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STUDIES ON AIRBORNE ACTINOMYCETES FROM EXTRA AND INTRAMURAL ENVIRONMENTS OF PUDUCHERRY CITY

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ABSTRACT

Similar to fungal spores, airborne actinomycete spores are significant pollutants in both indoors and outdoors of the workplaces and the homes. Their release into the air, aerodynamic and physical size while in the air, and survival following collection onto agar were investigated by gravitation plate method. The isolation of airborne actinomycetes was carried out by two different time intervals from extra and intramural environments of Puducherry city viz., Rock beach, Animal slaughter house, Flower market, Secretariat office and Hotel. The media plates were Potato Dextrose Agar, Starch Casein Agar, Actinomycetes Isolation Agar, Glycerol Asparagine Agar and Sabouraud Dextrose Agar. Glycerol Asparagine Agar (GAA) was recognized as one of the compatible media to isolate actinomycetes from air compared to Starch Casein Agar (SCA) and Actinomycete Isolation Agar (AIA). The findings demonstrated that the incubation conditions, temperature, duration and the media nutrients are necessary for the production of spores and their release into the atmosphere are distinct from those required merely for colony expansion. The cultures of actinomycetes were required more drying before spores could be released. An aerodynamic particle size was measured for the spores' aerodynamic diameters, which ranged from 0.50 mm to 1.20 mm. The physical sizes of the spores were smaller than those previously described in the literature, according to measurements made with a microscope and an image analysis system. On agar media, the spores recovered relatively in a range from 0.5 to 35%. The findings suggest that a number of factors, including the species and sampling flow rate, have a significant impact on the cultivability of the airborne actinomycete spores that were collected. As a result, alternatives to the usual cultivation techniques must be created in order to count actinomycete spores.

KEYWORD: Airborne actinomycetes, extra and intramural environments, Puducherry city, GAA, SCA, SDA, AIA and PDA.

INTRODUCTION

A broad group of gram-positive bacteria are known as actinomycetes. They resemble fungi because they can grow mycelium and dry spores like other fungi.[1] and because they have evolved to live on solid surfaces.[2] Actinomycete spores have recently drawn particular attention as markers of mould issues in buildings.[3] They are recognized to be significant air contaminants in occupational contexts, such as agricultural and waste composting plants.[4,5,6] They have been discovered in buildings with moisture and mould issues but do not belong to the typical microbial flora in indoor air.[7,8] Additionally, the prevalence of allergy alveolitis and other serious health issues have been linked to the airborne spores of various actinomycete species, including *Saccharopolyspora rectivirgula*, *Micropolyspora faeni*, *Thermoactinomyces vulgaris*, and *Streptomyces albus*.[9,10,11,12,13] The cellular mechanism of the health impacts brought on by actinomycete spores was examined. Their research demonstrates that lung macrophage responses, which can result in inflammation and tissue damage, can be triggered by Streptomyces species. Actinomycete spores can develop either endogenously or through the division of pre-existing hyphae through swelling or fragmentation. The sheath of the hyphae that break into spores may or may not remain in the spores following fragmentation.[9,10] As a result, there are three primary types of spores: endospores, aleuriospores, and arthrospores, which are divisions of sheathed hypha. Although the importance of the variations in spore structure is unknown, these variations are anticipated to affect how these spores survive and behave when in the air.

Although actinomycete spores have been found in air samples, it is unclear how they were released into the

atmosphere. Actinomycete spores can naturally become airborne by mechanically disturbing the material they are grown on, for as by using a farming tool or being exposed to strong winds.[14] Airborne actinomycete spores have only been used in a small number of laboratory experiments. While Madelin and Johnson.[15] released actinomycete spores from culture media by air currents, Lacey and Dutkiewicz.[16] released actinomycete spores from infected hay by mechanical handling. Because they are smaller than fungal spores, actinomycete spores are more challenging to aerosolize.[17] Aerodynamic diameter, agglomeration, and hygroscopicity of airborne actinomycete spores are three features that require more study because they have an impact on how these organisms behave in the air, in human respiratory systems, in air-purifying filters, and in aerosol samplers. There is a vast amount of literature on airborne actinomycetes since scholars and researchers now see study on aeroallergens as being of the utmost importance.[18,19,20] Over the past few years, numerous scientific investigations on outdoor microbes in various settings have been undertaken and reported on in national and international venues. These investigations have improved our fundamental understanding of the environmental diversity of airborne actinomycetes and have offered information on a number of significant public health-related topics.[21] Studying disease-causing airborne actinomycetes can aid in medical evaluations, the evaluation of health concerns, the creation of treatments, and the pro-active monitoring of air quality.[5]

In addition to negatively affecting human health by causing pathological disorders, allergies, and other health-related issues, air pollution also significantly slows down socioeconomic development in the majority of northern Indian cities.[22] There is currently few information on the variety of airborne actinomycetes comparing the various metropolitan environments in Puducherry, India.[23,24] By evaluating the diversity of cultivable strains and utilizing the open plate sampling method in various indoor and outdoor conditions, our study aims to advance our understanding of airborne actinomycetes in Puducherry. In five different Puducherry sampling sites, we discussed the variety, concentration, distribution, and relative frequency of cultivable airborne actinomycetes in Puducherry. We also looked at how various environmental factors affected the actinomycetes community of Puducherry that was the subject of our study. Results from the current study could benefit efforts to reduce pollution and safeguard the environment in urban settings.

MATERIALS AND METHODS

The research work was focused on the isolation of the airborne actinomycetes from the different locations and in different times in the extra and intramural environments of Puducherry city.

Isolation of Airborne actinomycetes

The isolation of airborne actinomycetes was carried out by two different time intervals i.e., 5 minutes and 10 minutes from extra and intramural environments of Puducherry city viz., Rock beach, Animal slaughter house, Flower market, Secretariat office and Hotel. Five different media plates like Potato Dextrose Agar, Starch Casein Agar, Actinomycetes Isolation Agar, Glycerol Asparagine Agar and Sabouraud Dextrose Agar were used in order to isolate the actinomycetes. These above said media were added with Cycloheximide 75µg/mL and Neomycin 75µg/mL to avoid the bacterial and fungal contaminations on the media plates. After collection, the media plates were incubated for 15 to 20 days in 37˚C. After 18 days the appeared actinomycetes colonies were underwent for the further process. Microscopic analysis was performed to segregate the actinomycetes among bacteria and fungi from the mixed cultures over the media plates. Gram staining was also accomplished to separate actinomycetes based on their gram positive characteristics.

Microscopic size measurements

A slice of actinomycete culture was cut from an agar plate with a sterilised knife, placed on a microscope slide (Superfrost/Plus; Fisher Scientific, Pittsburgh, Pa.), and left to air dry in order to assess the size distribution of actinomycete spores. After adding a drop of permount media (Fisher Scientific), a coverslip was placed on top. A colour video camera mounted to the trinocular head of a phase-contrast microscope was then used to digitally image the sample. A phase-contrast condenser (0.9 NA) with an oil immersion objective made up the phase-contrast optical system. A Matrox Millennium video board was linked to the control unit for the video camera. The Image-1/MetaMorph imaging software system operating on a Pentium 166 MHz CPU platform was used to analyze the actinomycete culture sample. The threshold-based boundary detection approach was used after the colour picture was decoded and the decoded image's "Lookup Table" was set up as a monochrome image for the purposes of the interactive morphometric analysis of the samples with the picture-1/MetaMorph system. The resulting morphological measures were then displayed using the "Show Regional Statistics" function once a calibration scale was activated. Before determining the actinomycete spore size, the microscope and image analysis system were calibrated using standard polystyrene latex particles (PSL) of two sizes.

RESULTS AND DISCUSSION

Air sampling was made by gravity petriplate method in indoors and outdoors of varied environments of Puducherry city employing five media plates viz., SCA, SDA, PDA, AIA and GAA by gravity plate technique. Altogether, 52 actinomycete were isolated and named as AA 1 to AA 52. They were pure cultured and maintained on GAA and PDA for future use (Table 1). During the Petri plate exposer timings, 10 minutes exposer was found to be good in order to count a comfortable number

of actinomycetes in comparison to 5 minutes. Glycerol Asparagine Agar (GAA) was recognized as one of the compatible media for actinomycetes from air compared to Starch Casein Agar (SCA) and Actinomycete Isolation Agar (AIA). Volumetrically, airborne actinomycetes were found to be very less but similar together among the indoor and outdoor environments of the city area. Fig 1 shows the occurrence of airborne actinomycetes in different environments of Puducherry city and Fig 2 displays the percentage of actinomycetes harbored in the environments.

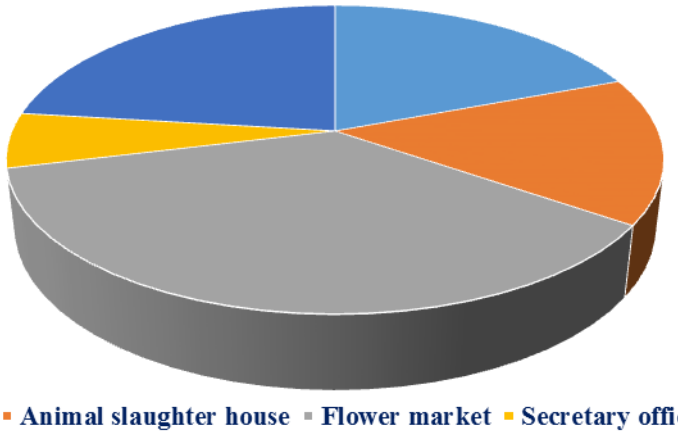
The aerodynamic particle size was measured to understand the aerodynamic diameters of actinomycetes isolates, which was calculated within 0.50 mm to 1.20 mm. The physical sizes of the spores were very smaller than those formerly described in the literature, according to measurements made with a microscope and an image analysis system.

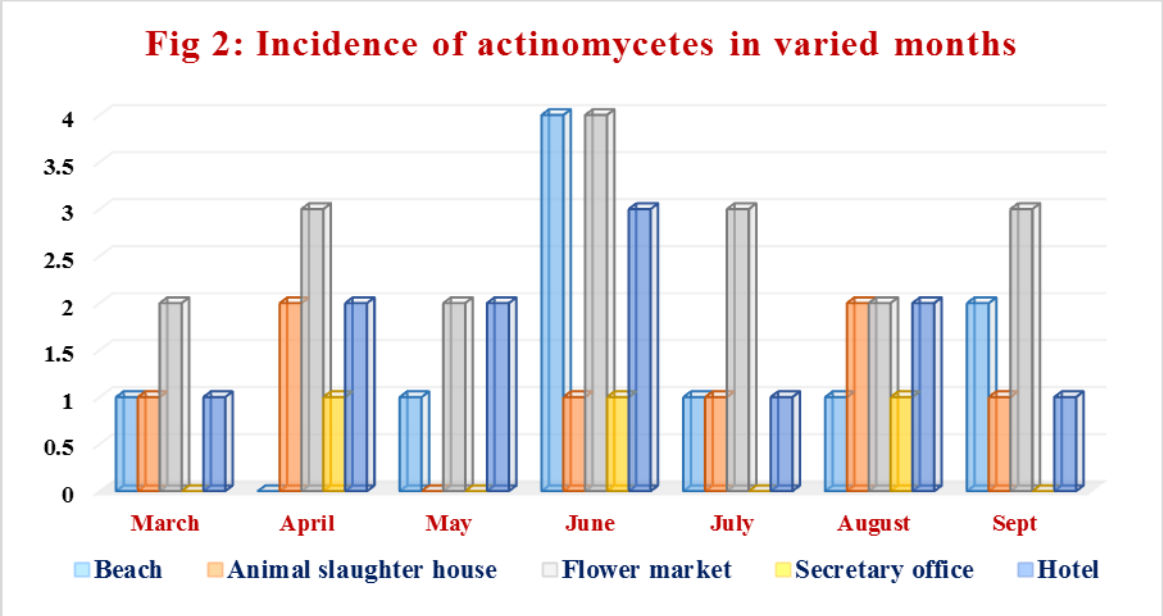
Table 1: Occurrence of airborne actinomycetes in different environments of Puducherry city.

Months	Beach	Animal slaughter house	Flower market	Secretary office	Hotel
March	1	1	2	-	1
April	-	2	3	1	2
May	1	-	2	-	2
June	4	1	4	1	3
July	1	1	3	-	1
August	1	2	2	1	2
September	2	1	3	-	1
Total	10	8	19	3	12

Based on the occurrence of the actinomycetes in different air, Flower market experienced with more number of spores (37%) and it was followed by hotel (23%), beach (19%), slaughter house (15%) and secretary office (6%). Secretary front office was found to be clean from the bio-pollution by actinomycetes among the studied environments (Fig 1). Month of June was recorded with the highest number of actinomycetes isolates in comparison to other months and it was followed by August, April and September among all the months studied (Fig 2). Maximum number of isolates found in June may be attributed to the north east early rainy season which has made the soil actinomycetes dispersed in the air in more quantity than other months. Due to proper cleaning and sanitization, secretary front office do not have more actinomycetes isolates in its air. More number of isolates in flower market may be corroborated with the deposition of organic materials as well as the dumped flower garbage.

Fig 1: Percentage incidence of airborne actinomycetes in varied environments





Only a limited information on the concentrations of actinomycete isolates in air is available at different environments in the literature. Madelin and Johnson.[15] aerosolized three different actinomycete species that were growing on a mixture of agar and hay and measured the airborne concentrations of their spores with an aerodynamic particle size. Angelin Buela and her coworkers.[23] worked on the prevalence of airborne actinomycetes in and around a pond in the garden environment and Senthamizhselvi et al.[24] isolated and enumerated airborne actinomycetes from indoor and outdoor environments of KMGIPSR college campus, Puducherry.

CONCLUSION

The results of this study showed that spore generation and release into the atmosphere require different incubation conditions, including temperature, length, and media nutrients, than colony growth alone. Before spores could be discharged, actinomycetes cultures needed to dry out more. Environments that had been cleansed and sanitized were discovered to be devoid of bio-pollutants such fungi, bacteria, and actinomycetes.

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