SCREENING OF ACTINOBACTERIA BY ENZYME PRODUCTION AND ANTIMICROBIAL PROPERTIES FROM MARINE ENVIRONMENT

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ABSTRACT

Many of the ecological factors of the marine environment were helpful in developing a strategy for discovering useful bioactive agents from marine microorganisms. Among them actinobacteria have gained special importance as they play a major role in recycling of organic matter, production of novel pharmaceuticals, cosmetics, enzymes, antitumor agents, enzyme inhibitors, immune-modifiers and vitamins. The present study was carried out to isolate actinobacteria, which have potential to produce enzymes from selected marine samples. A total of eleven isolates of actinobacteria were isolated and were characterized morphologically. Screening test was performed to determine analyse, protease and lipase activity. Almost all the isolates showed enzyme production from the potential actinobacteria. Then active isolates were selected for secondary screening by agar well diffusion method. The efficacy of potential antibacterial properties of all the strains of actinobacteria were tested against *E.coli, P.aeroginosa, K.pneumoniae* and *S.aureus* performed respectively. The maximum antibacterial activity of SVA3, SVA10 and SVA11 strain has been responsible against *P.aeroginosa* and *K.pneumoniae* bacteria.

Keywords: Actinomycetes, enzyme activity, Antimicrobial activity.

INTRODUCTION

Incorporation of biotechnology and marine environment has opened up new horizons for finding novel organisms for trapping their potential resources. The industrial sector in India is developing fast for meeting the needs of food processing, pharmacy and textile industries. Marine microorganisms have unique properties since they have to adapt to extreme marine environment conditions such as high or low temperature, alkaline or acidic water, high pressure and limited substrate in the deep-sea water. These distinctive characteristics have attracted many researchers to explore in depth since there is the potential of marine microorganisms used in industry (Baharum *et al*., 2018). Marine micro organisms are increasingly becoming an important source in the production of medical and industrially important enzymes. More than 4000 enzymes are known today, of which many are produced commercially. Majority of the industrial enzymes are microbial in origin. The need for microbial enzymes are increasing day by day due to their clean, ecofriendly and cost effective application in many of the biotechnological processes and also are becoming important for its technical and economical advantages. Recently, the rate of discovery of new compounds from terrestrial actinomycetes has decreased, whereas the rate of re-isolation of known compounds has increased (Berdy, 2017). Thus, it is very crucial for the isolation of new groups of actinomycetes from unexplored or under exploited habitats be pursued as sources of novel bioactive secondary metabolites. Marine environment is considered as a huge treasure-house of marine actinomycetes resources. Marine actinomycetes have gained special attention today as they are responsible for the production of about half of the discovered bioactive secondary metabolites, notably antibiotics, antitumor agents, immunosuppressive agents and enzymes by Arunachalam *et al*., 2016. Marine actinomycetes have a diverse range of enzyme activities that are capable of catalyzing various biochemical reactions. Different commercial enzymes viz. amylase, cellulase, protease, lipase have also been obtained from the marine actinobacteria. Cellulases are a group of hydrolytic enzymes which hydrolyze the glucosidic bonds of cellulose and related cello-digosaccharide derivatives. In the current industrial processes, cellulolytic enzymes are employed in the color extraction from juices, detergents causing color brightening and softening, biostoning of jeans, pretreatment of biomass that contains cellulose to improve nutritional quality of forage, and pretreatment of industrial wastes (Griebeler *et al*., 2011). Microbial diseases are increasing

day by day and becoming the big problem for human health. There are more than 200 known diseases which is transmitted by bacteria, fungi, viruses, prions and other microbes to human being. The emergence of drug and multidrug resistant pathogens is the biggest issues, therefore, novel antimicrobial agents from natural resources with novel mechanism of actions are required in biopharmaceutical industry. Many research works has been carried out to control the pathogens and to identify the novel antimicrobial agents. Microbes from the soil samples are the most common natural sources exhibiting the strong biological activities against various pathogens (Alexander,1961).

MATERIALS AND METHODS

Amylase assay (Aiyer, 2005)

Amylase production by using submerged state fermentation. A mineral broth medium (peptone 6 g/L; $MgSO_4$, 0.5 g/L; KCl 0.5 g/L and starch 1g/L) was prepared. From the broth medium,90mL was transferred into 150 mL capacity Erlenmeyer flasks and sterilized at 121°C for 15 min. A loopful of inoculum was transferred into five test tubes having a 10 mL of sterile nutrient broth. The test tubes were incubated at 37°C for 24 h.

Lipase assay (Wantanabe *et al*., 1997)

Lipase activity was determined titrimetrically on the basis of olive oil hydrolysis. One ml of the culture supernatant was added to the reaction mixture containing 1ml of 0.1M Tris-HCl buffer (pH 8.0), 2.5 ml of deionised water and 3 ml of olive oil. The reaction mixture was mixed well and incubated at 37 °C for 30 min. Both test and blank were performed. After 30 minutes the test solution was transferred to a 50 ml Erlenmeyer flask. 3 ml of 95% ethanol was added to stop the reaction. Liberated fatty acid was titrated against 0.1M NaOH using phenolphthalein as an indicator. End point is an appearance of pink color. A unit lipase is defined as the amount of enzyme which releases one micromole fatty acid per minute under specified assay conditions. Enzyme activity was expressed as units per gram of dry substrate.

Protease assay by Mitra and Chakrabartty *et al*., 2005)

Proteolytic activity was assayed using casein as the substrate. A 0.5 ml aliquot of the enzyme extract was incubated with 1 ml of 2.0% casein solution in 0.1 M Tris HCl buffer, pH 7.0 at 37˚C for 10 min. The

reaction was stopped by the addition of 5.0ml 5% trichloroacetic acid and incubated for 30 min. The mixture was filtered and 2.0ml of filtrate was added to 4.0ml of 0.1N NaOH and 0.5ml diluted Folin-Cocalteau reagent and incubated for 30 min and then the amount of tyrosine released into the filtrate was measured from its absorbance at 670 nm. Protein was estimated using BSA as the standard. One unit of protease activity is expressed as the amount of enzyme which converts 1µg of tyrosine per 1min at 37˚C.

Screening for antimicrobial activity (Ashu Srivastav and Prasad Pofali 2010)

The antimicrobial activity of isolated Actinomycetes was performed by cross streak method. The plates were prepared and inoculated with isolated by single streak at the centre of petridish and incubated at 30˚C for 3 days. The plates were then inoculated with test organism by a single streak the Actinomycetes strains and incubated at 37˚C overnight. The human bacterial pathogens such as *Escherichia coli, Staphylococcus aureus, K. Pneumoniae, and Pseudomonas aeruginosa* were used. The positive isolates were further screened against these pathogens by using agar well diffusion method. The pathogens were swabbed on NA plates and incubated at 37˚C for 24 hours.

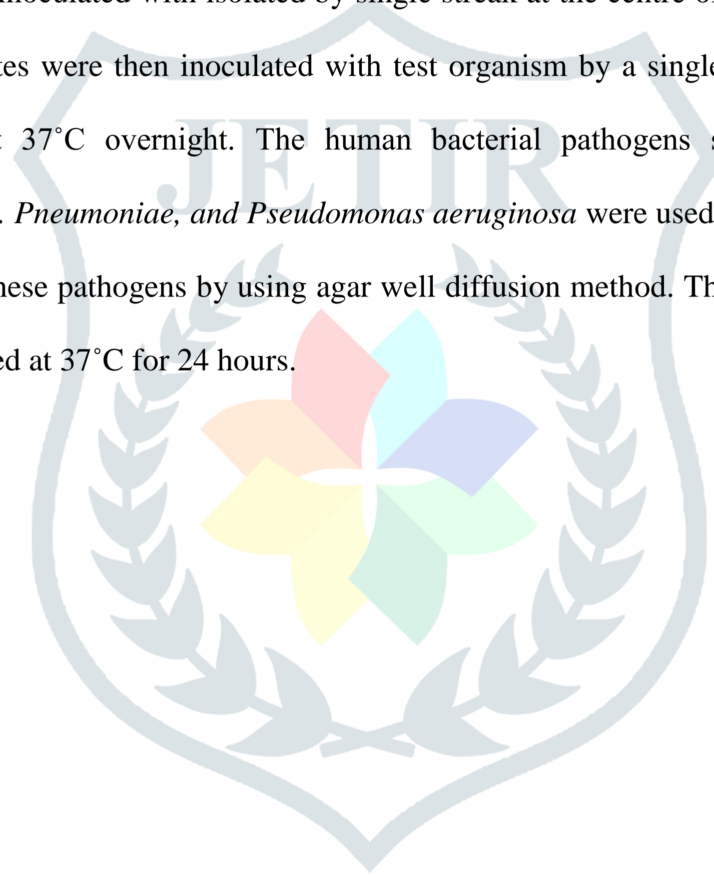

Table 1: Production of various enzymes by potential *Actinobacteria*

S.No	Strain code	**Quantity (mg/g)**		
		Amylase	Lipase	Protease
1	SVA3	+	+	+
2	SVA10	+	+	+
3	SVA11	+	+	+

Table 2: Efficacy of antibacterial activity of *Actinobacteria* by cross streak plate method

S.No	Strain code	Zone of inhibition (mm)			
		E.coli	*P.aeroginosa*	*K.pneumoniae*	*S.aureus*
1	SVA1	9.31±0.09	15.0±0.62	7.38±0.02	6.50±0.10
2	SVA2	-	5.08±0.12	-	-
3	SVA3	12.0±0.21	20.2±0.18	18.0±0.31	16.0±0.13
4	SVA4	-	6.00±0.22	8.44±0.21	-
5	SVA5	6.73±0.34	7.22±0.28	-	7.18±0.21
6	SVA6	7.02±0.11	8.12±0.41	8.09±0.47	-
7	SVA7	-	-	-	-
8	SVA8	6.07±0.26	-	-	7.61±0.71
9	SVA9	-	5.33±0.27	9.11±0.16	11.0±0.20

10	SVA10	7.07±0.04	10.3±0.08	15.2±0.24	21.3±0.33
11	SVA11	10.0±0.05	14.0±0.09	18.0±0.11	24.3±0.17

Standard deviation ±error

Table 3: Effect of antifungal properties of potential strain of *Actinobacteria* against fungi

S.No	Strain code	Zone of inhibition (mm)		
		A.flavus	*A.terreus*	*Penicillium* sp
1	SVA3	-	-	17.0±0.22
2	SVA10	-	19.0±0.32	22.0±0.22
3	SVA11	22.0±0.02	21.2±0.38	27.3±0.17

Standard deviation ±error

RESULT AND DISCUSSION

Marine microorganisms were proven already to have many beneficial bioactivities such as production of industrial enzymes, plant growth promotion potentials such as production of phytohormones, antibacterial and probiotic activity. Of that marine actinomycetes are potential producers of a variety of biologically active enzymes. The actinobcteria have gained enormous importance since they possess a capacity to produce and secrete a variety of extracellular hydrolytic enzymes. Studies on marine actinomycetes are very limited. The selective screening of these marine samples resulted in the isolation of 10 marine actinomycetes. All the cultures grow well in the Starch Casein Agar medium by Jenson and Lauro, 2008. In the present study, all the isolated strains were tested for their cellulase, lipase and protease activities. Enzymatic activities of the isolated strains revealed that out of 10 actinobacteria were possessing cellulase, protease and lipase activity respectively. The production of enzyme activity when comparing cellulase producing enzymes are more. Previous reports also revealed that actinomycetes are one of the known cellulose producers (Jang and Chenks, 2003).

In the current investigator suggested that the potential strains like SV3, SVA10, and SVA11 were performed for different enzyme amylase, lipase and protease for identically represented for the production of enzyme analysed. Those enzyme has industrially important application. So the efficacy of screening essential for scientific validation (Table-2)

Cross streak method were used to calculate the minimum inhibition concentration (MIC) value of isolates against different human pathogens. The test pathogens were *Escherichia coli, Staphylococcus aureus K.Pneumoniae,* and *Pseudomonas aeruginosa.* The Actinobacteria isolates grown at the centre of the plates and further perpendicularly the pathogen strains were incubated for 24 hours to observe their antibacterial effect (Table-1). The control plates without the Actinomycetes isolates were also prepared to compare the similar pattern of growth. Among the isolates the Actinomycetes there was a total of isolates showed antibacterial effect against one or more pathogens. This indicates that the potential for these two strains against *S. aureus* can lead to the further investigation towards multi-drug resistant *staphylococcus aureus*.

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