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Molecular Identification of Air Microorganisms from Municipal Dumping Ground

R. Shantha, K. Sarayu and S. Sandhya

National Environmental Engineering Research Institute,
CSIR-Complex, Chennai, India

Abstract: Quite often the presence of microbes contamination is caused by air borne biotic particles and may lead to fetal consequences. The traces of contamination need to be tracked down reliably and rapidly. Molecular phylogenetic sequence analysis has proved a new perspective on microbial communities by allowing the detection and identification of microorganisms in absence of cultivation. In this study we used broad specificity amplification of DNA genes to survey microorganisms present in municipal dumping ground. Survey was conducted from April to June 2008 on weekly basis. We have observed distinct set of microbial type present in air.

Key words: Bioaerosols · Molecular phylogenic sequence · DNA · Gases pollutant

INTRODUCTION

Air borne microorganisms are a potential source of a wide variety of public and industrial health hazards. Of particular significant are bioaerosols associated with wastewater treatment process [1], nosocomial infections [2], fermentation facilities [3], release of genetically engineered microorganisms to enhance agricultural productivities are of growing concern [4]. Municipal waste is the habitat of various microorganisms emitted into atmospheric air, when they exist in the form of bioaerosols. The kind of microorganisms emitted into air, the range of their occurrence, their number depend on many factors, such as the type and location of a facility, the weather condition and time of the year. Bioaerosols emitted by dumping sites may of viruses, bacteria, spores of fungi, protozoon's cyst and parasites ova. On this account municipal dumping grounds can be a potential source of many diseases [5]. Rapid urbanization of many cities in India has lead to the growth of populations near dumping grounds and may exacerbate the risks. In addition to respiratory tract infections, it has been suggested that associated enteric microorganisms common in dumpling ground may produce intestinal tract infections [6].

Effective monitoring of bioaerosols requires the efficient collection of microorganisms from the air. In addition, an appropriate technique for analysis of air samples must be selected. While monitoring for air borne microorganisms has traditionally focused on the collection of fungal spores and bacterial cells and the analysis of sample by total count and culture techniques, these methods have several limitations [7]. The total count enumeration methods are laborious and identification of microorganisms is problematic. Most of the organisms are not culturable under specific growth conditions imposed in the laboratory remain undetected yet may be capable of inducing adverse health effects [8, 9]. To reduce the constraints due to culture based methods and to monitor airborne microorganisms an alternative method for detection is required. The method based on polymerase chain reaction (PCR) permits detection of target nucleic acid sequences of DNA, thereby eliminating the requirement of growth to detect and identify the microorganisms. Previously, detection of genetically modified bacterium by PCR was shown in green house aerosolization experiments [10]. Agranovski *et al*. [11] have detected air borne viruses by personal bioaerosol sampler combined with PCR.

The aim of the study was to assess the degree of the atmospheric air microbial pollution in municipal dumping ground (MDG) in Chennai, India. The hazard was assessed on the ground of registered levels of bacterial aerosol concentration. The microorganisms were identified by 16S rDNA molecular approach.

Corresponding Author: S. Sandhya, National Environmental Engineering Research Institute, CSIR-Complex, Chennai, India

MATERIALS AND METHODS

Sampling Site: The dumpling ground was situated in southern part of Chennai at Perungudi almost 10 km from city centre. The sampling site was about 100 m from the garbage pouring site. Almost 1190 tonnes/ day waste is dumped on the site. The out door environment of the management office located near the entrance was sampled. Samples were collected from April to June 2008.

Sampling: Air borne microorganisms were collected by air sampler at 1.2 to 1.5 m height above the surface. Sampling was performed by liquid impingement method using AGI-30 Envirotech samplers operated at flow rate of 12.5 l/min for one hrs to yield the sampling volume of 750 liters. AGI-30 samplers each containing 25 ml of collection buffer (0.1M phosphate buffer) were operated outdoor. The solution volume was measured to evaluate evaporation loss and were adjusted to 25 ml by the addition of sterile phosphate buffer.

DNA Isolation, PCR Amplification: Genomic DNA was extracted from sampling buffer of pooled AGI-30 samples was processed by alkaline lysis methods and subjected to PCR amplification. Amplification of 16S rDNA fragment was performed with 20-30 ng of DNA template using 515 F(5'GTGCCAGCMGCCGCGGTAA3')and 1391R(5'GACGGGCGGTGWGTRC A3') eubacterial universal primers [12]. For each PCR, 2.0 µl_ of template DNA was added to 27.5µl of PCR master mix (1.5 mM $MgCl_2$, 0.5 mM of each primers, 0.2 mM dNTPs mix, 5 U Taq DNA polymerase, 1 µl_ Taq buffer and the rest water). Both the polymerase and the amplifying buffer were purchased Banglore Geneie, India. All reactions were performed in the 50 µl.The reactions were carried out in Eppendroff PCR system. The reactions were thermocycled for 2 min at 95°C followed by 30 cycles of 30 S each of 95°C, 45 S at 55°C with 2 min of 72°C with final extension of 10 min at 72°C. The amplified product was resolved on 1.2% (w/v) agarose.

16 S rDNA Sequence Analysis: 16 S rDNA (885bp) amplified products were purified and send for sequencing using 515 and 1391 universal primers in automated DNA sequencer using Big Dye terminator.

Bacterial Culturing: Air borne bacteria were plated on trypcase soy agar and colony forming units were counted after 24 hrs incubation.

RESULTS AND DISCUSSION

The amount of microbial count observed and the concentration of genomic DNA recovered varied greatly among the environmental samples. Optimization of time of air sampling was performed using AGI-30 sampler. The samples were collected every hour for four hours. The culturable microbial count during the sampling optimization is given in Table 1. DNA isolation was also performed in the samples. Cell culturing performed on samples collected shows bacterial increasing with the prolongation of time. Genomic DNA was extracted directly from the samples using alkaline lysis method. Almost all the samples were positive for DNA. The microbial count varied from 75 $x10^4$ to 155 $x10^4$ for 1 hr to 4 hr sampling, indicating not much variation in count with time. Therefore further sampling was performed for one hour duration only.

The concentration levels of microbial aerosol at the MDG are presented in Table 2. The air borne microorganism levels in MDG was usually higher during the warm days in April and May (10^6 CFU/ m^3)

Table 1: Optimization of sampling time and comparison of the sensitivity of DNA isolation protocols with culturable organisms

	Municipal dumping ground				
	Time, min				
Estimation	30	60	120	180	240
Culturable count, CFU/ m3	6.4x104	75x104	93x104	123x104	155x104
DNA	+	+	+	+	+

Table 2: Air microorganism concentration during air sampling at municipal dumping ground

Sampling schedule	April				May				June			
Week	1	2	3	4	1	2	3	4	1	2	3	4
Culturable count, CFU/m3	TNC	1.7x106	1x106	1x106	1.1x x106	10x x106	10x x106	10 x106	Nil	Nil	Nil	Nil
DNA	+	+	+	+	+	+	+	+	-	-	-	-

ND- Not detected

TNC- Too numerous to count

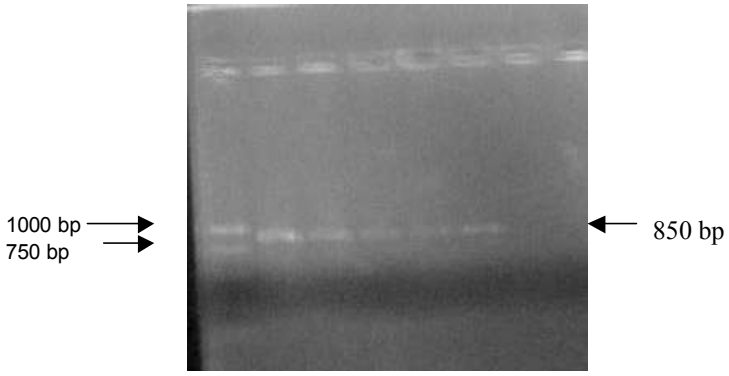

Fig. 1: PCR amplicons of 16S rDNA fragment for air microbes
Lanes: 1,PCR marker (1000 bp ladder, Bangalore Genei); 2,3,4,5,6 air microbes during sampling

Table 3: Climatic conditions during sampling near municipal dumping ground

Sampling schedule	April	May	June
Temperature,°C	26-35	28-41	26-36
Humidity, %	53-95	53-87	40-95
Dew °C	22-26	15-26	21-26
Wind Speed Km/h	0.073-3.09	0.25-3.8	0.41-3.38
Rainfall, mm	0	0	0--.35

when temperature was in the range of 28-41 °C (Table 3) than in June (7x10°CFU/ m^3). During this time humidity also recorded high between 53-95%.The results show densities ranging from $x10^5$ to $x10^6$ CFU/m^3, which is higher but in general accordance with previous studies, performed using culture based methods [14, 15]. Comparative analysis of sampling showed (Table 2) an influence of temperature, humidity and rain fall on the microbial count. The count was more when temperature was high (41 °C) and high humidity supporting air bacteria survival. In June, bacterial count was less because of settling of suspended particles as some rains are recorded. Huang *et al*.(15) have reported only 10^3 /m^3 bacterial count near closed municipal land fill site. In present study also we recorded higher bacterial count.

The samples were also processed for genomic DNA extraction and most of the samples were positive for DNA. To identify the possible reservoirs of microbes at MDG, DNA was amplified using universal eubacterial primers. Fig.1 shows the PCR amplicons of 16S rDNA fragment for air microbes. All the samples showed single band of 850 bp, indicating similar type of bacteria during sampling at MDG. This is further confirmed by subjecting the 16S rDNA amplified product to restriction fragment analysis.

The 16S rDNA gene, an 850 bp product, obtained from the isolates was sequenced using 27 F primers. These complete sequences were then probed using NCBI BLASTn program/Altuschul etal [13]. Pair wise alignment giving a close match of 99% with sequence analyzed were chosen. The sequences retrieved from NCBI data base giving the closest match in pair wise BLAST were identified as *Salmonella sp, Klebsiella pneumoniae* (89%), *Enterobactera, Pseudomonas sp.*

CONCLUSION

In present investigation air microbes were observed in all the three months. But their count varies because of fluctuation in temperature and rain. Most air borne bacterial concentration was higher than 10^6 CFU/ m^3. Streib *et al*. [14] also reported higher bacterial count (10^3 to 10^5 CFU/m^3) near waste transfer site.

Using air sampler with DNA amplification, a diverse group of bacterial species could be identified in various air samples. The 16S rDNA sequencing and blasting have shown that air bacteria belongs to *Salmonella sp, Klebsiella pneumoniae, Enterobactera, Pseudomonas sp.* with 89% sequence identity. The present result suggest that MDG harbor potential pathogens that can threaten human health. Therefore exposure to such environment should be minimized.

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