Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 10 [1] December 2020 : 146-150

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Antibacterial and Antioxidant Activity of Streptomyces Isolated From Soil

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ABSTRACT
Microorganisms are attractive sources of bioactive compounds with pharmaceutical importance. In the present study the potency of actinomycetes to produce antimicrobial substances has been studied in 28 strains isolated from different samples of agricultural land in Erode, Tamilnadu. Antibacterial activity of all the isolated actinomycete strains were checked by cross streak method against Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi. Solvent extracts showed significant result in pathogen inhibition. A4 isolate unveiled the efficient radical scavenging ability which was found to be 69.87% at highest concentration 500µg/ml, This extracts could be exploited to treat infection in future with the International Standard Experimental results.
KEYWORDS:- Actinomycetes , Streptomyces , Antibacterial , Antioxidant & Inhibitory zone

Received 21.10.2020 Revised 16.11.2020 Accepted 04.12.2020

INTRODUCTION

Actinomycetes, belonging to the order Actinomycetales, are Gram positive, filamentous eubacteria with high G+C content. They are responsible for the production of volatile compounds like geosmin responsible for characteristic earthy odor. They can degrade a variety of compounds such as lignocelluloses and many other polymers occurring in soil and litter, and a range of xenobiotic compounds. In addition, due to their metabolic diversity, actinomycetes are important source of lytic enzymes, antibiotics and other bioactive metabolites such as plant growth promoters, herbicides, insecticides, antitumor agents etc. These actinomycetes have been considered as biotechnologically and industrially valuable prokaryotes since they have produced a large number of compounds of pharmaceutical and agricultural importance [1]. Soils have been screened by pharmaceutical industry for about 50 years, only a small fraction of *actinomycetes* taxa have been discovered. Hence more focus needs to be shifted in exploring unexplored habitats [2].

Free radicals and oxidants play a dual role as both toxic and beneficial compounds, since they can be either harmful or helpful to the body. It has been implicated in the development of many human diseases. A few of them include arthritis, inflammatory diseases, kidney diseases, cataracts, inflammatory bowel disease, colitis, lung dysfunction; pancreatitis; drug reactions, skin lesions, and aging. Free radicals are also associated with liver damage due to alcohol consumption and the development of emphysema due to cigarette smoking. They are produced either from normal cell metabolism *in situ* or from external sources (like pollution, cigarette smoke, radiation and medication). When an overload of free radicals cannot gradually be destroyed, their accumulation in the body generates a phenomenon called oxidative stress. This process plays a major part in the development of several chronic and degenerative illness.

Moreover, it has been shown that antioxidants and free radical scavengers are crucial in the prevention of pathologies, in which reactive oxygen species (ROS) or free radicals are implicated. Synthetic antioxidants have been used in stabilization of foods. But their use is being restricted nowadays because of their toxic and carcinogenic effects. Thus, interest in finding natural antioxidants, without any undesirable effect, has increased greatly. Hence a study was planned to screen soil *actinomycetes* for antimicrobial and antioxidant secondary metabolites. The strain was isolated from soil sample of sathy, Erode. Our aim is toevaluate its antimicrobial and antioxidant activity.

MATERIAL AND METHODS

SAMPLE COLLECTION

A study was done by the collection of soil samples from different locations of agricultural land in sathy, Erode. The depth of 5cm below the surface of the soil was chosen and the soil samples were brought to the laboratory in sterile polythene bags and refrigerated.

Isolation of actinomycetes from farming soil:

MEDIA AND CULTURAL CONDITIONS:

Five actinomycete strains were isolated and obtained as pure culture by using standard microbiological method. From each soil sample, 1 gm of dried soil was suspended in 9 ml of sterile distilled water, and successive serial dilutions were made by transferring 1ml of aliquots to 2nd test tube containing 9 ml of sterile distilled water, and in this way dilutions up to 10^{-6} were prepared. Each time the contents were vortexes to form uniform suspension. An aliquot of 0.1 ml of each dilution was taken and spread evenly over the surface of nutrient agar medium supplemented with cyclohexamide (100µg/ml). Plates were incubated at 27°C and monitored for 7 days. The suspected colonies were purified using starch casein nitrate agar medium.

In vitro screening of isolates for antimicrobial activity

Primary screening

Isolated strains were inoculated on nutrient agar plates by single streak at the center. The plates were incubated at 30 °C for 3 days. Five bacterial pathogens *namely Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli, Salmonella typhi* were streaked perpendicular to the antagonist on the agar medium. The plates were incubated at 37 °C for 24 h. The microbial inhibitions were observed by determining the diameter of the inhibitory zone [3].

Fermentation and Extraction of Bioactive compounds [4].

The selected antagonistic isolates A1&A4 was inoculated into 500 ml of the fermentation medium, ISP4 (International Streptomyces-4) and incubated on rotary shaker at 200 rpm at 28°C for ten to fiftteen days. After fermentation the broth was filtered through sterile Whatmann No.1 filter paper. Then, the culture filtrate was centrifuged at 10,000rpm for 5 minutes to remove biomass and cell debris. After centrifugation, supernatant was separated and the antimicrobial compound was recovered from the filtrate by the solvent extraction method [5]. Equal volume of ethyl acetate was added to supernatant (1:1), shook vigorously and allowed to settle for 4 hours. The aqueous layer was collected in watch glass and kept in water bath at 70^0C – 80^0C until the solvent evaporates and the product was grabbed and stored in 4^0 C for further studies [6].

Secondary screening-Agar-well diffusion method [7].

The antibacterial activity of the isolates was analyzed by agar well diffusion method. The bacterial pathogens such as *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Bacillus cereus, Shigella flexneri., Streptococcus sp., Klebsiellapneumoniae.,* were swabbed onto the surface of Mueller-Hinton agar plates. The wells were made by using sterile cork borer. Then the extracted bioactive compound mixture was loaded into the wells. The anti bacterial plates were incubated at 37^0C for 24 hours and the diameter of inhibitory zones were measured.

DPPH radical scavenging assay:

The antioxidant activity of Actinomycetes was determined by DPPH scavenging assay [8]. Various concentration (100,200,300,400,500µg/ml) of crude extract were taken in separatetest tubes. Ascorbic acid was used as reference compound (100,200,300,400,500 µg/ml). A freshly prepared solution with 0.002% of 2 ml DPPH (1, 1, Diphenyl-2-Picryl hydrazyl) was added to each tube containing different concentrations of extract and standard solution respectively. DPPH in methanol without the ethyl acetate extract served as the positive control.The samples were incubated in dark place at 27°C for 20 min and read at 515 nm. The percentage of radical scavenging activity of the extract against the stable DPPH• was calculated using following equation (OD = optical density):

Radical Scavenging Activity (%) = (Control OD – Sample OD) / Control OD x 100

RESULTS

In the present study, 28 isolates were found from farming soil. Five actinomycete isolates were selected by screening . They were labeled as A1, A2, A3, A4 and A5. Out of these five isolates two isolates namely A1 and A2 (Fig 1) exhibited marked antagonistic activity against different bacterial pathogens.

Screening of isolates for antimicrobial activity:

Primary screening:

The isolates were subjected to cross streak method in order to assess antagonistic property against various bacterial pathogens in Actinomycete Isolation agar. Presence of clear zone or reduced growth of

test bacteria near the growth of actinomycetes was considered as positive for antagonistic activity (Fig 2).

Fermentation and Extraction of bioactive compounds:

During fermentation process, the growth rate of actinomycetes was high in first 3 days, after that a thick mass of cells were developed in the flasks. At the end of fermentation period a dense growth of actinomycetes was observed and the colour of medium changes from white to black. Bioactive compounds were separated from the culture broth by solvent extraction method.After the solvent extraction, the solvent phase was collected and evaporated in water bath at 70^0C for 1 hour. The product obtained was dry in nature and white in colour.

Secondary Screening of antimicrobial *Actinomycetes:*

Out of five isolates, two isolates (A1 and A4) showed significant antimicrobial activity against the selected bacterial pathogensinclude, *Escherichia coli, Staphylococcus aureus, Shigellaflexneri, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa, Bacillus cereus, Streptococcus pyogenes.* The zone of inhibition was measured and tabulated (Table 1). Similar results were observed by Usha *et al*., (2010) from mangrove soil against gram positive and gram negative pathogens [9]. This present study reveals the best isolates A1 and A4 posses broad spectrum activity against different pathogens. Similar results achieved by Juby *et al*., (2018). They isolated six isolates which showed antibacterial activity against at least one of the test bacteria. In perpendicular streak method, the isolates KUAJ1 and KUAJ5 possess broad spectrum activities against tested bacteria [10].

Antioxidant Activity of Solvent Extracts

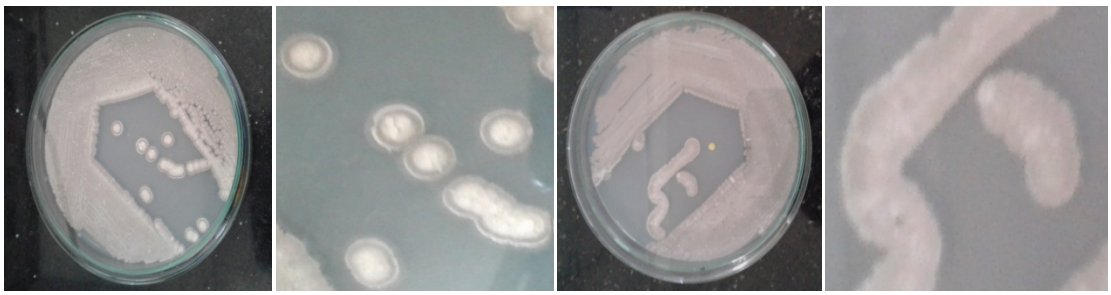
DPPH Radical Scavenging Activity:

The scavenging activity of different concentrations of Ethyl acetate extract of A1 and A4 isolates and *Ascorbic* acid (standard) was tested by DPPH free radical scavenging assay. In this study, the crude ethyl acetate extract of A1 and A4 produced a dose dependent DPPH radical scavenging activity and 79.99% of DPPH radicals have been scavenged by standard ascorbic acid at 500µg/ml concentration. On comparison with the extract of A1 isolate, A4 isolate unveiled the efficient radical scavenging ability which was found to be 69.87% at highest concentration 500µg/ml, whereas A1 isolate were able to scavenge only 65.24 % of the DPPH radicals at the same concentration (Fig 4). The scavenging ability of the extract was found to be dose dependent; the percentage of inhibition was increased with the increasing concentration for both the extracts.

The results were in accordance with Kekuda *et al*. [11] they revealed a dose dependent scavenging of DPPH free radicals by ethyl acetate extract of *Streptomyces* species isolated from Karnataka. Thus it can be concluded that, though the DPPH radical scavenging ability of the extracts was less than that of ascorbic acid, the study evidenced the potential proton-donating ability of the extracts and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. Moreover, the antioxidant activity of putative antioxidants have been attributed to various mechanism, which may be prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging [12].

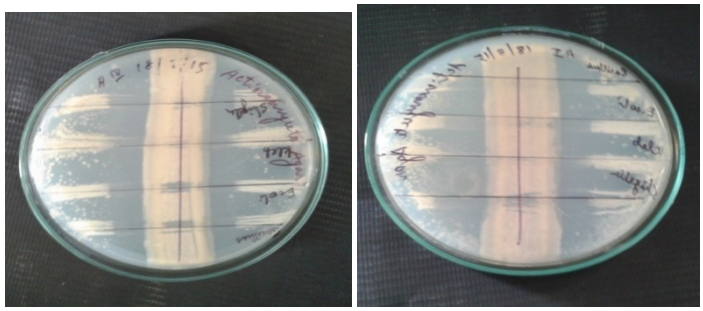
Table 1: Antagonistic activity of Actinomycetes (A1&A4) against bacterial pathogens

BACTERIAL PATHOGENS	ZONE OF INHIBITION (mm)	
	A1(200µl)	A4(200µl)
E.coli	24	24
S.aureus	18	20
S.flexneri	17	22
K. pneumonia	24	27
S. typhi	26	25
P. aueroginosa	21	16
B. cereus	30	24
S.pyogens	18	19



A1 **A4**

Fig 1: Cultural Characteristics Of Isolates On AIA



A1 **A4**

Figure 2: primary screening (Cross streak assay of the strain (A1&A4)

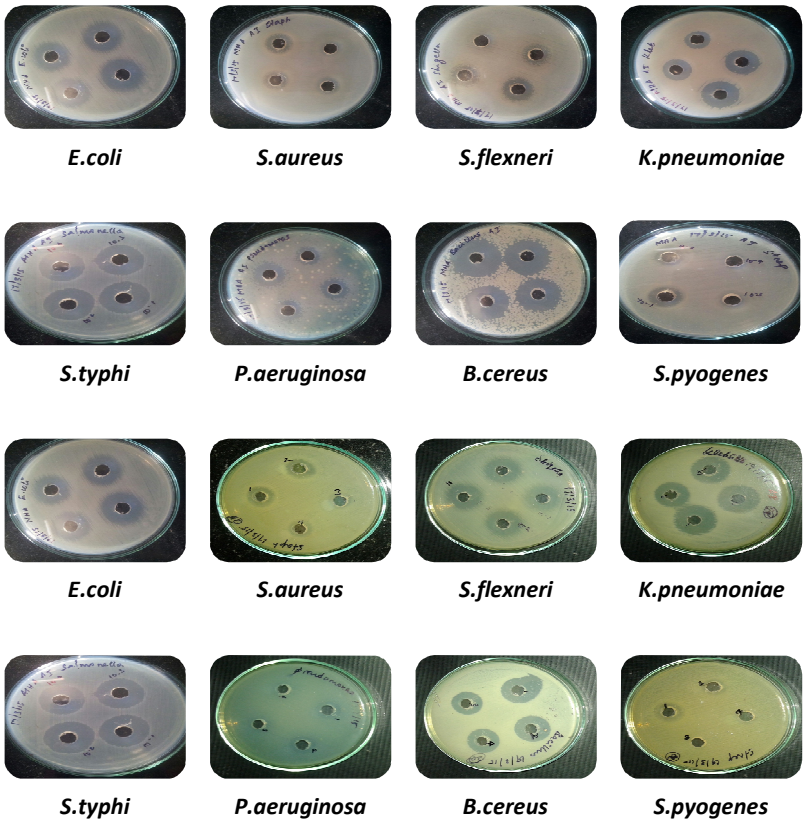


Fig 3: Antagonistic activity of Actinomycetes (A1&A4) against bacterial pathogens

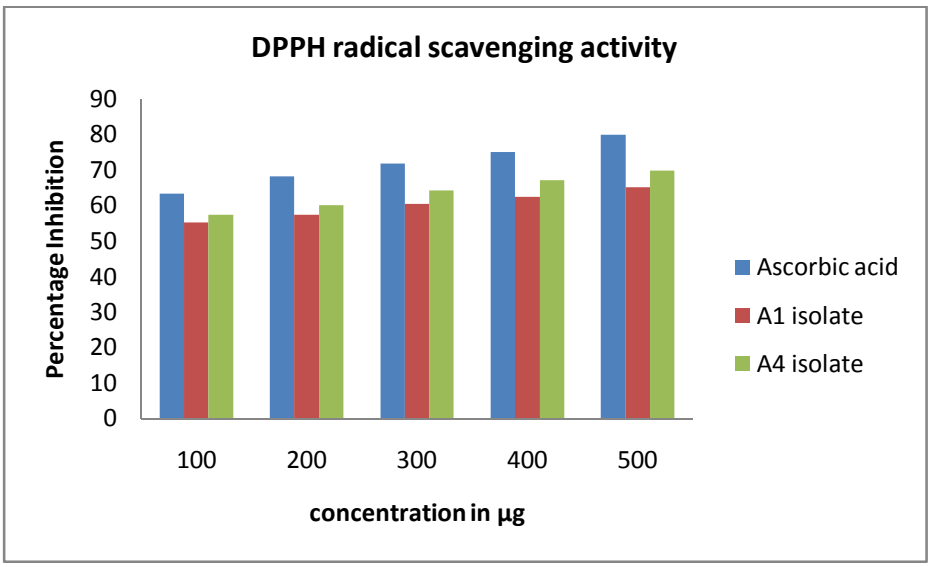


Fig 4: DPPH radical scavenging activity of A1 & A2 isolates

CONCLUSION

The study revealed antimicrobial and antioxidant potential of *Streptomyces* species tested. Screening of farming soil at erode should be carried out to isolate and characterize potential actinomycetes capable of producing bioactive metabolites. Further studies are needed to characterize the isolate, purify bioactive metabolites and determine antimicrobial and antioxidant efficacy.

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CITATION OF THIS ARTICLE

P. Muthukrishnan ,R. Dhanapalan , V. Suseela and R.Usha. Antibacterial and Antioxidant Activity of Streptomyces Isolated From Soil. Bull. nv. Pharmacol. Life Sci., Vol 10[1] December 2020 : 146-150