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STUDIES ON THE PREVALENCE OF AIRBORNE ACTINOMYCETES IN AND AROUND A POND IN THE GARDEN ENVIRONMENT

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ABSTRACT

Actinomycetes are the exciting and unique group of filamentous bacteria that are well documented for their metabolic adaptability among all microbes. Bioactive feasibility of these actinobacteria facilitates their survival even in unfavourable and anguish locations. Diverse unexplored locations often demand to researchers in the hope of succeeding novel actinomycetes with constant mission that has really led to find extraordinarily productive microbial strains. In our current study, actinomycetes were isolated from different outdoor environments of Dr. Abdul Kalam Eco-garden of our KMGIPSR college campus i.e., near the pond and 20meter away from the pond and at various altitudes viz., above the ground, 5ft above the ground by employing gravity petriplate method using five different media for 5 minutes and 10 minutes each. A total of 27 isolates of the actinomycetes were recorded from both the garden environments. The maximum number of actinomycetes were recorded form 20ft away from pond in comparison to near the pond. Among the Petri plate exposer timing, 10 minute exposer was found to be good to isolate a countable number of actinomycetes in comparison to 5 minutes. Starch Casein Agar (SCA) was documented as one of the well-suited media to isolate actinomycetes from air as compared to Potato Dextrose Agar (PDA), Glycerol Asparagine Agar (GAA) and Actinomycete Isolation Agar (AIA). Isolated airborne actinomycetes were found varied configuration in their concentrations more likely at away from the pond in comparison to near the pond. Based on the antagonistic nature on agar plates, five actinomycetes were tested for their antimicrobial potential, only one actinomycetes was found active against *Staphylococcus aureus, Vibrio cholera, Klebsiella pneumonia*e, *Pseudomonas aeruginosa* and *Candida albicans.*

KEYWORDS: Airborne actinomycetes, outdoor environments, KMGIPSR College campus, Filamentous bacteria, Gravity Petriplate method.

INTRODUCTION

Actinomycetes are one of the favourable microbes on the earth those are commonly Gram-positive bacteria and filamentous in nature like fungi but have tremendous adaptableness to sustain any types of substrates. These actinobacteria are extensively distributed in both the spheres, terrestrial as well as aquatic environments. In soil, they display an essential role in reutilizing refractory biomaterials by disintegrating complex mixtures of polymers in altered forms of materials. The antibiotic compounds developed by them reveals that many more functions like antibacterial, antifungal, anticancer and antiviral characterises. Actinomycetes contribute seventy percentages of the total antibiotics designed by these microbes on the earth.[1] Actinomycetes are known to be the strong sources of antibiotics as well as contributing towards enzymes and vitamins. Antagonistic actinomycetes of marine origin are being regularly described by varied authors for a time record.[1,2,3,4,5] Unevenly of the previous reports unveil that soil is the notable source of varied actinomycetes.[6,7,8,9] but very few studies are there about the actinomycetes of air. The investigation of original and novel antibiotics and other microbial bioactive metabolites is more significant to fight against new emerging microbial pathogens which are not able to be forbidden by the existing drugs in the market.[10,11,12,13] Therefore, it is very important to isolate and enumerate of microbes particularly actinomycetes from diverse ecological sources of highest potency that act as antimicrobial agents. Further, isolation of actinomycetes from exclusive and unmapped natural habitats is of recent interest to avoid re-isolation of strains that produce known bioactive metabolites. Desolate atmospheres are showing to be a good source of novel actinomycetes and bio-active compounds. The present inspection drives at outcome of better antibacterial

compound for monitoring the varied diseases with the help of bio-compounds extracted from the airborne actinomycetes isolated from the surroundings of the pond in garden environments of our K. M. Govt. Institute for Postgraduate Studies and Research campus, Lawspet, Puducherry, India.

MATERIALS AND METHODS

Isolation and enumeration of airborne actinomycetes was made by gravity petriplate method from the garden environments of our Institution, K. M. Govt. Institute for Postgraduate Studies and Research, Lawspet, Puducherry, India during 2019-20 academic year. The isolated airborne actinomycetes were tested for their antimicrobial potential against pathogenic bacteria and *Candida albicans*.

Collection of airborne actinomycetes

During the current work, isolation of airborne actinomycetes was made in different places of Dr. Abdul Kalam Eco-garden like near the pond and 20 meter away from the pond as well as at various altitudes in the garden viz., above the ground and 5ft above the ground by employing gravity petriplate method exposing the petriplates for 5 minutes and 10 minutes each. Different media (Potato Dextrose Agar, Glycerol Asparagine Agar, Actinomycetes Isolation Agar, Starch Casein Agar and Sabouraud Dextrose Agar) were used for the isolation of airborne actinomycetes. PDA slants and PDB were used for sub-culture in order to preserve in refrigerator as well as for further study. Five different media (PDA, SCA, AIA, SDA and GAA) were prepared and poured in the petriplates under sterile condition. These plates were exposed at the above said places and altitudes in order to collect the air samplings in triplicates. After 18 days of incubation actinomycetes were observed based on their morphology.

Enumeration of actinomycetes

After incubation, the colonies on the plates were count down and the total number of actinomycetes were plotted on the table and figure as number and percentage at different sites and altitudes. The isolated actinomycetes were undergone different antimicrobial analysis in order to find their potential and screened out for antimicrobial agents.

Preparation of Test microbes

Test microbes both bacteria and fungus were procured from Microbial type culture collection (MTCC), Chandigarh. Selected pathogens were Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Vibrio cholerae and Candida albicans. Four bacteria and Candida albicans were cultured in Nutrient broth at Ph-7 and stored in refrigerator at 4^{o}c. 24 hours culture was used for antibacterial activity for better results.[2]

Antagonistic activity (Preliminary screening)

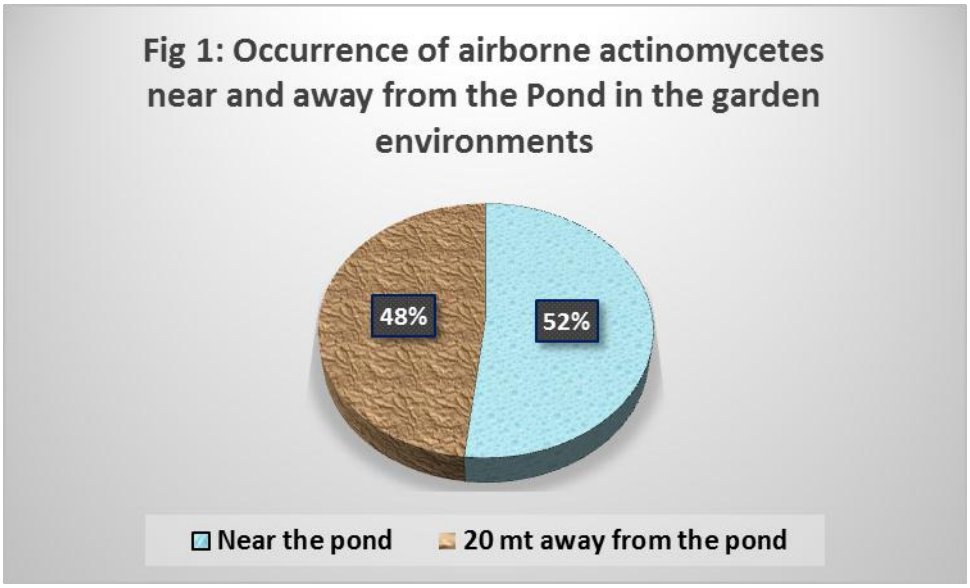
All the actinomycete isolates were screened for antibacterial activity by following Cross streak assay method. In cross streak assay method, single straight line (4–6 mm diameter) of the actinomycetes strains were streaked on the surface of the modified nutrient agar plates (50% NA + 50% SCA) (Glucose: 5g; Peptone: 5g; Beef extract: 3g; Sodium chloride: 5g; Agar: 15g; Distilled water: 1000ml; pH 7.0 ± 0.2; autoclaved at 15lbs for 15 min) and at room temperature (28 ± 2^{o}c) for 5-7 days. On the seventh day of incubation, the overnight culture of MTCC bacterial pathogens were streaked at right angles to the ribbon like growth of actinomycete isolates streaked at the centre of the plate and incubated at 28 ± 2^{o}c for 24 hrs. Inhibition zones of actinomycetes against MTCC bacterial pathogens were measured in mm after 48 h of incubation. Actinomycetes isolates which showed maximum zone of inhibition were selected for secondary screening.

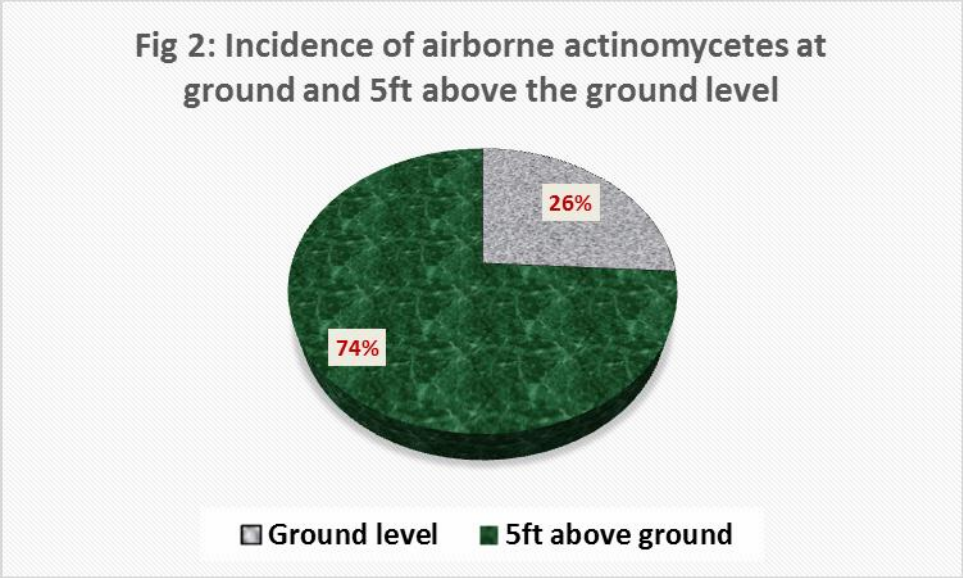
RESULTS AND DISCUSSIONS

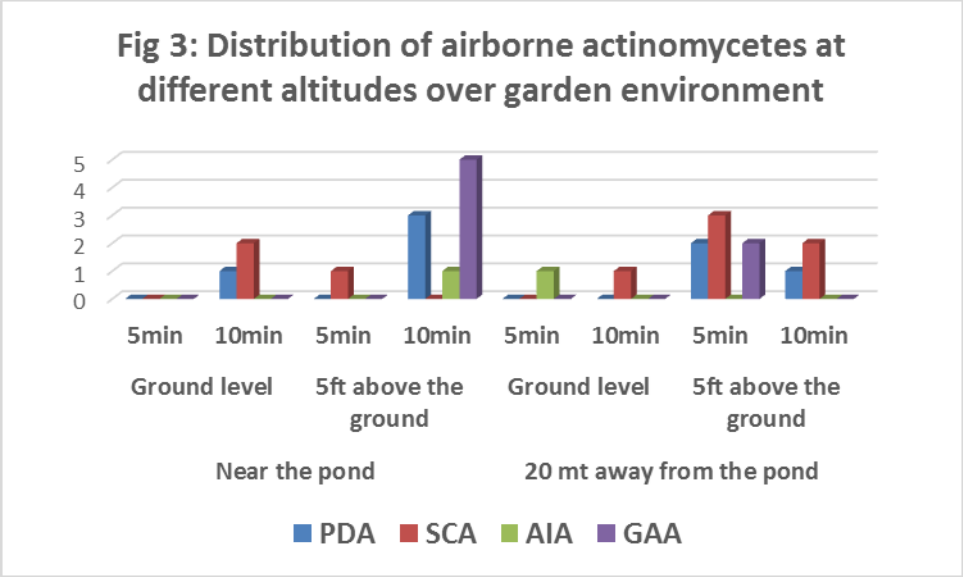
During the study period, altogether, 27 actinomycetes were isolated and named as GA 01 to GA 27. Out of the total actinomycetes isolated, the maximum isolates were recorded near the pond (52%) and it was followed by 20mt away from the pond (48%). Fig 1 shows the occurrence of airborne actinomycetes near and away from the Pond in the garden environments. It was observed that the maximum number of actinomycetes were recorded from 5ft above ground (74%) than that of above the ground level (26%) (Fig 2). Fig 2 displays the incidence of airborne actinomycetes at ground level and 5ft above the ground level in the garden environment. The amount of time required to isolate a countable number of actinomycetes was observed that 10minute was suitable in comparison to 5minute. Fig 3 shows the distribution of airborne actinomycetes at different altitudes over garden environment. Table 1 confirmed about the percentage occurrence of airborne actinomycetes at different places and altitudes in the garden environment.

Table 1: Percentage occurrence of airborne actinomycetes at different places and altitudes in the garden environment.

Media and Time	Near the Pond				20mt away from the Pond			
	Ground level		5ft above the ground		Ground level		5ft above the ground	
	5min	10min	5min	10min	5min	10min	5min	10min
PDA	-	25.0	-	33.3	-	-	28.6	33.3
SCA	-	50.0	100	-	-	50.0	42.8	66.6
AIA	-	-	-	11.1	100	-	-	-
GAA	-	-	-	55.5	-	-	28.6	-
SDA	-	25.0	-	-	-	50.0	-	-







Antibacterial assay of airborne actinomycetes

Based on the primary screening and antagonistic nature on agar plates, five actinomycetes were subjected to visualise their antimicrobial potential. Only one actinomycete was found active against *Staphylococcus aureus, Vibrio cholera, Klebsiella pneumonia*e, *Pseudomonas aeruginosa* and *Candida albicans* (Fig 2). The great majority of antibiotics that have been isolated in numerous screening programs concerned with the search for new therapeutic agents that have been tested primarily for their activity against different bacteria [15]. For the analysis of antimicrobial activity of 5 actinomycetes, primary screening was done antimicrobial assay method and its results is plotted in Fig 2. Most of the isolates were active against *Staphylococcus aureus, E. coli, Pseudomonas aeruginosa, Vibrio cholerae* and *Candida albicans*. Out of 5 isolates, one isolate was selected for secondary screening done by Agar well diffusion method due to their strong inhibitory activity in primary screening. In order to obtain better antibiotic production, liquid medium such as PDB was used. Antibacterial activity of both the isolates were done shown in Fig 2 confirmed the morphological view of the antagonistic behaviour of actinomycets against bacterial pathogens. The current work is agreed with other workers on the antibacterial belongings of actinomycetes [2, 3, 16,17,18] but our work relating to the isolation of actinomycetes from air is itself is unique and new in its work.

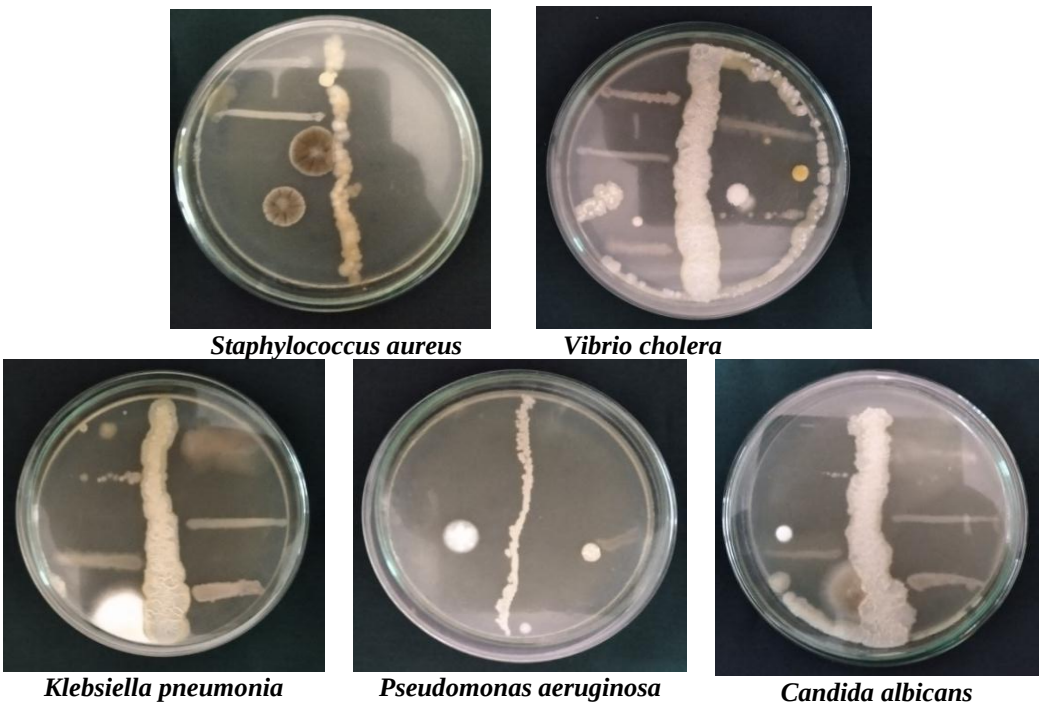


Fig. 4: Antimicrobial potential of airborne actinomycetes isolated from garden environments.

CONCLUSION

Resistance to multi drugs by bacterial pathogens converts it as global problem in order to treat diverse infectious diseases and it is necessary to counter to this feared situation. The present study was a goal to overcome this position by producing new bio-compounds from the actinomycetes isolated from an untouchd environmental source i.e., garden environment in the college campus which was found to be rich source of actinomycetes and shaped good metabolic inhibitory compounds against bacterial pathogens including *Candida albicans*. One of the airborne actinomycetes isolate showed good antibacterial activity against the bacteria and fungi while two of them showed best activity. Since the airborne actinomycetes are found to be potential source of antibiotics, these may be used in the pharmaceutical company to dvelop new drugs for bacterial infections.

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