Direct Extraction of Microbial DNA from Wastewater using Optimized Treatment Method

RWPM Rajapaksha and K Vivehananthan
Department of Biotechnology, Faculty of Agriculture and Plantation Management,
Wayamba University of Sri Lanka, Makandura, Gonawila (NWP), Sri Lanka

Abstract

Microbes that can be cultivated under standard laboratory conditions were detected as limited to only 1-4%. Culture independent direct microbial DNA extraction methods from environmental samples were identified as effective in unbiased microbial diversity analysis. A DNA extraction method using two different treatments including the supplementation of different aeration periods (5h, 10h, 12h, 14h and 20h) together with different glucose concentrations (5g/l, 10g/l, 15g/l and 20g/l) was tested on wastewater taken from five contaminated environments. The DNA extraction protocol was also optimized by adjusting the speed and time period of centrifugation facilitating the formation of the pellet. Extracted genomic DNA was evaluated using agarose gel electrophoresis and PCR amplification of 16S ribosomal RNA gene. It was identified that the optimized DNA extraction protocol supplied high quality DNA of sufficient quantity. The absence of differential amplifications, artificial PCR products and lack of problems such as inhibition of PCR amplification by co-extracted contaminants indicated the quality of DNA. Supplementation of glucose along with aeration increased the DNA yield and optimum conditions were identified as 12hr aeration with 10g/L glucose. The developed method could be effectively used in DNA extractions from different environmental samples for the detection of diversity of microbial species.

Key words: DNA extraction, 16S rRNA, Wastewater

Introduction

Interest in microbial diversity analysis has been catalyzed by the rapid advancement of molecular ecological methodologies. It has been observed that over the last decade, important advances in molecular biology led to the development of culture independent approaches to describing bacterial communities (Ranjard et al. 2000) and molecular approaches are now being used to characterize the nucleic acids of microorganisms contained in different environmental samples.

Microbial diversity studies conducted in complex ecosystems such as soil and sewage have often been found to be biased and it was identified that the reason was the inability to culture many microorganisms (Chaudhuri et al. 2006). Also, the reports indicated that only 1-4% of the microbes can be cultivated under standard laboratory conditions (Chaudhuri et al. 2006). The enrichment media used to culture microbes in the laboratory were identified as inherently selective and only a subpopulation of the microbes in an environmental sample will grow on any given medium. The analysis using incubation in the laboratory are indirect and produce artificial changes in the microbial community structure and metabolic activity, similarly, the growth of the microbial in culture media can be inhibited by antibiotic pretreatment and complex microbial nutrition requirements (Annon 2012). Therefore, using traditional culture techniques to measure the biodiversity of an environmental sample will underestimate species richness and skew measurements of species evenness (Annon 2012). However, molecular identification was detected as filling the dangerous gap of culture-negative microbials and, molecular technologies that enable the verification of broad range of microbial strain from environmental specimens are demanded.

Materials and Methods

Wastewater samples collected from a Rubber latex producing factory, stagnant water pool containing organic waste, Kelani River, Kandy Lake, coconut milling factory and a vehicle service station were treated with *different concentrations of glucose* (5 g/l, 10 g/l, 15 g/l and 20 g/l) *and different aeration time periods* (5 h, 10 h, 12 h, 14 h and 20 h).

Bacterial cells in the wastewater were collected by centrifugation 15 min at 13,000 rpm at 4°C and this step was repeated four times to concentrate the pellet (Ekanayake et al., 2010). The pellet was washed twice using the wash buffer (50 mM Tris HCl & 5 mM EDTA of pH 8.0) and re-suspended in lysis buffer (100 mM Tris HCl, 100 mM EDTA of pH 8.0 & 1.5 M NaCl). The suspension was centrifuged for 15 min at 13 000 rpm at 4°C and NaOAC and ice-cold isopropanol were added to the supernatant along the wall of the tube. It was centrifuged for 15 min at 13,000rpm at 4°C. The pellet was washed with 70% ethanol and re-centrifuged at 13,000 rpm for 10 min at 4°C. Ethanol was completely removed by air drying. The DNA was re-suspended in

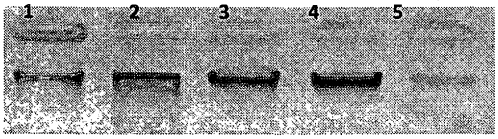

Figure 1. Agarose gel electrophoresis of the DNA samples extracted from Rubber effluent aerated at different times.
Lane 1: Wastewater, Lane 2: Wastewater aerated 5 hr, Lane 3: Wastewater aerated 10 hr, Lane 4: Wastewater aerated 12 hr, Lane 5: Wastewater aerated 20 hr.

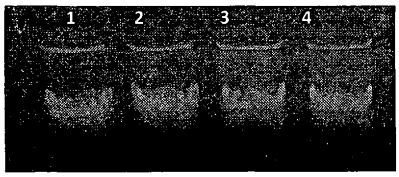

Figure 2. Agarose gel electrophoresis of the DNA samples extracted from Rubber effluent treated with different concentrations of glucose with 12 hr aeration.

Lane 1: Wastewater, 5 g/l glucose, Lane 2: Wastewater, 10 g/l glucose, Lane 3: Wastewater, 15 g/l glucose, Lane 4: Wastewater, 20

de-ionized water and each extracted genomic DNA sample was subjected to electrophoresis on a 0.8% agarose gel. The microbial DNA extracted from different wastewater samples was subjected to PCR, to amplify the 1484 bp fragment of the 16S rRNA gene using a set of universal primers, Amplified PCR products were first subjected to electrophoresis using a 1% agarose gel. DGGE method was then used for the separation of amplified PCR fragments of 16S rRNA region of microbial populations where the technique was used to separate the same length PCR amplified fragments of 16S rDNA according to the GC content.

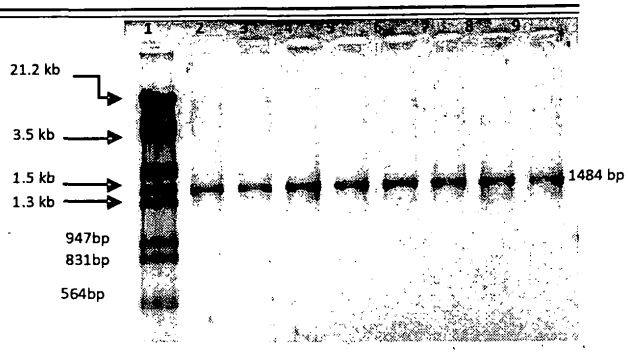

Figure 3 . Agarose gel electrophoresis of PCR products using 16S rDNA universal primers.

Results and Discussion

DNA Extraction: The optimized DNA extraction protocol gave sufficient amount of DNA from the samples collected from various contaminated sites (Fig 1). The yield was significantly higher in the aerated wastewater samples (150-200ng/μl) than the wastewater alone (75ng/μl), where the yield of DNA varied with the aeration time. The optimum aeration time was found to be 12h as the yield increment rate was not much significant with further increased aeration time. Supplementation of glucose along with aeration increased the quantity of DNA comparatively than the aerated samples (>500ng/μl). However, there were no significant differences in DNA yield among the samples treated with different concentrations of glucose (Fig 2). The DNA yields obtained from a series of treatment protocols indicated that the optimum treatment to obtain a higher yield of microbial DNA was found to be aeration of 12h with the glucose supplementation of 10g/l. The optimized protocol was used for extraction of DNA from all wastewater samples for further analysis.

PCR Amplification of 16S rRNA gene: PCR amplification of 16S rRNA gene using the 16S rRNA universal primers confirmed the presence of the expected band of 1484 bp length (Fig 3). The DNA extracted from all five wastewater samples had given the expected bands with good quantity. Differential amplifications and artifactual PCR products were not observed. The lack of problems such as inhibition of PCR amplification by co-extracted contaminants indicated the quality of the extracted DNA.

Conclusion

The best treatment for DNA extraction found to be 12h aeration supplemented with glucose, 10g/l and the method could yield high quality DNA with considerable quantity.

References

Anon 2012 researches identify non culturable microbes from blood stream infections. Accessed on 03.08.2012:http://www.molzym.com/products/dna-isolation-products/42-news-

Chaudhuri SR, Pattanayak AK and Thakur AR 2006 Microbial DNA extraction from samples of varied origin. Current Science, 91 (12), 1697-1700.

Ekanayake WMJY, Vivehananthan K and Wickramaarachchi T 2010 Identification & classification of xenobiotic degrading bacteria in different waste types. In proceedings of 10th Agriculture Research Symposium. 12th-13th August, 2010. Makandura, Wayamba University of Sri Lanka, 65-69.

Ranchard L, Franck P and Nazaret 2000 Mini review, Monitoring complex bacterial communities using culture independent molecular techniques: Application to soil environment. Res. Microbiol.151. 167-17