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ISOLATION AND ENUMERATION OF AIRBORNE ACTINOMYCETES FROM INDOOR AND OUTDOOR ENVIRONMENTS OF COLLEGE CAMPUS

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ABSTRACT

Actinomycetes, one of the exciting novel group of filamentous bacteria are well documented for their metabolic adaptability. The bioactive feasible of these actinobacteria enables their survival even in anguish and unfavourable environments. Different unexplored environments often demand to researchers in the hope of ensuing novel actinomycetes with nonstop mission which has really led to find remarkably industrious microbial strains. In our current study, actinomycetes were isolated from different indoor environments i.e., Botany staff room, Toilet, Library, Laboratory and outdoor environments like Basketball ground of our KMGIPSR college campus by Gravity Petriplate sedimentation method with the use of five media. A total of 16 actinomycetes isolates were recorded from both the environments. Among the Petri plate exposer timing, 60 second exposer was found to be good to isolate a countable number of actinomycetes in comparison to 30 second and 10 second. Glycerol Asparagine Agar (GAA) was recognised as one of the compatible media to isolate actinomycetes from air compared to Starch Casein Agar (SCA) and Actinomycete Isolation Agar (AIA). In concentration, isolated airborne actinomycetes were found to be same together in all the indoor and outdoor environments of the college campus. Based on the antagonistic nature on agar plates, 5 actinomycetes were tested for their antimicrobial potential, only two actinomycetes were found active against *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Candida albicans.*

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

INTRODUCTION

Actinomycetes are one of the promising microbes on the earth those are generally filamentous Gram-positive bacteria in nature but have tremendous adaptability to sustain any types of substrates. These actinobacteria are widely dispersed in both the spheres, aquatic and terrestrial environments. In soil, they show an essential role in reutilizing refractory biomaterials by decomposing complex mixtures of polymers in different forms of materials. The antibiotic compounds expounded by them exhibits many more functions like antiviral, antifungal, antibacterial and anticancer characterises. Actinomycetes contributes seventy percentage of the total antibiotics formed by the microbes on the earth.[1] Actinomycetes are known to be the strong sources of antibiotics as well as contributing towards vitamins and enzymes too. Antagonistic actinomycetes of marine derivation are being regularly reported by varied authors for a time record.[1,2,3,4,5] Roughly of the previous reports disclose that soil is the foremost source of varied actinomycetes[6,7,8,9] but a very few studies are there about the actinomycetes of air. The exploration of original and novel antibiotics and other bioactive microbial metabolites is more important to fight against new emerging microbial pathogens which are not able to be prohibited by the existing drugs in the market.[10,11,12,13] Henceforth, it is very important to isolate and enumerate of microbes from diverse ecological sources of highest potency to act as antibiotics. 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. Deserted environments are showing to be a good source of novel actinomycetes and bio-active compounds. The present examination purposes at outcome of better antibacterial compound for controlling the varied diseases with the help of bio-compounds extracted from the airborne actinomycetes isolated from indoor and outdoor 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 indoor and outdoor 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

Air samplings were made by gravity petriplate method employing five media plates viz., SCA, AIA and GAA at five indoor and outdoor environments of the Department of Botany viz., Laboratory, Toilet, Library, Office room and Basketball ground of our college campus in order to isolate airborne actinomycetes. Three replicates/ petriplates were exposed together at the study sites to find the mean of CFUs on agar plates. After exposer, the plates were brought to the Microbiology laboratory in sterile conditions and incubated at 37^0C in BOD incubator for the growth of actinomycetes for 21 to 30 days with evaluation of colony morphology at weekly intervals. Starch Casein Agar (SCA), Glycerol Asparagine Agar (GAA) and Actinomycete Isolation Agar (AIA) which were supplemented with cycloheximide 75µg/ml and nalidixic acid 75µg/ml to avoid non-actinomycete colonies.[14] Selected colonies were sub cultured and maintained in GAA and PDA.

Preparation of Test microbes

Test microbes both bacteria and fungus were procured from Microbial type culture collection (MTCC), Chandigarh. Selected pathogens were *Staphylococcus aureus, E. coli, 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^oc. 24 hours culture was used for antibacterial activity for better results.[2]

Screening by active agar plug on well diffusion method

Based on the result of primary screening and antagonistic nature on agar plates, five active isolates were selected and subjected to agar well diffusion method. All the selected colonies were cultured in Potato Dextrose Broth and incubated for 15 days at room temperature. After incubation, medium was filtered using Whatman No.1 filter paper. Nutrient agar plates Ph-7 were prepared and surface inoculated by test bacterial and *Candida albicans* culture. Six mm wells were created in medium using sterile corkborer. Each well was loaded active agar plugs of the actinomycetes and the plates were incubated at room temperature for 24 to 48 hours. After incubation, zone of inhibition was measured and the antibacterial activity was determined.

RESULTS AND DISCUSSIONS

Air samplings were made by gravity petriplate method in the indoor and outdoor environments of our college campus employing three media plates viz., SCA, AIA and GAA by pour plate technique. Altogether, 16 actinomycete were isolated and named as AA 1 to AA 16. They were pure cultured and maintained on GAA and PDA for future use (Table 1). Amid the Petri plate exposer timings, 60 second exposer was found to be good in order to count a comfortable number of actinomycetes in comparison to 30 second and 10 second. Glycerol Asparagine Agar (GAA) was recognised 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 same together in all the indoor and outdoor environments of the college campus. Fig 1 shows the growth of actinomycetes and their CFUs on agar plates isolated at indoor and outdoor environments of college campus.

Table 1: Isolation of airborne actinomycetes from indoor and outdoor environments of KMGIPSR college campus.

	Isolated actinomycetes (Indoors and Outdoors)		
Media used	**Laboratory (Indoor)**		
	10 second	**30 second**	**60 second**
AIA	-	-	1
GAA	-	-	2
SCA	-	-	1
	Toilet (Indoor)		
AIA	-	1	1
GAA	-	-	2
SCA	-	-	1
	Office room (Indoor)		
AIA	-	-	-
GAA	-	-	1
SCA	-	-	1
	Library (Indoor)		
AIA	-	-	1

GAA	-	1	-
SCA	-	-	1
	Basketball ground (Outdoor)		
AIA	-	-	1
GAA	1	1	1
SCA	-	-	1

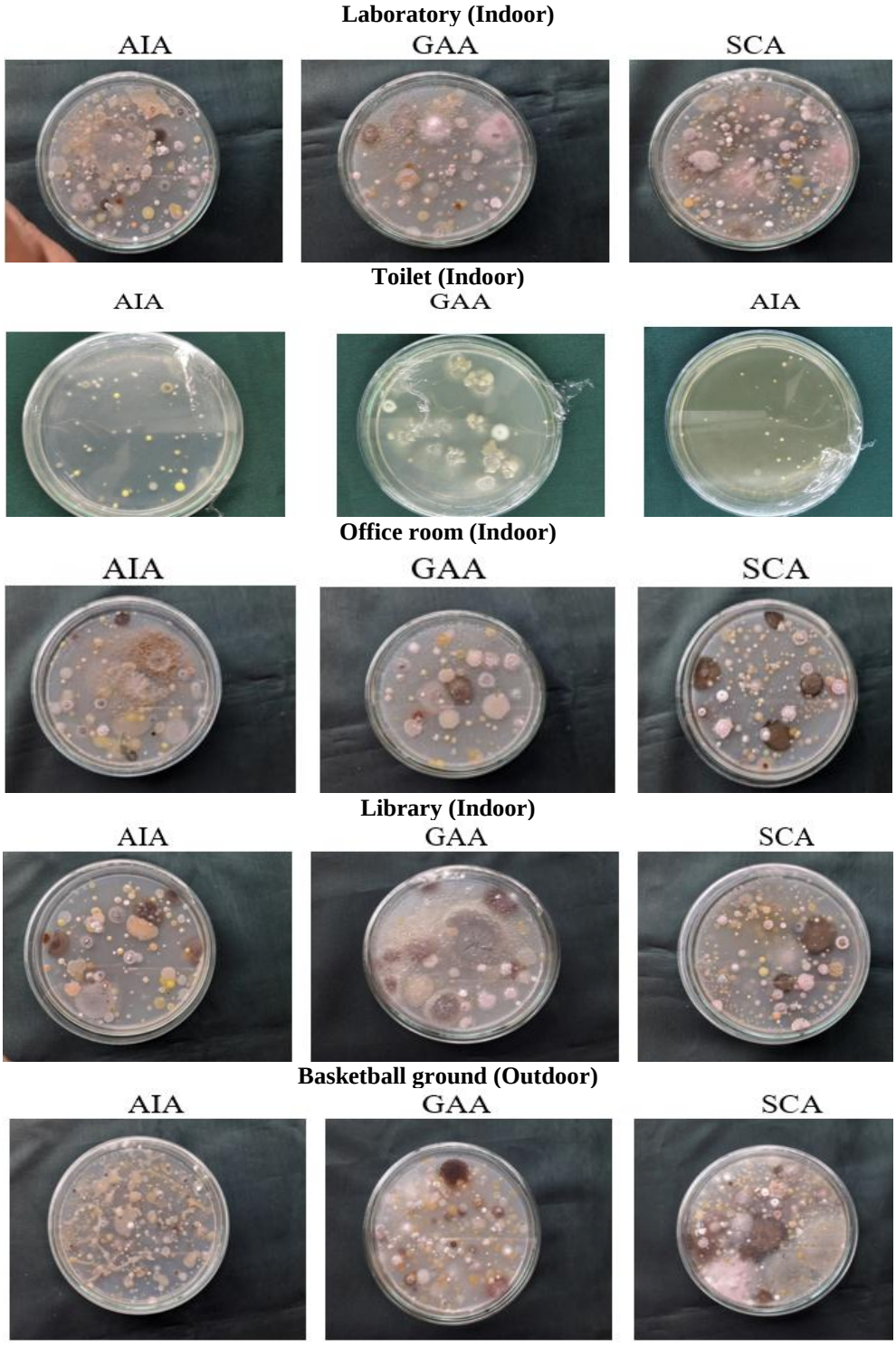
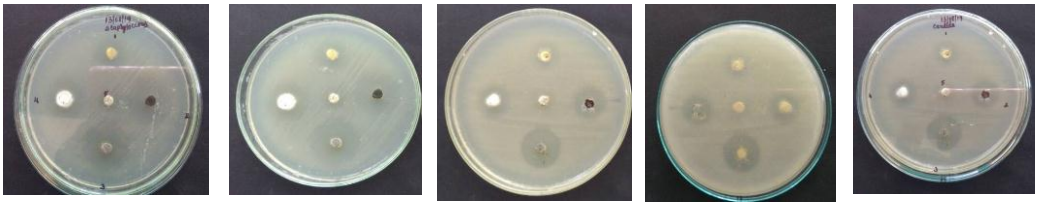


Fig 1: Actinomycetes on agar plates at indoor and outdoor environments of college campus.

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 two actinomycetes were found active against *Staphylococcus aureus, E. coli, Pseudomonas aeruginosa, Vibrio*

cholerae 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, two isolates were 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. Active isolates, AA-9 and AA-11 showed strong inhibition zones against the tested bacteria and *Candida albicans*. 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 present 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.



Staph. aureus ***Escherichia coli*** ***P. aeruginosa*** ***Vibrio cholerae*** ***C. albicans***

Fig 2: Antimicrobial potential of airborne actinomycetes isolated from indoor environments.

CONCLUSION

Multidrug resistance by bacterial pathogens becomes a global problem in order to treat varied infectious diseases and it is necessary to counteract to this dreaded situation. The present study was an aim to overcome this situation by producing new bio-compounds from the actinomycetes isolated from an untouchd environmental source i.e., indoor and outdoor air of the college campus which was found to be rich source of actinomycetes and produced good metabolic inhibitory compounds against bacterial pathogens including *Candida albicans*. Five of the airborne actinomycetes isolates 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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