Anaerobe 32 (2015) 24–31

Contents lists available at ScienceDirect

Anaerobe

journal homepage: www.elsevier.com/locate/anaerobe

Molecular biology, genetics and biotechnology

Methanogenic archaea diversity in hyporheic sediments of a small lowland stream

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ARTICLE INFO

Article history:
Received 12 September 2014
Received in revised form
14 November 2014
Accepted 16 November 2014
Available online 21 November 2014

Keywords:
Methanogen
Diversity
Hyporheic sediment
Denaturing gradient gel electrophoresis (DGGE)
Fluorescence in situ hybridization (FISH)

ABSTRACT

Abundance and diversity of methanogenic archaea were studied at five localities along a longitudinal profile of a Sitka stream (Czech Republic). Samples of hyporheic sediments were collected from two sediment depths (0–25 cm and 25–50 cm) by freeze-core method. Methanogen community was analyzed by fluorescence in situ hybridization (FISH), denaturing gradient gel electrophoresis (DGGE) and sequencing method. The proportion of methanogens to the DAPI-stained cells varied among all localities and depths with an average value 2.08×10^5 per g of dry sediment with the range from 0.37 to 4.96×10^5 cells per g of dry sediment. A total of 73 bands were detected at 19 different positions on the DGGE gel and the highest methanogen diversity was found at the downstream located sites. There was no relationship between methanogen diversity and sediment depth. Cluster analysis of DGGE image showed three main clusters consisting of localities that differed in the number and similarity of the DGGE bands. Sequencing analysis of representative DGGE bands revealed phylotypes affiliated with members belonging to the orders Methanosarcinales, Methanomicrobiales and Methanocellales. The knowledge about occurrence and diversity of methanogenic archaea in freshwater ecosystems are essential for methane dynamics in river sediments and can contribute to the understanding of global warming process.

1. Introduction

A great deal of attention has been focused at understanding the role of biodiversity in the natural environments [1]. In stream and river ecology, the biodiversity pattern has been probably influenced by River Continuum Concept (RCC) [2], which modeled spatial structure and function in streams in terms of gradual changes from headwaters to mouth. This concept predicts that for many common species, genetic diversity increases from upstream to downstream with a peak in mid-order streams [3]. In contrast to species like fish [4,5] or invertebrates [6,7], studies of the microbial biodiversity pattern along the longitudinal stream profile are still lacking. Although there is no clear definition of what "species" means for microbes [8], the short generation time of microbes permits rapid changes in population abundance and changes of microbial community structure are often the result of varying environmental conditions. In a short time, the habitat of microbial community may shift from oxygen supersaturation to anoxia and new microbial niches are created, filled, destroyed and refilled [1]. The variety of microbial niches could probably be a reason why the microorganisms are immensely diverse.

In aquatic ecosystems, microbial life is often dominated by benthic biofilms (matrix-enclosed communities including bacteria, archaea, algae, fungi, and protozoa), which are essential for the decomposition of organic matter, the uptake and transfer of material and the microbial foodweb [9,10]. These microbial biofilms are formed in rivers on submerged surfaces such as stones, roots, river bed sediments and also sediments beneath and beside streams - hyporheic sediments [9,11]. The hyporheic zone, defined as sediments hydrologically linked to the open stream channel, is a highly dynamic ecotone with active exchange of water, dissolved

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http://dx.doi.org/10.1016/j.anaerobe.2014.11.009
1075-9964/

material and organisms between river and groundwater [12,13]. The seemingly well-oxygenated hyporheic zone contains anoxic and hypoxic pockets ("anaerobic microzones"), which create a variety of microniches, allowing coexistence of a great diversity of microbial types [14–17]. The most useful way to consider the diversity of microbial types is in terms of functional groups – organisms that perform a particular process, for example converting complex organic compounds to carbon dioxide and methane [17]. Methanogenesis is a common process in habitats such as rice paddies [18], soils [19], lake sediments [20], as well as in the gastrointestinal tract of animals [21] and extreme habitats such as hydrothermal vents [22] and permafrost soils [23,24]. However, the production of methane is not a scarce process even in stream ecosystems, since a relatively high production of methane has been measured in river sediments [25–28].

Methane (CH_4) is produced mainly by methanogenic archaea as a final product of anaerobic respiration and fermentation [29,30]. Methanogenic archaea are obligate anaerobes, but anoxia is not the only factor influencing the process of methanogenesis. Other factors, which could stimulate the production of methane are low redox potential, increasing temperature and especially accessability of substrates - acetate and hydrogen [31,32]. The acetate is converted into CH_4 and CO_2 by acetoclastic methanogens while hydrogenotrophic methanogens convert CO_2 and H_2 to CH_4 [33]. Because methanogenic assemblages are in environmental habitats responsible for the most of methane production, it is very important to study methanogen density and diversity in environments [34]. There are several studies of methanogen abundance and diversity in lake and sea sediments, paddy fields, rice roots and peatlands [34], but studies of methanogen diversity in hyporheic sediments are lacking.

Since microbial diversity is difficult to estimate, it is necessary to use molecular markers such as 16S rRNA. There are a variety of techniques which could be used for an assessment of microbial diversity and composition in natural samples [35]. Fluorescence in situ hybridization (FISH) is a powerful technique which allows to detect and identify the community composition directly in environmental samples. FISH is based on the direct microscopic identification of single cells after hybridization with 16S rRNA targeted fluorescent dye-labelled oligonucleotide probes [36,37]. Denaturing gradient gel electrophoresis (DGGE) is used to determine the genetic diversity of microbial communities. The procedure is based on electrophoresis of PCR-amplified 16S rDNA fragments in polyacrylamide gels containing a linearly increasing gradient of denaturants. In DGGE, DNA fragments of the same length but with different base-pair sequences can be separated. Separation is based on the electrophoretic mobility of partially melted DNA molecules in a polyacrylamide gel and resulting into a band pattern [38,39].

The aim of this study was to reveal the methanogen diversity of hyporheic sediments along the longitudinal stream Sitka profile. We focused on the detection of methanogenic archaea richness (DGGE), identification (sequencing) and quantification (FISH) in two hyporheic sediment depths (0–25 cm and 25–50 cm sediment layers) at five localities alongside the stream, simultaneously with measuring of physicochemical parameters (dissolved methane and oxygen saturation, potential methane production, organic carbon content etc.). Sitka stream is a relatively short, some 35 km long second-order stream with a mostly natural meandering channel. Our previous measurements showed that with increasing distance from the spring channel the slope decreases while sedimentation of fine particles and accumulation of organic matter within sediments increase. Similarly, dissolved oxygen concentration in the interstitial water is highest in the upstream part of the stream, gradually decreases toward the stream mouth and sharply decreases with sediment depth. Based on these findings and the theory that microbial diversity is changing from headwaters to stream mouth along with River Continuum Concept, we hypothesized that the methanogen diversity and abundance would increase from upstream to downstream of the Sitka stream. Furthermore, based on our previous methane concentration measurements we hypothesized that methanogens occurring deep in the hyporheic sediments would exhibit higher abundance and taxonomic richness compared to those from upper sediment layer.

2. Material and methods

2.1. Study site

The sampling sites are located on the Sitka stream, Czech Republic. The Sitka is an undisturbed, second-order, 35 km long lowland stream originating in the Hrubý Jeseník mountains at 650 m above sea level. The catchment area is 118.81 km^2, geology being composed mainly of Plio-Pleistocene clastic sediments of lake origin covered by quaternary sediments. River bed sediments are composed of gravels in the upper parts of the stream while the lower part is characterized by finer sediment. Mean annual discharge is 0.81 m^3/s. For more detailed characteristics of the sampling sites see [28,40] and Table 1.

2.2. Collecting of sediment and water samples

Five localities (no. 1–5) alongside stream profile were chosen for sampling sediment and interstitial water samples. The locality no. 1 is situated in headwater of the stream, while locality no. 5 is the most downstream. The map showing the location of study sites 1–5 is available in our previous study [28].

Hyporheic sediment and interstitial water samples were taken during summer 2011. The sediment samples were collected with a freeze-core method using N_2 as a coolant [41]. At each locality three sediment cores were taken. After sampling, surface sediment layer (0–25 cm) and layer of 25–50 cm in depth were immediately separated from each core. The particular layer samples were then pooled. The samples were then stored at a low temperature (4–6 °C) during the transport to the laboratory. After thawing the wet sediment of each layer was sieved and only particles <1 mm were considered for the following measurements, since most of the biofilm microorganisms are associated with this fraction [42]. Six sediment subsamples from each locality (three from each sediment layer) were used for subsequent microbial analysis.

Samples for estimation of total cell numbers and, consequently, for FISH analysis were fixed with paraformaldehyde (2% final conc.). Samples for measurement of organic matter content and determination of methanogenic potential were used immediately and samples for DNA extraction were frozen (−20 °C) until used.

Interstitial water samples were collected using a set of mini-piezometers [43] from both sediment depths (0–25 cm and 25–50 cm) at each study site. Three randomly replicated interstitial water samples were taken for measurement of particular physicochemical parameters.

The values of selected physicochemical and microbial parameters are expressed as means ± SE.

2.3. Analysis of environmental parameters

Sediment organic matter content was determined by oven-drying at 105 °C and subsequent combustion at 550 °C for 5 h. Organic matter values were then converted to carbon equivalents and expressed as a percentage according to the protocol in [44]. Sediment grain size distribution was analyzed from air-dried sediment samples and descriptive parameters were computed