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Screening and antimicrobial prospect of marine Actinomycetes isolated from East Coast region of Tharangambapadi in Tamil Nadu, India

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Abstract

The aim of the current study was to isolate novel secondary metabolites actinobacteria from the marine soil samples of Tharangambadi. Isolation was performed by serial dilution and pour plate technique on Starch Casein agar medium. A complete often morphologically completely different actinobacteria were isolated and these isolates were screened for antimicrobial activity by disc diffusion technique on Mueller Hinton agar plates against 2 Gram Positive (*Staphylococcus aureus* and *B. subtilis*) and two Gram negative (*Pseudomonas aeruginosa*, and *E. coli*) bacterial cultures. Among 10 isolates just one isolate (T9) exhibited the great antimicrobial activity against the take a look at organisms. The crude extract showed most zone of inhibition against *B. subtilis* (23.33±0.57 mm), *staph aureus* (20.33±0.57 mm), and *E. coli* (18.33±0.57 mm) whereas, lowest against *P. aeruginosa* (15.00±1.72 mm). The inhibition potential of the actinobacteria isolate was compared with the quality medication. The minimum repressing concentration of the T9 isolate was found lowest against *E. coli* (25µg/ml), whereas, highest against *P. aeruginosa* (75 µg/ml). The potential isolate (T9) was characterised on the bases of microscopic, biochemical test. The isolated marine actinomycetes have potential bioactive compounds against gram positive and gram negative bacteria and also effective against multidrug resistant pathogens.

Keywords: primary screening, organic chemistry characterization, Actinomycete sp, antimicrobial activity, agar well diffusion technique.

Introduction

Actinobacteria are without cosmopolitan living, saprophytic gathering of microorganisms found in nature. They are Gram positive, stretched microbes, holding in high G+C proportion (>55%). They have attributes of both microbes and growths and perceived as prokaryotes. Actinobacteria are portrayed generally in physical living spaces and additionally oceanic natural surroundings. Actinobacteria is one of the real gatherings of soil small scale greenery and the phone tally fluctuates with the dirt sort. Soil is high in natural matter is most fitting for the strong development of actinobacteria. Actinobacteria present in prominent number in dry soil than that of wet soil [4].

Microorganisms in marine situations attractable a lot of consideration, because of their versatility to compelling situations and yield of novel characteristic mixes [14]. Marine natural conditions appearance extraordinary variety and are amazingly not the same as physical ones; henceforth marine actinomycetes have distinctive qualities from those of terrestrially partners. This permits the life forms to create

distinctive sorts of bioactive mixes with remarkably properties and applications [5]. For fruitful seclusion of actinomycetes from marine situations diverse techniques like decision of screening source, pre-treatment, particular medium, society condition and choice of potential provinces on an essential confinement plate are vital.

In the familiarize study, actinobacteria societies were confined from marine soil test place that is known for Tharangampadi, Nagapattinum (Dt),TN. India and were screened for antimicrobial action. Further the potential seclude was distinguished by utilizing morphological, biochemical methodologies.

Materials and Methods

Sample collection

Soil samples were collected from marine seashore of Tharangambadi, Nagapattinum (Dt) TN. Soil samples were collected at the depth of 10-25 cm. Samples were collected in sterilized plastic bags and brought to the Sri Rajendra Scientific & Surigicals (P) LTD., Pondicherry and stored in a refrigerator at 4°C until further processing.

Isolation of actinobacteria

Isolation of soil actinobacteria was performed by serial dilution and spread plate method on starch casein medium. One gram of soil test was serially weakened in disinfected refined water to get focuses raging from 10^{-1} to 10^{-5}. A volume of 0.1 ml of every dilution was exchanged aseptically to starch casein medium plates. The Petri plates were turned clockwise and anticlockwise to spread the sample consistently. The plates were hatched at room temperature for 7 days [15].

After brooding, the colonies of actinobacteria were separated taking into account their province morphology and pigmentation. The isolates were sub cultured into the starch casein agar plates and refined by streak plat method. The pure isolates were kept up at 4°C in icebox for further studies.

Primary and Secondary Screening:

In primary screening the antimicrobial activity of pure isolates were determined by cross streak method on ken knight agar. The test organisms used were *Pseudomonas aeruginosa, E coli, Staphylococcus aureus,* and *Bacillus subtilis*. Secondary screening was performed by agar well diffusion method against the standard test organisms.

Preparation of cell free extract

One loop full of actinobacteria isolate was inoculated in 100 ml of sterilized SS media [g/l, Soluble starch 25g; Glucose 10g; Yeast extract 2g; $CaCO_3$ 3g and Trace salt solution 1ml ($FeSO_4$, $CuSO_4$, $ZnSO_4$, $MnCl_2$)] in a 250 ml Erlenmeyer flask. The flask was incubated at 35°C and 110 rpm for 7 days in a rotary shaker (I.L.E. Co., India). After incubation, the medium was centrifuged at 5000 rpm for 15 min in a cooling centrifuge (C 24 BL, Remi, India). The supernatant was gathered and extracted with ethyl acetate derivation 1:1(v/v) by utilizing isolating channel.

Antibacterial activity assay

Antibacterial action of the crude actinobacterial extract was determined by taking after disc diffusion method. All the test bacterial cultures were vaccinated in Nutrient Broth and incubated at 37°C for 8 hours. The turbidity of the broth was ajusted at 0.5 (optical density) using spectrophotometer. The bacterial suspensions were seeded on MHA plates. In each of these plates stacked the ethyl acetate extract of actionomycetes by using a micropipette, 25µl, 50µl, 75µl and 100µl of ethyl acetate extract and positive control antibiotic was stacked in the disc. Plates were brooded for 24 hours at 37°C [1]. Antibacterial activity was assessed by measuring the zone of restraint. Triplicate samples were kept up in every analysis.

Determination of minimum inhibitory concentration (MIC)

The MIC of the cell free filtrate was dictated by modified disc diffusion method.

[12, 9] The ethyl acetic acetate extract was disintegrated in DMSO to get a fixation range of 25, 50, 75 and 100µg/ml. The test bacterial suspensions were seeded on MHA plates. In each of these plates were five disc set using forceps. Using a micropipette, 100µl of every dilution was added independently into plates. Plates were brooded at 37ºC for 24 hours. The minimum concentration of ethyl acetate extract of actinomycetes demonstrating an unmistakable zone of hindrance was thought to be MIC [12, 2].

Taxonomical identification of the Potential Strain

Morphological characteristics

Actinobacteria isolate was inoculated into five unique media and incubated for 5 days at room temperature. The colonies were see under a high power amplifying lens and colony morphology was noted as for shading, elevated mycelium, size, nature of province, opposite side shading and feeling the consistency with a sterile circle [3].

Microscopic portrayal

The actinobacterial isolate was morphologically characterized by Gram staining. Spore chain morphology was concentrated on by microscopic examination.

Biochemical portrayal

The capacity of actinobacterial strains to using different carbon sources *viz*., xylose, inositol, mannitol, fructose, rhamnose, arabinose, sucrose and raffinose as wellsprings of vitality was concentrated on [13].

Results and Discussion

The composition of a starch casein agar (SCA) is desirable for the selective isolation of aerobic Actinomycetes [4]. In this study, marine seashore soil samples were gathered from tharangambadi in Nagapattinum (Dt), TN, India. The examples were handled for the isolation of soil actinobateria and a sum of ten colonies of actinobacteria was acquired on SCA plate. Among them stand out morphologically unmistakable colony was separated and named as T9 (Table: 1) and utilized as a part of further study Figure 1. Another real turning point in the recognizable proof of Actinomycetes was the osmosis of carbon by Actinomycetes. Test incorporates ten carbon sources which are disinfected by layer filtration technique. All the strains have demonstrated exceptionally rich development. Pandey *et al*. demonstrated that for the ideal creation of anti-microbials certain carbon sources are required. In this study, the recommended that pH may play a vital variable for the generation of anti-infection agents by Actinomycetes.

Table 1: Morphological characteristics of the T9 isolate

Media name	Growth pattern	Aerial mycelium	Reverse pigmentation
Starch casein agar	Good growth	White	White
Kenknight agar	Good growth	White	Ash
Nutrient agar	Poor	Creamy	Creamy
Actinomycetes isolation agar	Good growth	White	White
ISP 1	Poor	Light yellow	Creamy

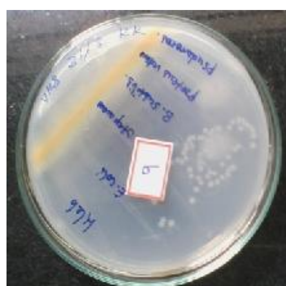

Figure 1 Primary screening of T9

Antimicrobial activity

Antimicrobial action of ethyl acetate extract of *Streptomyces sp*. with individual to positive control is recorded in Figure 2.ethyl acetate extract significant huge antibacterial action against all test creatures included *S. aureus, E. coli, B. subtilis,* and *P. aeruginosa,* with inhibition zone of 20.33±0.57, 18.33±0.57, 23.33±0.57 and 15.00±1.72 mm separately.

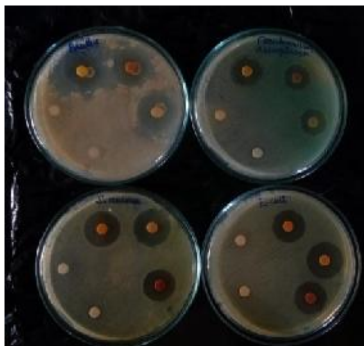

Figure: 2 antibacterial activity assay

As indicated by Kokare *et al*, amid the screening of the novel auxiliary metabolites, Actinomycetes disengages are frequently experienced which demonstrated more dynamic antimicrobial movement against gram positive microscopic organisms than gram negative microorganisms. *Streptomyces* species indicated critical antibacterial action against *Staphylococcus aureus* and *Pseudomonas aeruginosa* [9]. This was like the present discoveries. In the present concentrate, additionally the *Streptomyces* species demonstrated a decent antimicrobial movement against Staphylococcus species, *B. subtilis*, than gram negative *Pseudomonas* species and *E.coli*. The present study concurred with the before discoveries of Devi *et al* in which it has been accounted for that *Streptomyces* species demonstrated huge antimicrobial movement against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Vibrio cholera*[8].

In the present test, it has been watched that compared to different Actinomycetes, *Streptomyces* species indicated proficient hostile action. Just not very many records are accessible on the event and appropriation of opposing *Streptomyces* in the marine environment. The marine *Streptomyces* have not got much consideration. Late tests show that the huge capability of marine Actinomycetes, especially *Streptomyces* species items. In this manner, the consequences of the present examination uncover that the marine Actinomycetes from beach front environment are a powerful wellspring of novel anti-toxins. It is foreseen that confinement, portrayal and investigation of Actinomycetes can be helpful in the disclosure of novel types of Actinomycetes. Actinomycetes are the most imperative assets of these optional metabolites. Late advances of sub-atomic hereditary qualities in this class have empowered us to explain not just the association of biosynthetic qualities for their optional metabolites additionally administrative instruments firmly connected to the cell separation forms. Albeit such data has so far not been fruitful in adding to down to earth strain change, discerning methodology of combinatorial biosynthesis is required to be helpful in creating new mixes.

Microscopic studies

The T9 isolate was observed to be Gram positive, filamentous bacteria. Spore structure was concentrated on by microscopic analysis, spores were oval fit as a fiddle with smooth surface (Figure 3).

Figure: 3 T9 - Microscopic analysis.

Biochemical studies

The T9 isolate was checked for the sugar fermentation test against seven distinct sugars. The T9 isolate fermented the glucose, fructose, maltose, sorbitol and mannitole, though no fermentation was seen in Xylose and sucrose. The outcomes are shortlisted in Table 2.

But due to the lack of other tests, apart from proper identification of genera of Actinomycetes, besides morphological and physiological properties (Kuster, 1972) and by comparing all these results with the Bergey’s manual of Determinative Bacteriology , isolates were identified to belong to the genus Streptomyces.

Table 2: Utilization of different Carbone sources by T9isolate

S. No.	Carbone sources	Utilization of Carbone sources
1	Glucose	+
2	Fructose	+
3	Xylose	-
4	Maltose	+
5.	Sorbitol	+
6	Mannitol	+
7	Sucrose	-

+: Presence of growth; -: Absence of growth

Conclusion

A total of ten isolates of Actinomycetes from marine sediments of Tharangambadi at Nagapattinum were collected. Each isolate was tested against four pathogenic bacteria. Among the ten isolates, one of the isolates showed potent activity against all the bacteria. After performing some biochemical tests, all isolates were identified to belong to the genus *Streptomyces*. The spent broths which were taken from the antibiotic production media of T9 isolate are subjected to antimicrobial activity which showed potent activity against pathogenic bacteria. Further purification of this spent medium may gives the more activity than the standard antibiotics and also effective against some multidrug resistant pathogens.

References

1. Aravamuthan N, Kumar G, Karthik L, Bhaskara Rao KV, *In vitro* antagonistic activity of soil actinobacteria against multi drug resistant bacteria, Pharmacologyonline 2010; 2: 507-516.
2. Devi NKA, Jeyarani M, Balakrishnan K. Isolation and identification of marine actinomycetes and their potential in microbial activity. Pak J Biol Sci 2006; 9(3): 470-472.
3. Dhanasekaran D, Selvamani S, Panneerselvam A, Thajuddin N. Isolation and characterization of actinomycetes in Vellar Estuary, Annagkoil, Tamil Nadu. Afr J Biotechnol 2009; 8(17): 4159-4162.
4. El-Nakeeb MA, Lechevalier HA. Selective isolation of aerobic Actinomycetes. Appl. Environ. Microbiol 1962; 11:75-77.
5. Goodfellow M, Williams ST. Ecology of Actinomycetes. Annu Rev Microbiol 1983; 37:189-216
6. Goshev I, Gousterova A, Vasileva Tonkova E, Nedkov P. Characterization of the enzyme complexed produced by two newly isolated thermophilic actinomycete strains during growth on collagen rich materials. Process Biochem 2005; 40:1627–1631.
7. Kenneth SM, Walmsley MB, Adrian RW, Microbial and viral drug resistance mechanisms, Trends n in Microbiology 2002;10: S8-S14.
8. Kokare CR, Mahadik KR, Kadam SS, Chopade BA. Isolation, characterization and antimicrobial activity of marine halophilic Actinopolyspora species AH1 from the west coast of India. Curr Sci 2004; 86(4): 593-597.
9. Nonomura H, Key for classification and identification of 458 species of the *Streptomycetes* included in ISP. Journal of fermentation Technology 1974; 52: 78-92.
10. Okunji CO, Okeke CN, Gugnani HC, Iwu MM, An antifungal saponin from fruit pulp of *Dracaena manni*, International Journal of Crude Drug Research 1990; 28: 193-199.
11. Pandey. A., Shukla. A., Majumdar. SK. Utilization of carbon and nitrogen sources by *Streptomyces kanamyceticus* M27 for the production of an anti bacterial antibiotic. *Afr. J. Biotechol.*2005; 4: 909-910.

12. Raghunath D, Emerging antibiotic resistance in bacteria with special reference to India, J Biosci 2008; 33:593-603.
13. Rios JL, Recio MC, Vilar A, Screening methods for natural products with antimicrobial activity: A review of literature, J Ethnopharmacology 1988 23:127-149.
14. Shirling EB, Gottlieb D, Methods for characterization of *Streptomyces* sp. Int J Syst Bacteriol 1966; 16:313-340.
15. Solingen VP, Meijer D, Kleij WA, Branett C, Bolle R, Power SD, Jones BE. Cloning and expression of an endocellulase gene from a novel Streptomyces isolated from an East African soda lake. Extremophiles 2001; 5: 333-341.
16. Umasankar M, Kumar G, Karthik L, Bhaskara Rao KV, Exploration of antagonastic actinobacteria from Amirthi forest, International Journal of Current Pharmaceutical Research 2010; 2: 16-19.

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