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Antibacterial Activity of Actinomycetes Isolated from Different Habitats of Hadhramout- Yemen

Ali Mohammed Abdullah Bawazir[1], Manjula Shantaram*[2]
[1]Department of Microbiology, Mangalore University, Post Graduate Centre, Jnana Kaveri, Chikka Aluvara, Kodagu, Karnataka, India
[2]Department of Studies in Biochemistry, Mangalore University, Post Graduate Centre, Jnana Kaveri, Chikka Aluvara, Kodagu, Karnataka, India

Original Research Article

***Corresponding author**
Manjula Shantaram

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Abstract: A total of 70 actinomycetes strains were isolated from dams and caves of Hadhramout –Yemen and screened for their anti-bacterial activity based on their color and activity against bacterial pathogens. Twenty isolates have shown activities against pathogenic bacteria. Fourteen isolates (70%) showed activity against *Bacillus subtilis* by agar disk diffusion, twelve isolates (60%) showed activity against *Staphylococcus aureus* and fifteen isolates (75%) showed activity against *Pseudomonas aeruginosa.* The activity was minimal against *Escherichia coli*, having only 8 isolates (40%). Nine isolates were highly active with an inhibition zone more than 25 mm in diameter. Most of the isolates inhibited growth of tested Gram negative and Gram positive bacteria.

Keywords: Cave soil, Dam soil, antibacterial, actinomycetes, and pathogenic bacteria.

INTRODUCTION

The war continues between antibiotics and resistant bacteria. More the scientists produce new and effective antibiotics, more frequent will the bacteria change their genetic mutations and adjust their defenses according to the components of the new antibody and win it, every time. To solve the problem of antimicrobial resistance there is a need for the discovery of new drugs, which is a critical element in a coordinated response to antimicrobial resistance. The resistance of bacteria to antibiotics is one of the global health and economic problems, prompting researchers to look for new antibodies to overcome resistant bacterial strains that increase mortality and epidemics.

Health experts estimate that nearly 90,000 people die each year from antibiotic-resistant bacteria in the United States, and hospital-acquired disease develops in more than 2 million people annually, and that three-quarters of these occur as a result of at least one adverse common antibiotic. Antibiotic-resistant bacteria make the patient stay in the hospital for a long time, increase the risk of disease and also increase direct economic costs of antibiotic-resistant bacteria. For example, the infection of *Pseudomonas aeruginosa* in hospitalized patients costs $ 74,040, more than the treatment costs for a sensitive strain, ie., patients with *Staphylococcus aureus* methicillin resistance and may cost up to $ 4,000 more than the cost of treating patients with an antibiotic-resistant *S. aureus* strain. The cost of treatment with multidrug-resistant TB bacteria is $ 180,000 or more and the cost of treatment for sensitive strains is $ 2,000. In general, the total cost associated with antibiotic-resistant bacteria is estimated at $ 4-5 billion per year in the United States [1].

There is a look out for new antibiotics to treat diseases by searching for other sources such as actinomycetes to isolate raw antibiotics. Because, now two thirds of the antibiotics used in medicine are newly isolated from actinomycetes and many more are of medical importance, having been isolated from it [2]. Screening of actinomycetes for the production of new antibiotics has been intensively followed for many years by scientists. Antibiotics have been used in many fields including agriculture, veterinary and pharmaceutical industry. Actinomycetes have the ability to synthesize many different biologically active secondary metabolites such as antibiotics, herbicides, pesticides, anti-parasitic, and enzymes like cellulase and xylanase which are used in waste treatment [3].

Actinomycetes are vastly distributed groups of microorganisms naturally forming large part of the microbial population of the soil and aquatic environment such as rivers, lakes and other freshwater habitats [4]. The primary aim of the present study was to isolate actinomycetes having strong activity against pathogenic bacteria, especially against *Peudomonas aeruginosa*, because it is resistant to too many antibiotics.

MATERIALS AND METHODS

Sampling procedure

Between 2016 and 2017, eight soil samples were collected from 5-10 cms depth, into the sterile plastic bags from dams and caves of Hadhramout province of Yemen and its surroundings. Soil samples were air dried at room temperature.

Isolation of actinomycetes colonies

Isolation and enumeration of actinomycetes were performed by soil dilution plate technique using starch casein agar (SCA**).** It contained 10.0 g of starch; 1.0 g of casein; 0.02 g of $CaCO_3$; 0.01g of $FeSO_4.7H_2O$; 2.0 g of KNO_3; and 20.0 g of agar [5]. Isolation of actinomycetes was done by suspending 1 g of soil sample in 10 ml sterile water, which was vigorously shaken and allowed to settle for 5 min. The supernatant was serially diluted and incubated at 37 °C for 14 days.

Target pathogenic bacterial cultures

Antibacterial activities were tested against two Gram positive and two Gram negative bacteria which included *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa*.

Screening actinomycetes for antibacterial activities

The actinomycetes isolates were streaked on starch casein agar (SCA) of pH 7 and incubated at 28 °C for 7 days. After 7 days, the target organisms were seeded in nutrient agar (NA) of pH 7 and bacterial discs of 1.0 cm diameter with actinomycetes were transferred to the surface of NA plates. These plates were kept for one hour in refrigerator to facilitate diffusion then incubated at 37 °C for 24 hours. Antimicrobial activity was noted in terms of zone of inhibition around the agar discs of actinomycetes [6].

Identification of actinomycetes

The identification of actinomycetes was done by observing colony and performing biochemical tests according to Berge's manual, 2005.

RESULTS AND DISCUSSIONS

In the present study, actinomycetes were isolated from 1g of soil sample which was collected from dams and caves (Fig 1). Actinomycetes isolates were obtained from soil by serial dilution plates as done earlier by Vimal *et al*.[7]. Actinomycete isolates were screened for antibacterial activity by ADD (agar disc diffusion) method (Fig 2). From 70 actinomycetes isolates, 20 isolates were selected for the study according to color and their activity against bacterial pathogens (Fig 3). All the isolates were screened for antimicrobial activity, by ADD. Antimicrobial activity was studied against Gram negative and Gram positive bacteria. The target cultures used were *Escherichia coli*, *Pseudomonas aeruginos*a, *Staphylococcus aureus* and *Bacillus subtilis.* Out of 20 actinomycetes isolates, there were 14 isolates (70%) showing activity against *Bacillus subtilis*, *Staphylococcus aureus* 12 isolates (60%) and 15 isolates (75%) *Pseudomonas aeruginos*a. The activity was minimal against *Escherichia coli*; only 8 isolates (40%) had shown activity (Fig 4). All isolates showed strong activity against both Gram-positive and Gram-negative bacteria and in contradiction with the observation of Nkanga and Hagedorn [8] and AL-Mahdi [9], who reported that acidophilic actinomycetes showed more activity against Gram-positive bacteria compared to Gram-negative. These results prove that the actinomycetes are capable to produce a varied diversity of antibiotics with antibacterial activity. Thakur *et al*. [10] who reported that among the 110 isolates, 65 (59.09%) strains showed antibacterial activity, 47 (42.72%) strains showed antifungal activity and 33 (30%) strains exhibited a broad-spectrum activity against both test bacteria and fungi. Kumar *et al.* [11] also reported that a total of 117 actinomycetes isolates were isolated from the soil samples collected from the waste and alkaline and garden soil samples of the Ghaziabad, India, their results indicated that six isolates were highly active against *Staphylococcus aureus* strains. Seven isolates were highly active with an inhibition zone more than 20 mm in diameter. Most of the isolates inhibited growth of the tested Gram negative bacteria.

Our isolated strains were studied for culture characteristics (Table 1). This study agrees to our aim of isolation and screening of actinomycetes from the soils of dams and caves of Hadhramout region at 37℃ temperature and selecting the isolates with strongest antibacterial activity. All the isolates grown on starch casein agar media showed morphology typical of actinomycetes, since the colonies were slow growing, aerobic, glabrous, folded and with aerial and substrate mycelia of different colors.

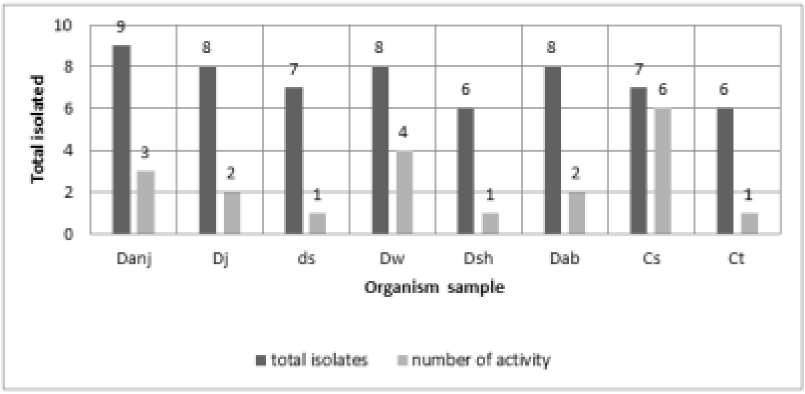

Fig-1: Selection of actinomycetes from different soils

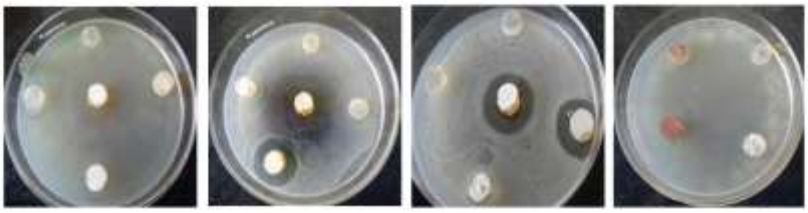

Fig-2: Antibacterial activity by ADD method *against- Staphylococcus aureus*; *Bacillus subtilis*; *Escherichia coli*; *Pseudomonas aeruginosa*

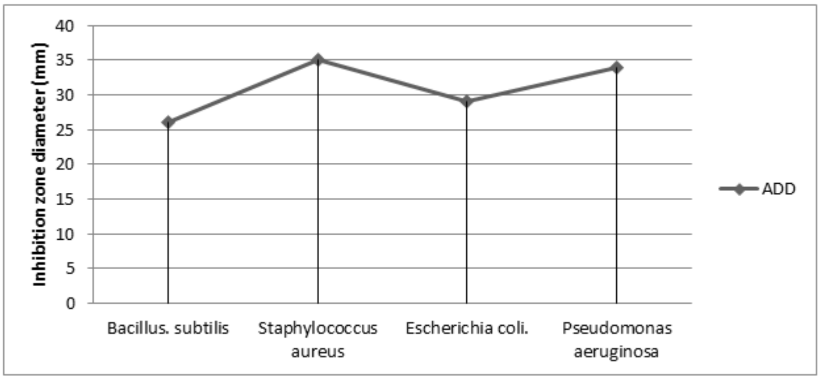

Fig-3: Maximum inhibition zone of antibacterial activity of actinomycetes isolates by Agar Disc Diffusion

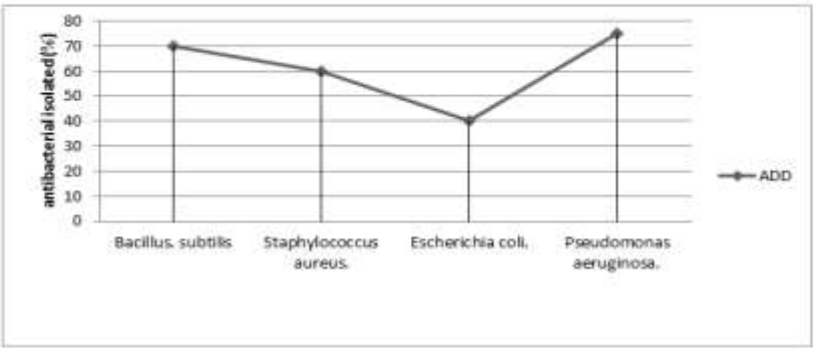

Fig-4: Percentage of antibacterial activity of actinomycetes isolates

Table-1: Culture characteristics of selective isolates on starch casein agar medium

Culture code	Colony shape	Colony consistency	Colony elevation	Colony visible in	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigment
Dnj_2	Irregular	Dry ash	Flat	48h	Good	White	Red orange	-
Dnj_3	Irregular	Hard	Convex	24h	Good	White	Melon yellow	-
Dj2	Irregular	Dry ash	Convex	24h	Good	White		
Ds_1	Irregular	Hard	Convex	120h	Moderate	-	Red orange	-
Dw_3	Irregular	Dry ash	Convex	24h	Good	White	Paige	-
Dw_4	Irregular	Hard	Convex	24h	Moderate	Yellow	Yellow orange	-
Dw_5	Irregular	Hard	Convex	72h	Moderate	Red orange	Red orange	Red orange
Dw_6	Irregular	Hard	Convex	24h	Moderate	Purple red	Beige red	-
Dsh_1	Irregular	Dry ash	convex	48h	Good	Grey to white	Brown	Brown
Dab_1	Irregular	Hard	Convex	48h	Good	White	Yellow white	
Cs_1	Filamento us	Dry ash	Convex	24h	Good	Grey white	Red orange	-
Cs_2	Irregular	Dry ash	Convex	24h	Good	Grey white	Brown yellow	-
Cs_3	Irregular	Dry ash	Umbonate	24h	Good	Grey white	Brown orange	Dark ivory
Cs_7	Irregular	Soft	Umbonate	24h	Good	White	Beige	-
Cs_{14}	Irregular	Hard	Convex	120h				
Ct_2	Irregular	Dry ash	Concave	24h	Good	White	White to yellow	-
Cb_1	Irregular	Soft	Convex	24h	Good	White	Yellow	Yellow
Cb_2	Irregular	Soft	Plan	24h	Good	White	Yellow	yellow
Ct_2	Irregular	Dry ash	Concave	24h	Good	White	White to Yellow	-

CONCLUSION

Isolation of actinomycetes from different environments like dams and caves are important for isolation of new antibiotics which contributes for treatment of several diseases. Some of them have more activity such as 34 mm inhibition zone against *Pseudomonas aeruginosa* which is resistant to most antibiotics.

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