ACTIVITY SCREENING AND OPTIMIZATION OF MARINE FUNGAL STRAINS FOR PROTEASE ACTIVITY

Ayona Jayadev*

Department of Environmental Sciences, All Saints’ College, Trivandrum, Kerala, India.

ABSTRACT

Marine fungal isolates were screened for protease enzyme activity in this study. Fungal strains were isolated from marine water samples using appropriate culture medium and culture techniques. All the strains isolated were first screened for protease production in skimmed milk agar and the positive strains were later assayed for protease enzyme activity. Three fungal strains (VF 1, VF 2 and VF 3) were found to be protease producing among the fungi isolated. Media conditions were optimized for these strains. It was found out that the strain VF 2 is a potent protease producer at a pH of 7 and at 37°C. The maximum protease activity was on 3rd day of incubation. In case of the other two strains, VF 1 showed good activity on the 9th day of incubation. The activity of VF 3 was almost comparable in all the pH, temperature and incubation period studied.

KEYWORDS: Protease, Assay, Marine fungi, optimization.

INTRODUCTION

Marine fungal isolates were screened for protease enzyme activity in this study. Fungal strains were isolated from marine water samples using appropriate culture medium and culture techniques. All the strains isolated were first screened for protease production in skimmed milk agar and the positive strains were later assayed for protease enzyme activity. Three fungal strains (VF 1, VF 2 and VF 3) were found to be protease producing among the fungi isolated. Media conditions were optimized for these strains. It was found out that the strain VF 2 is a potent protease producer at a pH of 7 and at 37°C. The maximum protease activity was on 3rd day of incubation. In case of the other two strains, VF 1 showed good activity on the 9th day of incubation. The activity of VF 3 was almost comparable in all the pH, temperature and incubation period studied.
MATERIALS AND METHODS

Sample Collection
The sampling site was Veli coast, Thiruvananthapuram, Kerala. The samples were collected in sterile bottles and were brought to laboratory maintaining a cold chain and refrigerated.

Enrichment and Isolation of Fungal strains
For enrichment, 1 mL of each selected sample was transferred to 100 mL of Potato Dextrose broth and incubated at 30°C for 2 days in shaker at 200 rpm. Isolation of fungal strains was done by the serial dilution and pour plate technique. A loopful of inoculum from the Potato Dextrose broth was streaked onto the Potato Dextrose Agar (PDA) and incubated at 30°C for 2 days. Single separated colonies were selected and the subcultures were maintained on petri plate at 40°C until further use.

Screening and isolation of protease producing fungal strains
The isolated fungal strains were screened for the presence of protease enzyme. Two stages enzymatic screening was done. Primary screening was done with all the isolated fungal strains. Secondary screening was limited to only those isolates which were positive to primary screening.

Primary enzymatic screening
For primary screening, the isolated strains were inoculated on skim milk agar media by spot inoculation method to screen the selected enzyme activity. The plates were incubated at 30°C for 7 days. The media as criteria for protease activity is given in Table: 1.

Table 1: Conditions for isolation of protease producing fungal strains.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Culture Medium</th>
<th>Positive Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protease</td>
<td>Skim milk agar</td>
<td>Clearing around the growth</td>
</tr>
</tbody>
</table>

Secondary enzymatic screening
Those fungal isolates which showed a positive activity for amylase, a further study for quantification of enzymatic activity at various temperature, pH and incubation times was done by using shake flask method.

Enzyme assay
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 as per standard reference, (Mitra and Chakrabartty, 2005). One unit of protease activity is expressed as the amount of enzyme which converts 1μg of tyrosine per 1min at 37˚C, (Kathiresan and Manivannan, 2007).

**Secondary enzymatic screening**

Those fungal isolates which showed a positive activity for enzymes, a further study for quantification of enzymatic activity at various temperature, pH and incubation times was done by using shake flask method.

**RESULTS AND DISCUSSION**

**Primary Enzymatic Screening**

The results of the primary screening are shown in the Table 2. Three of the isolated strains showed positive results for the protease activity. The strains were named VF 1, VF 2 and VF 3. These strains were selected for further enzymatic assay.

**Table 2: Fungal strains showing protease activity.**

<table>
<thead>
<tr>
<th>Fungal Strains</th>
<th>Protease Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>VF1</td>
<td>+</td>
</tr>
<tr>
<td>VF2</td>
<td>+</td>
</tr>
<tr>
<td>VF3</td>
<td>+</td>
</tr>
</tbody>
</table>

**Protease Assay**

**Effect of pH on Protease activity**

The protease enzyme production by isolated fungal strains was evaluated and the results are presented here. With regard to pH, (Table: 3 and Figure: 1), strain VF1 showed maximum production at pH 4 (56.3 U/mL) whereas Strain VF2 and VF3 showed maximum production at pH 7 (51.76 and 43.2 U/mL respectively).
Table 3: Protease enzyme activity at different pH and temperature.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Protease Enzyme Activity (Unit/mL)</th>
<th>pH</th>
<th>Temperature°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>VF1</td>
<td>56.3</td>
<td>30.15</td>
<td>43.5</td>
</tr>
<tr>
<td>VF2</td>
<td>27.99</td>
<td>51.76</td>
<td>35.69</td>
</tr>
<tr>
<td>VF3</td>
<td>35.81</td>
<td>43.2</td>
<td>38.18</td>
</tr>
</tbody>
</table>

Figure 1: Protease enzyme activity at different pH.

Effect of temperature on Protease activity

The influence of temperature on protease enzyme activity was studied and results are shown in Table: 3. Both strains, VF2 and VF3 showed maximum enzyme production at 37°C (51.76 and 43.20 U/mL respectively). Strain VF1 showed equal range of enzyme production both at 37 and 50 °C (30.15 and 31.45 U/mL respectively), (Figure 2).

Figure 2: Protease enzyme activity at different temperature.

Effect of incubation time on Protease activity

The protease enzyme production at different incubation period was checked and the results are shown in Table: 4. The results revealed that VF1 and VF3 strains showed equal range of
enzyme production from 3rd to 12th day of incubation. Maximum enzyme production was shown by strain VF1 (61.7U/mL) on 6th day of incubation. VF2 showed maximum enzyme production at 3rd day of incubation there after a decline in enzyme production was noticed (Figure: 3).

Table 4: Protease enzyme activities at different incubation period.

<table>
<thead>
<tr>
<th>Strains</th>
<th>3rd day</th>
<th>6th day</th>
<th>9th day</th>
<th>12th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>VF1</td>
<td>30.15</td>
<td>41.82</td>
<td>61.7</td>
<td>43.2</td>
</tr>
<tr>
<td>VF2</td>
<td>51.76</td>
<td>28.41</td>
<td>22.7</td>
<td>12.69</td>
</tr>
<tr>
<td>VF3</td>
<td>43.20</td>
<td>42.69</td>
<td>10.36</td>
<td>44.94</td>
</tr>
</tbody>
</table>

Figure 3: Effect of incubation time on protease activity.

There are so many works in which marine fungi were found to be good protease producers. In 2009, Haddar et al., isolated and purified two detergent stable alkaline protease from a fungal strain. In the current study, the fungal isolates were subjected to optimization in terms of pH, temperature and period of incubation based on the reports of earlier workers that the production of enzymes by fungi depends on their physiology as well on the composition of culture medium, (Baldrian and Gabriel, 2003). In their study, they found that the optimum temperature for protease activity ranged from 37 to 70°C and the pH from 3 to 9. The result of the current study also corresponds to their findings. Saeki et al., (2007) isolated a fungal strain which produced protease at very high alkaline condition. But in our study, one of the isolate (VF 1) showed maximum production at an acidic pH of 4. At alkaline pH such a hike was not observed in any of the isolates.

CONCLUSION

From the result of this study, it can be concluded that the fungal isolates from marine ecosystem can be potent protease producers and they can be posed as potent candidates for
isolation of protease enzymes for industrial applications. Also the production can be maximized by optimizing the culture conditions.

REFERENCES
