World Journal of Microbiology & Biotechnology 11, 347–348

Short Communication: Production of cellulases by thermophilic fungi grown on *Leptochloa fusca* straw

F. Latif,* M.I. Rajoka and K.A. Malik

Seven indigenous thermophilic fungi were screened for cellulase and xylanase production when grown on *Leptochloa fusca* (kallar grass) straw. *Aspergillus fumigatus* produced the highest activities of 0.4, 2.5, 3.5 and 0.14 U/ml of filter paper cellulase, CM-cellulase, xylanase and β-xylosidase, respectively. *Sporotrichum thermophile* produced 0.47 β-glucosidase/ml. *Chaetomium thermophile, Humicola grisea* and *Torula thermophila* had lower activities than the other thermophilic fungi.

Key words: Cellulase, lignocellulose, kallar grass straw, thermophilic fungi, xylanase.

Biomass (kallar grass) from saline lands, apart from other economic utilizations, appears to be a promising substrate for microbial conversion into fermentable sugars for biofuel production (Rajoka & Malik 1986; Latif *et al.* 1988). Lignocellulosic conversion to biofuel, which at present is far from economic, remains the most sought after process, given the abundance of raw material available. The search for novel, more efficient microbial strains and process development continues around the World. Use of some thermophilic cellulolytic species, with efficient, thermostable enzymes, high rates of cellulolysis, and ability to saccharify under non-aseptic conditions, might make the process more economic (Merchant *et al.* 1988). In the present study, seven thermophilic and cellulolytic fungal strains were grown on kallar grass straw as carbon source, in liquid fermentation, and screened for cellulase and xylanase production.

Materials and Methods

Thermophilic Fungi and Growth

Aspergillus fumigatus (thermotolerant), *Sporotrichum thermophile, Chaetomium thermophile, Humicola grisea, Torula thermophila, Malbranchea pulchella* and *Mucor pusillus* were grown on ball-milled cellulose/agar at 45°C using the medium of Eggins & Pugh (1962) at pH 5.0. Kallar grass was harvested at the Bio-saline Research Station (BSRS) at Lahore, dried to constant weight at 80°C, milled to 0.5-mm particle size and used in the medium at 20 g/l. KGS contained (% dry wt): lignin, 20; total polysaccharides, 56 (cellulose, 33; hemicellulose, 20); soluble sugars, 10; and ash, 11 (Latif *et al.* 1988).

The authors are with the National Institute for Biotechnology & Genetic Engineering, P.O. Box 577, Faisalabad, Pakistan; fax: 92 411 651472. *Corresponding author.

Shake-flask studies were carried out using 10% (v/v) seed inoculum. Flasks were incubated at 45°C and 120 rev/min in an orbital shaker. Samples were assayed for filter paper cellulase (FPase) (Mandels *et al.* 1976), CM-cellulase, xylanase, β-glucosidase and β-xylosidase (Rajoka & Malik 1986).

Results and Discussion

The use of saline lands for growing perennial grasses, like kallar grass, even under water-logged conditions, helps in their reclamation (Malik *et al.* 1986). Among various lignocellulosic substrates, kallar grass straw (KGS) is comparable with wheat straw or rice straw and is even better than bagasse and *Sesbania aculeata* (*dhancha*) for enzyme production (unpublished work). Pure cellulose and alkali-treated KGS have shown poor cellulase activities in *S. thermophile* compared with untreated straw. In the present study, thermophilic fungi grown on untreated KGS, were found to exhibit a lag phase of 2 days before cellulase and xylanase activities increase appreciably, up to a maximum after 5 to 7 days' incubation (Table 1). *Aspergillus fumigatus* and *C. thermophile* gave similar activities of CMCase and xylanase, (the highest observed) and *A. fumigatus* also produced the highest FPase and β-xylosidase activities. *Sporotrichum thermophile* produced the highest activity of β-glucosidase after 5 days. *Humicola grisea* and *T. thermophila* showed moderate activities and *Ma. pulchella* and *Mu. pusillus* exhibited low activities. Since cellulases act synergistically, the presence of each enzyme at optimal activities would give optimum saccharification (Warzywoda *et al.* 1992). The ratio of β-glucosidase to FPase should be 1:1.5 for efficient saccharification of cellulose to fermentable sugars (Breuil *et al.* 1992). It would therefore be worthwhile to tailor enzyme

Table 1. Maximal cellulase and xylanase activities produced by thermophilic fungi.

Fungus	Enzymatic activity (U/ml)*				
-	FP-ase	CMC-ase	β-Glucosidase	Xylanase	β-Xylosidase
A. fumigatus	0.40	2.5	0.40	3.5	0.14
C. thermophile	0.32	2.5	0.20	3.5	0.07
H. grisea	0.28	1.6	0.30	3.0	0.07
S. thermophile	0.26	1.8	0.47	2.4	0.05
T. thermophila	0.28	1.4	0.22	2.5	0.07
Ma. pulchella	0.15	0.5	0.04	2.3	0.04
Mu. pusillus	0.10	0.3	0.05	1.2	0.04

* Values are means of triplicate experiments. Standard deviations were 4% to 6% of these values.

concentrations from these fungi to achieve the maximum concentrations of hexoses and pentoses as the fermentable sugars.

The seven indigenous thermophilic fungi tested produce a variety of cellulases/xylanases when grown on untreated KGS. The ability of these fungi to use raw lignocellulosic substrate demonstrates their potential for large-scale, low-cost use.

References

Breuil, C., Chan, M., Gilbert, M. & Saddler, J.N. 1992 Influence of β-glucosidase on filter paper activity and hydrolysis of lignocellulosic substrates. *Bioresource Technology* **39**, 139–142.

Eggins, H.O.W. & Pugh, G.J.F. 1962 Isolation of cellulose decomposing fungi from soil. *Nature* **193**, 94–95.

Latif, F., Malik, K.A. & Puls, J. 1988 Effect of steam and alkali pretreatment on the enzymatic hydrolysis of plants grown in saline soils. *Biomass* **17**, 105–114.

Malik, K. A., Aslam, Z. & Naqvi, S.H.M. 1986 *Kallar Grass: a Plant for Saline Lands*. Faisalabad, Pakistan: NIAB.

Mandels, M.R., Andreotti, R. & Roche, C. 1976 Enzymatic conversion of cellulosic material: technology and application. In *Biotechnology and Bioengineering Symposium No. 6*, ed Gaden Jr, E.L., Mandels, M.H., Reese, E.T. & Spano, L.A. pp. 21–33. Chichester, UK: John Wiley.

Merchant, R., Merchant, F. & Margaritis, A. 1988 Production of xylanase by thermophilic fungus *Thielavia terrestris*. *Biotechnology Letters* **10**, 513–516.

Rajoka, M.I. & Malik, K.A. 1986 Comparison of different strains of *Cellulomonas* for production of cellulolytic and xylanolytic enzymes from biomass produced on saline lands. *Biotechnology Letters* **8**, 753–756.

Warzywoda, M., Larbre, E. & Pourquie, J. 1992 Production and characterisation for cellulolytic enzymes from *Trichoderma reesei* grown on various carbon sources. *Bioresource Technology* **39**, 125–130.

(Received in revised form 23 November 1994; accepted 26 November 1994)