042

Synthesis of ligninolytic enzymes from solid and aqueous growth of white-rot fungi on wheat straw

P. Pinto[1], R.M.F. Bezerra[1], A.A. Dias[1], C.V.M. Guedes[2], L.M.M. Ferreira[2], J.W. Cone[3], M.A.M. Rodrigues[2]
[1]CETAV-UTAD,, Vila Real, Portugal, [2]CECAV-UTAD, Vila Real, Portugal, [3]Wageningen University, Wageningen, Netherlands
Email:mrodrigu@utad.pt

Introduction There is an increased demand for raw materials that can be used as substrate for bio-ethanol production. The resultant by-products will have an important role in animal nutrition as possible feeds. Most of these potential feeds are high in cell walls with high lignin contents, limiting its nutritive value. The development and use of alternative enzyme methodologies can increase the availability of structural carbohydrates. Enzymes with the potential to break down cell walls, including lignin, are available in aerobic white-rot fungi. The aim of this study was to evaluate two white-rot cultivation procedures in wheat straw in order to determine if any differences were obtained between the ligninolytic enzyme concentrations. In addition, the susceptibility of the residual carbohydrates to hydrolysis with a commercially available cellulase was also evaluated.

Materials and methods Ten fungal strains were used to produce the enzymatic extracts, three strains of *Trametes versicolor* (TV1, TV2,TV3), *Bjerkandera adusta* (BA), *Fomes fomentarium* (FF), *Ganoderma applanatum* (GA), *Irpex lacteus* (IL), *Lepista nuda* (LN), *Phanerochaete crysosporium* (PC), and an unknown basidiomycet (EUC). Fungi were isolated as described by Rodrigues *et al*. (2007). Enzyme extracts were obtained from cultures containing 15 g of wheat straw with 45 ml of H_2O and 0.3 ml of a mineral solution, for the solid medium, and 4.5 g of wheat straw and 100 ml of citrate buffer 50mM and 1 ml of a mineral solution, for the aqueous medium. All media were adjusted to pH 5.0. Wheat straws from the fungi growth were submitted to cellulolytic hydrolysis with Onozuka R-10. This hydrolysis was processed to evaluate wheat straw cellulose accessibility of Onozuka R-10 cellulases. Incubations, enzyme activities and the released reducing sugars were determined as described by Rodrigues *et al*. (2007). Data were analysed using one-way Anova.

Table 1 Ligninolytic activities (U/ml) of fungi cultivated on solid and aqueous culture media.

Enzyme activities	Manganese peroxidase		Lignin peroxidase		Laccase	
	Solid	Aqueous	Solid	Aqueous	Solid	Aqueous
BA	1,27	0,01	0,11	0,00	0,00	0,00
TV1	0,34	0,14	0,01	0,00	0,04	0,18
TV2	0,20	0,06	0,04	0,00	0,03	0,15
TV3	0,90	0,08	0,00	0,00	0,05	0,07
FF	0,19	0,00	0,00	0,00	0,02	0,01
PC	0,64	0,09	0,00	0,00	0,00	0,00
EUC	0,22	0,08	0,03	0,00	0,02	0,21
LN	0,06	0,08	0,00	0,00	0,17	0,20
GA	0,25	0,08	0,00	0,00	0,07	0,03
IL	0,87	0,03	0,01	0,00	0,00	0,00

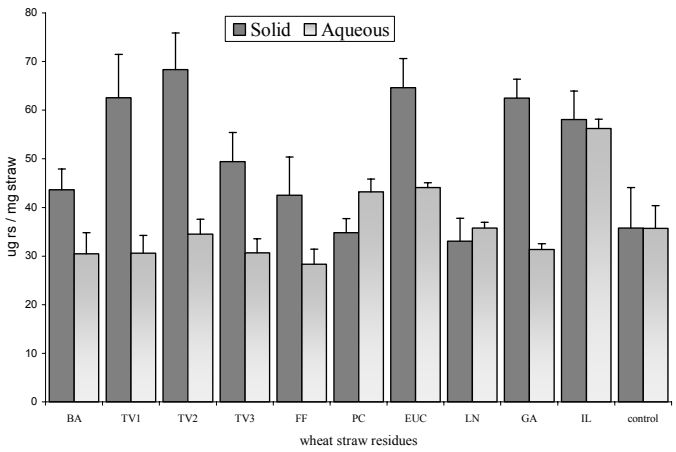

Figure 1 Reducing sugars (rs) released after incubation with a commercial cellulase.

Results The enzyme activities of fungi were quite different. All together the highest enzyme activities were detected in the solid medium, with the exception of Laccase.

In the solid growth medium (Table 1) all fungi, with the exception of LN, showed high manganese-peroxidase activity, with relevant values in BA. In the same medium an expressive activity of lignin-peroxidase was only found for BA.

For almost all fungal strains laccase activity was higher in the aqueous medium with relatively high values for TV1, TV2, EUC and LN (Table 1).

There was a higher increase ($P < 0.05$) in the amount of reducing sugars of wheat straw residues (Figure 1) isolated from the solid medium incubations.

Wheat straw residues of the solid growth trial from TV1, TV2, TV3, EUC, GA and IL released a higher amount ($P < 0.05$) of reducing sugars than the control, and IL, EUC and PC also showed higher values ($P < 0.05$) than the control, for the aqueous culture medium.

However, the data seem to indicate that other enzymes might be involved in the process of facilitating enzyme access to the cellulose molecules.

Conclusions There were considerable differences in enzyme activities in fungi, grown in a solid and an aqueous medium. These differences do not seem to have an effect on the enzymatic degradation of cellulose.

References

Rodrigues, M.A.M., Pinto, P., Bezerra, R.M.F., Dias A.A., Guedes C.V.M., Cardoso, V.M.G., Cone, J.W., Ferreira, L.M.M., Colaço, J., Sequeira, C.A. 2007. doi:10.1016/j.anifeedsci.2007.06.015.