WATER RESEARCH 41 (2007) 3407–3419

Available at www.sciencedirect.com

ScienceDirect

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

Seasonal and diel distributions of denitrifying and bacterial communities in a hypersaline microbial mat (Camargue, France)

Christelle Desnues[a], *Valérie D. Michotey*[a], *Andrea Wieland*[b], *Cui Zhizang*[c], *Aude Fourçans*[d], *Robert Duran*[d], *Patricia C. Bonin*[a,*]

[a]*Laboratoire de Microbiologie, de Géochimie et d'Ecologie Marines, CNRS-UMR 6117, Centre d'Océanologie de Marseille, Campus de Luminy, Case 901, 13288 Marseille cedex 9, France*

[b]*Institute for Biogeochemistry and Marine Chemistry, University of Hamburg, Bundesstr. 55, 20146 Hamburg, Germany*

[c]*State Key Laboratory for Infectious Disease Prevention and Control, Beijing, China*

[d]*Laboratoire d'Ecologie Microbienne EA 3525, Université de Pau et des Pays de l'Adour, Avenue de l'Université, BP 1155, F-64013 Pau cedex, France*

ARTICLE INFO

Article history:
Received 13 December 2006
Received in revised form
13 April 2007
Accepted 24 April 2007
Available online 1 May 2007

Keywords:
Denitrification
Diel cycle
Microbial mat
nirS and *nirK* genes
PCR-DGGE
Seasonal cycle

ABSTRACT

Changes in spatio-temporal distribution of bacterial and denitrifying communities were qualitatively studied in a microbial mat from Camargue (France). During a diel and a seasonal cycle, patterns of 16S rRNA and nitrite reductase genes (*nirS* and *nirK*) were compared by denaturing gradient gel electrophoresis (DGGE). Statistical analysis of DGGE profiles showed a significant seasonal shift in the community structure of the *nirS*-containing bacteria with a winter superficial population that extended in summer, whereas the *nirK*-containing bacteria seemed more affected by vertical gradients rather than by month-to month-changes. Denitrifying activities remained stable during these sampling times. The bacterial community at the surface of the mat also changed according to season, but appeared stable over a day. Finally, during a diel cycle *nirK* populations were localized in zones with large fluctuations of environmental parameters (oxygen, pH, and sulfur levels) while *nirS* populations seemed more restricted to the permanent anoxic layer of the microbial mat.

1. Introduction

The intertidal regions of many estuaries and coastal areas provide suitable habitats for the development of layered sediment communities called microbial mats. These mats represent a typical example of microbial consortia stratified on a microscale according to the physiology of the organisms and the physico-chemical gradients of the environment (Chaudhry and Chapalamadugu, 1991). Microbial mats are broadly distributed in marine and hypersaline coastal environments, estuary margins, alkaline lakes, streams and hydrothermal vents (Caumette et al., 1994; Ferris et al., 1996; Guerrero et al., 1993). They are highly productive systems and organic matter formed by oxygenic photosynthesis in the uppermost millimeter is almost completely recycled in deeper layers through aerobic and anaerobic metabolisms. Microbial mats consist of shallow structures of variable thickness ranging from 1 to 10 cm. At diel or seasonal scales, microbial mats are subjected to strong gradients and large fluctuations of light, temperature, oxygen concentrations and

*Corresponding author. Tel.: +33 4 91 82 93 36; fax: +33 4 91 82 96 41.
E-mail address: patricia.bonin@univmed.fr (P.C. Bonin).
0043-1354/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2007.04.018

salinity that have been found to influence the physiology of the microorganisms (Ferris and Ward, 1997; Norris et al., 2002; Ramsing et al., 2000). To assess spatial and/or temporal bacterial diversity, many studies were focused on the entire bacterial community using denaturing gradient gel electrophoresis (DGGE) analysis of 16S rRNA genes fragments (Muyzer et al., 1993) and/or cloning/sequencing approaches. Studies of the changes of the communities based on 16S rRNA genes can sometimes provide phylogenetic information about functional groups (such as sulfate-reducing bacteria that are a functional group close on a phylogenetic point of view). However for denitrifying bacteria, targeting 16S rRNA genes cannot reveal their presence and physiological role in microbial mats as they are spread among diverse phylogenetic groups. Then, analysis of the functional genes which are involved in denitrification represents a suitable alternative. Denitrification is mediated by heterotrophic anaerobic facultative bacteria, which can use nitrate (NO_3^-) or nitrite (NO_2^-) as a terminal electron acceptor for respiration, and reduce it to nitrous oxide (N_2O) or nitrogen (N_2). The first step of denitrification, the nitrate to nitrite reduction, is also common to dissimilatory nitrate reduction to ammonium. In contrast, the reduction of nitrite to gaseous products is a specific and well-known step of denitrification. This enzymatic reaction can be performed by two distinct nitrite reductases: a copper nitrite reductase (Cu-Nir) encoded by *nirK* and a cytochrome nitrite reductase (cd_1-Nir) encoded by *nirS*. Few studies have previously been dedicated to the analysis of the diversity of the denitrifying community, by using T-RFLP analysis on nitrite reductase genes (*nirS* and *nirK*) (Braker et al., 2001; Wolsing and Prieme, 2004) or nitrous oxide reductase gene (*nosZ*) (Scala and Kerkhof, 2000). DGGE has recently been used to examine bacterial diversity and distribution of functional groups in environmental samples, such as ammonia-oxidizing and diazotroph bacteria by targeting *amoA* and *nifH*, respectively (Piceno et al., 1999; Nicolaisen and Ramsing, 2002). In a previous study, we have reported the adaptation of the DGGE technique for the analysis of denitrifying bacteria biodiversity by targeting *nirS* of 89 marine isolates (Goréguès et al., 2005). Results of this study underlined the high level of intraspecific biodiversity among denitrification genes. Here, we present a spatio-temporal analysis of denitrifying diversity in a microbial mat during diel and seasonal cycles by DGGE analysis targeting functional genes (*nirS* and *nirK*). Our results clearly demonstrate effects of spatial and temporal changes on *nirS* and *nirK* denitrifying communities influenced by the previously described pH, oxygen, temperature and sulfide gradients (Wieland et al., 2005).

2. Materials and methods

2.1. Study site and sampling

For seasonal studies, mat samples were collected at 3:00 PM in May 2000, January 2001 and June 2001 in a pre-concentration pond of the Salin-de-Girauds salterns in the Camargue, France (43°27′35″N, 04°41′28″E) (Fourçans et al., 2004). The salinity of the overlying water (~20 cm) in this pond was 130, 95 and 120 psu (practical salinity units) at the sampling time for May 2000, January and June 2001, respectively. The in situ temperature was 20, 17, and 21 °C. Cores of 11 mm (i.d.) were collected with sterile tubes, transported at 4 °C and subsequently frozen at −20 °C. For daily studies, samples were collected in June 2001 at 3:00 PM, 10:00 PM and 4:00 AM.

2.2. In situ denitrification activities

Series of 1 cm^2 subsamples (0.5 cm thick) were placed, with the mat surfaces facing up, into 22 ml headspace vials containing 2 ml sterile filtered water of the sampling site containing chloramphenicol (1 $g\,l^{-1}$) to prevent *de novo* protein synthesis. The vials were closed with a rubber stopper. All processes were measured under ambient oxygen level without nitrogen bubbling. Denitrification rates were measured using the acetylene inhibition technique (Yoshinari and Knowles, 1976). Briefly, final acetylene partial pressure in each vial was adjusted to 20 kPa. The denitrifying activity corresponded to the rate of nitrous oxide accumulation after nitrate reduction measured with three independant flasks for each time point. The N_2O accumulation was followed during 10 h in an incubation chamber maintained at *in situ* water temperature. Denitrification rates were calculated from the changes in N_2O concentration, measured in triplicates, at each sampling time (0, 1, 3, 5, 7, and 10 h). The concentration was then plotted against time and fitted to the linear model ($A(t) = A \pm mt$) using the least-squares method, where t is the incubation time; A is the concentration at $t = 0$; and m is the slope of the linear curve. The rates were calculated from the initial linear slope of the curve. Rate uncertainties were calculated from the errors in the linear regressions. The concentration of N_2O in the water was calculated using the solubility coefficient of N_2O (Weiss and Price, 1980).

2.3. DNA extraction

For the 1 mm scale analysis, the frozen cores were sliced at 1 mm depth intervals between 0 and 3 and at 2 mm intervals for the deeper ones (3–5 mm) and lyophilized. DNA was extracted from 0.25 g (dry weight) of triplicate mat cores using MoBio Ultraclean™ Soil DNA isolation kits (MoBio Laboratories, California) according to the manufacturer's protocol. Extracted DNAs were pooled and the quality was analyzed on a 1% (w/v) agarose gel. Serial dilutions of genomic DNA (up to 10^{-2}) were made in sterile water and stored at −20 °C. For the 100 μm scale analysis, triplicate samples of mat cores (35 mm i.d.) were collected from June samples. The upper 10 mm of the mat core was sliced off aseptically, transferred into sterile Petri dishes, frozen in liquid nitrogen, transported on dry ice and finally stored at −80 °C until further analysis. These frozen samples were then sliced with a cryomicrotome (MICROM Gmbh, Walldorf, Germany) to ca. 100 μm thickness for the first 2 mm, and 200 μm thickness for the next 2 mm. Eleven layers within the top 4 mm from the surface were chosen. Three replicates from each layer were pooled and genomic DNA was extracted as previously described (Fourcans et al., 2006).