Short Communication

Product patterns of a feruloyl esterase from *Aspergillus nidulans* on large feruloyl-arabino-xylo-oligosaccharides from wheat bran

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**Article info**

**Abstract**

A purified feruloyl esterase (EC 3.1.1.73) from *Aspergillus nidulans* produced in *Pichia pastoris* was used to study the de-esterification of large feruloyl oligosaccharides consisting of 4 to 20 pentose residues and (xylose plus arabinose) and one ferulic acid residue. The feruloyl oligosaccharides were prepared from total oligosaccharidic hydrolysates from wheat bran treated with a purified endoxylanase from *Thermobacillus xylolyticus*. The feruloyl esterase showed similar specific activity but an affinity about 3.5-fold higher towards feruloyl oligosaccharides than towards methyl ferulate. Mass spectrometry analysis of the products after long-term enzymatic hydrolyses showed that the esterase was able to hydrolyze the largest feruloyl oligosaccharides and therefore could act alone on feruloylated xylans. Consequently, the feruloyl esterase from *A. nidulans* could be useful for the enzymatic deconstruction of xylans in plant cell walls.

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1. Introduction

Wheat straw and bran, corn stover and cobs are abundant renewable resources for lignocellulosic bioconversion. Major hemicelluloses are xylans, β-1-4 polymers of β-xylene residues which can be substituted at O-3 or O-2 by β-L-arabinose or at O-2 by α-D-glucuronic acid or its 4-O-methyl ether (Biely, 1985). By ester bonds, cinnamic and acetic acids can link the arabinose and xylose residues, respectively. Enzymatic depolymerization of these substrates involves endo- and exo-enzymes acting on xylans (Biely, 1985). The use of debranching enzymes including β-D-arabinofuranosidases (EC 3.2.2.55.), β-β xylidosidases (EC 3.2.1.37), α-D-glucuronidases (EC 3.2.1.139), feruloyl esterases (FAE, EC. 3.1.1.73) and acetyl-esterases (EC 3.1.1.6) together with endoxylanases (EC 3.2.1.8) can significantly improve the enzymatic hydrolysis of cell walls into pentoses from ammonium-fiber expansion pretreated corn stover (Banerjee et al., 2010). The synergic action of FAE-III from *Aspergillus niger* with a xylanase from *Trichoderma viride* leads to the removal of 95% of the ferulic acid (FA) from wheat bran (Faulds and Williamson, 1995). FAEs could play significant roles for the deconstruction of gramineaceous biomass but alone usually produce only small amounts of free FA from natural substrates (Shin et al., 2006). Studies on FAEs have generally used synthetic substrates, such methyl cinnamates, methyl sinapate, methyl caffeate or feruloyl oligosaccharides (Rale et al., 1994; Crepin et al., 2004). In the present study, the performance of a purified feruloyl esterase from *Aspergillus nidulans*. (AnidFAE) on large feruloyl-arabino-xylooligosaccharides isolated from enzymatic hydrolysis of wheat bran (degree of polymerisation, DP from 4 to 20 pentoses) was investigated. This enzyme was chosen since *A. nidulans* is an ubiquitous fungus often found on diverse plant materials and is therefore a good model for cell wall degrading enzyme studies and also because the enzyme was found to be active on wheat arabinoxylans (Bauer et al., 2006).

2. Methods

2.1. Production of *A. nidulans* feruloyl esterase

FAE from *A. nidulans* (AN5267.2) was produced in the yeast *Pichia pastoris* via a methanol inducible expression system (Bauer et al., 2006). The strain was obtained from the Fungal Genetics Stock Center (University Missouri, Kansas City). A culture of 150 ml was grown in Buffered Minimum Methanol medium (BMGY, Invitrogen) at 30 °C at 220 rpm. After 36 h, the cells were harvested by centrifugation (5000 g, 15 min) and resuspended in the same volume of BMGY (Buffered Minimum Methanol medium). After 72 h at 30 °C at 220 rpm in baffled flasks, the culture was centrifuged and the supernatant recovered. Proteins from the supernatant were precipitated with 90% ammonium sulfate at 4 °C. After centrifugation (10,000g, 20 min, 4 °C), the pellet was resuspended in 2 ml of Tris buffer pH8.2, 300 mM NaCl, 50 mM imidazole and dialysed for 12 h against 1 L of the same buffer. The feruloyl esterase was purified to homogeneity by metal chromatography (HiTrap Chelating HP columns, GE Healthcare) according to the manufacturer’s protocol. Analysis by SDS–PAGE of the purified protein

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