Development of fungal cellulolytic enzyme system suitable for hydrolysis of agricultural residue

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ABSTRACT:

Lignocellulosic biomass, in particular agricultural residues such as rice straw, sugarcane bagasse and corn stover,isa potential sustainable feedstock for expanding ethanol and commodity chemicals production. An enzymatic hydrolysis of the cellulose and hemicellulose components to fermentable sugars is a key step in the bioconversion of biomass,however, it has been pointed out that the enzyme cost in the commercialization of ethanol production from biomass is still high. The composition and structure of biomass substrate varies widely depending on the feedstock and a pretreatment process that increases the enzymatic hydrolysis yield. This diversity complicates the development of a cellulolytic enzyme preparation suitable for the hydrolysis of target biomass substrate.

We have developed a cellulase-producing fungus *Talaromyces cellulolyticus*by using two strategies to reduce enzyme cost: one is on-site enzyme production using pretreated biomass; and another way is an improvement of cellulase performance to reduce enzyme loading. To establish on-site production, the cellulase productivity of *T. cellulolyticus* was improved in UV and chemical mutagenesis. The obtained mutant strain, CF-2612,secreted a large amount of cellulase using a pretreatedrice straw as a carbon source. We further isolated and identified a hemicellulase-producing fungal strain, *Trichoderma asperellum* KIF125, to combine with CF-2612 in on-site production. KIF125 hemicellulase prepared using the rice straw showed high abundance of β-xylosidase, and was effective for the improvement of xylose yield in the hydrolysis of the rice straw by CF-2612 cellulase. In the meantime, to understand the cellulolytic enzyme system of *T. cellulolyticus*, six core enzymes from CF-2612 were purified and identified as β-glucosidase (Bgl3A), two endoglucanases (Cel5A and Cel7B), cellobiohydrolase II (Cel6A), cellobiohydrolase I (Cel7A), and xylanase (Xyl10A). The effect of supplementation of purified enzyme with *T. cellulolyticus*cellulaseand the hydrolysis of target biomass usinga core enzyme mixture revealed the deficient core enzyme in *T. cellulolyticus* cellulase. Based on these results, a recombinant strain producing cellulase mixturesuitable for the hydrolysis of pretreated corn stover was successfully constructed using CF-2612.

KEYWORDS:

Cellulase; Hemicellulase;Lignocellulosic biomass;On-site enzyme production; *Talaromyces cellulolyticus*;*Trichoderma asperellum*.