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STUDIES ON ANTIBACTERIAL ACTIVITY OF MUD WASP NEST ACTINOMYCETES AGAINST MTCC BACTERIAL STRAINS BY SCREENING METHODS

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ABSTRACT

During the study period, actinomycetes were isolated from mud wasp nest by serial dilution pour plate method. All the actinomycetes were curtained for their antagonistic proeprties against pathogenic bacterial strains procured from IMTECH, Chandigarh, India. Initially the actinomycetes were primarily screened by cross streak method and only two potential actinomycetes were tested subjected to secondary screening which was done by Agar well diffusion method. Most of the isolates were found active against *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Vibrio cholerae*. Most of the actinomycetes (around (80%) showed good antagonistic effect against most of the tested pathogens and 20% of the total actinomycetes were found non-effective against the test pathogens.

KEYWORDS: Antibacterial activity, Wasp nest actinomycetes, Well diffusion method, Cross streak method, MTCC. Strains.

INTRODUCTION

Actinomycetes are one of the strongest antagonists among microbes and ruled over time immemorial. The antibiotic substances elaborated by them display antibacterial, antifungal, anticancer, antiprotozoal and antiviral properties. Of the ten thousand known antibiotics produced by microbes over a decade ago, about 70% are of actinomycete origin; of them, representatives of the genus *Streptomyces* account for two thirds.[1] Actinomycetes are known to be the potent sources of antibiotics, too with vitamins and enzymes and such of antagonistic actinomycetes of marine origin are being regularly reported by varied authors for a time record.[1,2,3,4,5] Some of the reports reveal that soil is the major source of actinomycetes.[6,7,8,9] The search of new and novel antibiotics and other bioactive microbial metabolites is more important for the fight against new emerging pathogens which are not able to be prevented by the existing drugs in the market.[10,11,12,13] It is very important to isolate and enumerate of microbes from different environmental sources of highest potency to act as antibiotics. Further, isolation of actinomycetes from unique unexplored natural habitats is of interest to avoid re-isolation of strains that produce known bioactive metabolites. Neglected habitats are proving to be a good source of novel actinomycetes and bio active compounds. The present investigation aims at finding better antibacterial compound for controlling the bacterial diseases with the help of bio-compounds extracted from the actinomycetes isolated from mud wasp nest soil collected from our K. M. Govt. Institute for Postgraduate Studies and Research campus, Lawspet, Puducherry, India.

MATERIALS AND METHODS

Collection of mound wasp nest soil

Mud wasp nest soil was collected from our K. M. Govt. Institute for Postgraduate Studies and Research, Lawspet, Puducherry, India and the soil samples were transferred to the sterile polythene bag and brought into the laboratory. The pH of the fresh soil sample was determined.[14] Further, the soil samples were grounded and sieved to remove the debris.

Isolation of wasp nest actinomycetes

One gm soil sample was poured into 9ml (10^{-1} distilled water and serially diluted up to 10^{-5}. For the isolation of the actinomycetes, 10^{-5} dilution was taken and pour plate technique was done,[15] using Starch Casein Agar [16] which was supplemented with cycloheximide 75µg/ml and nalidixic acid 75µg/ml to avoid non-actinomycete colonies. All the plates were incubated at room temperatures for 15-30 days and the plates were examined for appearance of actinomycete colonies. Selected colonies were sub cultured and maintained in SCA and PDA.

Preparation of Test bacteria

Test bacteria were procured from Microbial type culture collection (MTCC), Chandigarh. Selected pathogens

such as *S. aureus, S. putrefaciens, Pseudomonas vulgaris, P. aeruginosa, Vibrio cholera, E. coli, K. pnuemoniae, Bacillus subtilis*. Test bacteria were cultured in Nutrient broth at Ph-7 and stored in refrigerator at 4°c. 24 hours culture was used for antibacterial activity for better results.[2]

Screening test for antibacterial assay

Primary screening by cross streak method

All the actinomycetes were primarily screened by cross streak method.[17] A loop of actinomycetes colonies were streaked as a thin line on SCA medium and allowed to grow for 10 days for better antibiotic production. After incubation, 24hours bacterial cultures were streaked perpendicular to the line of grown actinomycetes and incubated for 24hours at 30°c. After incubation, the zone of inhibition was measured and recorded.

Secondary screening by Agar well diffusion method

Based on the result of primary screening, two active isolates were selected and subjected to agar well diffusion method. All the selected colonies were cultured in Potato Dextrose Broth and incubated for 15days 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 culture. Six mm wells were created in medium using sterile corkborer. Each well was loaded with 100µl of culture filterate and the plates were incubated at room temperature for 24hours. After incubation, zone of inhibition was measured and the antibacterial activity was determined.

RESULTS AND DISCUSSIONS

Isolation and maintenance of mud wasp nest soil actinomycetes

Mud wasp nest soil was collected and the soil samples were inoculated on SCA, PDA, AIA media plates by serial dilution-pour plate technique. 15 actinomycete colonies were isolated and pure cultured and maintained on SCA and PDA for future use. The great majority of antibiotics that have been isolated in numerous screening programs concerned with the search for new therapeutic agents have been tested primarily for their activity against different bacteria.[18] All the actinomycetes were subjected for screening test using MTCC bacterial cultures.

Antibacterial assay of mud wasp nest soil actinomycetes

For the analysis of antibacterial activity of 15 actinomycetes, primary screening was done by cross streak method and its results is plotted in Table 1. Most of the isolates were active against *S. aureus, S. putrefaciens, Bacillus subtilis, P. aeruginosa, E. coli, P. vulgaris, K. pnuemoniae, Pseudomonas* sp. *and V. cholera*. Out of 15 isolates, two isolates were selected for secondary screening done by Agar well diffusion method due to their strong inhibitory activity in primary screening (Table 1). In order to obtain better antibiotic production, liquid medium such as PDB was used. Active isolates, WN-9 and WN-11 showed strong inhibition from both primary and secondary screening (Table 1). Antibacterial activity of actinomycetes by secondary screening (Agar well diffusion assay) is given in Table 2, which depicted the antibacterial potency of the two best actinomycetes strains showing good activities against five pathogens. Antibacterial activity of both WN-9 and WN-11 isolates were done by Cross streak method and is given in Fig 1, which shows the morphological view of the antagonistic behaviour of actinomycets against bacterial pathogens. The present work is agreed with other workers on the antibacterial properties of actinomycetes[2,3,13,14] but our work peratining to the isolation of actinomycetes from mud nest soil is itself is unique and new in its work.

Table 1: Primary screening of antibacterial activity of actinomycetes isolated from mud wasp nest by cross streak method.

Sl. No.	Identification Code of the Isolates	Zone of inhibition in mm								
		S. aureus	***S. putrefaciens***	***B. subtilis***	***P. aeruginosa***	***E. coli***	***P. vulgaris***	***K. pneumonia***	***Pseudomonas vulgaris***	***V. cholerae***
1	WN-1	04	04	03	03	05	04	03	03	04
2	WN-2	11	10	15	12	11	14	10	07	11
3	**WN-3**	**13**	**13**	**12**	**12**	**10**	**18**	**10**	**02**	**15**
4	**WN-4**	**09**	**16**	**17**	**13**	**10**	**13**	**13**	**03**	**09**
5	WN-5	14	12	15	07	04	10	12	12	13
6	**WN-6**	**08**	**13**	**12**	**16**	**07**	**04**	**14**	**10**	**08**
7	WN-7	14	15	13	14	13	16	08	07	14
8	WN-8	04	04	07	10	03	03	05	10	10
9	**WN-9**	**14**	**11**	**13**	**11**	**10**	**09**	**05**	**10**	**10**
10	WN-10	11	12	14	13	15	01	13	01	10
11	**WN-11**	**17**	**17**	**18**	**17**	**10**	**17**	**15**	**02**	**14**

12	WN-12	11	16	14	11	12	02	14	02	08
13	WN-13	22	19	22	21	9	21	16	09	01
14	WN-14	14	11	12	13	10	11	11	06	11
15	**WN-15**	**12**	**13**	**14**	**13**	**12**	**11**	**14**	**05**	**13**

Table 2: Antibacterial activity of actinomycetes by secondary screening (Agar well diffusion assay).

Isolate code	Diameter of inhibition zones by Agar well diffusion methods(mm)				
	S. aureus	***B. subtilis***	***Pseudomonas vulgaris***	***V. cholerae***	***E. coli***
WN-9	**14.0**	**14.5**	**13.5**	**12.0**	**9.0**
WN-11	**14.0**	**14.0**	**17.5**	**14.0**	**10.0**

Fig. 1: Antibacterial activity of WN-9 and WN-11 by Cross streak method.

CONCLUSION

Bacterial resistance to multidrug is a global problem in the treatment of infectious diseases and to counteract to this situation is very tough and critical. The present study was an aim to overcome this situation by producing new bio-compounds from the actinomycetes isoleted from an untouchd environmental source viz., Mud wasp nest soil which was found as a rich source of actinomycetes and produced good metabolites inhibitory compounds against bacterial pathogens. Mostly (80%) of all the actinomycetes isolates showed good antibacterial activity against all the bacteria while 20% showed least activity. Since the mud wasp nest soil actinomycetes are potential source for producing antibiotics for bacteria, these can be used in the pharmaceutical field to find novel drugs for bacterial infections.

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