6th International Conference on Multidisciplinary Research (ICMR-2019)
Osmania University Campus, Hyderabad (India)
30th-31st May 2019 www.conferenceworld.in
ISBN : 978-93-87793-89-7

Role of Amplified rDNA Restriction Analysis (ARDRA) in the study of the Diversity Analysis of the Microbial Coterie in Wastewater

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Abstract

Activated sludge, a common biological treatment technique for both municipal and industrial waste water, symbolizes an intricate microbial community. Owing to complex interactions within the microbial community, process control of waste water treatment plants can be hard. Population changes within the microbial community may results from the variations in the plant operating conditions and lead to sludge quality problems for example poor sludge settling, compaction and dewatering. Supervising of the microbial populations may aid in the diagnosis and rectification of such sludge problems. In the study, a PCR-based 16S rDNA, amplified rDNA restriction analysis (ARDRA) method was used to illustrate the microbial community structure in waste water.Inoculum or seed sludge was collected from sewage treatment plant (Full scale 72 MLD UASBR), Indrapuram, Ghaziabad, City, India. Each PCR product was acquired by PCR with eubacteria 16S rDNA. After amplification, the 16S rDNA PCR products were digested with 4-base site specific restriction endonucleases. Restriction pattern was examined with four endonucleases (AluI, MspI, HhaI, and HaeIII). The result of the bacterial community examination, by ARDRA showed that the two wastewater treatment plants carry considerably different microbial population; however the diversity among the samples of same plant is not much. These results recommended that Amplified rDNA restriction analysis (ARDRA) is a tremendously valuable tool for evaluating the diversity from waste water treatment plants.

Key words: ARDRA, Microbial coterie, Wastewater

1. Introduction

Biological treatment with activated sludge is the most common and appropriate technology for the wastewater treatment process. Activated sludge uses micro organisms to break down organic material with

aeration and agitation. Even though several microorganisms are usually found in different waste water treatment plants, differences in microbial community have been described. Hence, it is vital to examine the microbial community present in specific wastewater treatment plants. Examination of the structure and function of activated sludge microbial communities could result in identification of the microbial wastewater composition, wastewater treatment plant (WWTP) operation, or alterations to be done in the activated sludge.

The arrival of molecular tools has been evidenced extremely valuable in evaluating the variations in microbial community structure in intricate environmental samples. Traditionally, the detection of pathogens in water, wastewater, and other environmental samples is controlled by the capability to culture such organisms. The application of molecular techniques to the study of natural and engineered environmental systems has augmented our insight into the immense diversity and interaction of microorganisms existing in complex environments. Of the numerous methodologies for the understandings of microbial community structures in nature, comparative analysis of 16S rRNA sequence of microorganisms has been generally applied, because of the ubiquity of ribosomal RNA molecules in all microorganisms, to conclude relationships among organisms (Pederson *et al*., 1996; Wise *et al*., 1999; Lee *et al*., 2000). The rRNA molecules are encompassed with highly conserved sequence domains, spread with more adjustable regions. In general, the crucialrRNA domains are preserved across all the phylogenetic domains, thus general tracts of sequences can be recognized (Olsen *et al*., 1986). Amplified ribosomal DNA restriction examination (ARDRA) is a simple method based on restriction endonuclease digestion of the amplified bacterial 16S rDNA. As, ARDRA utilizes universal 16S rRNA gene primers, it is probable to be applicable to the identification of most bacterial species from any kind of environmental sample. ARDRA identifies inter species and inter strain as well as inter operations

Variability and allows a relatively fast multiple strain evaluation (Heyndrickx et al. 1996). This technique is suitable to obtain indicative phylogenetic and taxonomic information. Hence, ARDRA can be labeled as a common approach for a rapid molecular characterization based on the generation of so- called “genetic fingerprints”.

ARDRA method has been effectively tested to identify alterations in activated sludge bacterial communities fed on domestic or industrial wastewater, and subject to different operational conditions (Gich et al. 2000). The aim of this study was to develop comparable approach to evaluate the practicability of the method in wastewater systems and to identify the differences in microbial in Inoculum or seed sludge was collected from sewage treatment plant (Full scale 72 MLD UASBR), Indrapuram,

Ghaziabad.

Materials and Methods

Sample collection

Two samples were selected from each site; influent water (water entering the system) and activated sludge samples (combination of raw sewage and microorganisms. Sterile bottles were used to collect the samples and stored in the dark at 4°C until used (1–2 days).

DNA isolation

DNA isolation was performed by using Potassium Ethyl Xanthogenate: 1 ml volume of homogenous cell culture was pelleted and suspended in freshly made Xs buffer (1% Potassium ethyl Xanthogenate, 100 mMTrisHCl, pH-7.4,20mMEDTA,pH-8.0,1%SDS,800
mM Ammonium Acetate). Pellet was incubated at 65°C for 2 h, mixed and then incubated on ice for 30 min. The mixture was centrifuged for 10 m at 10,000 rpm. The supernatant was taken to which 1 volume of 100% isopropanol was added. The DNA was precipitated and pelleted, and washed with 70% ethanol. Finally the pellet was resuspended in TE buffer pH-7.4, Tillett & Neilan(2000).

16S rDNA amplification

PCR amplification was performed to obtain a
1.5 kb fragment of 16S rRNA gene. Reaction volumewas25µlwith50ngofextractedDNA,
200 µM of each dNTPs, 1 U Taq polymerase (Bangalore Genei), 10X Taq buffer and 1.5 mM $MgCl_2$, both supplied with the enzyme, and 20 pmol of each primer: forward 5'-GAGTTGGATCCTGGCTCAG -3' and reverse 5'-AAGGAGGGGATCCAGCC-3'. The PCR parameters were 5 min initial denaturation at 94°C followed by 30 cycles of 1 min denaturation at 94°C, 45 s annealing at 65°C, and 1 min extension at 72°C, finishing with 7 min extension at 72°C. PCR products were electrophoresed in 1.5 % agarose gel for 1 h at 60V.

Amplified ribosomal DNA restriction analysis (ARDRA)

Four limiting enzymes were used for the restriction digestion of the amplified DNA samples. *AluI, HaeIII, HhaI*and *MspI*were used. *HaeIII, HhaI*and *MspI*were used in single digestion. However*AluI+ MspI*were used for double digestion. The protocol was standardized for restriction digestion to acquire the best possible results, for all the enzymes for their highest efficiency. The incubation of the reaction mixture was performed in the PCR Thermal Cycler at 37°C for 4 h. Table 1. Shows the optimized conditions for each enzyme reaction. After the incubation the samples were electrophoresed on 2% agarose gel at 50V for 1h.

ISBN : 978-93-87793-89-7

Data analysis

The patterns of each sample were compared by identifying, from different samples, fragments of identical size in the same digestion. Pair wise comparison of the band pattern was manually performed, and a presence/absence matrix was constructed. NTSYS software was used to organize summaries of relationships using cluster analysis to get the phylogenetic tree.

2. Results and Discussion

Activated sludge systems are extensively used as a method of biological wastewater treatment. Microbial population existing in the activated sludge can significantly affect the treatment of the waste. Hence, it is tremendously crucial to comprehend the structure of the microbial community. In the last decade, a set of molecular tools have been developed and applied for the examination of the microbial community composition and dynamics in activated sludge systems, in both cultivation dependent and independent manners (Pike and Carrington, 1972; Wagner et al., 1993; Juretschko et al., 2002). ARDRA is a prompt, precise and trust worthy procedure to evaluate the microbial diversity. The band pattern attained specifies the structure of the community present in the environmental system. The results notice ably showed differences in the microbial community composition amongst the activated sludge systems studied. Considerable differences have been detected between the restriction patterns of the two waste water treatment plants. All the enzymes used for the digestions indicated the difference in the banding pattern of the two plants. But, not much difference was observed among the samples from the two sites except for the restriction pattern obtained from *HhaI*(Fig 2). This enzyme indicated

very different patterns for sample 1-1 and sample 1- 2 where as a very similar patterns are observed for sample 2-1 and sample 2-2 except the lack of some bands in sample 2-1. The nonexistence of differences between the patterns does not safeguard that the composition of the communities is exactly the same. Yet, substantial composition changes in the community should be noticed with the restriction enzymes used (Moyer et al. 1996). The phylogenetic tree (Fig 4) represent the analogous results that are indicative of the fact that there is difference between the microbial community composition of sample 1-1 and sample 1-2 that is observed in the distribution of the nodes for both the samples at a point however the microbial community composition of sample 2-1 and sample 2-2 are alike. However, the two plants carry different microbial populations as both the samples from the two plants branched out. Restriction analysis from *HaeIII* display almost akin patterns for sample 1-1 and sample 1-2 except one band (Fig 2.). This shows that most of the bacterial population is similar in the two samples, except for one community that is characterized by that single band in 1-1. The restriction patterns were very analogous in sample 2-1 and

ISBN : 978-93-87793-89-7

sample 2-2. The phylogenetic tree (Fig 5) suggests that similar microbial biota exist in sample 2-1 and sample 2-2. The phylogenetic tree shows a complete contrast among the sample 1-1 and sample 1-2 as a completely different node represents sample 1-1, this suggests that there is a deviation in the microbial population among these two samples. There is a slight difference observed among sample 1-2, sample 2-1 and sample 2-2 but the distance from the node of sample 1-1 and the rest of the samples display that the microbial community composition of sample 1- 1 is very different from rest of the samples. Also, the intensity of the band present in sample 1-1 recommends the dominancy of that microbial population existing in the sample. Very remarkable results were detected in the restriction patterns that were attained after the restriction digestion of the samples by enzyme *MspI*(Fig 2). The result illustrate that the sample 1-1 and sample 1-2 share similar microbial biota as shown by the similar restriction patterns for both the samples. Similarly the identical restriction patterns show the correspondence of the microbial community of sample 2-1 and sample 2-2. The phylogenetic tree attained equivalent to the restriction bands validate the interpretation that sample 1-1 and sample 1-2 have similar bacterial community and also that the sample 2-1 and sample 2-2 have similar community composition. Also the similarity amongst sample 1-1, sample 1-2 sample 2-1 and sample 2-2 can be observed. The origination and the no difference in the distance among the samples from two The restriction patterns attained after restriction digestion of the samples by restriction enzyme *Msp I* + *Alu I* clearly illustrates the difference between the populations in the two plants. However, in accordance to the band patterns the results of the phylogenetic tree (Fig 6) attained by the comparison amid the samples display that sample 1-1 and sample 1-2 have similar microbial biota as well the sample 2-1 and sample 2-2 have identical bacterial community. The cluster examination also characterize that there is resemblance amongst the bacterial community composition of sample 1-1, sample 1-2, sample 2-1 and sample 2-2. Previous works established that double restriction endonuclease digestions are sensitive enough to identify important composition variations in the community (Acinas et al. 1997; Martínez-Murcia et al. 1995; Moyer et al. 1996). In their study, the nonexistence of differences between the patterns of the among the samples from the same site resulted in the conclusion that there were probably no substantial vicissitudes among the microbial communities , but, in this study clear alterations were attained among the samples from two different sites. This advises that the two wastewater treatment plants vary in their microbial population. ARDRA has been used formerly to evaluate the microbial diversity. Gich et al. presented the difference among the industrial and domestic wastewater treatment plant communities by using ARDRA. They assessed the appropriateness of this technique to identify differences in activated sludge bacterial communities fed on domestic or industrial wastewater, and subject to different operational conditions. In their study the alterations in the community structure as a result of influent characteristics and temperature were observed, however, no differences were observed between the oxic and anoxic reactors of each of the three MLE configurations. Alike

conclusions were drawn by Ehlers and Cloete (1999). They used protein fingerprints to assess the modifications among the microbial community structures among P-removing, non-P-removing and N-removing systems. The similarity of endonuclease restriction patterns between the samples agrees with the high similarity of protein fingerprints in bacterial communities of different activated sludge systems. Their study showed no change in the community, which they elucidated as given the residence times and the internal recycle values of the systems studied, the generation times of the microorganisms are perhaps too long to observes substantial variances in community composition between the anoxic and oxic reactors. This suggests that examination of the microbial community structure is vital for understanding the role of microorganisms in relation to the treatment processes that arise within wastewater treatment plants.

Figure 1. PCR products of extracted DNA by a 16S

rDNA primer

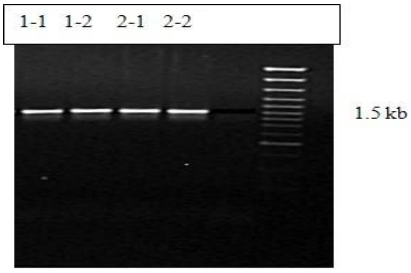


Figure 2. Restriction pattern of PCR-amplified fragment of 16S rDNA genes digested with *Msp*I and *Hae*III, and *HhaI*. 1-1 for influent water, 1-2 for activated sludge site 1; 2-1 for influent water, and 2-2 for activated sludge site 2

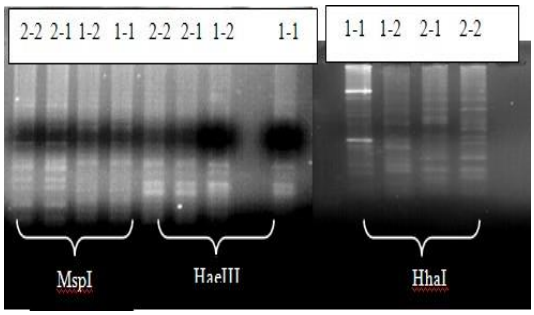


Figure 3. Restriction pattern of PCR-amplified fragment of 16S rDNA genes digested with *Alu*I and *Msp*I. 1-1 for influent water, 1-2 for activated sludge site 1; 2-1 for
Influent water, and 2-2 for activated sludge site 2

ISBN : 978-93-87793-89-7

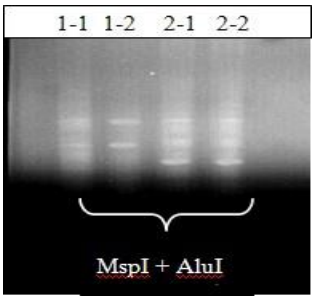


Figure 4.Dendrogram of genetic similarity matrix value of 16S rDNA genotypes analyzed by PCR-ARDRA using enzyme *HhaI*. 1-1 for influent water site 1, 1-2 for activated sludge site 1; 2-1 for influent water, and 2-2 for activated sludge site 2

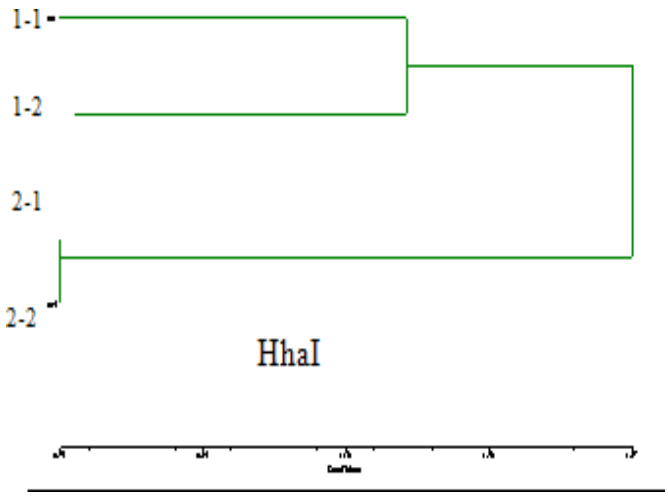


Figure 5.Dendrogram of genetic similarity matrix value of 16S rDNA genotypes analyzed by PCR-ARDRA using enzyme *HaeIII*. 1-1 for influent water, 1-2 for activated sludge site 1; 2-1 for influent water, and 2-2 for activated sludge site 2

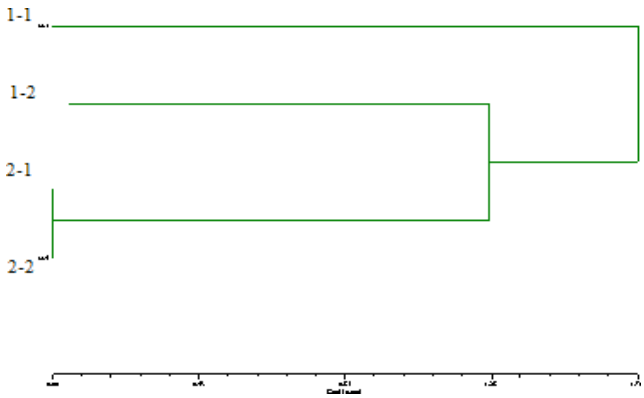
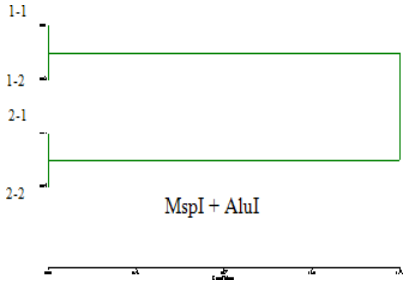


Figure 6.Dendrogram of genetic similarity matrix value of 16S rDNA genotypes analyzed by PCR-ARDRA using double digestion with enzymes *AluI*and *MspI*.1-1 for influent water, 1-2 for activated sludge site 1; 2-1 for influent water, and 2-2 for activated sludge site 2

ISBN : 978-93-87793-89-7



3. Conclusion

The results specified that the sewage treatment plant (Full scale 72 MLD UASBR), Indrapuram, Ghaziabad share some common microbial population but, the total microbial community is equitably different. The samples from the same treatment plant were alike in community structure. In spite of the limited sampling, the study notice ably discovered the broad diversity of bacteria involved in two plants. In order to better classify the microbial populations of the two plants, more examinations are required. But, in conclusion, we can understand that ARDRA is a potent molecular biology tool to identify differences between activated sludge communities and to examine the microbial diversity in wastewater treatment plants.

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