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Research Article

Isolation of Marine Microbugs from Mangrove Ecosystem and Screening of Industrially Important Enzymes

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Abstract

Mangrove ecosystem are known for their diverse and rich microbiota. Here human influence is limited, so novelty in bacterial diversity is more common as mangroves ecosystem comes under conservative area and human interference in restricted here. In this present study, mangrove sample like mangrove associated Soil, Coastal water, Roots were collected from Borivali coastal area of manori creek and Jhow Island, Maharashtra under prior permission from CCF, Mangrove Cell, Maharashtra government. Halophiles are in demand in many Biotech based company and their enzymes are equally in demand due their properties. Here in the present experiment, with the help of enriched media 30 samples were processed from which 8 samples were from Jhow Island and 22 samples from borivali coastal-line. All the samples were screened with 10% salt and 20% salt concentration (NaCl). Sample were also screened for 7 pH and 9 pH growth parameters from Borivali and Jhow Island respectively. From above mention samples, total 62 Isolates were obtained, which were screened for Bio-Industrially important enzymes like Amylase, Protease and Cellulase. These enzymes have great demand when they are active in high salt concentration or alkaline pH. These enzymes may emphasize more Scientific-Entrepreneur in India.

Keywords: Mangrove Ecosystem; Halophiles; Bio-industry; Scientific-entrepreneur

Introduction

Mangrove ecosystem is known for it's diverse microbiota. The high nutritional load in mangrove sediments is due to mechanisms such as mangrove microbial activity and remineralization. This process is smoothly carried out as associated microbiota produce hydrolytic enzymes. Many researcher claim to find microorganism rich in producing crucial enzymes like Protease, Cellulase, Amylase. These enzyme play Important role in nutritional cycle of land and associated biotic forms [1]. The parameters of commercial production activities are associated to enzymatic activity in contexts with temperature, pressure, pH, and salinity changes. Industrial biotechnology is frequently used to manufacture enzymes of bacterial origin; the enzymes produced contain hydrolytic thermostable enzymes such as amylases, cellulases, proteases for the generation of bio-fuel [2]. Water covers approximately 71% of the earth's surface area, making it a magnificent blue planet. Because the salter ocean is so vast, it contains a diverse range of life forms. Halophiles are a widely distributed organism; 'Hal' means salt, and 'philos' means lovable. Their growth is affected by salt concentration. They are classified according to salt concentration and represent all three life branches, Archaea, Eukarya and Bacteria. Based on salinities, they are classified as Slight-Halophiles, Moderate-Halophiles, and Extreme-Halophiles, with sodium chloride (NaCl) concentrations of nearly 1-5 %; 5-20 %; 20-30 %, respectively. Nonhalophiles, which grow in less than 0.2M salt, and Halotolerants, that can grow

in either absence or high salinity, are two more classifications. Both halophiles and halotolerant can survive in salt(salinity) [3]. This study will lead to increasing valorization of enzyme from microbial sources and open new prospective for Bio-based Industries.

Materials and Methods

Sample processing and isolation

The bioactive molecules, enzymes have received a lot of attention. Microbial sourced enzymes are in high demand in industrial domains, especially to their use in clean, eco friendly and cost-effective biotechnological applications. The worldwide economy for industrial enzymes is now valued at over $ 2 billion and is predicted to increase at a 3.3% yearly rate. However, the majority of the enzymes come from bacteria that flourish in low-temperature, neutral-pH environments. Research of extremophiles and their bio-catalyst machinery has gathered considerable attention. Halotolerant or also called as halophilic organisms are the key category with better biocatalytic potential [4].

Screening of protease

Isolates were screened for Protease. Medium contain gelatine-10g/l, Gelatine-10g/l, Pepton-5g/l, Agar-20g/l, Nacl-10% and 20%, pH -7 and 9. Inoculated plates were incubated for 48h at 34°C then plates will be observed for zone of hydrolysis by overlaying plateby Frazier reagent [5].

Screening of amylase

Isolates were screened for Amylase. Medium have 1g/l Starch, 0.5 g/l $NaNO_3$, 1g/l K_2HPO_4, 0.5 g/l $MgSO_4 \cdot 7H_2O$ and 0.001 g/l $FeSO_4 \cdot 7H_2O$, 1g/l yeast extract and 15 g/l agar, Nacl-10% and 20%, for pH -7 and 9 for Borivali and Jhow.incubation for 48h at 34°C and then was checked for hydrolysis zone 0.2 g/l potassium iodide was poured on plate to observe hydrolysed zone [6] .

Screening of cellulase

Isolates were screened for Cellulase. Medium comprises 1g/l carboxymethylcellulose (CMC), 0.5 g/l $NaNO_3$, 1g/l K_2HPO_4, 0.5 g/l $MgSO_4 \cdot 7H_2O$ and 0.001 g/l $FeSO_4 \cdot 7H_2O$, 1g/l yeast extract and 15 g/l agar, Nacl-10% and 20%, pH-7 and 9. Inoculated plates were incubated for 48h at 34°C then Gram's Iodine was flooded in the plate to observe zone of clearance [7].

Results and Discussion

Mangrove ecosystem was selected for sampling. Samples were collected in sterile containers. From site of Jhow Island soil and water sample was collected, while from manori creek in Borivali coastal area water, root, soil sample were collected. Soil and root sample were collected from mangrove plants like, *Avicennia marina, Bruguiera cylindrical, Salvadora persica, Sonneratia alba* etc. Sample were stored in cooling temperature till they were processed. Then 1g soil was suspended in flask containing 50ml enrichment broth, which were kept at 100RPM shaking condition for 48h. From them 30 samples were processed from which 8 samples were from Jhow Island and 22 samples from Borivali. Each sample inoculated flask contain NaCl concentration of 10% and 20% (2 flask for each sample). Jhow Island samples were screened with 9 pH while Borivali samples were screened with 7 pH growth medium. After 48hr serial dilution was carried out and culture was spread on complete medium plate.

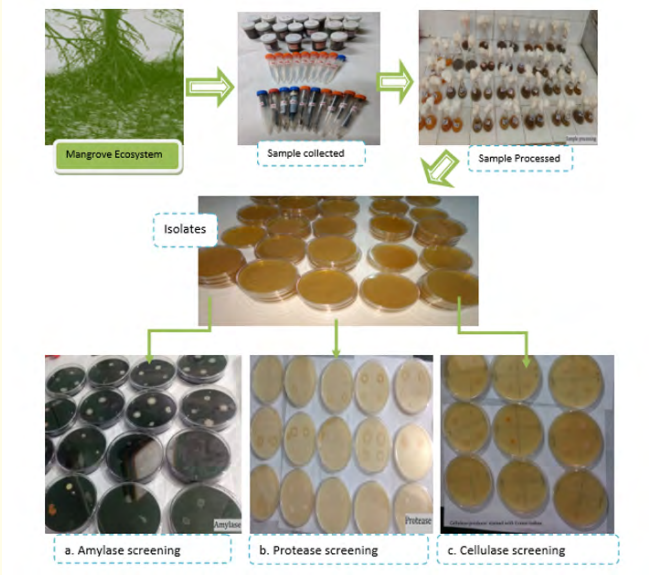
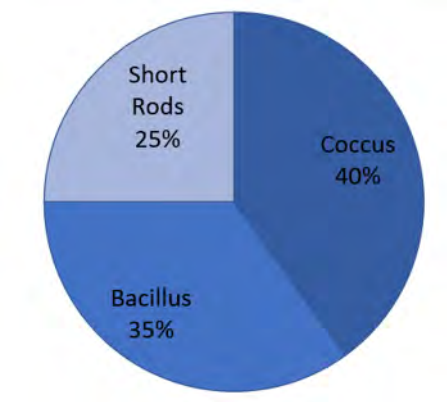


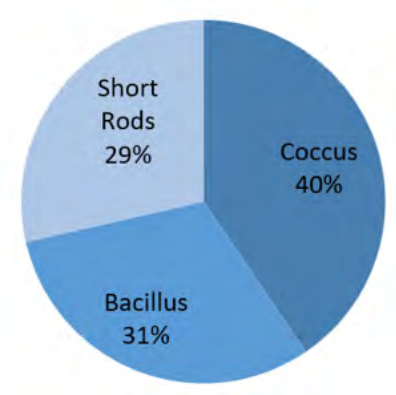
Figure 1

Result obtained from 30 sample were, 62. These 62 isolates were further screened for industrially important enzymes like Amylase, Protease and Cellulase as shown in above images flow of the process is shown. Here figure 1a is Amylase producing bacterial zone of starch hydrolysis, figure 1b is Protease producing bacterial zone and figure 1c is Cellulase enzyme producing bacteria.

Detail description is given in below chart.



Graph 1: Isolates from Jhow Island.



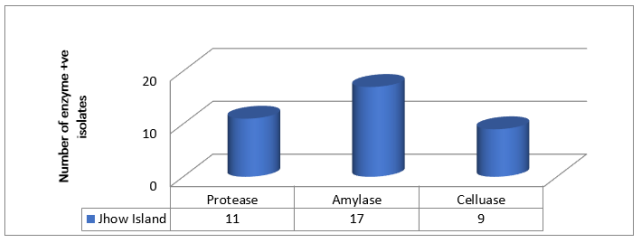
Graph 2: Isolates from Borivali Coastline.

SR.	Isolates	Morphology	Gram Reaction	Medium salt conc.	Protease	Amylase	Cellulase
1	Jho S1B1	Cocci	Gram +ve	10% NaCl	+	+	-
2	Jho S1B2	Short rod	Gram +ve	10% NaCl	-	+	-
3	Jho S1B3	Bacilli	Gram +ve	10% NaCl	+	+	-
4	Jho S5B4	Cocci	Gram +ve	10% NaCl	-	++	+
5	Jho W16B5	Bacilli	Gram +ve	10% NaCl	+	-	-
6	Jho S3B6	Cocci	Gram -ve	10% NaCl	+	+	-
7	Jho S3B7	Short rod	Gram +ve	10% NaCl	-	+	-
8	Jho S5B8	Bacilli	Gram +ve	10% NaCl	-	+	-
9	Jho S5B9	Cocci	Gram +ve	10% NaCl	-	+	+
10	Jho S6B10	Bacilli	Gram +ve	10% NaCl	-	++	+
11	Jho S6B11	Short rod	Gram +ve	10% NaCl	++	-	-
12	Jho W16B12	Bacilli	Gram -ve	10% NaCl	+	+	-
13	Jho S5B13	Bacilli	Gram +ve	10% NaCl	-	+	+
14	Jho S8B14	Bacilli	Gram +ve	10% NaCl	+	+	++
15	Jho S10B15	Cocci	Gram +ve	10% NaCl	++	+	+
16	Jho S8B16	Short rod	Gram +ve	10% NaCl	++	++	+
17	Jho S9B17	Cocci	Gram -ve	10% NaCl	-	+	+
18	Jho S9B18	Cocci	Gram +ve	10% NaCl	-	-	-
19	Jho S10B19	Short rod	Gram -ve	10% NaCl	+	+	+
20	Jho S10B20	Cocci	Gram +ve	10% NaCl	+	+	-
21	Bor R1B1	Short rod	Gram -ve	10% NaCl	-	-	-
22	Bor R1B2	Cocci	Gram -ve	10% NaCl	+	-	-
23	Bor S1B3	Bacilli	Gram -ve	10% NaCl	++	+	-
24	Bor S1B4	Cocci	Gram +ve	10% NaCl	+	-	-
25	Bor S2B5	Short rod	Gram -ve	10% NaCl	-	+	-

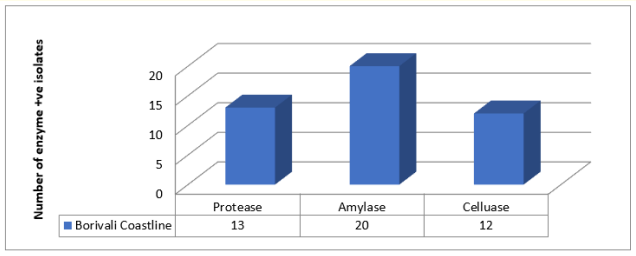
26	Bor S8B6	Short rod	Gram -ve	10% NaCl	+	-	-
27	Bor S9B7	Bacilli	Gram +ve	10% NaCl	+	+++	-
28	Bor R3B8	Bacilli	Gram -ve	10% NaCl	+	-	-
29	Bor S7B9	Bacilli	Gram -ve	10% NaCl	-	-	-
30	Bor S9B10	Short rod	Gram -ve	10% NaCl	-	-	-
31	Bor S16B11	Cocci	Gram -ve	10% NaCl	-	+++	-
32	Bor S16B12	Bacilli	Gram +ve	10% NaCl	+	++	-
33	Bor S17B13	Bacilli	Gram +ve	10% NaCl	+++	+	+
34	Bor S1B14	Bacilli	Gram +ve	10% NaCl	+	++	-
35	Bor R3B15	Bacilli	Gram +ve	10% NaCl	+++	-	+
36	Bor R8B16	Cocci	Gram -ve	10% NaCl	-	-	-
37	Bor R14B17	Bacilli	Gram +ve	10% NaCl	+	++	-
38	Bor W3B18	Cocci	Gram +ve	10% NaCl	-	+	-
39	Bor W3B19	Bacilli	Gram +ve	10% NaCl	-	-	+
40	Bor S14B20	Cocci	Gram -ve	10% NaCl	+	+	-
41	Bor S13B21	Short rod	Gram -ve	10% NaCl	-	-	+
42	Bor W4B22	Short rod	Gram +ve	10% NaCl	-	-	+
43	Bor S12B23	Short rod	Gram -ve	10% NaCl	-	-	+
44	Bor S13B24	Cocci	Gram +ve	10% NaCl	-	+	-
45	Bor S12B25	Cocci	Gram +ve	10% NaCl	-	-	+
46	Bor S17B26	Cocci	Gram +ve	10% NaCl	-	++	++
47	Bor S12B27	Short rod	Gram +ve	10% NaCl	+	+	+
48	Bor W4B28	Short rod	Gram -ve	10% NaCl	-	-	+
49	Bor W2B29	Cocci	Gram +ve	10% NaCl	-	+	-
50	Bor S10B30	Short rod	Gram -ve	10% NaCl	-	+	-
51	Bor S10B31	Cocci	Gram +ve	10% NaCl	-	-	-
52	Bor W1B32	Short rod	Gram +ve	10% NaCl	-	-	+
53	Bor S8B33	Cocci	Gram -ve	10% NaCl	-	+	-
54	Bor S5B34	Cocci	Gram -ve	10% NaCl	-	+	-
55	Bor S4B35	Cocci	Gram +ve	10% NaCl	-	++	-
56	Bor S5B36	Bacilli	Gram +ve	10% NaCl	-	-	-
57	Bor W8B37	Cocci	Gram +ve	10% NaCl	-	-	-
58	Bor R14B38	Cocci	Gram -ve	20% NaCl	-	-	-
59	Bor R14B39	Short rod	Gram -ve	20% NaCl	-	+	-
60	Bor S15B40	Bacilli	Gram +ve	20% NaCl	-	-	-
61	Bor S18B41	Cocci	Gram +ve	20% NaCl	-	+	-
62	Bor S18B42	Bacilli	Gram +ve	20% NaCl	-	-	+

Table 1: Indicates primary screening of Amylase, Protease, Cellulase.

Observation - = No Growth, + = Low Growth, ++= Moderate/Medium Growth, +++ = Optimum/Best Growth.



Graph 3: Total enzyme +ve isolates from Jhow Island.



Graph 4: Total enzyme +ve isolates from Borivali Coastline.

Conclusion

Result obtained from above experiment primary screening of enzymes according to Table-1, 62 Isolates obtained from both the sites from which total 20 Isolates were from Jhow Island and 42 Isolates were from Borivali. Graph 1 and 2 indicate percentage of Coccus, Short rod and Bacillus from obtained samples, were coccus is about 40% of total isolates in both sites of isolation, while Graph 3 and Graph 4 represent number of enzyme producer. Few isolates produces all three type of enzyme and have Bio-Industry based potential future. These data shows large number of producers of bio-industrial important enzymes obtained from mangrove rich eco-system. Isolates obtained may have halophilic and alkaline properties as they were screened at high salt and alkaline pH concentration.

Bibliography

1. I Dewiyanti., *et al.* "Cellulase enzyme activity of the bacteria isolated from mangrove ecosystem in Aceh Besar and Banda Aceh". *IOP Conference Series: Earth and Environmental Science* 951.1 (2022): 012113.

2. B O De Veras., *et al.* "Screening of protease, cellulase, amylase and xylanase from the salt-tolerant and thermostable marine *Bacillus subtilis* strain SR60". *F1000Research* 7 (2018): 1704.

3. P Sawant and J Thumar. "Amalgamation of technologies and scientrepreneur prospective of electrochemically active halophilic microbugs - a review paper". *Vidya-A Journal of Gujarat University* 16.2 (2021): 125-156.

4. JT Thumar and SP Singh. "Secretion of an alkaline protease from a salt- tolerant and alkaliphilic, Streptomyces clavuligerus strain Mit-1". *Brazilian Journal of Microbiology* 38.4 (2007): 766-772.

5. F William and K Catherine. "Microbial Enzymes and Biotechnology". *Microbial Enzymes Biotechnology BOOK* (1990).

6. R Zalak., *et al*. "Isolation and Screening of Multivariant Enzyme Producing Endophytic Bacteria from Citrus limon". *Acta Scientific Microbiology* 5 (2022): 2581-3226.

7. R C Kasana., *et al.* "A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine". *Current Microbiology* 57.5 (2008): 503-507.