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Production of cellulases and xylanases by white-rot fungi cultured in corn stover media for ruminant feed applications

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ABSTRACT

Growth rate and fungal aggressiveness were measured in *Phanerochaete chrysosporium*, *Sporotrichum pulverulentum*, *Bjerkandera adusta*, *Pleurotus ostreatus*, *Trametes zonatus*, and *Trametes trogii* in solid culture media with corn stover hybrid (subtropical and High Valleys germplasm). Endoglucanase, exoglucanase, β-glucosidase, and xylanase activities were assayed at 39 °C, pH 6.0 in liquid culture media with H324, AS822 and AS951, reflecting the average temperature and pH in ruminants. The enzyme activities in enzyme extracts of *B. adusta* and *T. trogii* in culture medium with neutral detergent fiber, acid detergent fiber, and acid detergent lignin fractions were also analyzed. H324, AS822, and AS951 tended to have more neutral detergent fiber than other corn stover hybrids: AS951 had more cellulose, AS822 more hemicellulose, and H324 more acidic detergent lignins. Although *P. chrysosporium* and *S. pulverulentum* had the highest growth rate in AS822, H324, and G766 culture medium, *B. adusta* and *T. trogii* were more aggressive. Growth rate was not clearly related to fungal adaptation or enzyme activity. *B. adusta* enzyme extracts showed the highest endoglucanase activity, *T. trogii* the highest xylanase activity, and *S. pulverulentum* the highest β-glucosidase activity. Increasing the lignin proportion in the culture medium (H324 and ADL) improved endoglucanase and xylanase peaks and the endoglucanase:xylanase ratio. Culture medium composition and fungus could promote useful enzyme activity proportions for corn stover fiber degradation.

1. Introduction

Fungus and culture medium composition could promote specific enzyme activity proportions in enzyme extract products for improving neutral detergent fiber degradability under certain ruminal environment conditions. Cell walls are primarily composed of cellulose and hemicellulose, whose potential energy sources are limited not only by the β-1, 4 bonds linking

Abbreviations: ADF, Acid detergent fiber; ADL, Acid detergent lignin; CSH, Corn stover hybrid; NDF, Neutral detergent fiber.

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the monomers to form polymers, but also because of the lignin that restricts the degradation of the other cellular polysaccharides. During maturation of the leaves and stems in plants, lignin strengthens cell walls by cross-linking polysaccharides, thereby providing structural resistance to enzymatic attack (via the phenylpropanoid pathway) (Grabber, 2005; Hatfield and Fukushima, 2005). This process involves the formation of strong, barely hydrolyzable cross-links between ferulate and *p*-cumarate, with arabinoxylan, and lignin (Jung and Engels, 2002; Jung and Casler, 2006a, 2006b).

White-rot fungi produce cellulases (endo-β-glucanases, *exo*-β-glucanases, cellobiohydrolases, and β-glucosidases) and xylanases (arabinofurosidases, acetyl-xylan esterases, glucuronidases, β-xylosidases, and *endo*-β-xylanases) to access and utilize the energy from cellulose and hemicellulose in lignocellulosic substrates for their adaptation, growth, and fructification (Obodai et al., 2003). The media and culture period affect the growth environment, thereby affecting the proportion and diversity of fungal enzymes (Galhaup et al., 2002; Xiao et al., 2006; Kumar and Wyman, 2009).

Fungal extracellular enzyme isoforms also differ in their domains, the temperature and pH ranges in which they are stable, the affinity of their active sites, their isoelectric points, molecular weights, spectral characteristics, and sugar content (Farrell et al., 1989; Glumoff et al., 1990; Galhaup et al., 2002; Xiao et al., 2006; Zhou et al., 2007). These factors affect their ability to remain active after being secreted outside the cell and to be used in different industrial processes (Moriya et al., 2003; Mikán and Castellanos, 2004; Cheng et al., 2005). Cellulases and xylanases have been studied in animal feedstuffs since 1980, with the objective of increasing the degradability of cell walls (neutral detergent fiber; NDF) and the digestible energy of diets (Bhat, 2000). The optimal conditions for the activity of these enzymes may also be different from that expected; for ruminant feed applications, the temperature and pH used in assays should be 39 °C and between 5.8 and 6.8, respectively, similar to the ruminal conditions (Colombatto et al., 2003a,b Colombatto and Beauchemin, 2003).

2. Materials and methods

2.1. Biological material

2.1.1. Fungal strains

Bjerkandera adusta (UAMH 8258), *Pleurotus ostreatus* (UAMH 8258), *Phanerochaete chrysosporium* (UAMH 3642), *Sporotrichum pulverulentum* (UAMH 340), *Trametes trogii* (UAMH 8156), and *Trametes zonatus* (UAMH 8158) were obtained from the Microfungus Collection and Herbarium of the University of Alberta, Edmonton, Canada. Strains were grown for 7 d in potato-dextrose agar (PDA, pH 4.5) and stored at 4 °C until required for use.

2.1.2. Corn stover hybrids

Culture media were prepared with extracts of the corn stover hybrids AS951, AS498, and AS822 (subtropical germplasm) and G766, H324, and Rio Grande (High Valleys germplasm), cultivated by the Forestry, Agricultural, and Livestock Research Mexican Institute (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, INIFAP).

2.1.3. Bromatological analysis of corn stover hybrids

After harvesting (170 d), samples of corn stover hybrids were dried in a forced air oven at 60 °C until they reached a constant weight (dry matter: DM). The corn stover hybrid samples were ground in a Thomas-Wiley Mill 4 (Thomas Scientific, Swedesboro, NJ), with a 1-mm sieve. The NDF, acidic detergent fiber (ADF), and acidic detergent lignin (ADL) fractions were analyzed according to Van Soest et al. (1991), expressed with the inclusion of the residual ash. NDF and ADF methods were adapted to use the reagents and F57 filter bags of the Ankom (Ankom and Technol., 2016), and the Ankom^{200} Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY, USA). Hemicellulose (Hem) and cellulose (Cel) percentages were calculated by subtracting the ADF from the NDF, and ADL from ADF percentages. Total ashes (Ashes) were obtained according AOAC method (942.05), samples were complete ignited in a muffle (FELISA, Fabricantes Feligneo S.A. de C.V., Jalisco, México) on calcining of the samples by treatment at 520 °C for 4 h.

2.2. Fungal radial growth rate and adaptability

Radial growth rate, fully invaded petri dishes, and contamination by bacterial growth were measured for *B. adusta, P. ostreatus, P. chrysosporium, S. pulverulentum, T. trogii,* and *T. zonatus* cultures. Corn stover hybrids were used as the carbon source in solid culture media (2% wt/vol) dissolved in potassium phosphate buffer (pH 6.0, 60 mM). The media also contained bacteriological agar (15 g/L), 125 mL/L of mineral solution (16 g/L KH_2PO_4, 4 g/L Na_2SO_4, 8 g/L KCl, 2 g/L $MgSO_4.7H_2O$, 1 g/L $CaCl_2.2H_2O$), 8 mL/L of trace mineral solution (30 mg/L H_3BO_3, 70 mg $MnCl_2$, 200 mg/L $ZnCl_2$, 20 mg/L $Na_2MoO_4.2H_2O$, 50 mg/L $FeCl_3$, 200 mg/L $CuSO_4.5H_2O$), and Na_2NO_3 (0.15% wt/vol of N). After adjusting the pH to 6.0, media were sterilized by autoclaving at 1.5 atm (121 °C) for 15 min. After the petri dishes were inoculated, they were incubated under constant conditions of temperature (28 °C) and darkness. The diameter of growth was measured every 24 h until the fungi completely filled the petri dishes (area = 50.266 cm^2), and the invaded area was calculated ($A = \pi \times r^2$). When fungal invasion formed an oval shape, the area was calculated measuring the larger and the smaller growth radius, using the formula $A = \pi \times r1 \times r2$. Growth rate was adjusted to the linear trend, and R^2 and slope data were analyzed.