Xylanolytic activity of thermophilic *Sporotrichum* sp. and *Myceliophthora thermophilum*

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Abstract. Extracellular xylanase of 5 isolates of thermophilic *Sporotrichum* sp. and *Myceliophthora thermophilum* was studied in media containing wheat straw, wheat bran, paddy straw, paddy husk, sugar cane bagasse and local grass (*Cyanodon dactylon* L). Maximum D-xylanase (0.92 IU/ml) was secreted on 6th day by *Sporotrichum* sp. strain 1 in a basal medium that contained 5% (w/v) wheat straw; treatment of straw by 4% NaOH prior to fungal fermentation did not alter xylanase yields appreciably. Temperature and pH optima for enzyme production were 45°C and 5.0 respectively. Enzyme activity showed a temperature optima of 70°C, pH maxima of 5.5 and a substrate level of 3% larch wood xylan. Xylanase of *Sporotrichum* sp. strain 1 was inducible and highly thermostable; nearly 70% of activity was retained even after 5 min exposure at 80°C. Associated cellulolytic activity (0.12 IU/ml) was detected in the crude enzyme preparation.

Keywords. Xylanase; thermophilic; lignocellulose; fungus.

1. Introduction

In the search for alternate sources of energy considerable work has now been undertaken on agricultural residues and wastes (Clarke *et al* 1969; Flannigan and Sellars 1977; Carrol and Petrini 1983). However, it is the cellulosic fraction of the plant material that has received most attention. Degradation of lignin even today presents a considerable problem but efforts are underway to overcome this. The major component of lignocellulosic waste is cellulose (40–60%), but, in addition to this, lesser but significant amounts of hemicelluloses (20–30%) and lignin (15–30%) are also present (Dekker 1983). Hemicellulose also occur as a waste in pulp and paper industries and thus this material provides a good substrate for enzymatic degradation. In fact, it has recently been suggested that enzymatically hydrolyzed wood hemicelluloses can be used for producing liquid fuel, 2,3-butanediol, ethanol and acetic acid (Saddler *et al* 1983).

Xylan is a major constituent of hemicellulosic residues and can comprise ~2–51% of the total component (Thaysen and Bunker 1927). Its degradation is brought about by xylanases which are produced among others by thermophilic moulds. The enzymatic abilities of thermophilic moulds are perhaps better by way of their high thermostability, greater production and commercial applications. Among these, species of *Acremonium, Chaetomium, Humicola, Sporotrichum, Thermoascus, Torula*...
and Talaromyces are known to possess xylanolytic activity (Flanningan and Sellars 1972; Yoshioka et al. 1981; Satyanarayana and Johri 1983). Recently, Maheshwari and Kamlam (1985) have described a thermophilic fungus, Melanocarpus albomyces which possesses high xylanolytic activity.

In view of the scanty information available on xylanase of thermophilic fungi, the aim of the present study was to (i) isolate a good xylanase—producing organism, (ii) determine a suitable substrate for xylanase production, and (iii) optimise conditions for xylanase activity.

2. Materials and methods

2.1 Organisms

Myceliophthora thermophilum van Oorschot and 5 strains of thermophilic Sporotrichum sp. were used; M. thermophilum was an isolate from paddy straw compost. The 5 strains of Sporotrichum sp. were isolated from different habitats but belong to the same species. These strains were designated as strains 1–5 which were respectively isolated from paddy straw compost, forest litter, forest soil, Chirmiri coal mine and Korba coal mine (Chouhan 1985). They were maintained on YpSs medium: yeast extract, 4.0 g; soluble starch, 15.0 g; KH$_2$PO$_4$, 1.0 g; MgSO$_4$, 0.15 g; agar, 20.0 g; distilled water, 1000 ml; pH prior to sterilization adjusted to 7.0.

2.2 Cultural conditions

Basal medium used for growth of the organism contained (per l): KH$_2$PO$_4$, 1.0 g; MgSO$_4$, 0.5 g; Peptone, 2.0 g; yeast extract, 1.0 g; and microelement solution, 1.0 ml [FeSO$_4$ (NH$_4$)$_2$ SO$_4$, 6H$_2$O, 0.212 g; ZnSO$_4$,7H$_2$O, 0.0439 g; MgSO$_4$, 0.203 g; distilled water, 1,000 ml]. The pH before sterilisation was adjusted to 7.0. The carbon source was added to it as powdered wheat straw, paddy straw, paddy husk, sugar cane bagasse, wheat bran and local grass; particle size in all cases was approximately 0.5 mm in diameter.

For growth in liquid medium, untreated or alkali-treated hemicellulosic substrate was dispersed at the rate of 5% (w/v) in 50 ml basal medium into a 250 ml Erlenmeyer flask. After sterilisation at 121°C for 20 min, each flask was inoculated by a actively sporulating mycelial disc (10 mm in diameter) culture of the test isolate grown in YpSs. Cultures were incubated at 45°C for 6 days.

Alkali treatment was performed by soaking the powdered substrate in 4% NaOH and autoclaving it for 30 min. Treated material was subsequently washed under running tap water until it attained a normal pH and finally washed in double distilled water. The resulting material was oven dried at 90°C.

2.3 Enzyme preparation and xylanase assay

Culture broth was passed through Whatman No. 1 filter paper to remove any debris and the filtrate was used as crude enzyme preparation. For enzyme assay, D-xylan from larch-wood (Sigma) was dissolved in 0.2 M McIlvaine’s citrate phosphate buffer
(pH 6.0) to prepare a 1% (w/v) solution. The reaction mixture contained 0.5 ml D-xylan and 0.5 ml crude enzyme: this was incubated at 50°C for 30 min in a shaking water bath. The liberation of reducing end groups was estimated by dinitrosalicylic acid (Miller 1959). A standard curve was prepared using D-xylose in the concentration range 20–200 μg/ml. The level of reducing end groups released by enzyme action was computed from this standard curve. A unit of D-xylanase refers to the amount of enzyme required to liberate reducing end groups equivalent to 1 μmol of D-xylose per min per ml. Specific enzyme activity refers to xylanase units per mg of protein. Protein was estimated by Lowry’s Folin phenol method (Lowry et al 1951).

2.4 Selection of isolate and substrate

A comparison of enzyme produced by M. thermophilum and 5 isolates of Sporotrichum sp. was made in various liquid media. On the basis of enzyme produced, Sporotrichum sp. strain 1 and untreated wheat straw were selected for further experimentation.

2.5 Optimisation of conditions for D-xylanase production by Sporotrichum sp. strain 1

Culture flasks were withdrawn at intervals 3 and 9 days and D-xylanase activity was assayed. Optimum initial pH of the culture medium was determined by preparing culture broths at pH 4.0–9.0. Sporotrichum sp. strain 1 was grown in these broths under conditions as described earlier followed by D-xylanase assay. Optimization of incubation temperature was carried out using a series of flasks containing wheat straw and basal medium inoculated with Sporotrichum sp. strain 1. These were incubated at 35–50°C for 6 days when the xylanase activity was assayed. Inducible nature of D-xylanase was established through observation of enzyme production in the presence and absence of pure xylan.

2.6 Optimization of conditions for D-xylanase activity

Effect of pH, temperature and reaction time on D-xylanase activity was studied in order to determine optimum values for enzyme activity. Optimum pH was determined by carrying out the reaction at pH 4.0–9.0 under standard assay conditions. For temperature optimization enzyme activity was assayed over the range 30–95°C. The reaction mixture was incubated for different periods ranging between 10–60 min in order to determine optimum period for D-xylanase reaction.

For characterization of thermal stability crude D-xylanase preparation was exposed to temperature of 40–80°C for 5–30 min before assaying the activity.

3. Results and discussion

3.1 Selection of suitable isolate and substrate

As shown in table 1, enzyme activity for all isolates of Sporotrichum sp. and M. thermophilum was greatest after growth on wheat straw (0.84–0.92 IU/ml);
Table 1. Xylanase activity* (IU/ml) of *Sporotrichum* sp. and *M. thermophilum* on different natural sources.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Grass (C. dactylon)</th>
<th>Sugarcane bagasse</th>
<th>Wheat bran</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sporotrichum</em> sp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strain 1</td>
<td>0.92</td>
<td>0.71</td>
<td>0.66</td>
</tr>
<tr>
<td>Strain 2</td>
<td>0.88</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Strain 3</td>
<td>0.88</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Strain 4</td>
<td>0.84</td>
<td>0.66</td>
<td>0.66</td>
</tr>
<tr>
<td>Strain 5</td>
<td>0.84</td>
<td>0.66</td>
<td>0.66</td>
</tr>
<tr>
<td><em>M. thermophilum</em></td>
<td></td>
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</tr>
</tbody>
</table>

*Xylanase activity estimated after 6 days at 45°C and is expressed in terms of IU/ml. One xylanase unit refers to the release of reducing end groups equivalent to 1 μmol of xylose per ml per min.

Table 2. Specific enzyme activity (SEA)* of *Sporotrichum* sp. and *M. thermophilum* in liquid medium containing different hemicellulosic substrates.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Grass (C. dactylon)</th>
<th>Sugarcane bagasse</th>
<th>Wheat bran</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sporotrichum</em> sp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strain 1</td>
<td>0.30</td>
<td>0.24</td>
<td>0.31</td>
</tr>
<tr>
<td>Strain 2</td>
<td>0.33</td>
<td>0.24</td>
<td>0.26</td>
</tr>
<tr>
<td>Strain 3</td>
<td>0.31</td>
<td>0.24</td>
<td>0.22</td>
</tr>
<tr>
<td>Strain 4</td>
<td>0.30</td>
<td>0.24</td>
<td>0.43</td>
</tr>
<tr>
<td>Strain 5</td>
<td>0.30</td>
<td>0.22</td>
<td>0.38</td>
</tr>
<tr>
<td><em>M. thermophilum</em></td>
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</tbody>
</table>

*SEA refers to enzyme units/mg of protein
minimum activity was noted in a wheat bran containing medium (0.03-0.09 IU/ml). Among various isolates, maximum xylanase activity (0.93 IU/ml) was shown by Sporotrichum sp. strain 1 on wheat straw. The variation in xylanase production on various natural carbon sources could be due to accessibility of the hemicellulose which in turn is influenced by lignin, silica and other complexes in these substrates. According to Bisaria et al (1987), the hemicellulose content of wheat straw is greater than that in rice straw and sugar cane bagasse, but the lignin content is less. Thus, a higher hemicellulose content and also possibly greater accessibility to this because of lower lignin content, might have caused greater D-xylanase induction in wheat straw. A medium containing wheat bran did not support good D-xylanase production which is in conformity with the observations made by Satyanarayana and Johri (1983) for other thermophilic strains. Yoshioka et al (1981), however, reported good D-xylanase production in a wheat bran medium which was supplemented with D-xylan that would help in the induction of this enzyme.

The specific enzyme activity (IU/mg of protein) in paddy husk was 2.5-5 X higher than paddy straw. This would indicate that much more of the protein produced in paddy husk medium represented D-xylanase. In the case of wheat bran culture filtrate, specific enzyme activity estimated was higher than on other substrates with 3 strains suggesting that xylanase formed the major fraction of total protein in that culture filtrate.

A remarkable decrease in the total enzyme production after alkali treatment of the substrate was recorded. This may be due to removal of readily solubilized (arabino) xylans, resulting in depletion of the level of inducing substrate. Singh (1982) has reported lower D-xylanase activity for Sporotrichum thermophilum on alkali-treated bagasse. Protein production in alkali-treated substrate decreased to a considerable extent. This could be due to removal of some other components which might have promoted enzyme synthesis in untreated substrate.

Based on the above observations, Sporotrichum sp. strain 1 and wheat straw were selected for further investigation.

3.2 Production studies

Figure 1 represents effect of the period of incubation on enzyme production. Maximum protein (mg/ml) was produced after incubation for 9 days, while the maximum D-xylanase activity was observed after 6 days. The pH of the culture filtrate increased to 7.8 from initial 7.0, but after 5 days it decreased slightly and reached 7.5 after 9 days.

Figure 2 indicates that D-xylanase production initially increased with the pH of the culture medium up to pH 7.0 (0.84 IU/ml); and thereafter decreased markedly. However, extracellular protein production increased with increasing pH over the entire pH range, 4.0-9.0. The final pH of the culture filtrate was towards the alkaline range (7.15-8.0) irrespective of the initial pH.

As is evident from figure 3, the production of D-xylanase improved with rising temperature from 35-45°C and attained a peak value (1.0 IU/ml) at 45°C, which was also the optimum for the growth of the isolates. Although total extracellular protein production increased between 35 and 40°C, it remained constant from 40-45°C and increased between 45 and 50°C. The final pH of the culture filtrate at all temperatures was 7.5.
Figure 1. Effect of period of incubation on production of D-xylanase of *Sporotrichum* sp. strain 1.

Figure 2. Effect of initial pH of basal medium on production of D-xylanase by *Sporotrichum* sp. strain 1.

Figure 3. Effect of temperature of incubation on production of D-xylanase by *Sporotrichum* sp. strain 1.
3.3 Optimization of conditions for D-xylanase activity

With increased temperature, D-xylanase activity increased (figure 4) and attained a maximum at 70°C (0.75 IU/ml). At 80°C it was 92% of this value, 45% at 90°C and there was no detectable activity at 95°C, in line with the findings of Yoshioka et al (1981).

The optimum pH of the reaction mixture for D-xylanase activity was 5.5 (figure 5), falling within the range reported in the literature for most of the D-xylanase (Dekker and Richard 1976). D-xylanase activity of Sporotrichum sp. strain 1 improved with increased substrate concentration and reached a maximum at 3% level.

3.4 Thermal stability of D-xylanase

There was no deleterious effect of temperature on D-xylanase activity up to 40°C, and when exposed to 50°C, the enzyme activity increased after 15 min, from 0.86 to 0.96 IU/ml (figure 6). This may possibly be due to some conformational change at the active site of the enzyme leading to enhancement of enzyme action (hysteresis). Thermal denaturation of D-xylanase appeared to start after exposure to a temperature of 60°C; 10 min exposure at this temperature brought activity down to ~90% of the control, exposure for 30 min decreased activity to 84% of the control. With further increases in temperature, the extent of thermal denaturation increased, but D-xylanase of Sporotrichum sp. strain 1 appeared highly thermostable as it retained about 42% activity even after 30 min exposure of crude enzyme to 70°C. So much so that a 5 min exposure at 80°C decreased enzyme activity by only 30%; 30 min exposure at this temperature resulted in complete denaturation of protein and the resultant activity at this stage was only about 5% of control.

Figures 4 and 5. Effect of temperature (4) and pH (5) of reaction mixture on activity of D-xylanase from Sporotrichum sp. strain 1.
Figure 6. Effect of previous exposure to heat for varying periods on subsequent activity of D-xylanase from Sporotrichum sp. strain 1.

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