Isolation, Characterization and Screening of Marine Actinomycetes for Bioactive Compounds

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***Abstract*: Marine ecosystem is a wide source for industrially important microorganism. Among the marine microorganisms, actinomycetes are a group of bacteria that are widely distributed. They have characteristics in common to both bacteria and fungi and yet they possess sufficient distinctive features to classify them into a separate category. The present study reports bioprospecting of marine actinomycetes. Our findings suggest potential of these isolated actinomycetes as industrially and biotechnologically important enzymes**.

Keywords: Actinomycetes, amylase, protease, biosurfactant, lipase

I. INTRODUCTION

The marine biosphere is one of the richest habitats of microorganisms. The oceans cover around 70% of the earth's surface and present themselves as an unexplored area of opportunity [1].

Among the microorganisms, marine actinomycetes have been a great source of new compounds and their isolation all around the globe, from shallow coastal sediments to the deepest sediments [2]. Actinomycetes are a group of Gram positive bacteria, which comprise a group of branching unicellular microorganism [3]. Actinomycetes are becoming increasingly important as a source of novel products and are routinely screened for new bioactive substances like, antibiotics, enzymes and biosurfactants [4]. Enzymes like amylase and protease have wide applications in food, fermentation, textile, paper, detergent, pharmaceutical, leather, waste processing and silk industries [5, 6]. The actinomycetes are the best common source of antibiotics and provide approximately two thirds of naturally occurring antibiotics [7].

Due to the advancement in industrial, agricultural and biotechnological fields, there is an increase in the need microorganisms with novel characteristics that can be used for scientific and industrial purposes [8]. Considering the fact that Antarctic ocean is cold and saline in nature, it could be a reservoir of microorganisms producing rare and unique bioactive compounds, beneficial for humans.

The need for new microbial products with better properties and stability is ever increasing. Hence, the aim of our study is to identify and characterise marine actinomycetes having potential of producing industrially and biotechnologically important bioactive compounds.

II. MATERIALS AND METHODS

1). Isolation of actinomycetes

Isolation of actinomycetes was carried out from Antarctic water samples. The isolates were maintained on Starch Casein Nitrate Medium (SCN) for further studies. (Composition per litre: soluble 9.0g starch, 0.3g casein, 2.0g KNO_3, 2.0g NaCl, 0.5g $MgSO_47H_2O$, 2.0g K_2HPO_4, 0.02g $CaCO_3$, 0.01g $FeSO_47H_2O$, 20.0g Agar, 50.0mg Nystatin, 0.8mg Benzyl Penicillin with pH adjusted to 7.0 ± 0.2).

2). Effect of temperature

Effect of temperature on the growth of organisms was studied by cultivating the isolates in SCN medium at temperatures between 18°C to 45°C for 5 to 7 days.

3). Effect of pH

Effect of pH was studied by incubating the isolates in SCN medium with varying pH range from pH 4 to 10 at 18°C for 5 to 7 days.

4). Effect of salt concentration

Effect of salt concentration was studied by incubating the isolates in media containing different concentrations of NaCl (0% to 3.0%) at 18°C for 5 to 7 days.

5). Screening for the production of enzymes

The isolated actinomycetes were screened for amylase production by starch iodine plate method [9]. Briefly, the isolates were streaked on starch agar plates and incubated at 22°C for 7days. Iodine solution was spread on the plate and left for 5 minutes. The organisms that secreted amylases produced a zone of clearance or decolourization against the blue colour background.

The protease activity was checked using method described by Jeyadharshan [10]. The isolated actinomycetes were inoculated on skim milk agar medium and kept for incubation at 22°C for 5 days. Further the plates were flooded with 15% mercuric chloride and 20%HCl and left for 5 min. The plates were then observed for the development of clear zone.

The screening of the isolates for cellulase production was studied by inoculating them on Czapek-mineral salt agar medium. The plates were incubated at 22°C for 5 days. Iodine-potassium iodide solution was spread on the plate and left for 5 minutes for zone of clearance.

The lipolytic activity of the isolates was examined using tributyrin medium. The organisms were streaked and incubated at 22^{o}C for 5days. After incubation the plates were observed for zone of clearance surrounding the colonies [11].

The isolates were tested for urease production by method described by Meena *et al* [11]. The actinomycetes were streaked on urea agar medium and incubated at 22^{o}C for 5 days with phenol red as an indicator.

6). Screening for antimicrobial activity

The isolates were screened for antimicrobial activity by agar well diffusion method. Isolates were inoculated in 50ml of starch casein nitrate broth and kept on rotary shaker incubator (90-100rpm) at 22^{o}C for 7days. Centrifugation at 10,000rpm for 15 minutes was done to obtain the supernatant.

The pathogenic organisms used were *Staphylococcus aureus***,** *Salmonella typhi, E. coli, K. pneumoneae, P. mirabilis, Aspergillus niger, S. paratyphi* and *Candida albicans*. Test organisms were spread over nutrient agar plates and wells were aseptically bored and filled with 50µl of the supernatant. Further the plates were incubated at 28^{o}C for 48 hours for fungal species and at 37^{o}C for 24 hours for bacterial species.

7). Screening for biosurfactant production

The isolates were examined for the production of biosurfactant by the method described by Carilo *et al.* (1996) [12]. The isolates were streaked on blood agar plates supplemented with 5% fresh human blood. Observations were made for α, β and γ hemolysis. Plates were then incubated at 28^{o}C for 7 days. Haemolytic activity was correlated with the ability to produce biosurfactant.

Drop collapsing test was also used to examine the ability of the isolates for biosurfactant production according to method described by Youssef *et al* (2004) [13]. In a 96 microtitre plate mineral oil was added. The plate was equilibrated for one hour at 37^{o}C. After equilibration, 5µl of the culture supernatant was added to the surface of the oil in the well and the shape of the oil was observed after 5 minutes. Distilled water was used as a negative control.

III.RESULTS

1).Effect of temperature on the growth of the isolates

The isolates were grown at various temperatures range from 18^{o}C to 25^{o}C. None of the isolates could grow above 30^{o}C. The optimum temperature for growth for all the isolates was 22^{o}C, as shown in Table 1.

2).*Effect of pH on the growth of the isolates*

All the isolates could grow over a pH range of 6 to 9 with the exception of RGI-4 and RGI-5 which showed the growth at pH 4 and 9. However maximum growth was observed between pH 7.0 to 8.0.

3).*Effect of salt concentration on the growth of the isolates*

All the isolates showed growth at concentrations of NaCl (0.2% to 3.0%). At 1.0% NaCl concentration, growth of all the isolates was optimum.

4).Screening of isolated actinomycetes for enzyme production

All the isolated actinomycetes were evaluated for their ability to synthesize various enzymes. Our results indicated that four (RGI-1 to RGI-4) out of five isolates were positive for amylase production. Two (RGI-1 and RGI-2) isolates screened positive for protease activity. Two (RGI-1 and RGI-5) were positive for cellulase activity. None of the isolates were positive for urease or lipase activity. Significantly one of the isolate (RGI-1) could produce four different types of enzymes. However, all the isolates could produce one industrially important enzyme, as shown in Table2.

5).Screening for antimicrobial and biosurfactant activity

None of the isolates showed significant bio-surfactant and antimicrobial activity.

Table 1. Effect of various physiological parameters on the growth of actinomycetes*

Isolates	RGI-1	RGI-2	RGI-3	RGI-4	RGI-5
	Effect of pH				
4.0	++	+++	-	-	-
6.0	+++	+++	+++	-	++
7.0	+++	+++	+++	+++	+++
8.0	++	+++	+++	+++	++
9.0	++	++	++	-	-
Effect of Temperature (^{o}C)					
18°C	+++	+++	+++	+++	+++
22°C	+++	+++	+++	+++	+++
25°C	+++	+++	+++	+++	+++
30^{o}C	+	+	+	+	+
37°C	-	-	-	-	-
45°C	-	-	-	-	-
Effect of NaCl (%)					
0.2%	++	+++	+	++	++
0.5%	+++	+++	++	+++	+++
1.0%	+++	++	++	+++	++
2.0%	+++	++	+	+	+
3.0%	++	+	+	+	+

*+++Maximum growth, ++Moderate growth, +Poor growth, - No growth

Table 2. Summary of enzyme assays*

	RGI-1	RGI-2	RGI-3	RGI-4	RGI-5
Amylase	+	+	+	+	+
Protease	+	+	-	-	-
Cellulase	+	-	+	-	+
Urease	-	-	-	-	-
Gelatinase	+	+	-	-	-
Lipase	-	-	-	-	-

*+ positive - negative

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