IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 16, Issue 2 Ser. I (February 2022), PP 06-12
www.iosrjournals.org

Identification of Culturable Airborne Bacteria Associated with $PM_{2.5}$

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Abstract
The present study aimed at identifying culturable bacteria (Bio-Aerosol) which are associated with particulate matter of the size 2.5 microns and less, by analyzing $PM_{2.5}$ ambient air samples of quartz filter paper using classical culture techniques. The sampling was conducted in various areas of Delhi in both summer and winter seasons. Samples taken from the filters were placed in tubes containing nutritive broth BHI (Brain Heart Infusion) and incubated at 37 ˚C for 18 to 24 hours. This was followed by spread plating of appropriate dilutionsof inoculums onto the surfaces of trypticase soy-agar (TSA). The results indicated a predominance of Gram positive, rod shaped, catalase positive bacteria associated with $PM_{2.5}$ for both seasons. There was a predominance of endospores in the winter samples as compared to summer samples. Bacillus was found to be the predominant genus on the basis of their micro- and macro morphological characteristics, and standard taxonomic keys such as the Bergey's manual of determinative bacteriology. The genus Bacillus includes endospore forming, Gram positive, rod shaped, catalase positive bacteria. Since there was predominance of Gram-positive culturable bacteria in air samples, these results were consistent with the previous work of other authors.

Keywords-*$PM_{2.5}$,Bio-Aerosols, Gram-Positive Bacteria, Bacillus.*

Date ofSubmission:01-02-2022 Date of Acceptance:13-02-2022

I. Introduction

The ambient air consists not only of gases but also of bio-aerosols and particulate matter(PM). Particles with both biological and non-biological origins are transported together with air currents in the atmosphere. Particles originate from various natural and anthropogenic sources, and affect visibility, climate, air quality, and human health [1]. Particle concentrations are influenced by meteorological conditions, long range transport of pollutants, and new particle formation in the air [2]. Particles are removed from the air either by sedimentation orprecipitation [3]. The quality of air has been a cause of concern all over the world since concentrations of various *criteria* pollutants often breach the ambient standards, especially in developing countries. Particulate matter, which comprises a complex mixture of different elements and compounds, is one of the six *criteria* pollutants. $PM_{2.5}$ (diameter $\leq$ 2.5μm) can penetrate into the lungs more readily and are therefore more likely to have short- and long-term effects such as decreased lung functions and alterations in lung tissues, premature death and increased respiratory symptoms and disease[4].

Biological particles/bio-aerosols are particles of biological origin suspended in the air such as: bacteria, fungi, viruses, microbial toxins, proteins and enzymes [5]. Such particles may be suspended in the air either as individual organisms or attached to dust particles or tiny droplets of water. Bio-aerosols tend to attach in coarser PM fraction, however fungal spores, fragmented pollen, and non-agglomerated bacteria are found in the fine fraction as well, due to the mechanism of reaction between biological agents and PM.Biological particles have received less attention in the atmosphere than other aerosol particles such as: sulfates, mineral dust and ash, because its average concentrations have been assumed to be insignificant compared to non-biological particles [6]. Although bacteria are ubiquitous in the near-surface atmosphere and they can have importanteffects on human health, airborne bacteria have received relatively little attention.Studies of aerosols in the near-surface atmosphere typically focus on non-biological particles despite increasing recognition that biological particles may represent a significant portion of the particulates suspended in the atmosphere.

Of those studies examining biological aerosols, most have focused on the quantification and identification of fungal spores, pollens and dust mite allergens. Although there is a history of research examining bacterial distributions in the near-surface atmosphere, the majority of this work has largely been restrictedtosurveysofculturablebacteria(thosebacteriawhichcanreadilybegrownandisolatedunder

laboratory conditions). As most bacteria cannot be readily cultured, the vast majority of airborne bacteria have effectively been excluded from these culture-based studies and comprehensive surveys of airborne bacterial diversity are uncommon. Our limited understanding of bacterial diversity in the atmosphere represents a critical knowledge gap in atmospheric research given that bacteria are ubiquitous in the atmosphere with concentrationstringing between 104 and 108 cells per m^3 of air [7].

The focus of the present study is characterization and identification of the kind of culturable bacteria that is associated with $PM_{2.5}$, the sampling of which was done in areas around Delhi. Delhi, the capital city of India with over 14 million populations, is experiencing health risks from air pollution, especially the respirable particulate matters. Sources of PM include road side dust, vehicles, industries, trans-boundary migrations, power plants, solid waste and local sources. Particulate matters from these sources may contain hazardous pollutants and forms of bio-aerosol associated with it which can have carcinogenic and mutagenic effects [8]. There is a lack research about the bio-aerosol associated with $PM_{2.5}$, in the studied area therefore it was aimed to identify the kind of bacteria that could be found along with $PM_{2.5,}$ for better air qualitymanagement.

II. Materials andMethods

2.1 SampleCollection

Sample collection for $PM_{2.5}$ was conducted in various areas of Delhi for both winter and summer seasons in 2014. Winter sampling was done in the month of January and summer sampling was done in May. Delhi, the Capital of India,spread over an area of approximately 1500 sq. km, is situated between latitudes 28˚24'17" and 28˚53'00N and longitudes 76˚50'24" and 77˚20'37"E, with an elevation above sea level of 216 meters. The duration of the sampling was 24-hours with a sampling cycle of 12- 12 hrs. (i.e. morning 8 am to 8 pm and night 8 pm to 8 am). The $PM_{2.5}$ Sampler (Ecotech Instruments Pvt. Ltd., APM 550), worked on WINS impactor and ran at a constant flow rate of 16.67 L/min. Quartz fiber filter papers were used for sampling and further analysis.Pre-conditioning of the filters was done for 2 days in a controlled room (temperature: 20˚C ± 1˚C, relative humidity: 50% ± 5%) before and after the sampling and then they were weighed using an analytical balance (Sartorius, CPA2PF). Following sampling, the sampled filters are sealed in aluminum foil bags and stored in a freezer (−20˚C) prior to analysis[8].

2.2 Bacteriologicalanalysis

An appropriate portion of the filter was cut aseptically and placed in tubes containing nutritive broth BHI (Brain Heart Infusion)and incubated at 37 ˚C for 18 to 24 hours.The overnight culture was then serially diluted 10^7 times in micro-centrifuge tubes of1 ml volume. The serial dilutions were made in BHI broth.10µl of each serial dilution was spread plated on a Trypticase soy agar(TSA) plate.These plates were then kept for incubation at 37˚C for 18 to 24 hours, Overnight.Examination of colony morphology of the obtained culture was done for each sample, the next day, from the incubated plates.Colonies with similar morphology were sub-cultured on another TSA plate and incubated overnight in similar conditions as above.Colonies were selected according to morphological differentiation and Gram stainingwas used to differentiate cell morphology. This process was done for isolation of pure culture. Further procedures such as the Gram staining, the catalase test, streaking on MacConkey Agar Medium, observation under oil immersion was done using the isolated culture.The identification of the isolated microorganisms was done by the biochemical tests recommended in Bergey's Manual (9^{th} edition ISBN 0-683-00603-7) [9].

III. Results andDiscussions

The results of this study indicated that most of the bacteria associated with $PM_{2.5}$ were Gram positive,rod shapedand catalase positive,for both winter and summer samples.They showed no growth on MacConkey agar medium. There was difference in the colony morphology of bacteria between summer and winter samples. Except for the difference in colony morphology on TSA plate the cell morphology was rod shaped with Gram positive staining. Each sample showed homogenous population of bacterial colonies i.e. similar colony morphology was obtained on plate for each single sample.

The winter samples mostly showed large creamy white colonies with irregular margins which lay flat on the plate with a certain fluid filled centeror a network pattern of fluid filled channels when the colonies merged to form a bio-film on the plate. The summer samples mostly showed medium sized(small in comparison to winter samples) flat, creamy white colonies with round margins. When viewed under oil immersion predominance of endospores in the winter samples was found as compared to summer samples. Also, one particular winter sample which was found to have distinct round, flat and yellow bacterial colonies,had a pattern of chains of rod-shaped cells when viewed under oil immersion microscopy. The other samples mostly showed pairs of Gram-positive rod-shaped cells under oil immersion microscopy.*Bacillus* was identified as the predominant genus based upon micro- and macro morphological characteristics, and standard taxonomic keys suchastheBergey'smanualofdeterminativebacteriology. AccordingtotheBergey'smanualofdeterminative

bacteriology, the genus *bacillus* includes, Gram positive, rod shaped, catalase positive bacteria with oval (sometimes round or cylindrical) endospores and are very resistant to many adverse conditions. There is not more than one spore per cell and sporulation is not repressed by exposure to air. The cells of thesebacteria (of the size 0.5-2.5×1.2-10µm) are often arranged in pairs or chains with rounded or squared ends[9].

According to the book, Medical Microbiology (4th edition) *bacillus* species are aerobic, sporulating, rod-shaped bacteria that are ubiquitous in nature. *Bacillus anthracis*, the agent of anthrax, is the only obligate *Bacillus* pathogen in vertebrates. *Bacillus larvae, B lentimorbus, B popilliae, B sphaericus, and B thuringiensis* are pathogens of specific groups of insects. A number of other species, in particular *B cereus*, are occasional pathogens of humans and livestock, but the large majority of *Bacillus* species are harmless saprophytes [10].

Since there was predominance of Gram-positive culturable bacteria in air samples these results were consistent with the previous work of other authors. The identified bacterial genus was *Bacillus,* a member of the phylum Firmicutes. Many previous authors for example Robert M Bowers et al. (2011) [7], Chen Cao et al. (2013) [11], GholamrezaGoudarzi et al. (2013)[12] have shown a dominance of Gram positive bacteria of the phylum Firmicutes, in their results. Kellogg and Griffin (2006) and Osman et al. (2008) have shown that culturable bacteria from atmospheric aerosols mainly constitute Gram positive *Bacillus* species. R.S. Singh et al.(2014) identified *bacillus subtilis*in samples of PM 2.5 and PM 10, in Varanasi, India [13]. Thus it can be safe to say that the results of this study were quite similar to many previous studies where association of the Gram positive bacteria of the phylum Firmicutes and *bacillus* species was found with particulate matter. The primary thing about the present study is that only the very fine fraction of particulate matter i.e. of diameter ≤2.5µm was considered.

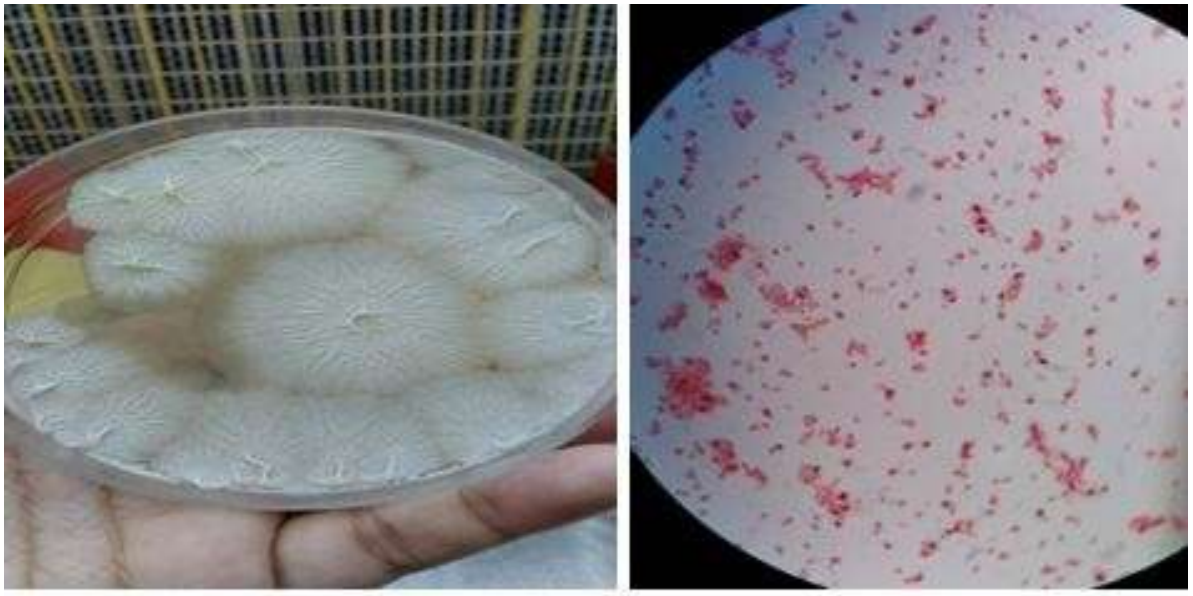

Figure 1(a)(left)- Winter sample Q1 on TSA plate at 10^4 dilution
1(b)(right)Gram stained slide of sample Q1

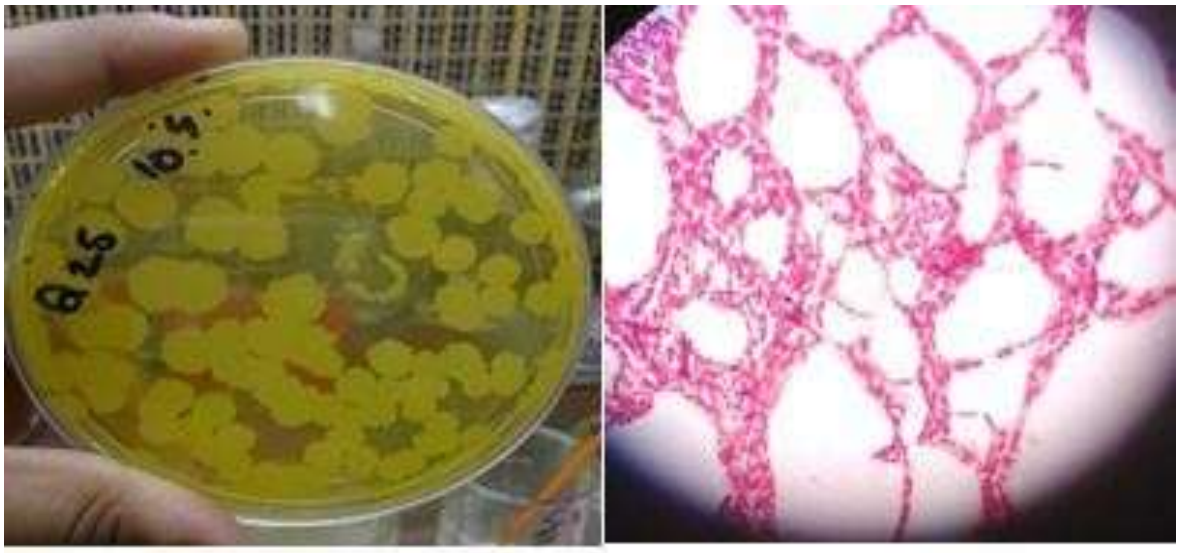

Figure 2(a)(left)- Winter sample Q25 on TSA plate at 10^4 dilution
2(b)(right)Gram stained slide of sample Q25

Figure 3(a)(left)- Winter sample Q28 on TSA plate at 10^5dilution
3(b)(right)Gram stained slide of sample Q28

Figure 4(a)(left)- summer sample QCS003 on TSA plate at 10^4 dilution
4(b)(right)Gram stained slide of sample QCS003

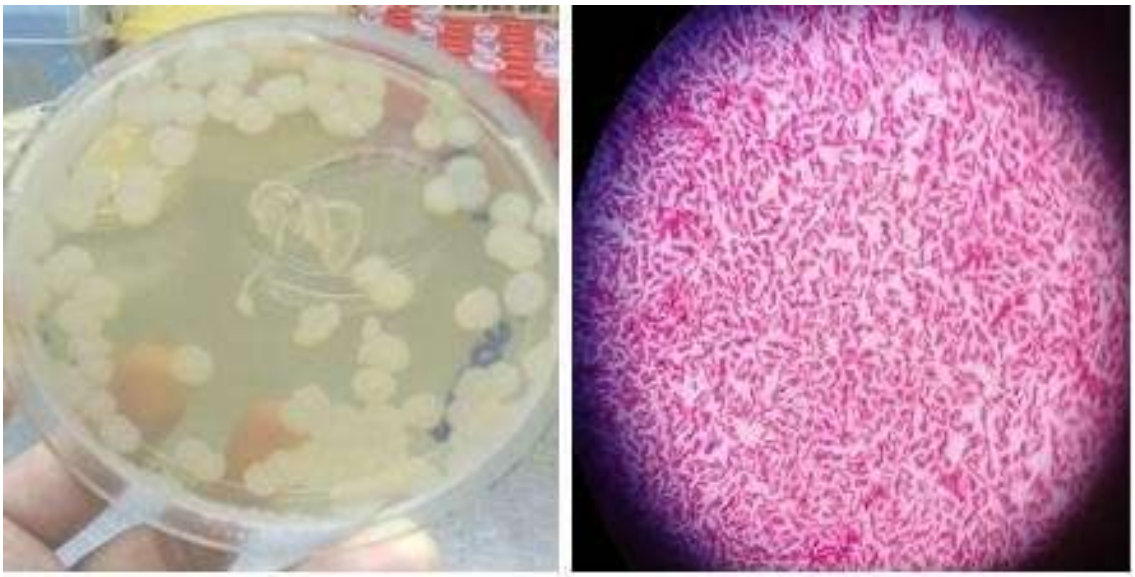

Figure 5(a)(left)- summer sample QCS007 on TSA plate at 10^4 dilution
5(b)(right)Gram stained slide of sample QCS007

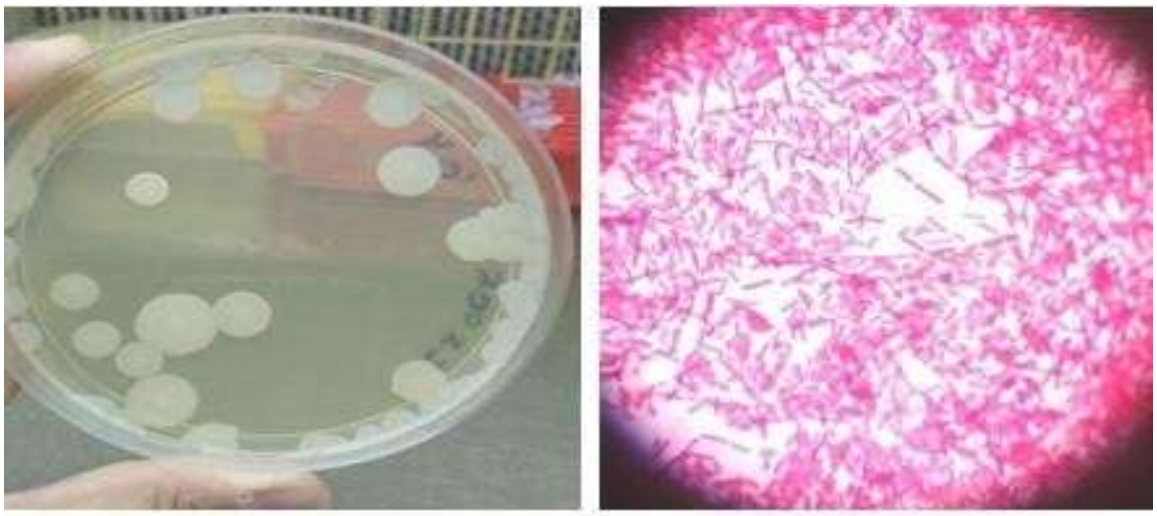

Figure 6(a)(left)- Summer sample QCS005 on TSA plate at 10^4 dilution
6(b)(right)Gram stained slide of sample QCS005

IV. Conclusion

This study examined the culturable bacterial community found in association with $PM_{2.5}$ by using ambient air samples of quartz fiber filter paper and classical culture techniques. A diversity of bacterial colonies was expected from the cultures of the ambient air samples, but contrary to this expectation, it was discovered that the bacterial community was mostly homogenous for each sample. The identified genus of bacteria was *bacillus.* A distinct difference between the colony morphology of these bacteria was seenbetween winter and summer samples, as can be seen in the figures above. Also, presence of endospores was seen mostly in the winter samples. It was concluded, that the results of this study were fairly alike to the results of many previous authors. Further studies of air filters from the same or different locations will be required to determine the variability in bacteria in particular air filters, and whether these bacterial communities in particular air filters have enhanced fitness for survival in thatenvironment.

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Sonakshi Garg, et. al. "Identification of Culturable Airborne Bacteria Associated with $PM_{2.5}$." *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, 16(02), (2022): pp 06-12.