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Fusarium verticillioides secretome as a source of auxiliary enzymes to enhance saccharification of wheat straw

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ABSTRACT

Fusarium verticillioides secretes enzymes (secretome), some of which might be potentially useful for saccharification of lignocellulosic biomass since supplementation of commercial cellulases from *Trichoderma reesei* with the *F. verticillioides* secretome improved the enzymatic release of glucose, xylose and arabinose from wheat straw by 24%, 88% and 68%, respectively. Determination of enzymatic activities revealed a broad range of hemicellulases and pectinases poorly represented in commercial cocktails. Proteomics approaches identified 57 proteins potentially involved in lignocellulose breakdown among a total of 166 secreted proteins. This analysis highlighted the presence of carbohydrate-active enzymes (CAZymes) targeting pectin (from glycoside hydrolase families GH5, GH27, GH28, GH43, GH51, GH54, GH62, GH88 and GH93, polysaccharide lyase family PL4 and carbohydrate esterase family CE8) and hemicelluloses (from glycoside hydrolase families GH3, GH10, GH11, GH30, GH39, GH43 and GH67). These data provide a first step towards the identification of candidates to supplement *T. reesei* enzyme preparations for lignocellulose hydrolysis.

1. Introduction

Second generation bioethanol produced from various lignocellulosic materials, such as wood, agricultural or forestry residues, is one of the most promising renewable energies. Wheat straw is a feedstock of particular interest since it represents a potentially large and readily available agricultural residue resource of low value. Current costs of enzymatic saccharification are among the largest contributors to the overall cost of lignocellulosic ethanol production. A major challenge is the development of an efficient and economically viable hydrolysis process step (Margeot et al., 2009).

The filamentous fungus *Trichoderma reesei* remains one of the most effective producers of cellulases. For the conversion of cellulose into glucose, three types of cellulolytic enzymes are secreted by *T. reesei*: endoglucanases (EG, EC 3.2.1.4), cellobiohydrolases (CBH, EC 3.2.1.91) and β-glucosidases (BGL, EC 3.2.1.21). Compared to other filamentous fungi, this model fungus was shown to be lacking many hemicellulase and pectinase families (Martinez et al., 2008). In order to overcome the recalcitrant structure of lignocellulose and release the locked polysaccharides, enzymes altering the interaction between lignin, hemicellulose and cellulose or those involved in degradation of hemicellulose and lignin are required. Previous studies demonstrated that supplementation of cellulases with various hemicellulases or other polysaccharide- and lignin-modifying enzymes can have a beneficial effect on enzymatic hydrolysis, presumably by improving cellulose accessibility (Couturier et al., 2011; Gao et al., 2011).

Many filamentous fungi gain nutrition from the breakdown and the decay of plant biomass and therefore are particularly interesting producers of cell wall-degrading enzymes (CWDEs) including cellulases, hemicellulases, ligninases and pectinases (Van den Brink and de Vries, 2011). Exploration of fungal biodiversity for additional auxiliary enzymes that could be added to *T. reesei* cellulases preparations to improve biomass degradation is of great interest. With the increasing availability of fungal genome sequences and the development of proteomic methods, it has become possible to identify the secreted enzyme pools involved in lignocellulosic biomass degradation. Several descriptive and/or differential analyses of secreted proteins by fungi grown on lignocellulose and related carbon sources have been reported (for a review see Bouws et al. (2008)). However, only a few studies have highlighted the relationships between distribution and prevalence

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