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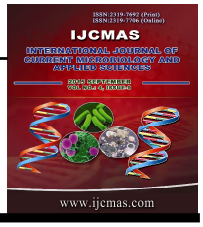

Original Research Article

Antimicrobial Activity of Estuarine Actinobacteria against Selected Human Pathogens

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K e y w o r d s

Estuarine soil, Actinomycetes, Antimicrobial activity, Agar well diffusion method.

A B S T R A C T

A Collection of 50 Actinomycetes strains were isolated from estuarine sample of Puducherry – India. The strain isolated from estuarine habitat is capable of producing novel secondary metabolites which strongly inhibit the growth of G+ve, G–ve bacteria and Yeast. Out of 50 isolates only seven strains exhibited antimicrobial activity towards one or more test organisms. Among them, the strain PAS-9 showed broad spectrum antimicrobial activity.

Introduction

Actinomycetes are G +ve Bacteria having high G+C content (>55%). Actinomycetes were originally considered to be an intermediate group between bacteria and fungi but now are recognized as prokaryotic organisms. The most important genus in Actinobacteria is Streptomyces, five hundred and thirty three species of Streptomyces species were described in Bergy's Manual of Systematic bacteriology (Kampfer, 2012). Marine Actinomycetes are rich source for production of secondary metabolites like enzyme, antibiotics etc. In general Estuary is rich in organic matter due to its reverse action, and it results in high flora and fauna. Estuary environment is largely untapped source for discovery of novel secondary metabolites or bio products (Tara Devi Gurung *et al.*, 2009). The purpose of discovering new antibiotics is more in now a day due to the development of antibiotic resistant form of bacteria for the existing antibiotics (Ashok *et al.*, 2012). The present study is aimed to isolate the Actinomycetes from Estuary of river Sankarabarani – Ariyankuppam – Puducherry (East coast region) and screening of its antimicrobial activity against human pathogenic bacteria.

Materials and Methods

Sample collection

The Estuary sediment samples were collected from river Sankarabarani, Ariyankuppam in Puducherry (East coast region). Sediments collected from 10 to15 cm depth using sterile scoop and stored in sterile polythene bag (sandy soil appeared in black color) (Eman Mohammd Jarallah and Noor Haidar Rahman, 2014). The collected Estuary soil sediment sample were air dried for seven days in dark condition and then the sample were gently crushed by using mortar and pestle followed by sieving to remove coarse particles and stored in sterile condition for further use (Mohan Remya *et al.,* 2008).

Physical and chemical analysis of sediment sample

Air dried sample was sent to Sugarcane research institute at Cuddalore to analyse the physical and chemical properties of Estuary soil sediment sample.

Pretreatment of Estuarine soil sample

The collected marine soil sample was shade dried for seven days in aseptic condition. Then the sample was gently crushed by using sterile mortar and pestle, sieved and stored. In this study two pre treatment methods have been used for isolation of novel (rare) actinomycetes: SDS method and phenol treatment method.

SDS Treatment

In the 10^{-2} dilution contains yeast extract 6% +SDS 0.05%.After adding the sample, the 10^{-2} dilution was kept at 40ºC for 20 minutes (Masayuki Hayakawa and Hideo Nonomura, 1989). Yeast extract activates spores and SDS will lyse the bacterial cells, so that this treatment will facilitate the growth of actinomycetes.

Phenol Treatment

In serial dilution, the 10^{-1} dilution is added with 1.5% of phenol. This was left undisturbed for 10 minutes which results in bactericidal effect.

Media and its composition

In this study three different media were used for isolation. Starch casein agar (SCA), Humic acid vitamin agar (HVA) and Water agar (WA) (Yeast extract - 0.25 gm, Agar- 20 gm, Water- 700ml distilled water+300ml sea water, pH- 7.2) were prepared, each with the addition of 50% sea water (Vijayakumar *et al.,* 2012). Naldixic acid and cycloheximide were added to the media (50µg/L) after sterilization to control the bacterial and fungal contamination respectively.

Isolation of Actinomycetes

Isolation of Actinomycetes was done by pour plate technique. After sterilization the medium was cooled to warm temperature and it was poured in to the respective labelled plates and swirled uniformly to obtain even distribution of microbes (10^{-3}, 10^{-4}, 10^{-5}). The solidified plates were incubated at laboratory conditions for 30-60 days. After the period of incubation, the suspected colonies of Actinobacteria were sub cultured in PDA slants which contains 50% sea water.

Screening the antimicrobial activity against bacteria

Morphologically differed 50 Actinomycetes isolates were selected for screening. Those

strains were named PAS 1 – PAS 50. Screening can be done by two methods namely agar streak method (Primary) and well diffusion method (Secondary).

Agar streak method

All the 50 isolates of Actinomycetes were inoculated as a single streak at one end of the respective petridish containing Potato Dextrose Agar(PDA) and incubated for 10 - 15 days at laboratory condition to permit the growth and Antibiotic production. After the attainment of complete growth of Actinomycetes, the test bacteria were inoculated by streaking perpendicular to the growth of Actinomycetes (Muharram *et al.*, 2013) isolate. Then the plates were incubated for 24 hrs at 37ºC. The inhibition of test bacteria around the growth of Actinomycetes was taken as positive for antimicrobial activity.

Well diffusion method

From the agar streak method, only one potent Actinomycetes (PAS 9) was selected and used for further study which shows broad spectrum activity. The selected (PAS 9) strain was grown in Potato Dextrose Broth (PDB) for 15 days at laboratory condition (Gulve and Deshmukh, 2012). After incubation period the cells are removed by centrifugation process (5000 rpm for 10 minutes). The supernatant only used for screening the antimicrobial activity against bacteria (Shoba *et al.*, 2014) by well diffusion method. Ten MTCC bacterial cultures [*Bacillus subtilis* (MTCC121), *Staphylococcus aureus* (MTCC96), *Proteus vulgaris* (MTCC744), *Pseudomonas aeruginosa* (MTCC424), *Klebsiella pnemoniae* (MTCC4031), *Salmonella typhi* (MTCC3220), *Shigella flexneri* (MTCC1457), *Vibrio cholera* (MTCC3906), *Bordetella bronchiseptica* (MTCC6837), *Candida albicans* (MTCC183) were used for screening. All the cultures were bought from Microbial Type Culture Collection Centre - Chandihargh (MTCC).

Results and Discussion

The soil sediment sample analysis report says that the given sample was rich in potassium and nitrogen (Subhajit Das *et al.*, 2012). Presence of more Nitrogen indicates that the sediment sample was more fertile as a result it facilitates the more numbers of microbes in the estuarine environment.

Table.1 Physico chemical characteristics of estuarine sediment soil

Macro nutrients	**Kg/acre**	**Micro nutrients**	**Quantity(ppm)**
Nitrogen	60	pH	6.1
Phosphorus	5	EC (dSm-)	11.00
Potassium	81	Ferrous	16.28
Calcium carbonate	nil	Manganese	7.65
*values given in ppm except pH and EC		Zinc	0.71
		Copper	1.75

Table.2 Anti microbial activity of *Streptomyces* species

Organism	**Zone of Inhibition in mm**	**Result**
Bacillus subtilis	20	Sensitive
Staphylococcus aureus	18	Sensitive
Proteus vulgaris	24	Sensitive
Pseudomonas aeruginosa	21	Sensitive
Klebsiella pneumoniae	16	Sensitive
Salmonella typhi	17	Sensitive
Shigella flexneri	20	Sensitive
Vibrio cholerae	21	Sensitive
Bordetella bronchiseptica	13	Less Sensitive
Candida albicans	17	Sensitive

Table.3 Biochemical test for strain PAS 9

Test	**Result**
Amylase	Positive
Lipase	Positive
Gelatin	Negative
Pectinase	Positive
Nitrate Reduction	Positive
Citrate	Negative
Cellulose	Negative
Urease	Positive
Indole Production Test	Negative
H_2S Production	Positive
Blood Agar	No Haemolysis
Protease	Negative
Phosphatase	Positive
Pigment Production	Positive (Reddish Violet)

Fig.1 Anti microbial activity of *Streptomyces* crude Extract

Plate.1 a- *Staphylococcus aureus,* **b-** *Bacillus subtilis,* **c-** *Proteus vulgaris,* **d-** *Pseudomonas aeruginosa* **e-** *Vibrio cholera,* **f-** *Shigella flexneri,* **g-** *Candida albicans***, h-** *Bordetella bronchiseptica,* **i-** *Klebsiella pneumoniae,* **j-** *Salmonella typhi*

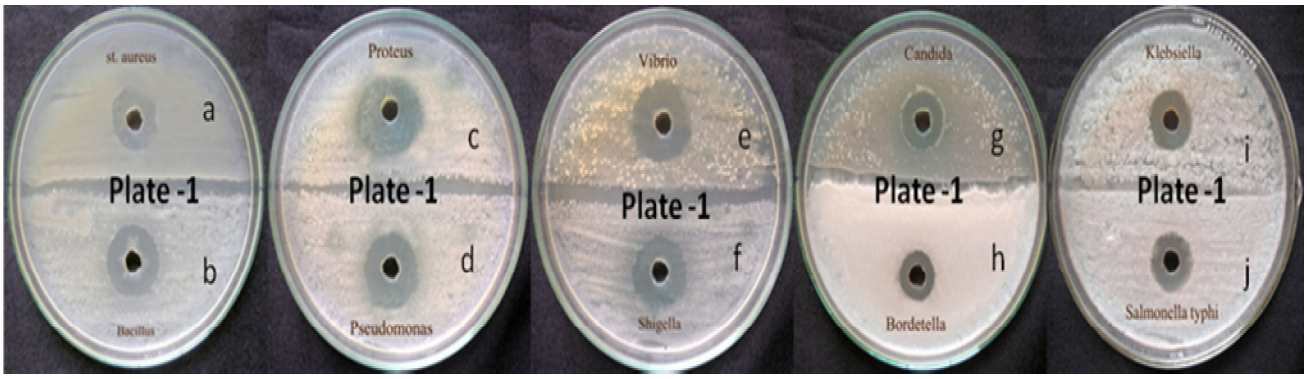

Fig.2 Pure Culture of selected *Streptomyces* strain in ISP -2

Biochemical characterization of strain PAS 9

Different biochemical tests were performed for Strain PAS 9 and the results were recorded.

In this study a total of 50 actinomycete strains were isolated. All the fifty isolates were subjected to antibacterial screening by Agar streak method and Agar well diffusion method against a panel of Gram +ve (*Bacillus subtilis, Staphylococcus aureus Bordetella bronchiseptica*), and Gram -ve [(*Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumoniae, Salmonella typhi, Shigella flexneri, Vibrio cholerae,* bacteria and *Candida albicans* (Yeast)].Out of fifty isolates five isolates exhibited antibacterial activity, and only one isolate evinced anti-candidial activity. Further it was noticed that Gram negative bacteria were inhibited by more number of isolates (n=5), than gram positive bacteria (n=3). Within the gram positive bacteria *Bacillus subtilis* was more sensitive than *Staphylococcus aureus,* similarly Gram negative bacteria *Proteus vulgaris* was more sensitive. Only one isolate PAS-9 showed broad spectrum antimicrobial activity and therefore it was selected for further study. The isolate was identified as member of the

Genus *Streptomyces* based on biochemical and physiological characteristics and spore chain morphology.

In conclusion, secondary metabolites such as antibiotics from estuarine actinomycetes show strong inhibitory activity especially towards Gram negative bacteria which indicates their selective target specificity. This is may be due to the presence of some special structural and chemical features in estuarine secondary metabolites (antibiotics) which is not present in terrestrial forms. Therefore this habitat deserves further detailed study.

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