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Comparative studies on the extraction of metagenomic DNA from the saline habitats of Coastal Gujarat and Sambhar Lake, Rajasthan (India) in prospect of molecular diversity and search for novel biocatalysts

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ABSTRACT

Extraction of total DNA from a given habitat assumes significance in metagenomics, due to the requirement of inhibitor free and high quality metagenome in good quantity for applications in molecular biology. DNA extraction and its quality assessment for PCR applications from saline soils of Coastal Gujarat and Sambhar Soda Lake, Rajasthan in India is described in a comparative manner. The mechanical and soft lysis methods were simple and efficient for rapid isolation of PCR amplifiable total genomic DNA. The results are significant as only few extreme environments, particularly saline habitats are explored for their metagenomic potential.

1. Introduction

Current estimates indicate that approximately 99% of the microorganisms in nature cannot be cultivated by standard techniques. Isolation and further applications of total genomic DNA (metagenomic DNA) from soil microorganisms without cultivation [1,2], is a recent approach in molecular biology [3–5]. Marine environment has enormous microbial biodiversity that remains largely unexplored. The metagenomic approaches have highlighted the population heterogeneity and phylogenetic status of a habitat, in entirety [6,7]. This novel approach has been further aided by bioinformatics based software for analyses and interpretations [8–10].

Among the key factors for the successful metagenomics, the isolation of quality environmental DNA in appreciable amount from a given habitat holds significance [10,11]. While the isolation of total DNA is highly significant, it remains as one of the bottlenecks in metagenomic studies [12,13], as the extracted DNA should be of high quality in good yield to pursue molecular biological applications [14–16].

During the last 10 years, number of protocols for DNA extraction from environmental sample is reported [17–19]. Some commercial soil DNA extraction kits are also available [20–21]. These kits and most of the published methods have improved the original Direct DNA extraction procedures mainly in terms of DNA yield and ease of application [22–24]. These protocols are broadly classified as direct and indirect methods. The variability among the methods is viewed on account of the degree of shearing, purity and yield of DNA [25].

Direct DNA isolation methods involve cell lysis within the sample matrix followed by the separation of DNA from cell debris [4,20]. However, in indirect methods, cells are extracted from the environmental sample before lytic release of DNA [14]. Direct DNA extraction protocols involve soft and harsh lysis methods. Soft lysis is based on the disruption of microorganism by enzymatic and chemical means. The enzymatic lysis treatment relies on enzymatic digestion of microbial cells to release DNA [22,23]. The treatment of soil with surfactants and chelating agents resulted into inhibitors free high quality DNA in good quantity, where as harsh lysis approaches involve the mechanical cell disruption by bead beating, sonication, freeze-thawing and grinding. The indirect DNA extraction protocols are based on blending and cation-exchange [26].

Standardization of total DNA extraction technique is desirable as the composition of different habitats varies with respect to their matrix, organic and inorganic compounds and biotic factors [27–29]. Improved DNA extraction techniques should also ensure a metagenomic library adequately representing the entire community's genome without inhibitory substances [30–32].

As an extension of our on-going research on haloalkaliphilic bacteria form Coastal Gujarat [33–35], the present study aims at the optimization, assessment and comparison of the extraction methods for total environmental DNA from two different habitats; saline soil near salt pan of Okha Madhi, Gujarat Coast and Samb-

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har Soda Lake, Rajasthan, both in India. The protocols described in the present report signify the extraction of DNA from different saline habitats without any prior treatment. The methods proved wide amenability of the extracted DNA for further molecular biology applications, such as capturing functional genes from the total habitat and assessment of diversity and phylogeny in entirety.

2. Materials and methods

2.1. Environmental soil sampling and storage

Two soil samples, designated as Ok.M.3.6 and Ok.M.3.7 were collected from Coastal region of Okha Madhi (Latitude 22.20 N, Longitude 70.05 E) Gujarat and SL1.1 from Sambhar Lake (Rajasthan), India. They represent a typical saline soil with heavy deposition of salt with pH; 9–11. From the site of collection, a block of soil was removed and transported to laboratory in sterile plastic bags for storage at 4 °C. Total DNA extraction and further analyses were carried out from these samples within 7 days.

2.2. Direct DNA extraction methods

2.2.1. Soft lysis method

DNA extraction using lysis buffer

Soil samples (1 g) in duplicate were suspended in 10 ml of extraction buffer (100 mM Tris–HCl (pH 8.2); 100 mM EDTA (pH 8); 1.5 M NaCl) and incubated at 37 °C for 10–12 h under shaking at 150 rpm. Samples were re-extracted in 1 ml of extraction buffer and supernatant were collected by low speed centrifugation (5000 rpm) for 10 min. A 4 ml of Lysis buffer (20%, w/v) SDS; Lysozyme 20 mg/ml; ProtinaseK10 mg/ml; N-lauroyl sarcosine 10 mg/ml; 1% (w/v) CTAB (cetyltrimethylammonium bromide) was added and incubated at 65 °C for 2 h with vigorous shaking at every 15 min. Samples were centrifuged at 10,000 rpm for 10 min at 4 °C. The upper aqueous phase was extracted with equal volume of P:C:I phenol:chloroform:isoamylalcohol (25:24:1) at 1000 rpm for 20 min at 4 °C. Upper aqueous phase was again extracted with equal volume of chloroform:isoamylalcohol (C:I) (24:1) at 10,000 rpm for 10 min at 4 °C. DNA preparation was further treated by adding 1/10 volume of 7.5 M potassium acetate and subsequently precipitated by adding 2 times of chilled ethanol. DNA precipitates were collected by centrifugation at 10,000 rpm for 10 min, air dried and suspended in 20–50 μl sterile D/W.

2.2.2. Harsh lysis method

A. DNA extraction using bead beating method

Soil samples (1 g) in duplicate were suspended in 10 ml of extraction buffer and incubated at 37 °C for 10–12 h with shaking at 150 rpm. Samples were re-extracted in 1 ml of extraction buffer and supernatants were collected by low speed centrifugation (5000 rpm) for 10 min. To the supernatants, glass beads (1 g) were added and the sample blended for 15 min followed by incubation at 65 °C for 2 h. Samples were then centrifuged at 10,000 rpm for 10 min at 4 °C. Further steps of the extractions were then continued as per soft lysis method.

B. DNA extraction by sonication treatment

Soil samples (1 g) in duplicate were suspended in 10 ml of extraction buffer and incubated at 37 °C for 10–12 h with shaking at 150 rpm. Samples were re-extracted in 1 ml of extraction buffer and the supernatants were collected by low speed centrifugation (5000 rpm) for 10 min. The supernatants were sonicated using a high intensity ultrasonic processor (Sartorious) with a standard 13 mm horn solid probe for 3 pulses of 30 s each in a chilled ice bath. The sample was cooled in ice and repeatedly sonicated (6 cycles of 30 s) followed by incubation at 65 °C for 10 min. Samples were then centrifuged at 10,000 rpm for 10 min at 4 °C. Further extraction was continued as per soft lysis method described above.

2.2.3. DNA extraction by combination of soft and harsh method

A. DNA extraction using bead beating combined with lysis buffer treatment

Soil samples (1 g) in duplicate were suspended in 10 ml of extraction buffer and incubated at 37 °C for 10–12 h with shaking at 150 rpm. Samples were re-extracted in 1 ml of extraction buffer and supernatant were collected by low speed centrifugation (5000 rpm) for 10 min. Glass beads (1 g) were added and the sample blended for 15 min. A 4 ml of lysis buffer was added and incubated at 65 °C for 2 h with vigorous intermittent shaking at 15 min intervals. Samples were centrifuged at 10,000 rpm for 10 min at 4 °C. Extraction was then continued as per soft lysis method.

B. DNA extraction by a combination of bead beating with sonication treatment

Soil samples (1 g) in duplicate were suspended in 10 ml of extraction buffer and incubated at 37 °C for 10–12 h with shaking at 150 rpm. Samples were re-extracted in 1 ml of extraction buffer and supernatants were collected by low speed centrifugation (5000 rpm; 10 min). The preparations were then blended with glass beads (1 g) for 15 min followed by sonication using a high intensity ultrasonic processor (Sartorius) with a standard 13 mm horn solid probe for 3 pulses of 30 s each in a chilled ice bath. The sample was cooled in ice and sonicated repeated (6 cycles of 30 s) followed by incubation at 65 °C for 10 min. The preparations were centrifuged at 10,000 rpm for 10 min at 4 °C. The upper aqueous phase was extracted with equal volume of P:C:I (25:24:1) at 10,000 rpm for 20 min at 4 °C. Further extraction was continued as per soft lysis method.

C. DNA extraction using sonication treatment combined with lysis buffer

Soil samples (1 g) in duplicate were suspended in 10 ml of extraction buffer and incubated at 37 °C for 10–12 h with shaking at 150 rpm. Samples were re-extracted in 1 ml of extraction buffer and supernatant were collected by low speed centrifugation (5000 rpm) for 10 min. The supernatants were sonicated using an ultrasonic processor with similar conditions as described in above methods. Extraction was further continued from lysis buffer treatment as per soft lysis method.

2.3. Determination of purity and yield of DNA

Co-extracted humic acids are the major contaminant when DNA is extracted from soil. These compounds absorbs at 230 nm, DNA at 260 and protein at 280 nm. To evaluate the purity of the extracted environmental DNA (eDNA), absorbance ratios at 260 nm/230 nm (DNA/humic acid) and 260 nm/280 nm (DNA/protein) were determined. A high A_{260} to A_{230} ratio of greater than 2 indicates purity of DNA.

2.4. Gel electrophoresis

DNA extracts (10 μl) from each method were mixed with 5 μl loading buffer and analyzed on 0.8% agarose gels using TAE as electrophoresis buffer. Gels were stained with ethidium bromide and analyzed by syngene gene genius Bio-imaging system. A DNA marker of middle range from Banglo Genei, India was included in each run.