Marine Pollution Bulletin xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Taxonomic profiling of bacterial community structure from coastal sediment of Alang–Sosiya shipbreaking yard near Bhavnagar, India

Vilas Patel [a], Hitendra Munot [b], Varun Shah [a], Yogesh S. Shouche [b], Datta Madamwar [a,*]

[a] Environmental Genomics and Proteomics Lab, BRD School of Biosciences, Vadtal Road, Satellite Campus, Post Box No. 39, Sardar Patel University, Vallabh Vidyanagar 388 120, Anand, Gujarat, India
[b] Molecular Biology Unit, National Center for Cell Science, Ganeshkhind, Pune 411 007, Maharashtra, India

ARTICLE INFO

Article history:
Received 7 August 2015
Received in revised form 14 September 2015
Accepted 4 October 2015
Available online xxxx

Keywords:
Alang–Sosiya shipbreaking yard
Bacterial diversity
16S rRNA gene
Proteobacteria

ABSTRACT

The Alang–Sosiya shipbreaking yard (ASSBY) is considered the largest of its kind in the world, and a major source of anthropogenic pollutants. The aim of this study was to investigate the impact of shipbreaking activities on the bacterial community structure with a combination of culture-dependent and culture-independent approaches. In the culture-dependent approach, 200 bacterial cultures were isolated and analyzed by molecular fingerprinting and 16S ribosomal RNA (r-RNA) gene sequencing, as well as being studied for degradation of polycyclic aromatic hydrocarbons (PAHs). In the culture-independent approach, operational taxonomic units (OTUs) were related to eight major phyla, of which Betaproteobacteria (especially *Acidovorax*) was predominantly found in the polluted sediments of ASSBY and Gammaproteobacteria in the pristine sediment sample. The statistical approaches showed a significant difference in the bacterial community structure between the pristine and polluted sediments. To the best of our knowledge, this is the first study investigating the effect of shipbreaking activity on the bacterial community structure of the coastal sediment at ASSBY.

1. Introduction

The shipping industry contributes >80% to international trade. Ships owned and used for trade by developed countries are often scrapped and disposed in developing countries such as India, China, and Bangladesh. The Alang–Sosiya shipbreaking industry is the largest of its kind, which is known as the graveyard of ships, where aging vessels are broken down by unskilled laborers and the scrap metal is then sold. This industry is located in India along the Gujarat coast of the Arabian Sea. However, the coastal area surrounding this industry is heavily polluted due to these shipbreaking activities. Many pollutants such as petroleum hydrocarbons (PHCs), heavy metals, chlorinated compounds, and others are released into the environment during these activities. Due to their hydrophobic nature, these pollutants sink and settle in the marine coastal sediment (Reddy et al., 2003). This has resulted in serious health problems of industrial workers and people in the surrounding slums. Therefore, bioremediation technologies must be developed to reduce the levels of anthropogenic pollutants and in turn their impact in the vicinity of such marine ecosystems. Marine microorganisms are fundamental to the maintenance of marine ecosystems. They are directly involved in the transformation of nutrients, photosynthesis, nitrogen fixation, methanogenesis, phosphate solubility, sulfate reduction, other biogeochemical cycles, bioprospecting, and bioremediation. Bioremediation is the most practical approach wherein microorganisms transform or degrade anthropogenic pollutants in the ecosystem. However, the implementation of effective bioremediation strategies relies on the dynamics, structure, and function of the innate microbial community (Desai et al., 2010). Depending on the biotic and abiotic factors, microorganisms adapt to the environment and accordingly that environmental conditions select microorganisms with specific capabilities.

Our perspective on microbial diversity in the environment is based primarily on studies of pure cultures in the laboratory. However, >99% of microbes in the environment cannot be cultivated easily; therefore, most of these microbes have not been described and accessed using the culture-dependent approach (Handelsman, 2004). The advent of molecular technologies over the past few decades has led environmental microbiologists to identify microbial communities using culture-independent approaches. With both molecular and culture-based methods, information on the composition of microbial communities can be collected and pollutant-degrading microorganisms can be isolated for purposes of bioremediation. In many coastal environments, the bacterial community structure has changed due to anthropogenic pollutants as well as due to seasonal fluctuation (Paisse et al., 2008; Feng et al., 2009; Håvelsrud et al., 2012; VandeWalle et al., 2012; Powell et al., 2013; Sintes et al., 2013; Pinto et al., 2015).

The Alang–Sosiya shipbreaking yard (ASSBY) has received attention for the pollution of the sediment of the coastal region with PHCs, polycyclic aromatic hydrocarbons (PAHs), and heavy metals from

* Corresponding author.
E-mail addresses: patelva1986@gmail.com (V. Patel), datta_madamwar@yahoo.com (D. Madamwar).

http://dx.doi.org/10.1016/j.marpolbul.2015.10.003
0025-326X/

shipbreaking activities (Reddy et al., 2003, 2005; Basha et al., 2007). We have developed a PAH-degrading consortium (Patel et al., 2012, 2013), and Ghevariya et al. (2011) also isolated chrysene-degrading *Achromobacter* sp. from the sediment of ASSBY. In addition, we recently studied the effect of shipbreaking activities on the coastal water of ASSBY. To the best of our knowledge, the bacterial community structure at ASSBY has not been studied; however, this information is crucial in understanding the link between prokaryotic community composition and environmental variables and in developing knowledge-based strategies to accelerate the ecological repair process. The aim of the present study was to characterize the bacterial community structure in the coastal sediments of ASSBY as well as in the pristine sediment.

2. Materials and methods

2.1. Sample collection

The ASSBY (N 21′ 5° 21′29°; E 72′ 5° 72′15°) is situated in the Gulf of Cambay, Gujarat, on the western coast of India. The yard runs for about 14 km from north to south, encompassing a total area of approximately 67 km^2, bifurcated into two nearly parts by a small creek. As the southern part is known as Alang and the northern part as Sosiya, it is popularly known as "Alang–Sosiya shipbreaking yards." In the present study, samples were collected from six different plots (three from the southern part and three from the northern part of ASSBY) of the coastal sediment of ASSBY. Metagenomic DNA was extracted from each of these six samples. Furthermore, individual metagenomic DNA from three different samples (samples collected from the southern part of ASSBY) were pooled to obtain one DNA sample; similarly, the remaining three metagenomic DNA samples (samples collected from the northern part of ASSBY) were pooled to obtain another DNA sample. Finally, two different metagenomic DNA samples were obtained for analyzing the microbial community structure of polluted coastal sediment samples. To evaluate the effect of pollution on the bacterial community structure at Alang–Sosiya, pristine samples were collected from 10 km away (where no anthropogenic activities take place) and denoted as "DVPSD." The concentration of toxic metals and organic carbon in the sediment samples was determined using an ICP Optima-3300RL (Perkin Elmer, Norwalk, CT, USA) and a TOC-V analyzer (Shimadzu, Japan), respectively.

2.2. Bacterial community structure based on culture-dependent approach

2.2.1. Isolation of bacterial strains

The sediment samples were serially diluted and spread on (1) high-nutrient media (nutrient agar, Luria agar, Pikovskayas agar, A1 Agar, and plate count agar) and incubated for 2–5 days at 20, 30, and 37 °C and on (2) low-nutrient media (Zobell agar, seawater agar, soil extract agar, glycerol agar, and Reasonar's agar) and incubated for 15 days at 20, 30, and 37 °C. As the coastal environment was contaminated with anthropogenic pollutants, bacterial strains were also isolated on different media supplemented with PHCs (benzene, phenanthrene, and diesel fuel (0.1% w/v)) and salt (3% w/v). Microorganisms were also isolated on minimal media (Bushnell–Hass agar, BHA) containing PHCs (benzene and phenanthrene) as the sole carbon source. Bacteria isolated from BHA containing polycyclic aromatic hydrocarbons were also analyzed for PAH degradation (Patel et al., 2013)

2.2.2. Extraction of genomic DNA from isolates

DNA was extracted from freshly grown cultures using the standard phenol:chloroform method (Sambrook, 2000). The isolates were identified by 16S ribosomal RNA (rRNA) gene sequencing using the universal primer set 8F (5′-CCAGAGTTTGATCGTGGCTCAG-3′) and 1492R (5′-TTC TGC AGT CTA GAA GGA GGT GWT CCA GCC-3′) (Desai et al., 2009). All bacterial isolates were analyzed using ARDRA. Based on their ARDRA banding pattern, bacterial cultures with a unique banding pattern from all samples were selected and sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction Kit v 3.1 in an automated 3730xl DNA analyzer (Applied Biosystems Inc., USA).

2.3. Bacterial community structure using culture-independent approach

2.3.1. Extraction of metagenomic DNA from sediment samples

For metagenomic DNA, 50 g of each sediment sample was used for total DNA preparation using the protocol described by Patel et al. (2014) but with some modifications. In the present study, a pretreatment step with washing buffer (1 mM dithiothreitol (DTT) and 10 mM ethylenediaminetetraacetic acid (EDTA)] and an additional polyvinylpolypyrrolidone (PVPP) column purification step were performed. The DNA concentration was measured by means of a NanoDrop spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA) and analyzed by gel electrophoresis.

2.3.2. 16S rRNA clone library preparation

To characterize the bacterial community structure from all three samples, the 16S rRNA gene was amplified from metagenomic DNA using the universal primer set 8F (5′-CCAGAGTTTGATCGTGGCTCAG-3′) and 1492R (5′-TTC TGC AGT CTA GAA GGA GGT GWT CCA GCC-3′). The polymerase chain reaction (PCR) products were purified from the gel (NucleoSpin, Düren, Germany), ligated into a pGEM-T vector (Promega, Madison, WI, USA), and transformed into *Escherichia coli* DH5α competent cells. Plasmids from positive clones were isolated using the alkaline lysis method. A total of 1000 16S rRNA gene clones (~350 from each sample) were randomly selected and subjected to 16S rRNA gene sequencing using the Big Dyes Terminator v3.1 cycle sequencing kit (Applied Biosystems). The sequences from each of the clone libraries were compared with the current database of 16S rRNA gene sequences at the Ribosomal Database Project II (Cole et al., 2007).

2.3.3. Phylogenetic analysis

The sequences were assembled and contigs were obtained using ChromasPro software. Subsequently, the sequences were aligned using CLUSTAL *X*2 and edited manually using DAMBE to obtain an unambiguous sequence alignment. All sequences were checked for chimeric artifacts using the Mallard program (*E. coli* U000096 was used as the reference sequence) (Ashelford et al., 2006). Appropriate subsets of 16S rRNA gene sequences were selected and subjected to further phylogenetic analysis using DNADIST of Phylip (version 3.61). The number of operational taxonomic units (OTUs) (>97% similarity grouped together as one OTU) was obtained by the DOTUR program (version 1.53) using the furthest neighbor algorithm (Schloss and Handelsman, 2005). Representative sequences from each of the OTUs were retrieved and checked against the previously determined 16S rRNA gene from the RDPII release 10 version of the database, and these sequences were downloaded in the FASTA format. Phylogenies were constructed with the Molecular Evolutionary Genetics Analysis v3.0 program (Kumar et al., 2004) using the Kimura two-parameter model and the neighbor-joining algorithm. The significance of the branching order was determined using bootstrap analysis with 1000 resampled data sets.

In order to determine the significant differences between the sediment samples, multivariate principal component analysis (PCA) was conducted with quantitative data of 16S rRNA gene sequence phylotype distributions, using Paleontological Statistics software (PAST) version 2.17c (Hammer et al., 2001). The diversity indices and richness were determined, as described by Desai et al. (2009).

2.3.3.1. Sequences. The sequences have been submitted to NCBI. Accession numbers KF463336-KF463658 (DVASD_D); KF464664-KF464965 (DVBSD_D); and KF465315-KF465638 (DVPSD)]. Bacterial cultures from culture dependent study were also deposited to Microbial culture collection center having accession number MCC2367-MCC2449.

Please cite this article as: Patel, V., et al., Taxonomic profiling of bacterial community structure from coastal sediment of Alang–Sosiya shipbreaking yard near Bhavnagar, Ind, Marine Pollution Bulletin (2015), http://dx.doi.org/10.1016/j.marpolbul.2015.10.003