Academic Sciences

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 4, Issue 2, 2012

Research Article

ISOLATION AND CHARACTERIZATION OF ANTIBIOTIC PRODUCING ACTINOMYCETES FROM MARINE SOIL SAMPLES

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Received: 21 February 2012, Revised and Accepted: 20 March 2012

ABSTRACT

Marine environment is a potential source of secondary metabolites which provides an encouraging source for development of novel natural pharmaceuticals. Among the marine organisms, actinomycetes are a group of bacteria that are widely distributed and are known to play a very supporting role in the degradation of organic matter. These microbes have characteristics in common to both bacteria and fungi and yet they possess sufficient distinctive features to classify them into a separate category. The current research focuses on isolation and study of antimicrobial effects of actinomycetes collected form marine and soil samples. A total of thirty soil samples were collected from konark and western terrestrial sea. Actinomycetes were isolated by serial dilution and plating method on starch casein agar media. Total 20 species were isolated on the bases of colony characteristics on starch casein agar and all the isolates were primarily screened for antibacterial activity by cross streak method. And the Potent strains (AC-6, AC-7 and AC-8) were further screened for their antibacterial activity by agar well diffusion method.

Keywords: Marine Source, Actinomycetes, Antibiotic, Antibacterial activity, Starch casein agar.

INTRODUCTION

The marine environment is becoming increasingly appreciated as an exceptional reservoir of bioactive natural compounds, which exhibit structural / chemical features not found in terrestrial natural products[1]. Oceans cover 70% of the Earth's surface and harbor most of the planet's biodiversity[2]. Many compounds which are highly biologically active have been isolated from microorganisms, plants and animals. These active compounds can be further explored as new drugs or antibiotics. Actinomycetes are best known for their ability to produce antibiotics and are gram-positive bacteria, which comprise a group of branching unicellular microorganism[3]. The colonies have pastel colors, soil-like odour, hard and stick into agar. Recent reports show that this group of microorganism still remains vital source of antibiotics. As a result of the increasing prevalence of antibiotic resistant pathogens and the pharmacological limitation of antibiotics, there is an exigency for new anti microbial substances from bacteria and fungi. Around 100 antibiotics have been commercially used to treat human, animal and plant diseases. The genus, *Streptomycete* is responsible for the formation of more than 60 % of known antibiotics.

The present research study aims in collecting various soil and marine samples from konark and western terrestrial sea, isolation of actinomycetes colonies and to evaluate their antibiotic producing efficacy by performing antibacterial studies.

MATERIALS AND METHODS

Collection of soil samples

The soil samples were collected from various locations in and around konark and western terrestrial sea India. Soil sample (approx. 500 g) were collected using some clean, dry and sterile polythene bags along with sterile spatula, marking pen rubber band and other accessories. Samples are dried separately at 45 °C for 1 hour in a hot air oven and then cooled to room temperature. Each of the soil sample collected (1g) was taken in a conical flask containing 100 ml of sterile water and few drops of Tween 80 was added. The flasks were shaken for 30 min in an orbital shaker incubator at 27 °C and their contents are designated as stock cultures[4,5].

Isolation of actinomycetes from soil samples

Isolation of actinomycetes was performed by serial dilution and plating technique using starch casein agar medium. One gram of this soil sample was suspended in 25 ml sterile water in a conical flask, stirred thoroughly with the help of a glass rod and left for some time. Distilled water (9ml) was taken in each of the 7 test tubes and labeled from 1 to 7. The supernatant liquid from the dissolved soil sample was transferred into the test tubes so as to achieve the serial dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}. 1 ml of the diluted sample was inoculated in the starch casein agar medium plates from each dilution. The Petri plates are then rotated to spread the sample uniformly. Plates were then incubated at room temperature (28 to 30ºC) for 84hrs[6,7,8].

After 72h, white pin-point colonies, characteristic of actinomycetes with a clear zone of inhibition around them were seen. The pinpoint colonies with inhibitory zone of inhibition were selected and purified into actinomycetes agar slants. The selected strains were further purified by multiple streaking methods. The stock cultures of each selected strain was prepared and maintained in actinomycete agar slants at +4 °c. The actinomycete colonies isolated from the crowded plate were selected for the further studies and labelled as AC-6, AC-7, and AC-8.

Preliminary screening of crude antibiotic

Agar streak method

The microbial sensitivity of the soil isolates was analyzed by agar streak method. Each of the isolate was streaked as a straight line on soyabean casein digest (SBCD) medium. medium and incubated at 27 °C for 6 days (144 h). After the 6th day, different strains of microorganisms were streaked at right angle, but not touching each other, and then incubated at 37 °C for 24 h in the case of bacteria and 27 °C for 48 h in the case of fungi. If the organism is susceptible to the antibiotic produced by actinomycetes, then it will not grow near the actinomycetes. The zone of inhibition against each test organism was noted. The isolated actinomycetes were screened against some microorganisms Staphylococcus aureus and Escherichia coli[9]. Based on the antimicrobial properties, isolates were chosen for the further biochemical characterization.

Table 1: Shows Antimicrobial Activity Of Promising Isolates

Name of the Pathogen	Zone of inhibition (mm)		
	AC-6	G-Soil	APJ-US
Escherichia coli	13	9	11
Staphylococcus aures	10	7	8

Antimicrobial efficacy

The intense antimicrobial compound producing actinomycete strain is selected and its antimicrobial spectrum was tested against the pathogenic bacteria. The selected isolate is inoculated into Starch casein broth, and shaken at 28-30°C at 250 rpm for seven-ten days. After incubation the staling substance is centrifuged and the supernatant liquid is used to test the antibacterial activity. Antibacterial activity of actinomycetes is determined by using well diffusion method[10,11]. Two different pathogenic bacteria were inoculated on to nutrient broth agar and incubated at 37°C. After 24-48h of incubation the zone of inhibition was measured to evaluate the antimicrobial activity of actinomycetes isolates.

Isolates having large inhibition zones was selected for further study. Based on the above results isolate AC-6 were considered for further study. Antimicrobial efficacies were tested.

Table 2: Shows Antimicrobial Efficacies of Actinomycete Isolate Ac-6

No. of days	Zone of inhibition (mm)		
	Escherichia coli	*Staphylococcus aures*	Gentamycin (std)
1	-	-	13
2	-	-	13
3	11	-	13
4	11.5	11	13
5	12	11.5	13
6	12.5	12	13
7	12.5	12	13
8	12.3	11.7	13

Characterization and Identification of Actinomycetes

Microscopic observation

Gram staining, acid fast staining was performed to check the morphology of the cells and spore chain morphology was identified by cover slip culture technique.

Biochemical characterization

Actinomycetes isolates are characterized using citrate utilization, starch hydrolysis, casein hydrolysis, urease production, indole production, methyl red, voges prauskouer, nitrate reduction, H_2S production, catalase, and oxidase and gelatin liquefaction tests according to International *Streptomyces* [12] project.

Physiological and cultural characterization

The ability to grow at various temperatures (10-40°C), range of P^H (7-9) and in different concentrations of Nacl (2-16g/l) on medium was also tested. The organism was also tested for its ability to utilize carbon sources such as dextrose, fructose, glucose, inositol, lactose, maltose, mannitol, rhamnose, starch, sucrose and xylose in modified Bennett broth[13]. Cultural characteristics of the strain were determined following incubation for 10-15 days at 28-30°C on actinomycetes isolation agar, sabauraud dextrose agar, corn meal agar, oat meal agar and potato dextrose agar media. After incubation the growth, colour of spore mass and diffusible pigment production were observed.

Chemotaxonomy

Isomers of diaminopimelic acid (DAP) in cell wall hydrolysates and whole cell sugars of actinomycete were determined by thin layer chromatography following the standard methods of Waksman and Henrici[14] and Boone and pine[15].

RESULTS AND DISCUSSION

Total 20 actinomycetes were isolated from the soil samples. They had pinpoint colonies with zone of inhibition. The presence of relatively large populations of actinomycetes in the soil samples of Konark and western terrestrial sea indicates that the source is an eminently suitable system. Out of the 20 isolated actinomycetes screened for antimicrobial activity against Staphylococcus aureus and Escherichia coli agar streak method, three, namely, AC-6, AC-7 and AC-8showed significant antimicrobial activity (Table-1). However, AC-6 showed a good spectrum with the highest scores and its antimicrobial efficacy is calculated (Table 2).

The chemotaxonomic studies showing the presence of L-DAP in cell wall and absence of characteristic sugars in their cell, which is useful to categorize the cell wall of the strain, belongs to the cell wall type-I hence, the above characteristic features of the strains AC-6, AC-7 and AC-8 justifiably make to place the strain under the genus *Steptomyces* (Table 3).

The strains growed well at temperature of 30°C, P^H 8, Nacl Conc 2-4g/l. Cultural studies revealed that the strains growed well on several media where Actinomycetes isolation agar and corn meal agar showed better growth when compared to others. Thus the biochemical (Table-4), cultural (Table-5) and physiological characteristics (Table-6) such as tolerance to varied temperatures, P^H, Nacl and utilization of carbon sources confirm the identification of the strains as *Steptomyces sps* (AC-6, AC-7 and AC-8).

Table 3: Shows Chemotaxonomic Properties

Name of the test	Properties		
	AC-6	G-Soil	APJ-US
Di – aminopimelic acid	+	+	+
Cell wall sugars	-	-	-

Table 4: Shows Biochemical Properties

Name of the test	Properties		
	AC-6	G-Soil	APJ-US
Citrate utilization	-	-	+
Starch hydrolysis	+	+	+
Casein hydrolysis	+	+	+
Urease production	+	+	+
Indole production	-	-	-
Methyl red	+	+	-
Voges prauskouer	+	+	+
Nitrate reduction	+	+	+
H_2S production	-	-	-
Catalase	+	+	+
Oxidase	-	-	-
Gelatin liquefaction	+	+	+

Table 5: Shows Cultural Properties

Growth medium	Name of the isolate		
	AC-6	G-Soil	APJ-US
Actinomycetes isolation agar	Excellent	Excellent	Excellent
Sabauraud dextrose agar	Good	Good	Good
Corn meal agar	Moderate	Moderate	Moderate
Oat meal agar	Excellent	Excellent	Excellent
Potato dextrose agar	Poor	Poor	Poor

Table 6: Shows Physiological Properties And Carbon Source Utilization

Name of the test	Properties		
Growth temperatures (˚c)	**AC-6**	**G-Soil**	**APJ-US**
4	-	-	-
20	+	+	+
30	++	++	++
42	++	++	++
55	+	+	+
Growth P^H			
5.8	-	-	-
7	++	++	++
9	+	+	+
Nacl tolerance (%)			
2.5	++	++	++
5	-	+	+
7	-	-	+
Carbon Source Utilization			
Arabinose	-	-	-
Galactose	-	-	-
Glucose	+++	+++	+++
Inositol	-	-	-
Sucrose	+++	+++	+
Fructose	+++	-	-
Maltose	+++	+++	-
Xylose	-	+	-

CONCLUSION

The present investigation concludes that the biochemical and physiological characteristics of actinomycetes varied depending on the available nutrients in the medium and the physical conditions. Present study was an attempt to identify and pick out versatile strains of streptomyces from the regions of konark and western terrestrial sea displays antimicrobial activity against the tested organisms. Further the purification and characterization of the secondary metabolites can be carried out.

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