A comparison of selected enzyme activities by marine fungal and mangrove actinomycete isolates

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Abstract— Marine and Mangrove ecosystems are two of the most unique ecosystems on the earth. In the current study, the production and optimization of one enzyme, Cellulose was studied from two different categories of microbes from two environments: marine fungi and mangrove actinomycetes. A total of three fungal strains were isolated from the selected marine sample (VF1, VF2, VF3) from Veli, coas and 4 strains were isolated from the Veli Mangrove samples (M1, M2, M3 and M4). For the activity of enzyme studied, the marine fungi showed the following results: neutral pH was found to be better for the production at 37°C and activity of the enzymes (VF2 and VF3) and 12th day showed the maximum production. One of the isolates (VF1) showed highest production. In case of mangrove actinomycetes, a total of Of the three strains of actinomycete isolates from mangrove ecosystem (M1, M2 and M4), showed Cellulase activity. M1 showed maximum activity at pH4, 50°C and 9th day of incubation. Strain M3 and M4 showed maximum production at 4 pH, 37°C and 3rd day of incubation.

Index Terms— Actinomycetes, Cellulase, Fungi, Marine, mangrove, optimization

1 Introduction

Marine ecosystem is important due to the fact it is an ever changing environment as far as living conditions to organisms are considered. There are frequent changes in salinity, temperature, nutrient flux etc. to which the marine organisms need to adjust to. Oceans are provided with immense of treasure to be used for research. Mangroves are also equally important as it is considered as a huge treasure-house of so many organisms which can undertake degradation of organic materials accumulated there. This ecosystem also changes the water quality frequently and the organisms need to adapt to this. One of the candidate microbe preset in mangrove ecosystems are actinomycetes. Two most important categories of microbes on earth are fungi and actinomycetes. Since these are isolated from marine and mangrove ecosystems respectively, they are expected to be highly potent as far as their activities are considered. The marine environment is found to be extremely complex and contains a broad spectrum of fungal diversity. So many of fungi [1] and fungal-derived metabolites [2], [3], [4] have been identified and isolated. The marine natural product was found to be very successful in industries as these are produced by organisms exposed to diverse harsh conditions.

Actinomycetes are a genus of actinobacteria. They are related to bacteria physiologically and fungi as well morphologically. Potent actinomycetes have been isolated by earlier researchers from various ecosystems [5]. Since mangroves are intertidal zones located in the changeover between land and sea, these environments are characterized by periodic flooding.

These make environmental factors such as salinity and nutrient availability is extremely variable, resulting in precise characteristics [6]. A similar work was done earlier in the same laboratory [5] which dealt with the screening of marine actinomycetes for the production of industrial enzymes. More than 2000 actinomycetes were isolated by earlier worker [7] from mangrove and their secondary metabolites which showed anti-infection. Actinomycete strains were isolated from mangrove sediments [8] which gave positive results for amylase (25%), Protease (21%), Lipase (16%), Esterase (17%) and Gelatinase (21%).

In the present study, marine fungal isolates and isolated mangrove actinomycete strains were screened for the production of the industrially important enzymes, cellulase and the production of enzymes were studied at different temperature, pH and incubation periods.

2 Materials and Methods

2. 1 Screening of fungal isolates for enzyme production

The isolated fungal strains were screened for the presence of an enzyme: cellulases. Two stages of enzymatic screening were done. All the isolated strains were subjected to primary screening for enzyme production while secondary screening was done for those isolates which showed enzymatic activity in primary screening.

In primary screening, all the isolated strains were inoculated on specific media by spot or streak inoculation method to screen the selected enzyme activities (cellulases). The plates were incubated at 30°C for 7 days. The media as criteria for enzyme activities are given in Table: 1.

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Table: 1 Methods for the Primary enzymatic screening of fungal isolates

Sl. No	Enzyme	Medium	Incubation (days)	Criteria for positive enzyme activity
2	Cellulase	Carboxy-methyl cellulose media	4	Clear yellow zones after flooding with iodine

To those fungal and actinomycete isolates which showed a positive activity for the said enzyme, a further study for quantification of enzymatic activity at various temperature, pH and incubation times was done by using shake flask method.

2.2 Enzyme assay

L-Asparaginase assay

Cellulase assay

Fifty mL of Carboxy Methyl Cellulose (CMC) broth was taken for each positive strain and inoculated with the strain. The strains were incubated for 48- 72hrs and then centrifuged in refrigerated centrifuge for 10min at 2,000 rpm. The cell free supernatant was collected and enzymatic assay was performed. 0.1mL of crude enzyme extract was added to 1 mL of carboxy methyl cellulose substrate and made up to 3mL with distilled water. The mixture was incubated at 60˚C for 20min and then reaction was stopped by addition of DNS reagent. The absorbance was measured at 540nm4. 1 unit (IU) is defined as the amount of enzyme that released 1µmole of glucose from carboxy methyl cellulose per minute at pH 7.0 at 60˚C [9].

2.3 Optimization of enzyme production

In order to find out the effect of incubation period on enzyme production each of the selected enzyme activity was checked for 24, 48, 72 and 96hrs of incubation. The effect of various parameters like pH (4, 7 and 9) and temperature (20°C, 37°C and 50°C) in the activity of the enzymes were also determined.

3. Results and Discussion

3. 1. Primary Enzymatic Screening

In primary screening the marine fungal strains and mangrove actinomycete isolates were inoculated on specific media by spot inoculation method and the results are shown in the Figure 1 and 2. All the marine fungal isolated strains showed positive results for the selected enzyme activity. The strains which showed positive enzyme production were selected for further enzymatic assay. In the case of mangrove actinomycetes, three isolates, M1, M2 and M4 showed positive cellulase activity.

3. 2. Enzyme Assay

Effect of pH and temperature

The effect of pH on the cellulase enzyme activity of marine fungal isolates was studied and the results are shown in Table: 2. Strain VF3 showed highest production at neutral pH (9.53 U/mL). The result revealed that all the isolated strains showed similar range of enzyme activity both at pH 7 and pH 9. Strain VF2 showed least enzyme activity at pH 4 (6.53U/mL). The effect of temperature on cellulase enzyme production was studied and is also shown in Table: 2. VF2 and VF3 strains showed maximum production at 37°C, (8.46 and 9.53U/mL respectively) whereas VFI showed maximum activity at 50°C (9.46 U/mL). VF1 showed least enzyme production at 20°C.

Table: 2. Effect of pH on fungal cellulase activity

Strains	Cellulase Enzyme Activity (Unit/ mL)					
	pH			Temperture°(C)		
	4	7	9	20	37	50
VF1	7.4	7.83	8.42	7	7.93	9.46
VF2	6.53	8.46	7.62	8.1	8.46	7.61
VF3	6.82	9.53	8.33	8.23	9.53	7.33

In case of mangrove actinomycetes, all strains showed almost equal range of enzyme production (Figure: 1). Strain M4 showed maximum production at pH 7 followed by strain M3 (10.60 U/mL and 8.53 U/mL respectively). Strain M1 showed maximum enzyme production at pH 4 and minimum at pH 7 (8.66 and 3.33 U/mL respectively).

Table: 3. Actinomycete cellulase activity at different pH

Sl No.	Strains	pH 4	pH 7	pH 9
1	M1	8.66	3.33	6.66
2	M3	7.46	8.53	6.80
3	M4	7.93	10.60	8.00

The values showing fungal enzyme activity indicates that these organisms can be the most potent enzyme producers as neutral pH. Hence they would be under stress at the fluctuating pH of the marine environment. Since the acidic as well as alkaline pH did not cease the production of enzyme and its activity, they can be posed as potential candidates from an industrial point of view. Compared to marine fungi, mangrove actinomycetes were better producers of enzyme and the activity are also higher at the different pH conditions analysed.

The cellulase activity of the isolated actinomycetes at different temperatures (20°C, 37°C and 50°C) is shown in Table: 5. M1 Strain showed maximum activity at 50°C and minimum activity at 20°C whereas M3 and M4 shows maximum activity at 370C (10.60 U/mL) and minimum activity at 20°C (7.66U/mL). The activities of the actinomycetes were found to be better at the different incubation temperatures as well.

Table: 4. Actinomycete cellulase activity at various temperatures

Sl. No.	Strains	20(°C)	37(°C)	50(°C)
1	M1	7.06	3.33	8.10
2	M3	7.72	8.53	7.80
3	M4	7.53	10.60	7.66

Effect of incubation time

The influence of incubation period on cellulase enzyme activity by marine fungi is shown in the Table: 5. The enzyme activity seemed to get increased from 3rd to 12th day of incubation. All the strains showed a similar trend. Maximum enzyme production was shown by strain VF2 (11.46 U/mL) VF1 and VF3 showed almost equal range of enzyme activity (10.8 and 10.33 U/mL respectively).

Table: 5. Fungal cellulase activity at different incubation periods

Strains	3rd	6th	9th	12th
VF1	7.93	7.73	8.26	10.8
VF2	8.46	7.93	6.53	11.46
VF3	9.53	8.26	8.13	10.33

The optimization of cultural parameters for cellulase enzyme production from fungi was carried out [12]. In their study they describe the optimization of process parameters for the production of cellulases by different fungal species. They found that cellulase production was maximum at a pH of 5. The optimum temperature of cellulase production was found to be 40°C and 72 hours of incubation gave the best result. In the present study, a neutral pH was fund to be better that the other pH analyzed. 37°C was found to be optimum for strains VF2 and VF3. But in the case of strain VF1, 50°C was the optimum temperature. The isolates showed better production at the 3rd hour of incubation. After that the production decreased and then increased at the 12th hour.

In the case of cellulase enzyme produced by mangrove actinomycetes, among the isolated strains M1 showed maximum activity in 9th day of incubation whereas strains M3 and M4 showed maximum activity in 3rd day of incubation. All the strains showed almost similar range of enzyme production. The results are shown in the table: 6

SL. No.	Strains	Incubation period (hours)			
		3rd	6th	9th	12th
1	M1	3.33	6.86	8.13	7.2
2	M3	8.53	6.73	8.33	6.53
3	M4	10.60	7.00	9.80	10.46

4 Conclusion

The study was conducted to screen fungal isolates from marine system and actinomycete from a mangrove system in terms of cellulase. The results of the study indicated that as far as cellulase enzyme production is concerned, mangrove actinomycetes were found to be potent over marine fungal isolates.

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