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Research Article

SCREENING AND ISOLATION OF GELATINASE PRODUCING BACTERIA FROM VARIOUS REGIONS IN BANGALORE

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Abstract: This work has been undertaken for the Screening and isolation of Gelatinase producing strains of Bacteria were carried out from Eleven soil samples, collected from various regions of Bangalore and used to screen for Gelatinase production by using Gelatin agar plate assay. In the present study, an attempt was made to isolate efficient Gelatinase producing bacteria from diverse environmental samples. Different isolates were screened for possessing the ability to produce Gelatinase. About 19 bacterial isolates were found to be promising to produce Gelatinase. The organisms were tested for various biochemical tests, which leads to their identification as *Bacillus subtillus*, *Bacillus sphaericus*,*Bacill pasteurii* and *Staphylococcus aureus.*

Key words: Gelatinase, Soil samples, Screening, Gelatin agar, Environment ,Bangalore.

INTRODUCTION

Enzymes are delicate protein molecules necessary for life. Proteases are the single class of enzymes which play an important part in the metabolism of almost all organisms (Plants, Animals, Fingi, Bacteria and Viruses).Investigation of proteases is a central issue in enzymology due to their wide applications in Landry detergents, Pharmaceutical. Leather products, Photography, Food, Agricultural products and Bioremediation process[2]. Among the various proteases, bacterial extracellular proteases are the most significant, compared with animal,Plants,viruses and fungal extracellular proteases. Extracellular proteases produced by Bacillus and cocci species are of main interest from a biotechnological perspective, and are not only in scientific fields of protein chemistry and protein engineering but also in applied fields such as detergents, foods, tannery, pharmaceutical and leather industries. These proteases account for 60% of the total worldwide production of enzymes. The genus Bacillus and cocci contains a number of industrially important species and approximately half of the present commercial production of bulk enzymes derives from the srtains of Bacillus and cocci. Gelatinases are important metalloproteases and their action are very specific, i.e., they acts only on gelatin substrates. Gelatinases are hydrolyze both native and denatured collagens (gelatin), and the enzymes are widely used not only in chemical and medical industries but also in food and basic biological science[3] .In this study an attempt was made for the screening and isolation of gelatinase producing bacteria from soil samples collected from various regions in Bangalore.

MATERIALS AND METHODS

Collection and isolation of sample

Samples were collected from dump yards of beef, chicken, fish and milk centers at Solddevanahalli , Chikkabanavara, Devasandra, K.R.puram, Tannary road, Yashwanthpur and at Tumkur Diary in and around Bangalore, Karnataka, India. The samples were labeled after collected. These were spread onto isolation media (Gelatin agar) and incubated at 37^{o}C for 48 hours after serial dilution of 10^{-1} to $10^{-6.}$

Screening of Gelatinase production by plate assay

The isolates were screened for gelatinase activity. This was done by inoculating the organisms on the agar plates containing 0.4 % gelatin incubated at 37^{o}C for 48 hours. the plates were flooded with 10 % HCl-15% $HgCl_2$ solution and incubated for 15 min at 37^{o}C[4]. A clear zone around the growth of the bacteria was indicated to gelatinase activity

Identification of Bacteria

The isolated bacteria were identified based on cellular morphology, growth condition, grams staining, endo spore staining, capsule staining and biochemical tests (Pokorny, M., LJ. Vitale;1979).[1]

Table -1:Tabulation for Samples Description

S.NO	DESIGNATION OF SAMPLE	SAMPLE COLLECTED AREA	SAMPLE COLLECTED LAND MARK	SAMPLE NATURE	SAMPLE PH
1	ABMRCP-1	Shivaji Nagar	Opposite to Maszid at Beef center	Semisolid sticky Seems to Brown in colour	7.64
2	ABMRCP-2	Tannery Road	Near to Bus stop at Beef center	Semisolid Seems to Black in colour	7.60
3	ABMRCP-3	Tannery Road	Slaughter house inside	Semisolid Seems to Brown in colour	7.72
4	ABMRCP-4	Tannery Road	Slaughter house opposite canal	Hard consist of sand and clay seems to Brown in colour	7.65
5	ABMRCP-5	Solddevanahalli	Near to Bus stop Chiken Center	Semisolid Seems to Brown in colour	7.62
6	ABMRCP-6	Chikka Banavara	Near to Bus stop Chiken Center	Sticky consist of sand and clay seems to Brick red in colour	7.44
7	ABMRCP-7	K.R.Puram	Devasandra lake Beef dump	Semisolid Seems to red in colour	7.71
8	ABMRCP-8	Tin Factory	Opposite to Maszid at Chiken center	Semisolid Seems to red in colour	7.60
9	ABMRCP-9	Tumkur Dairy	Tumkur Dairy Milk packing area	Hard consist of sand and clay seems to Black in colour	7.26
10	ABMRCP-10	Tumkur Dairy	Tumkur Dairy Milk treatment area	Sticky consist of sand and clay seems to Brown in colour	6.59
11	ABMRCP-11	Yashwanth Pura	Fish market Near to Railway station	Sticky consist of sand and clay seems to Black in colour	7.34

ABMRCP DENOTES ACHARYA &B.M REDDY COLLEGE OF PHARMACY

RESULTS AND DISCUSSION

Ninteen bacterial isolates were obtained(Table:3) from soil samples of ABMRCP 1 to ABMRCP 11 (Table:1)and identified as *Bacillus subtillus, Bacillus sphaericus, Bacill pasteurii* and *Staphylococcus aureus.* Morphologically and biochemically. The colonies were subjected to grams staining,capsule staining and endospore staining.The colonies which were positive and negative for Grams staining,Capsule and endospore staining were considered for further studies(Table 3&4).The selected colonies were streaked on Gelatin agar plates.The plates were subjected to incubation for a period of 48 hours at 37^{o}C.The plates which showed clear zone around the streaked area of test organism was selected as gelatinase producing strain.The organisms named(Table2) showed the inhibition zone and was subjected to various biochemical tests(Table4). G.D isolates (Table2) showed the following results for the biochemical tests.These were positive for Methyl red test,Starch hydrolysis,Citrate utilization test.Oxidase test,gelatin hydrolysis test.urease test and nitrate reduction test ,and few isolates were shows negative for Voges Paskauer test,Indole test and Catalase test.After biochemical tests these organisms were confirmed to belong to the Bacillus and Cocci species (*Bacillus subtillus, Bacillus sphaericus, Bacill pasteurii* and *Staphylococcus aureus*)producing protease.

Table -2:Tabulation for results of colony characteristics which shows Gelatinase activity.

STRAIN NO.	COLONY SURFACE	COLONY COLOUR	VISUAL CHARACTERISTICS	SHAPE OF THE COLONY	HEIGHT OF THE COLONY	PROTEASE /GELATINASE ACTIVITY
G.D-1	Smooth	Brown	Opaque	Irregular	Raised	Positive
G.D-16	Smooth	Off white	Translucent	Circular	Raised	Positive
G.D-20	Smooth	Brown	Translucent	Irregular	Flat	Positive
G.D-31	Smooth	Off white	Opaque	Irregular	Raised	Positive
G.D-32	Smooth	Brown	Translucent	Circular	Raised	Positive
G.D-55	Smooth	Off white	Opaque	Circular	Flat	Positive
G.D-65	Smooth	Off white	Translucent	Irregular	Raised	Positive
G.D-72	Smooth	Brown	Opaque	Circular	Raised	Positive
G.D-75	Smooth	Off white	Translucent	Circular	Flat	Positive
G.D-78	Smooth	Off white	Opaque	Circular	Raised	Positive
G.D-89	Smooth	Off white	Opaque	Circular	Flat	Positive
G.D-91	Smooth	Off white	Translucent	Irregular	Raised	Positive
G.D-97	Smooth	Off white	Translucent	Irregular	Raised	Positive
G.D-104	Smooth	Off white	Translucent	Circular	Flat	Positive
G.D-105	Smooth	Brown	Translucent	Circular	Raised	Positive
G.D-107	Smooth	Brown	Opaque	Irregular	Flat	Positive
G.D-113	Smooth	Brown	Opaque	Irregular	Raised	Positive
G.D-115	Smooth	Brown	Translucent	Irregular	Flat	Positive
G.D-116	Smooth	Brown	Translucent	Irregular	Flat	Positive

G.D DENOTES GOLI DIVAKAR

Table -3: Tabulation for results of Staining Techniques

STRAIN NO.	GRAM STAINING	MORPHOLOGY (BACILUS/COCCI)	ENDOSPORE STAINING	CAPSULE STAINING
G.D-1	Positive	Cocci	Positive	Positive
G.D-16	Negative	Cocci	Positive	Positive
G.D-20	Negative	Cocci	Positive	Positive
G.D-31	Positive	Cocci	Positive	Positive
G.D-32	Positive	Cocci	Positive	Positive
G.D-55	Positive	Cocci	Positive	Positive
G.D-65	Positive	Cocci	Positive	Positive
G.D-72	Positive	Cocci	Positive	Positive
G.D-75	Positive	Cocci	Positive	Positive
G.D-78	Negative	Cocci	Positive	Positive
G.D-89	Negative	Cocci	Positive	Positive
G.D-91	Negative	Cocci	Positive	Positive
G.D-97	Negative	Cocci	Positive	Positive
G.D-104	Positive	Cocci	Positive	Positive
G.D-105	Positive	Cocci	Positive	Positive
G.D-107	Negative	Bacillus	Positive	Positive
G.D-113	Positive	Bacillus	Positive	Positive
G.D-115	Positive	Bacillus	Positive	Positive
G.D-116	Positive	Bacillus	Positive	Positive

Table -4: Tabulation for results of Various Biochemical tests

S.No.	SAMPLES	INDOLE	MR	VP	AMYLASE	NITRATE	OXIDASE	CATALASE	UREASE	GELATINASE	CASEIN
1	G.D-1	-Ve	-Ve	-Ve	+Ve	-Ve	+Ve	+Ve	-Ve	+Ve	+Ve
2	G.D-16	-Ve	-Ve	+Ve	+Ve	+Ve	+Ve	-Ve	+Ve	+Ve	+Ve
3	G.D-20	-Ve	+Ve	-Ve	+Ve	+Ve	+Ve	-Ve	+Ve	+Ve	+Ve
4	G.D-31	-Ve	+Ve	-Ve	+Ve	+Ve	-Ve	-Ve	-Ve	+Ve	+Ve
5	G.D-32	-Ve	-Ve	+Ve	+Ve	-Ve	+Ve	-Ve	-Ve	+Ve	+Ve
6	G.D-55	-Ve	-Ve	+Ve	-Ve	+Ve	+Ve	+Ve	-Ve	+Ve	+Ve
7	G.D-65	-Ve	-Ve	+Ve	-Ve	+Ve	+Ve	+Ve	-Ve	+Ve	+Ve
8	G.D-72	-Ve	-Ve	+Ve	+Ve	-Ve	+Ve	-Ve	-Ve	+Ve	+Ve
9	G.D-75	-Ve	-Ve	+Ve	+Ve	-Ve	+Ve	-Ve	-Ve	+Ve	+Ve
10	G.D-78	-Ve	-Ve	+Ve	+Ve	-Ve	+Ve	-Ve	-Ve	+Ve	+Ve
11	G.D-89	-Ve	-Ve	+Ve	+Ve	-Ve	+Ve	-Ve	-Ve	+Ve	+Ve
12	G.D-91	-Ve	-Ve	+Ve	-Ve	+Ve	+Ve	+Ve	-Ve	+Ve	+Ve
13	G.D-97	-Ve	-Ve	+Ve	+Ve	-Ve	+Ve	-Ve	-Ve	+Ve	+Ve
14	G.D-104	-Ve	-Ve	+Ve	+Ve	-Ve	+Ve	-Ve	-Ve	+Ve	+Ve
15	G.D-105	-Ve	-Ve	+Ve	+Ve	-Ve	+Ve	-Ve	-Ve	+Ve	+Ve
16	G.D-107	-Ve	-Ve	+Ve	+Ve	-Ve	+Ve	-Ve	-Ve	+Ve	+Ve
17	G.D-113	-Ve	-Ve	+Ve	-Ve	-Ve	+Ve	+Ve	-Ve	+Ve	+Ve
18	G.D-115	-Ve	-Ve	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	+Ve	+Ve
19	G.D-116	-Ve	-Ve	-Ve	-Ve	+Ve	-Ve	+Ve	-Ve	+Ve	+Ve

Figure 1: isolated Pure culture

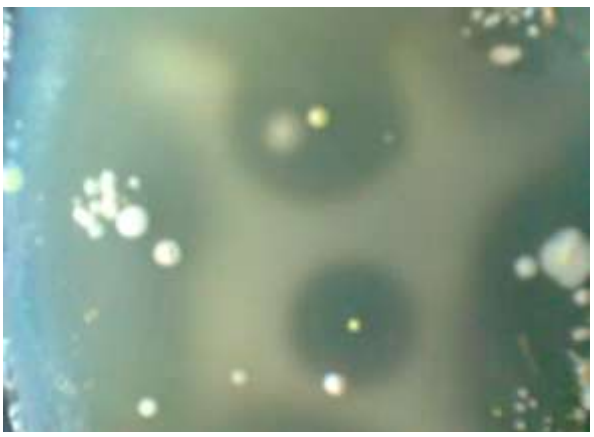

Figure 2: Gelatinase production

CONCLUSION

The search for promising strains of Gelatinase producers is a continuous process. The isolates which shows higher gelatinase activity were selected for biochemical characterization and identification. The organisms were identified as *Bacillus subtillus, Bacillus sphaericus, Bacill pasteurii* and *Staphylococcus aureus*. on the basis of data obtained in the present work it can be concluded that Bacillus and cocci species isolates can be employed in the production of Gelatinase.

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