Soil Biology & Biochemistry 42 (2010) 601–610

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

High levels of spatial heterogeneity in the biodiversity of soil prokaryotes on Signy Island, Antarctica

Chun Wie Chong [a,*], David A. Pearce [b], Peter Convey [b], G.Y. Annie Tan [a], Richard C.S. Wong [c], Irene K.P. Tan [a]

[a] Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
[b] British Antarctic Survey, NERC, High Cross, Madingley Road, Cambridge CB3 OET, UK
[c] Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

ARTICLE INFO

Article history:
Received 23 October 2009
Received in revised form
12 December 2009
Accepted 16 December 2009
Available online 24 December 2009

Keywords:
16S rRNA gene
DGGE
Environmental impact
Human impact
Heavy metals

ABSTRACT

In a previous study, soil bacterial diversity at environmentally distinct locations on Signy Island was examined using denaturing gradient gel electrophoresis (DGGE) profiling, and a range of chemical variables in soils was determined in order to describe variations between them. The dominant bacterial communities of all locations were found to be significantly different, although higher levels of similarity were observed between locations with similar physico-chemical characteristics, such as at penguin rookeries, seal wallows and vegetated soils. Extending this study, here soil prokaryote biodiversity was compared between 15 distinct locations in order to elucidate any interaction between four general habitat types on Signy Island (South Orkney Islands, maritime Antarctic) and any influence of previous human impacts at these sites. Specific sites were selected to represent the range of different soil environments present and to cover a range of environmental factors present in the maritime Antarctic which are known to influence bacterial community composition in soils elsewhere. A diverse prokaryote community is described, again with the majority of excised and sequenced bands belonging to the Bacteroidetes. Although DGGE profiling identified significant differences in prokaryotic biodiversity between all sampling sites, aggregations of banding patterns were also apparent across the different soil environments examined. Correlations between specific DGGE profiles and 10 selected soil parameters suggested that much of this variation could be explained by differences in the levels of environmental disturbance and soil pH. In particular, a greater proportion of variation in soil bacterial diversity was explained by differences in soil properties at human-disturbed locations than at undisturbed locations, with higher explanatory values by edaphic factors in the former and soil metal content in the later. In general, our data indicate that small-scale variation is an important factor in understanding patterns of prokaryotic distributions in soil habitats in the maritime Antarctic environment.

1. Introduction

Knowledge of prokaryotic biodiversity remains very patchy across Antarctica (Tindall, 2004; Wall, 2005; Steven et al., 2006). However, in recent decades, several studies using both culture dependent and culture-independent methodologies have focused on Signy Island (Bailey and Wynn-Williams, 1982; Wynn-Williams, 1990; Pearce, 2003, 2005; Pearce et al., 2003; Moosvi et al., 2005; Chong et al., 2009a), as a benchmark location within the maritime Antarctic, whose terrestrial ecosystems are representative of the region (Smith, 1990). Studies are also starting to emerge from other locations along the Antarctic Peninsula, such as that of Yergeau et al. (2007a,b, 2008) which investigated the prokaryotic communities of a series of Antarctic terrestrial habitats along a latitudinal gradient as part of a larger regional microbial diversity study spanning between the Falkland Islands (~50°S) and Mars Oasis, Alexander Island (~72°S). From the restricted habitats examined to date, a relatively large bacterial diversity has been suggested (Holdgate, 1977; Pearce, 2003, 2005; Pearce et al., 2003; Moosvi et al., 2005; Chong et al., 2009a).

There is a consensus that spatial variation amongst soil organisms is not random but exhibits predictable patterns over different spatial scales, with small-scale variation exhibiting greater diversity than large scale variation (Weins, 1989; Ettema and Wardle, 2002; Fraschetti et al., 2005). Such small-scale variation could be more susceptible to local environmental influences such as areas of

* Corresponding author. Tel.: +6012 7148890; fax: +603 7967 4699.
E-mail address: cchhoonngg81@yahoo.com (C.W. Chong).

0038-0717/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2009.12.009

increased substrate availability (Horner-Devine et al., 2004). Bailey and Wynn-Williams (1982) reported that organic content (loss on ignition), total N, and water content showed significant direct correlations with microbial counts from soil at 6 locations on Signy Island, while pH showed an inverse relationship. However, Wynn-Williams (1990) proposed carbon as the limiting factor for cyanobacterial and algal colonization of frost-sorted soil polygons at Jane Col. In addition, recent culture-independent studies have also shown the direct influence of soil properties such as soil nutrients, moisture and pH on bacterial diversity (Barrett et al., 2006a,b; Aislabie et al., 2008, 2009) and these parameters also showed close relationship to specific functional genes such as glutamate dehydrogenase and nitrate reductase (Yergeau et al., 2007b). In the preliminary study soil microbial biodiversity on Signy Island (Chong et al., 2009a), pH, conductivity, copper and lead content were found to correlate most strongly with soil prokaryote biodiversity. In addition, substantial overlap was observed across sites visibly affected by seals, penguins and the presence of vegetation. Here, we examine further the links between soil prokaryote biodiversity and a range of biological, disturbance and soil chemistry factors across a larger set of 15 disparate locations on Signy Island.

2. Materials and methods

2.1. Study sites

A general description of the physical and biological environment of Signy Island (60° 43′S 45° 36′W) is given by Smith (1990), and placed within the context of soil microbiological studies by Chong et al. (2009a). The soils of the island are predominantly gelisols, with a prevalence of psammoturbels, haplorthels, haploturbels, and psammorthels. In addition, histoturbels, historthels, and fibristels are present in low altitude areas, especially along the island's western coast (Guglielmin et al., 2008). The vegetation of the island, typical of the maritime Antarctic, is predominantly cryptogramic (Smith, 1990; Bokhorst et al., 2007; Guglielmin et al., 2008).

We investigated the dominant soil bacteria community structure and soil chemical profiles from 15 locations across the island (Table S1; Fig. 1), specifically targeting sites expected to maximize spatial heterogeneity. Each location was visually inspected for potential human disturbance and classified according to one of the four major environmental influences that govern soil type on Signy Island: (a) association with vertebrate activity such as penguin rookeries and seal wallows (vertebrates) (Gourlay Peninsula, North Point, Cummings Cove, Elephant Flats, Cemetery Flats 1 & 2); (b) presence of well-developed vegetation (vegetated) (Deschampsia Point, Berntsen Point) or (c) absence (barren) (Skua Terrace, Jane Col, Knob Lake, Pumphouse, Signy Station); (d) close proximity to the coast with low visible animal influence (shore) (Cummings Shore, Factory Shore). Additionally, we considered the spatially restricted consequences of human occupation.

Signy Station (Fig. 1) currently operates only during the austral summer period, housing a maximum of 9 personnel, although previously it operated year-round between its establishment in 1944 and 1995, with a larger contingent of up to 25 personnel. Soils within a 5 m radius of the station buildings were considered to be subject to intense human activity. Samples were collected outside the main domestic building (Signy Station) and from the supralittoral area approximately 2 m in front of the station (Factory Shore). Three further sampling locations targeted areas of previously intense human activity. Berntsen Point was in the vicinity of the main buildings of the now-removed wintering Signy Station, Cemetery Flats was a location of whaling activities in the 1920s, and Pumphouse was the location of a whaling era water pumping station, around which corroded metal engine parts and traces of coal remain visible.

2.2. Soil sampling

Surface soil samples were collected during the austral summer between 10 December 2006 and 18 February 2007. At each location, six replicate samples of approximately 50 g were collected from the top 5 cm of the soil profile using sterile falcon tubes. Samples were kept at 4 °C prior to DNA extraction (within 24 h of collection) and frozen (−20 °C) at the earliest opportunity after DNA extraction.

2.3. Soil chemical analyses, DNA extractions and PCR amplifications

Soil chemical (carbon, hydrogen, nitrogen and heavy metal) contents and properties (water content, pH, salinity) were measured and DNA extractions and PCR amplifications were carried out as described by Chong et al., (2009a).

2.4. Denaturing gradient gel electrophoresis (DGGE)

DGGE profiles of dominant soil bacterial diversity were obtained using the D-Code Universal Mutation Detection System (Bio-Rad, USA) using electrophoretic conditions as described by Powell et al. (2005). In short, 45 μL of the secondary PCR products were loaded on a 6% acrylamide gel with a denaturing gradient of 35–60% (where 100% denaturant is 7 M urea and 40% formamide). Gels were pre-run at 80 V, 60 °C in 1× TAE for 30 min before the samples were loaded, and later at 80 V for 15 h. Gels were stained in 1:10,000 Sybergold in the dark for 60 min and then rinsed with distilled water prior to viewing on a UV transilluminator (Syngene Bio Imaging, UK).

2.5. Sequencing of DGGE bands

Dominant bands in the DGGE were excised using a sterile scalpel blade and incubated at 4 °C overnight in sterile distilled water before they were re-amplified using the secondary primers. The positions of the excised bands in the DGGE gel were confirmed by repeating the DGGE. Bands showing the expected melting position were amplified with the secondary primer without GC-clamp (341F, 907R). The PCR products were purified using PCR quick-spin™ PCR Product Purification Kit (iNtRON Biotechnology, Korea) and sequenced with ABI Big Dye Terminator V3.1 kit in ABI377-96 upgrade and ABI3100 Genetic Analyzer. Taxonomic identities of the partial 16S rRNA gene sequences were obtained using the Sequence Match search tool in the Ribosomal Database Project II (RDP)-Release 9 and BLAST search in the GenBank database. Genetic distance of the sequences within replicates of each location was analysed by MEGA 4 (Tamura et al., 2007) under the Jukes Cantor calculation model which averages the pairwise genetic distance of “unique” sequences (bands from different melting positions). The data were then used to compare the genetic variation between locations, with higher values representing greater taxonomic diversity.

2.6. Statistical analyses

Differences in soil chemical properties between locations and aggregated data were analysed using univariate and multivariate analysis of variance (ANOVA/MANOVA), and the Tukey HSD test for *post hoc* analysis.

The DGGE bands were detected and transformed into an absence/presence binary matrix using Quantity One 4.6.5 (Bio-Rad, USA). The detection was first automatically carried out by the software and subsequent addition or subtraction of bands was