Available online at www.sciencedirect.com
ScienceDirect
Bioresource Technology 98 (2006) 761–768
BIORESOURCE TECHNOLOGY

Extraction of inhibitor-free metagenomic DNA from polluted sediments, compatible with molecular diversity analysis using adsorption and ion-exchange treatments

Chirayu Desai, Datta Madamwar *

Postgraduate Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar 388 120, Gujarat, India

Received 9 February 2006; received in revised form 1 April 2006; accepted 1 April 2006
Available online 27 June 2006

Abstract

PCR inhibitor-free metagenomic DNA of high quality and high yield was extracted from highly polluted sediments using a simple remediation strategy of adsorption and ion-exchange chromatography. Extraction procedure was optimized with series of steps, which involved gentle mechanical lysis, treatment with powdered activated charcoal (PAC) and ion-exchange chromatography with amberlite resin. Quality of the extracted DNA for molecular diversity analysis was tested by amplifying bacterial 16S rDNA (16S rRNA gene) with eubacterial specific universal primers (8f and 1492r), cloning of the amplified 16S rDNA and ARDRA (amplified rDNA restriction analysis) of the 16S rDNA clones. The presence of discrete differences in ARDRA banding profiles provided evidence for expediency of the DNA extraction protocol in molecular diversity studies. A comparison of the optimized protocol with commercial Ultraclean Soil DNA isolation kit suggested that method described in this report would be more efficient in removing metallic and organic inhibitors, from polluted sediment samples.

Keywords: Polluted sediments; Metagenomic DNA; Powdered activated charcoal (PAC); Ion-exchange; 16S rDNA; ARDRA

1. Introduction

It is well-established in microbial ecology that diversity in one gram of soil accounts for 10 billion microbes of possibly thousands of different species (Roselló-Mora and Amann, 2001). The soil metagenome accommodates 6000–10,000 *Escherichia coli* genomes in unperturbed organic soils, and 350–1500 genomes in perturbed or heavy metal polluted soils (Torsvik and Øvreas, 2002). To date, despite the existence of such microbial diversity, only 5% has been cultured and studied in the laboratory. Given the importance of microbes and their crucial role in ecosystem processes, there is a great need to develop metagenomic DNA extraction methods that will aid in identifying and isolating bacteria that have so far eluded culture because of lack of suitable cultivation methods and/or because they have entered a non-cultivable stage in their growth cycle and/or there is no knowledge of their presence in the ecosystem under investigation (Amann et al., 1995; Torsvik et al., 1990; Hugenholtz et al., 1998). A large number of methods have been published for the extraction of metagenomic DNA from unpolluted soils (Picard et al., 1992; Smalla et al., 1993; Holben, 1994; Zhou et al., 1996; Burgmann et al., 2001; Kauffmann et al., 2004; Bertrand et al., 2005). In all cases, humic acid has been identified as a major inhibitor of PCR and concentrations as low as 10 ng can inhibit the reaction (Tsai and Olson, 1992). Polyvinylpolypyrrolidone (PVPP) (Frostegard et al., 1999), hexadecyltrimethylammonium bromide (CTAB) (Cho et al., 1996), DNA excision from agarose gels (Zhou et al., 1996), cesium chloride centrifugations (Leff et al., 1995) and size exclusion spin columns (Tsai

* Corresponding author. Tel.: +91 2692 234402; fax: +91 2692 226865.
E-mail addresses: datta_madamwar@yahoo.com, energymethane@yahoo.com (D. Madamwar).

0960-8524/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2006.04.004

and Olson, 1992) have been used in metagenomic DNA extraction protocols to remove such inhibitors.

The metagenomic DNA of polluted environments is a potential genetic resource from which phylogenetic affiliation of uncultured bacterial species could be determined and their genetic potential can be tapped by identifying novel biocatalyst, xenobiotic and metal detoxifying genes with utility in bioremediation processes. Despite this, polluted ecosystems remain poorly studied, given the bottleneck of isolating inhibitor-free metagenomic DNA. In polluted sediments, humic and fulvic acids along with a large number of other organic pollutants, metal ions, and chemical impurities co-precipitate with metagenomic DNA and interfere in all the subsequent molecular diversity analysis (Fortin et al., 2004; Hinoue et al., 2004).

Consequently, the metagenomic DNA extraction protocols used in preparations from unpolluted environments are not as effective for polluted environments. In this paper, we describe a new protocol for the extraction of high quality, high yield and PCR inhibitor-free metagenomic DNA from polluted sediments. In our protocol, we have avoided the use of chelating agents which decrease DNA yields (Braid et al., 2003) but have instead used inert materials such as activated charcoal and amberlite resins which are efficient removers of metal ions, organics and humic acids. Activated charcoal has been shown to bind and remove resinous and colored compounds, which would otherwise co-purify with genomic DNA extracted from cotton, coffee, rubber tree, cassava and banana, and this treatment also increases the efficiency of RAPD amplification of recalcitrant plant DNA (Vroh et al., 1996). Powdered activated carbon (PAC) has a vast application in the removal of various contaminants due to its extraordinarily large surface area and pore volume that gives it a unique adsorption capacity (Baker et al., 1992). Humic acids have a metal binding capacity and have been reported to form complexes with Pb and Cu (Logan et al., 1997). Non-biodegradable organic substances such as humic acid, lignin sulfonate, tannic acid, arabic gum and other biodegradable organic substances have been removed by adsorption onto activated carbon from waste waters (Seo and Ohgaki, 2001). Heavy metals, namely Cu(II), Pb(II), Ni(II), Zn(II) and Cr(VI) have also been adsorbed onto hydrous activated carbon surface (Corapcioglu and Huang, 1987; Perez-Candela et al., 1995; Seco et al., 1997). Humic substances and their metal complexes have also been removed by bone char (Katsumata, 2004). Amberlite IRA-400 resin used here is a macroporous Styrene-DVB Gel based strong anion exchanger. Macroporous strong base resins have been reported to remove humic acids from waste waters (Brattebo et al., 1987). Amberlite ion-exchangers are known to absorb the metal cations like Zn^{2+} and Cd^{2+} under alkaline conditions, and metal oxyanions like CrO_4^{2-} under acidic pH. The aim of this work was to demonstrate the efficiency in recovering higher yields and inhibitor-free metagenomic DNA, which was compatible with PCR, cloning and ARDRA analysis, taking advantage of the gentle treatment regimes for adsorption of PCR inhibitors onto PAC and ion-exchange chromatography with amberlite-IRA 400 resin.

2. Methods

2.1. Analysis of texture and contaminants in the sediments

Sediments were collected from the contaminated discharge streams and landfill sites receiving toxic industrial discharges and solid wastes from several dyestuff and chemical industries of Vatva and Gorwa industrial estates, Gujarat State, India. Texture classification of four sediments from Gorwa and Vatva industrial estate is presented in (Table 1). For the analysis of contaminants the sediments were dried and total organic carbon (TOC) was determined by TOC-V analyzer (Shimadzu). Metals were analyzed from the sediments by Perkin–Elmer ICP Optima 3300RL and Pt–Co APHA Units/g of sediments were used to describe apparent colour, where 1 colour unit equaled 1 mg/l Pt as chloroplatinate ion, were determined using datalogging spectrophotometer HACH DR/2010 at 455 nm. Analysis of pollutants at the sampling sites is shown in (Table 2).

2.2. Optimization of suitable lysis and pretreatment procedure for metagenomic DNA extraction

Optimization of a suitable lysis and pretreatment procedure to extract high quality DNA from the sediments was performed by incorporating modified treatments using bead lysis (Smalla et al., 1993), sonication based lysis using an Ultrasonic Processor (Vibra Cell) with 30 pulses of 10 s each in a chilled ice bath (Picard et al., 1992), high lysozyme (4–15 mg/ml) concentrations (Tsai and Olson, 1991; Hinoue et al., 2004), removal of inhibitors on pretreatments with 0.05% Triton X-100 and successive prewashes with 5 mM EDTA (Fortin et al., 2004; Watson and Blackwell, 2000). All these pretreatments were then followed by SDS-based DNA extraction (Zhou et al., 1996), phenol: chloroform: isoamylalcohol treatments and precipitation by isopropanol and a final clean-up with sephadex G150. A combination of gentle bead-beating treatment followed by SDS/proteinase K based lysis in presence of 10% (w/v) sucrose and treatment with powdered activated charcoal efficient in removing color, metal ions, organic and humic substances and amberlite IRA 400 based anion-exchange chromatography to remove metal ions were subsequently chosen based on the contaminants present in the sediments.

Table 1
Analysis of texture of polluted sediments

Sediment sample	Soil texture (%)			Texture classification	pH
	Silt	Clay	Sand		
Gorwa Industrial Estate	10.25	6.75	75.35	Loamy sand	8.5
Vatva Industrial Estate	14.79	23.5	59.5	Sandy clay loam	7.5