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Research article

PCR BASED IDENTIFICATION OF MICROBIAL COMMUNITY IN USAGE WATER OF SLUM AREAS IN HYDERABAD

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ABSTRACT: Due to rapid urbanization in a context of economic constraints, the majority of urban residents in Hyderabad live in slums often characterized by a lack of basic services such as water and sewerage. Consequently, the urban poor often use inexpensive pit latrines and at the same time may draw domestic water from nearby local taps. Overcrowding in slums limits the adequate distance between taps and pit latrines so that micro-organisms migrate from latrines to water sources. Sanitary practices in these overcrowded slums are also poor, leading to contamination of this tap water. The DNA sequencing results indicated the microbial diversity, revealing that the dominant bacteria present in Khairathabad slum area of Hyderabad is *Acinetobacter* sp. whereas the dominant bacteria in Varasiguda slum area of Hyderabad is *Alpha-proteobacteria*. Futhermore, cluster analysis of the DGGE profiles indicated significant diversity in the bacterial community by depicting two distinct clusters for each waste water treatment plant. These data endorse the ability of PCR-DGGE method to identify and characterize bacterial community from Usage water.

Key words: Usage water, PCR-DGGE, Bacterial diversity

INTRODUCTION

Rapid urban growth in a climate of economic constraints has resulted in the majority of residents in India's large cities, and an increasing proportion of Indians overall, living in overcrowded slums and shantytowns. In these slums and shantytowns, health conditions and livelihood opportunities are poor. The need for clean water is increasing and wastewater treatment can be used as a cost-effective solution for purification of organically polluted industrial waste streams (Watanabe and Baker, 2000). Moreover, the presence of slums and their unhealthy environment within Rajshahi City Corporation is an ever-present threat of public health. They have no proper arrangement of water supply and sanitation system which has created an adverse effect on city's environment. A few numbers (13%) of the households use apparently good latrines, which are not fully hygienic. Among the rest households 15% use open latrines and 72% use ring slab latrine without water seal. Some organizations are trying to improve the condition of water supply and sanitation facilities for urban poor in Rajshahi City with different approaches. But the crisis of water supply and sanitation facilities is a common feature in daily life of urban slum poor. So, it is an immediate concern to study the approaches of different organizations related to water and sanitation facilities for urban poor in this city. Application of molecular biology techniques allows us to detect and enumerate microorganisms in their natural habitat and so to determine the structure, function and dynamics of bacterial communities. Of the various approaches for the understandings of microbial community structures in nature, comparative analysis of 16S rRNA sequence of microorganisms has been universally applied, due to the ubiquity of ribosomal RNA molecules in all microorganisms, to infer relationships among organisms (Pederson *et al*., 1996; Wise *et al*., 1999; Lee *et al*., 2000).

The rRNA molecules are comprised of highly conserved sequence domains, interspersed with more variable regions. In general, the essential rRNA domains are conserved across all the phylogenetic domains, thus universal tracts of sequences can be identified (Olsen *et al*., 1986). Denaturing gradient gel electrophoresis (DGGE) is perhaps the most commonly used among the culture-independent fingerprinting techniques (Muyzer *et al.* 1993). It is based on the separation of polymerase chain reaction (PCR) amplicons of the same size but different sequences. In this work, an attempt has been made to open the "black box" of the usage water to evaluate the bacterial diversity in water of slum areas located in Hyderabad.

MATERIAL AND METHODS

Sampling Sites

The two slum areas selected for our study were Khairathabad and Varasiguda, located in Hyderabad, Telangana. Two samples, usage water were collected from tap for a period of six months.

DNA isolation

For the isolation of DNA from sludge samples, 1 ml volume of homogenous cell culture was pelleted and suspended in freshly made Xs buffer (1% Potassium ethyl Xanthogenate, 100 mM Tris HCl, pH -7.4, 20 mM EDTA, 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 and Neilan 2000).

PCR Amplification

PCR were standardized to precisely amplify the 16S conserved region (1.5 kb) for each sample. The universal primer sequences were used for 16S rDNA amplification,

Fwd: 5'-GAGTTGGATCCTGGCTCAG -3' and Rev: 5'-AAGGAGGGGATCCAGCC-3'. The variable V3 region of 16S rDNA was PCR amplified to obtain a PCR product of 220 bp with primers to conserved regions of the 16S rRNA genes. The nucleotide sequences of the primers were primer1: 5'- CCTACGGGAGGCAGCAG-3', primer 2: 5' ATTACCGCGGCTGCTGG-3', andprimer 3:5'CGCCCGCCGCGCGCGGCGGGCGGGGCGGGG GCACGGGG GGCCTACGGGAG, that contained the same sequence as primer 1 but has at its 5' end an additional 40-nucleotide GC-rich sequence (GC clamp). A combination of primers 1 and 2 or primers 3 and 2 was used to amplify the 16S rDNA.

DGGE Analysis

PCR products were resolved on 8% (w/v) polyacrlamide gels in 0.5X TAE using denaturing gradients ranging from 40% to 80% (where 100% denaturant contains 7M urea and 40% formamide). For each sample 10 µl of PCR product was loaded after mixing with equal volume of loading dye to the bottom of the well. Electrophoresis was carried out at low voltage (20V) for 20 min and then at 200 volts for 3 hrs at a constant temperature of 60^0C. The gels were stained for 20 min with ethidium bromide and washed twice for 5 min with Milli-Q water prior to UV transillumination in UVI gel documentation system (UVItec, Cambridge, United Kingdom). The DGGE bands were excised and subsequently sequenced.

RESULTS AND DISCUSSION

Khairathabad Microbial analysis

PCR-DGGE analysis was done for the usage water for six months. In these six months, the usage water sample produced a total of six bands whereas nine bands were observed in the usage water (fig.1).

A total of six bands were produced. All of these six bands were common to all samples of usage water. For the determination of the more specific community structure traits, a sequencing analysis of the bands was performed. The results of the alignment of the obtained sequence, using the BLAST suggested up to ~ 90% similarity with *Acinetobacter* sp. These results suggest that the dominant bacterial population in this usage water is *Acinetobacter* sp. The class of bacteria identified in present study is in agreement with the previous studies. The *Acinetobacter* species have been identified from waste water treatment plants since early 1990s (Blackall *et al.* 1989). *Acinetobacter* organisms which are heterotrophic works on enhanced biological phosphorus removal. These organisms release phosphorus, thereby obtaining the energy to uptake readily biodegradable organics. This ability enables *Acinetobacter* to become dominant. *Acinetobacter* species is also known to be predominant micro-organism involved in enhanced phosphorus uptake

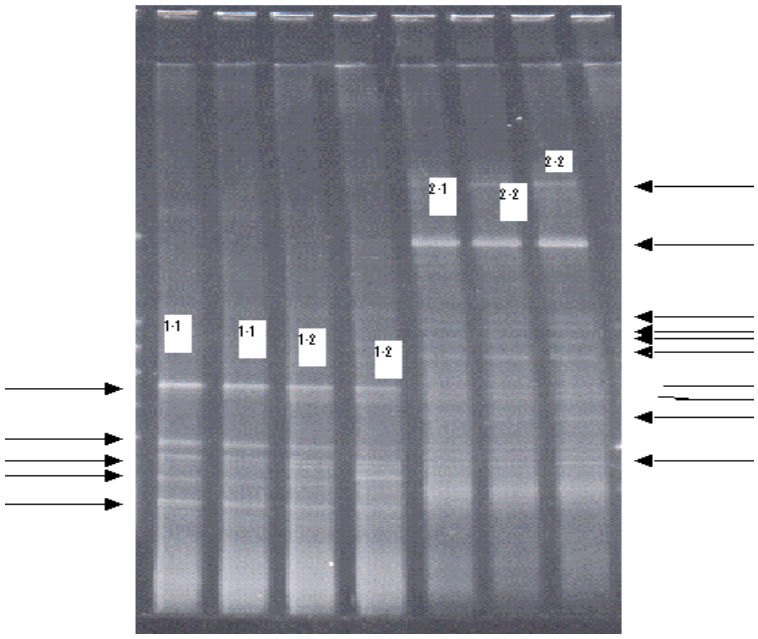

Fig 1: PCR amplification of Khairathabad sludge sample

Other researchers reported that *Acinetobacter* spp. were predominant when enumerated using the analytical profile index method. For example, Hart and Melmed (1982) estimated *Acinetobacter* spp. at 56% to 66% of the total population, Buchan (1983) reported 48% to 66%, Lötter (1985) 56% to 66%, Lötter and Murphy (1985) ca. 60% to 70% and Kerdachi and Healey (1987) 73%. Khairathabad water basically deals with textile wastes and bacteria of this genus are known to be involved in biodegradation, leaching, and removal of several organic and inorganic man-made hazardous wastes that are known to be produced by textile dyes. Also, among microbial communities involved in different ecosystems such as soil, fresh water, wastewater, and solid waste, several strains belonging to the genus *Acinetobacter* have been identified. Thus, the presence of *Acinetobacter* sp as dominant bacteria seems justified.

Varasiguda Microbial analysis

For the Varasiguda location ten bands were observed for the influent water, and for the usage water, three dominant and three faint bands were observed.

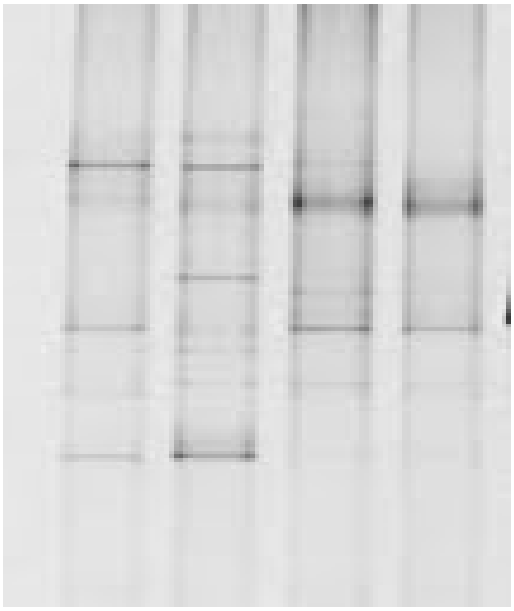

Fig 2: PCR amplification of Varasiguda sludge sample

A similar band pattern was produced by both, the usage water samples. Sequence analysis of the excised bands revealed up to 100% similarity with *alpha proteobacteria.*

This location basically deals with mixed wastes, domestic and industrial. *Alphaproteobacteria* is known to be associated with bulking in industrial waste water treatment plants (Levantesi *et al.* 2004). Large population of *Alphaproteobacteria* has been observed in waste water treatment plants. Wagner *et al.* (1993) studied bacterial community structure in activated sludge samples using group specific oligonucleotide probes for in situ analysis. Probing activated sludge with fluorescently labeled oligonucleotide probes specific for the alpha, beta and gamma subclasses of the *proteobacteria* had revealed that the microbial consortia are dominated by the *Proteobacteria* (approximately 80%), a phylum containing a majority of the traditional gram negative bacteria. Arroyo *et al.* 2010, provided information about bacterial community structure in natural wastewater treatment systems treating different types of wastewater using the direct sequencing of the 16S ribosomal RNA codifying genes. They concluded that the municipal wastewater treatment system presented a high diverse community in both macrophytes with *gammaproteobacteria* and *Alphaproteobacteria*, respectively, as the most abundant groups. This is in agreement with our findings. Reid *et al.* 2008, studied the bacterial composition of a waste water treatment system reliant nitrogen fixation, they confirmed that despite changes in wastewater composition and dissolved oxygen levels, the bacterial community composition appeared stable and was dominated by *Alphaproteobacteria* and *Betaproteobacteria.* Thus, it can be inferred that alpha proteobacteria is one of the dominant bacterial species found in waste systems. The Proteobacteria kingdom is the largest and most diverse in the domain bacteria. As a group, these organisms show extreme metabolic diversity and represent the majority of known gram-negative bacteria of medical, industrial, and agricultural significance. This is an evolutionarily, geologically, and environmentally important group. This is in agreement with our findings for Varasiguda location, since this water basically deal with non specific wastes.

Cluster analysis

Cluster analysis of the DGGE profiles depicted that the two waste water treatment plants carry different microbial populations (fig 3).

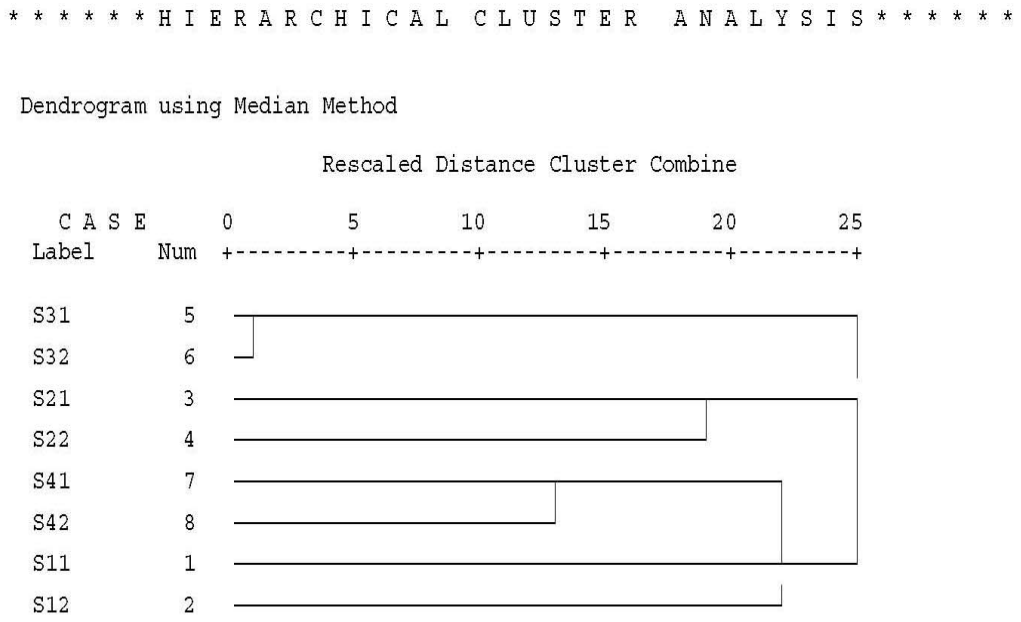

Fig 3: Cluster analysis

The dendrogram depicts two distinct clusters for each of the waste water treatment plants suggesting that the two waste water treatment plants carries different bacterial population. However, no significant difference was observed over the period of six months. This suggests that diversity of bacterial community did not change much over a period of six months. One striking observation was that usage water of bacterial population was similar in both the locations. This suggests that no specific bacteria are being used by these waste water treatment plants. Table-1

Proximities

Case Processing Summary[a]

Cases					
Valid		Missing		Total	
N	Percent	N	Percent	N	Percent
11	78.6%	3	21.4%	14	100.0%

a. Binary Squared Euclidean Distance used

Proximity Matrix

	Matrix File Input							
Case	S11	S12	S21	S22	S31	S32	S41	S42
S11		4.000	6.000	5.000	7.000	7.000	5.000	3.000
S12	4.000		6.000	7.000	5.000	5.000	5.000	3.000
S21	6.000	6.000		3.000	7.000	7.000	9.000	7.000
S22	5.000	7.000	3.000		6.000	6.000	8.000	8.000
S31	7.000	5.000	7.000	6.000		.000	6.000	8.000
S32	7.000	5.000	7.000	6.000	.000		6.000	8.000
S41	5.000	5.000	9.000	8.000	6.000	6.000		2.000
S42	3.000	3.000	7.000	8.000	8.000	8.000	2.000	

Median Linkage

Agglomeration Schedule

	Cluster Combined			Stage Cluster First Appears		
Stage	Cluster 1	Cluster 2	Coefficients	Cluster 1	Cluster 2	Next Stage
1	5	6	.000	0	0	7
2	7	8	2.000	0	0	4
3	3	4	3.000	0	0	6
4	1	7	3.500	0	2	5
5	1	2	2.875	4	0	6
6	1	3	4.594	5	3	7
7	1	5	4.086	6	1	0

Table-1: Proximate analysis

CONCLUSION

In conclusion, it can be said that since the two slum areas deal with different kinds of wastes and thus the dominant bacteria present in each plant are different. To the best of our knowledge, this is the first study assessing the bacterial population in these two slum areas. The results from present study indicates that even though the bacterial community structure is different in the Khairathabad and Varasiguda slum areas, the usage water does not carry much bacterial diversity. This implies that the two working waste water treatment plants are not using specific bacteria to ensure the maximum efficiency of the plant. The results from this study would be beneficial for the operators and engineers of the slum areas to further improve on the process and increase the efficiency of the standard living conditions.

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