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Studies on the Bio-Active Alkalophilic Actinomycetes From Terrestrial Substrates.

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ABSTRACT

Twenty four actinomycetes were isolated and tested against antimicrobial, amylolytic and proteolytic activities. The extracellular protease production potential was studied for one of the best isolate which resulted in highest yield (235 μ g/ml) of protease.

Keywords: Alkalophilic actinomycetes, cross-streak, amylolytic, proteolytic and extracellular protease

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INTRODUCTION

Hydrogen ion concentration is considered as one of the most fundamental factors affecting the growth and multiplication of micro-organisms. Extremely low or high concentration of hydrogen ion is toxic to most forms of life. However a very few micro-organisms are able to thrive well under such extremely high acidic or alkaline p^H .

There is a diverse group of micro-organisms which thrive in alkaline environments and these are known as alkalophiles, which grow in the p^H range of 8.0 to 12.0 but fail to grow at P^H 7.0. Alkalophiles have been isolated from both man-made alkaline environments such as effluents from food and leather industries , potato processing plant effluents, detergents, etc. and from natural environments such as soda lakes, deserts, some dilute alkaline springs, soils containing decaying proteins, etc.

The literature indicates that bacteria are the predominant alkalophiles which can be commercially exploited for the production of various alkaline enzymes [1]. Very few reports are found on the alkalophilic actinomycetes and their metabolites. There is almost no report on the alkalophilic actinomycetes from Indian soil, which prompted us to screen the alkaline terrestrial substrates for the detection of alkalophilic actinomycetes and their metabolites.

The present investigation deals with the selective isolation of alkalophilic actinomycetes from alkaline soils and evaluation of their antimicrobial, amyolytic and proteolytic activities. In view of the importance of alkaline proteases, a detailed study was conducted on the production of extracellular alkaline proteases which have application in industrial processes such as pharmaceutical, leather, detergent, etc. and for degrading the industrial effluents.

MATERIALS AND METHODS

A few alkaline soil samples collected near Hyderabad, were screened for alkalophilic actinomycetes employing starch-casein-agar medium [2] with p^H 8.5. Altogether about 60 alkalophilic actinomycetes were isolated. After preliminary screening, 24 isolates were selected and the following parameters were investigated.

p^H

The ability of the isolates to grow at various p^H values was studied by growing them on starch-casein-agar medium (p^H :7.5,8.0,8.5, and 9.0).

Antibacterial activity

The antibacterial activity was tested on nutrient agar medium by the conventional cross-streak method [3].

Antifungal activity

The antifungal activity was studied on yeast extract malt extract agar medium by cross-streak method.

Amyolytic activity

The amyolytic activity was tested with starch-casein-agar medium [4]. The inoculated plates were incubated at $28^{\circ}C$ for five days, flooded with weak iodine solution and hydrolyzed zones were measured.

Proteolytic activity

The proteolytic activity was studied with milk-casein agar medium [4] by measuring the hydrolyzed zones after incubating the inoculated plates at

28° C for five days .The extracellular protease activity of the isolates was determined qualitatively and the ratio of the diameter of hydrolyzed zone and that of the growth on milk casein agar.

Extracellular protease production

The extracellular protease production potential was studied by shake-flask method. A synthetic medium with the following ingredients was used(g/l): Potassium nitrate:1.0,Dipotassium hydrogen phosphate: 0.5,Magnesiumsulphate:0.5, Sodium chloride:0.5, Ferrous sulphate:0.01, Calcium carbonate:0.1,Glucose:20.0, pH.8.0. The protease activity was assayed by casein hydrolysis [5,6].

Unit: One protease unit (PU) is defined as the amount of enzyme that releases 1 µg of tyrosine per hour under the reaction conditions.

RESULTS AND DISCUSSION

All selected isolates showed good growth in the pH range of 8.0 to 9.0 indicating that the isolates are alkalophilic.

Antibacterial activity

Twenty four isolates were tested for their antibacterial activity which show that (i) 11 isolates have very good activity (ii) 7 isolates are active against both Gram-positive and Gram –negative bacteria.(iii) 2 isolates are predominantly active against Gram-positive bacteria; and (iv) 2 isolates are predominantly active against Gram-negative bacteria.

Antifungal activity

The antifungal activity of the isolates show that (i) 4 isolates are active against fungi and yeast (ii) 3 isolates are active against only fungi and (iii) one isolate is predominantly active against yeast.

Amylolytic activity

Extracellular amylases were produced by majority of the isolates (Table.1). The highest amylase activity was exhibited by AA-33 isolate followed by AA-25 and AA-34.No amylase activity was observed with 5 isolates.

Proteolytic activity

The data on the extracellular protease activities are given in Table.1. Twelve isolates showed casein hydrolyzed zones on milk-casein agar medium plates suggesting their ability to secrete extracellular proteases. The results indicate that AA-33 was the highest protease producer and the moderate producers being AA-34,AA-23 and AA-19. Twelve isolates did not show any proteolytic activity. Isolate AA-33 exhibited maximum enzymatic activities of both amylases and proteases.

The productivity of proteases by the isolates in submerged culture is shown in Table.2. The isolate AA-33 yielded the highest amount of protease (235µg/ml) followed by AA-34 (215µg/ml). The results obtained by the plate method (milk casein agar medium) are comparable with the protease production by submerged culture technique.

The present investigation indicates that alkalophilic actinomycetes are the potent sources for the production of alkaline proteases. The isolate showing maximum enzyme activities in solid and liquid media was selected for further detailed studies. The optimization parameters and strain improvement studies of the best protease producer are under investigation.

Table 1: Amylolytic and Proteolytic Activities of Isolates

Isolate no	Hydrolysing zone/Growth zone	
	Amylolytic Activity	Proteolytic Activity
AA-13	1.13	1.93
AA-14	1.30	1.83
AA-15	1.66	-
AA-16	1.71	-
AA-17	2.05	-
AA-19	2.17	2.00
AA-21	1.55	1.85
AA-22	1.25	1.70
AA-23	2.13	2.11
AA-24	1.58	-
AA-25	2.23	1.52
AA-27	1.53	1.70
AA-29	1.40	1.73
AA-31	1.35	-
AA-32	1.85	-
AA-33	2.26	2.50
AA-34	2.21	2.33
AA-35	1.38	1.40
AA-36	1.44	-

Table 2: Production of Proteases by the Selected Alkalophilic Actinomycetes

Isolate no	Caseinase activity (PU)
AA-13	145
AA-14	80
AA-19	150
AA-22	95
AA-23	105
AA-25	80
AA-27	100
AA-29	100
AA-33	235
AA-34	215
AA-35	75

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