



# Functional characterization and synergic action of fungal xylanase and arabinofuranosidase for production of xylooligosaccharides

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## HIGHLIGHTS

- ▶ Hypersecretion of xylanase and arabinofuranosidase by fungal expression system.
- ▶ Biochemical and biophysical characterization of both enzymes.
- ▶ Depiction of the mode of operation of both enzymes.
- ▶ Synergistic breakdown of wheat xylan and sugar cane bagasse.
- ▶ Enzymatic production of xylooligosaccharides from hemicellulosic feedstock.

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## ABSTRACT

Plant cell wall degrading enzymes are key technological components in biomass bioconversion platforms for lignocellulosic materials transformation. Cost effective production of enzymes and identification of efficient degradation routes are two economic bottlenecks that currently limit the use of renewable feedstocks through an environmental friendly pathway. The present study describes the hypersecretion of an endo-xylanase (GH11) and an arabinofuranosidase (GH54) by a fungal expression system with potential biotechnological application, along with comprehensive characterization of both enzymes, including spectrometric analysis of thermal denaturation, biochemical characterization and mode of action description. The synergistic effect of these enzymes on natural substrates such as sugarcane bagasse, demonstrated the biotechnological potential of using GH11 and GH54 for production of probiotic xylooligosaccharides from plant biomass. Our findings shed light on enzymatic mechanisms for xylooligosaccharide production, as well as provide basis for further studies for the development of novel enzymatic routes for use in biomass-to-bioethanol applications.

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## 1. Introduction

Lignocellulosic biomass has great potential as a renewable energy source and is considered the most promising feedstock for producing biofuels due to its versatility, availability and low cost. Biomass hydrolytic enzymes are key technological components for the efficient use of renewable feedstocks through an environmentally friendly bioconversion routes. Bottlenecks in this process include the cost effective production of enzymes and efficient deg-

radation of recalcitrant plant cell wall polymers into fermentable sugars (Pauly and Keegstra, 2008). Therefore, development of new routes for plant feedstock bioconversion into simple sugars, along with strategies for production of enzymes at high yield, are main foci in the biofuel research field.

Xylans can be broadly classified as homoxylans, arabinoxylans, glucuronoxylans, and arabinoglucuronoxylans (Polizeli et al., 2005). Arabinoxylans are the main hemicellulose forms in plant cell walls, especially in cereal grains such as wheat, and consist of a xylose backbone with arabinose residues linked to its O-2 or O-3 (Polizeli et al., 2005). One of the critically important enzymatic activities required to break down the xylan backbone are provided by endo-1,4- $\beta$ -xylanases. These enzymes cleave the  $\beta$ -1,4 glycosidic linkage between xylose residues in the backbone. Xylanases have been classified into glycoside hydrolases (GH) families 5, 7, 8, 10, 11, and 43 on the basis of their amino acid sequences,

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