and 7 October in Chikugo) in 2003. The field managements were described previously in detail (Watanabe et al., 2006). Soil samples of plow layer (0–10 cm) were collected from the center of 4 rice plant hills during rice cultivation periods and from the center of rows of wheat plants during upland cropping periods by using a trowel. Approximately 1 kg of soil sample was collected from three or four points that were randomly selected in one plot, and mixed well in a polyethylene bag on site. Then the sample was immediately transferred into disposable 15-ml sterilized polypropylene tubes and stored in a cooler box with dry ice. The soil samples were transferred to the laboratory and deep-freezed at −80 °C until use.

Two types of air-dried soil were also used to examine the survival of methanogenic archaea. One was taken from Anjo on 10 May 2005 during wheat cultivation (air-dried Anjo soil) and another was from WS plot in Chikugo on 8 November 1990 before wheat seeding (air-dried WS soil). Both samples were air-dried after sampling, passed through a 2-mm mesh sieve and stored at room temperature.

2.2. Methanogenic activity in paddy field soil and air-dried WS soil

Soil samples, which were collected from Anjo and Chikugo paddy fields under flooded (20 July in Anjo and 24 July in Chikugo) and drained (30 May in Chikugo) conditions in 2006, were used for the measurement of methanogenic activities in paddy field soils. The soils were

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