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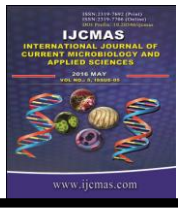

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Isolation and Scrutinize of Marine *Actinomycetes* Metabolites against Clinical Pathogens

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A B S T R A C T

To isolation and identification proof of optional metabolites producing marine *Actinomycetes* against clinical bacterial pathogens. To scrutinize the bioactive compound from isolated actinomycetes. Marine *Actinomycetes* isolated from seashore samples from different places of Nagapattinam, Velankani, and Karaikal in India. Antimicrobial substances from marine *Actinomycetes* were identified by cross streak method and agar plate method. The potent *Actinomycetes* species were inoculated on production medium and extracted. The extracted compound was screened for antibacterial activity. Fifteen *Actinomycetes* species were isolated from the selected marine soil sample. All the fifteen isolates were active against the test organisms. Chemical screening strongly suggested that presence of alkaloids, Flavonoids, Carbohydrates and Glycosides. The result of marine *Actinomycetes* is composed of potent secondary metabolites. Isolation, screening of marine *Actinomycetes* can be useful in discovery of novel metabolites.

Introduction

Actinomycetes are aerobic, reproductive structure forming gram-positive bacteria, happiness to the order *Actinomycetales* characterised with substrate and aerial mycelium growth (Lechevalier and Lechevalier, 1981). It's a high G+C magnitude relation of the desoxyribonucleic acid (>55 mol %), that are phylogenetically connected from the proof of 16S ribosomal cataloguing and DNA:rRNA pairing studies (Goodfellow and Williams, 1983; Korn-Wendisch and Kutzner, 1992).

It represents one in every of the biggest taxonomical units among the 18 major lineages presently recognized inside the domain bacteria (Ventura *et al.*, 2007). Nature acts as a distinguished reservoir for brand new and novel medical specialty. By using subtle techniques in varied screening programs, the speed of discovery of natural compounds exceeds one million. Out of 22,500 biologically active compounds that are extracted so far from microbes, 45% are made by Actinobacteria, 38th by fungi and terrorist organization by living thing bacteria (Demain and sanchez, 2009). Sadly, the

emergence of drug-resistant pathogens and also the increase in diseases touching the system have greatly intense the necessity to analyze new bioactive metabolites for potential pharmaceutical and industrial applications (Demain and sanchez, 2009; Wise, 2008).

The look for new antimicrobials has not been restricted to the medicative field, however conjointly extends to crop protection. Advancement of fungicide-safe plant pathogens and in addition over the top and unpredictable utilization of engineered agrochemicals has prompted biological irregular characteristics in soil and human wellbeing (Thind, 2008). The bioactive secondary metabolites created by *Actinomycetes* embody antibiotics, antitumour agents, immunological disorder agents and enzymes. These metabolites are known not antibacterial, antifungal, cell reinforcement, neutrogenic, hostile to growth, against algal, hostile to helmintic, hostile to malarial and mitigating (Kekuda *et al.,* 2010; Ravikumar *et al.,* 2011). This study evaluates isolation, identification; chemical screening and antibacterial activity of living thing compounds against different types of infectious agent that were gift in *Actinomycetes*, the pathogens were used *Pseudomonas aeruginosa, E coli, staphylococcus aureus, Klebshilla pneumonia* and *Bacillus subtilis*.

Materials and Methods

Sample Collection

Soil samples were collected 10 cm below the surface of seashore soil from the areas of Nagapattinam, Velanganni and Karaikal. Soil samples were air-dried under room temperature for about 10 days before isolation.

Isolation of Actinomycetes

Soil tests gathered were pretreated by drying hot air broiler at 40°C for 2 days. Tests of 1 g each were blended with 10 ml of clean refined water and hatched at room temperature (25 ± 2°C) for 1 h on orbital shaker with vivacious shaking. Soil suspension was then pipetted and unfolds onto humic substance B-Vitamin (HV) agar (Hayakawa *et al.,* 2004) and incubated at 30°C for seven days.

Characterization of Actinomycetes

The actinomycetes selected after secondary screening, were characterized by morphological and bio chemical method. Morphological characterizations were done by microscopic method. The microscopic characterization was done by cover slip culture method (Kawato and Sinobu, 1959).

The mycelium structure, color and arrangement of conidiospore and arthrospore on the mycelium were observed through the oil immersion (100X). The observed structure was compared with Bergey's Manual of Determinative Bacteriology, Ninth edition (2000) and the organism was identified. Various biochemical tests performed for the identification of the potent isolates are as follows: starch hydrolysis, Indole, methyl red, temperature tolerance, NaCl resistance.

Screening of Actinomycetes for Antimicrobial Activity

The screening procedure was finished by following techniques procedure described by Liu *et al.,* (2011).

Primary and Secondary Screening

In essential screening the antimicrobial

movement of isolated disconnects were controlled by cross streak technique on ken knight agar. The test living beings utilized were *Pseudomonas aeruginosa, E coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Bacillus subtilis*. Auxiliary screening was performed by agar well diffusion methods against the standard test organisms.

Chemical Screening

Chemical tests were carried out on the ethyl acetate extract of *Actinomyctes* using standard procedures to identify described by Harborne *et al.,* (1973); Trease *et al.,* (1989).

Test for Carbohydrates

To 2ml of concentrate, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were included. Purple shading arrangement showed the vicinity of carbohydrates.

Test for Flavonoids

To 5 ml of weaken ammonia solution was added to a segment of the fluid filtrate of concentrate took after by option of focused sulphuric acid. Look of yellow colouration demonstrated the vicinity of flavonoids.

Test for Alkaloids

To 2 ml of extract, 2 ml of focused hydrochloric acid was added. Then few drops of Mayer's chemical agent were added. Presence of green color indicated the presence of alkaloids.

Test for Glycosides

To 2ml of extract, 3ml of chloroform and 100 percent ammonia resolution was added. Pink color formation indicated the presence of glycosides.

Test for Phenols

To 1ml of the extract, 2ml of H_2O followed by few drops of 100 percent metallic element chloride was added. Formation of inexperienced color indicated the presence of phenols.

Test for Tannins

To 1ml of extract, 2ml of 5% metallic element chloride resolution was added. Formation of chromatic black color indicated the presence of tannins.

Test for Saponins

To 2 ml of extract, 2 ml of H_2O was added and jolted in an exceedingly graduate for 15minutes lengthwise. Formation of 1cm layer of froth indicated the presence of saponins.

Results and Discussion

A total of fifteen isolates were isolated from soil samples. The quantity of samples and isolates in every sample were given in Table 1. Out of 15 isolates, 5 cultures i.e. N3, N11, V5, V6, and K9 were the 5 strains elite for any analysis, since they showed important medicinal drug activity against check organisms and chemical screening Table 2 and 3. As for because the 5 cultures involved, the 2 cultures N11 and K9 have most activity against check organisms. Then N11 and K9 were known and confirmed by microscopic and macroscopical examination. N11 strain could be a gram positive, cocci in nature, long reproductive structure chain, and filamentous bacterium. K9 strain could be a gram positive, Cocci with spiral reproductive structure chain bacterium. The macroscopical look of the isolate N11 showed leather like, white powdery colonies in starch casein agar

media whereas K9 showed inexperienced powdery colonies. The isolates conjointly created antimicrobial compounds. Two elite isolates i.e. N11 and K9 were tested for the antimicrobial activity against test organisms are given Table 4 among the two isolates, the very best inhibition was shown by the cultures N11 and K9 against *Staph aureus*. Cultures K9 showed activity against *Pseudomonas aeruginosa, E. coli, staphylococcus aureus, Klebseilla pneumoniae* and *bacillus subtilis* Figure 1(a,b,c,d and e).

Actinomycetes comprise 100% of the overall microorganism colonizing marine aggregates. Marine natural surroundings have been demonstrated as a remarkable and captivating asset for developing new and strong bioactives creating microorganisms. Marine microbes are notably engaging as a result of they need the high efficiency needed for bioactive compounds to be effective within the marine surroundings, attributable to the diluting result of ocean water. Members of the *Actinomycetes*, that board marine surroundings, are poorly understood and solely few reports are on the market.

Table.1 Isolation of *Actinomycetes* from Marine Soil Samples

S.No.	Geographical locations	Sampling spots	Types of sample	Number of isolates
1	Nagapattinam,	Sea shore soil	Soil sample with water	4
2	Velanganni	Sea shore soil	Soil sample	8
3	Karaikal	Sea shore soil	Soil sample with water	3

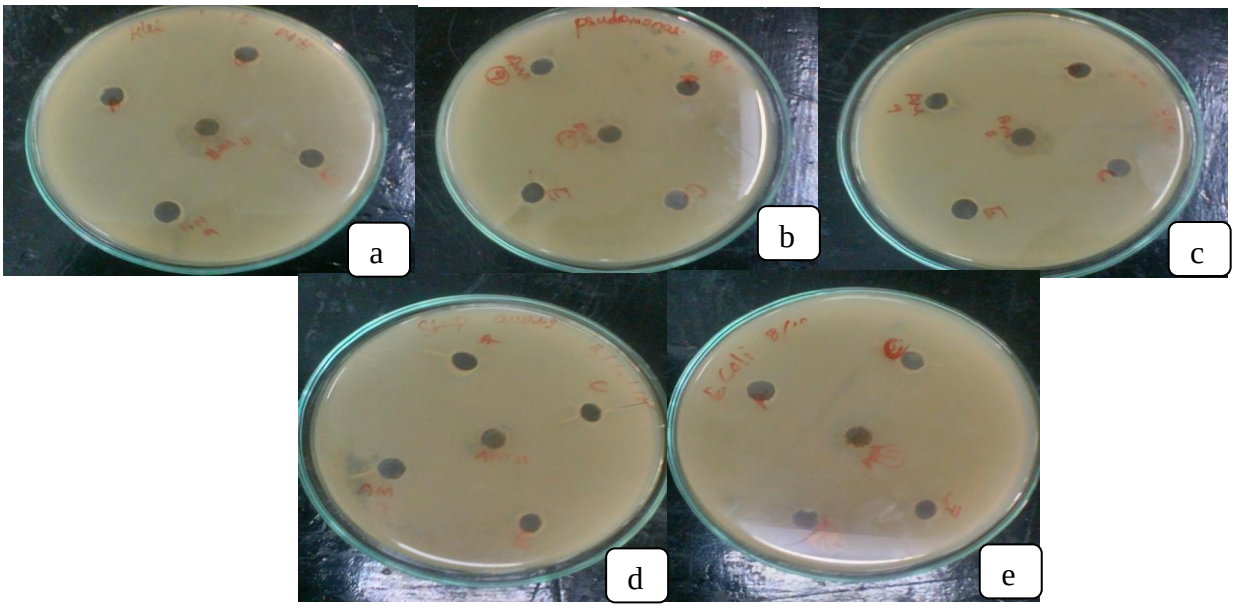
Table.2 Preliminary Screening of *Actinomycetes* for Antimicrobial Activity by Cross-Streak Method

Isolates	*Pseudomonas aeruginosa*	*E. coli*	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Bacillus subtilis.*
V1	-	+	-	-	-
V2	+	-	-	-	+
V3	-	-	-	-	+
V4	+	-	-	-	+
V5	-	-	-	-	-
V6	-	+	-	-	-
V7	-	-	-	-	+
V8	-	-	+	-	+
K9	+	-	+	+	+
K10	-	+	-	-	-
N11	+	+	+	+	+
N12	-	-	-	-	+
N13	-	+	-	-	-
N14	-	-	-	-	+
N15	-	+	-	-	-

Table.3 Chemical Screening for Isolated *Actinomycetes*

Test	Response	V 1	V 2	V 3	V 4	V 5	V 6	V 7	V 8	K 9	K1 0	N1 1	N1 2	N1 3	N 14	N1 5
Carbohydrates	Purple colour	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	Yellow colouration	-	-	+	-	+	-	+	-	+	-	+	-	+	-	+
Alkaloids	Presence of green colour.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	Pink colour	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	Formation of green colour.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	Formation of block colour.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saponins	Formation of foam layer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Figure.1 Antibacterial Activity of Marine *Actinomycetes*



a. *Kleb. pneumonia*, b. *Psudomonous auroginosa*, c. *Bacillus subtilis*, d. *Staph. aureus* and e. *E. coli*

Table.4 Antimicrobial Activity

Sample No	*Staph. aureus*	*E. Coli*	*Kleb. pneumoniae*	*B. subtilis*	*Psudo. auroginosa*
N11	19mm	8mm	12mm	10mm	11mm
K9	20mm	15mm	18mm	13mm	19mm

Actinomycetes account 70th of the earth's surface and represent engaging supply for isolation of novel microorganisms and production of potent bioactive secondary metabolites (Usha *et al.,* 2011). This study was aimed to isolate *Actinomycetes* from marine surroundings and screen them for the assembly of secondary metabolites.

In the current study the medium was supplemented with amphotericin B to eliminate the fungus contamination. Constant methodology was antecedently done by Remya and Vijayakumar (2008) Production of antibiotic substance is ocean water dependent. Within the present study additionally, the Actinomycetes isolation agar medium was ready victimization sterile ocean water. Okazaki and Okami (1972) ascertained that compared to alternative *Actinomycetes*, *Actinomycete* species showed economical antagonistic activity. This was like the current investigation that additionally showed economical antagonistic activity of *Actinomycete* species. The isolated *Actinomycetes* were known supported the colony morphology and Gram staining (Holt *et al.,* 1994). Within the present work, we've got known the *Actinomycetes* by the presence of pulverized colonies on the surface of agar plate. *Actinomycetes* are gram positive and thin in nature. According to Kokare *et al.,* (2004) throughout the screening of the novel secondary metabolites, *Actinomycetes* isolates are usually encountered that showed additional active antimicrobial activity against gram positive bacterium than gram negative bacterium. *Actinomycete* species showed important antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This was just like this finding. Within the current study, conjointly the *Actinomycete* species showed an honest antimicrobial activity against *Coccus species, B. subtilis*, than gram negative *Pseudomonas* species and *Klebseilla pneumoniae*. this study united with the sooner findings of Devi *et al.,* (2006) within which it's been reported that *Actinomycete* species showed important antimicrobial activity against *Staph aureus, Pseudomonas aeruginosa*, *Klebseilla pneumoniae* and *Vibrio cholera*.

In conclusion, According to marine *Actomycetes*, which have specific secondary metabolites from the isolated colonies (K9 and N11).

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References

Demain, A.L., Sanchez, S. 2009. Microbial drug discovery: 80 years of progress. *J. Antibiot.*, 62: 5e16.

Devi, N.K.A., Jeyarani, M., Balakrishnan, K. 2006. Isolation and identification of marine actinomycetes and their potential in microbial activity. *Pak. J. Biol. Sci.,*

9: 470-472.

Goodfellow, M., Williams, S.T. 1983. Ecology of Actinomycetes. *Annu. Rev. Microbiol*., 37: 189-216.

Harborne, J.B. 1973. Phytochemical methods. Chapman and Hall Ltd., London, pp. 49-188.

Hayakawa, M., Yoshida, Y., Iimura, Y. 2004. Selective isolation of actinomycetes belonging to the *Streptomyces violaceusniger* phenotypic cluster. *J. Appl. Microbiol.*, 96: 973e981.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., Williams, S.T. 1994. Bergey's manual of determinative bacteriology. 9th ed. Baltimore: Williams and Wilkins; p. 518-537.

Kawato, M., Shinobu., R. 1959. A simple technique for the microscopical observation, memoirs of the Osaka University Liberal Arts and Education. 114.

Kekuda, T.R.P., Shobha, K.S., Onkarappa, R. 2010. Studies on antioxidant and anthelmintic activity of two *Streptomyces species* isolated from Western Ghat soils of Agumbe, Karnataka. *J. Pharm. Res.*, 3: 26-9.

Kokare, C.R., Mahadik, K.R., Kadam, S.S., Chopade, B.A. 2004. Isolation, characterization and antimicrobial activity of marine halophilic Actinopolyspora species AH1 from the west coast of India. *Curr. Sci.*, 86: 593-597.

Lechevalier, H., Lechevalier, M.P. 1981. Introduction to the order *Actinomycetales*. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG,Editors. The Prokaryotes. Germany: *Springer-Verlag. Berlin.*, 2: 1915-22.

Liu, Q.G., Ren, P.G., Wang, L.X., Zhao, Y. 2011. Statistical optimization of key medium components by response surface methodology to promote ganoderic acid formation by medicinal mushroom Ganoderma sinense in submerged culture. *J. Med. Plant. Res.*, 5(3): 425-431.

Okazaki, T., Okami, Y. 1972. Studies on marine microorganisms II. Actinomycetes in Sagami Bay and their antibiotic substances. *J. Antibiot.*, 25: 461-466.

Ravikumar, S., Inbaneson, S.J., Uthiraselvam, M., Priya, S.R., Ramu, A., Banerjee, M.B. 2011. Diversity of endophytic actinomycetes from Karangkadu mangrove ecosystem and its antibacterial potential against bacterial pathogens. *J. Pharm. Res.*, 4: 294-6.

Remya, M., Vijayakumar, R. 2008. Isolation and characterization of marine antagonistic actinomycetes from west coast of India. *Med. Biol.*, 15: 13-19.

Thind, T.S. 2008. Fungicide resistance: a perpetual challenge in disease control. *J. Mycol. Plant. Pathol*., 38: 407e418.

Trease, G.E., Evans, W.C. 1989. Pharmacognsy 11th edition Brailliae Tiridal Can. Macmill and Publishers.

Usha, Y., Koppula, S., Vishnuvardhan, Z. 2011. Bioactive metabolites from marine sediments (Streptomyces species) of three coastal areas. *Drug. Invent. Today.*, 2: 114-117.

Ventura, M., Canchaya, C., Tauch, A., Chandra, G., Fitzgerald, G.F., Chater, K.F., *et al*. 2007. Genomics of Actinobacteria: Tracing the evolutionary history of an ancient phylum. *Microbiol. Mol. Biol. Rev.*, 71: 495-548.

Wise, R. 2008. The worldwide threat of antimicrobial resistance. *Curr. Sci.*, 95: 181e187.

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