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ENHANCEMENT OF Trichoderma harzianum CFAM-422 FOR CELLULASE AND HEMICELLULASE PRODUCTION BY DELETION OF THE CARBON CATABOLITE REPRESSOR GENE cre1

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ABSTRACT

Carbon catabolite repression (CCR) is a mechanism by which microorganisms can utilize preferably highly energetic compounds over those of difficult degradation. For Trichoderma reesei, the protein that acts as repressor in the presence of glucose is CRE1. In this project, we aim to delete cre1 gene in Trichoderma harzianum CFAM-422 and obtain mutants with enhanced production of biomass degrading enzymes. Disruption of cre1 in T. harzianum CFAM-422 was performed by gene replacement of cre1 for hph (hygromycin B phosphotransferase) via homologous recombination. Hygromycin resistant mutants and parental strains enzyme production was evaluated in both inductive and repressive conditions in four different carbon sources. Enzymatic indexes (EI) were determined and compared. All genetically stable transformants showed increased enzymatic index under inductive conditions and modest inhibition under repressive conditions for most carbon sources, indicating that the deletion of cre1 in T. harzianum can be beneficial to cellulase and hemicellulase production with reduced product inhibition.

1. INTRODUCTION

Cellulases and hemicellulases are groups of enzymes that act in synergism to degrade different portions of the plant cell wall and, therefore, are key components for biomass hydrolysis into fermentable sugars. This process, however, requires substantial quantities of enzymes due to their low turnover and high sensitivity to product inhibition, making the process less efficient and more expensive (Tomme et al., 1995).

One mechanism that contributes to cellulose product inhibition is the carbon catabolite repression (CCR), which allows microorganisms adapting to environments with variable nutritional conditions by suppressing the expression of enzymes required to utilize hardly degradable compounds when a readily useable carbon source is present (Gancedo, 1961). In this context, Strauss et al. (1995) investigated CCR in *Trichoderma reesei*, the current reference for cellulase production, and identified the repressor protein Cre1, an orthologue of *Aspergillus nidulans* CreA. A few years later, Ilmén (1997) demonstrated that the Cre1 regulates the expression of several different genes encoding hydrolytic enzymes, including four cellulase and six hemicellulase genes.

In this project, we aim to improve lignocellulolytic enzyme production of *Trichoderma harzianum* CFAM-422, a promising cellulolytic strain isolated from Amazonian soil (Souza et al., 2011), by *cre1* gene replacement via homologous recombination.

2. MATERIALS AND METHODS

2.1. Genetic transformation of *T. harzianum* CFAM-422

Cassettes consisting on hph gene (hygromycin B phosphotransferase gene from Escherichia coli, which confers resistance to hygromycin B) and 500 bp flanking regions of the cre1 gene were obtained by double joint PCR and inserted in CFAM-422 cells via protoplast transformation at 25% polyethylene glycol (PEG) treatmeant. The putative transformants obtained were cultivated in Potato Dextrose Agar (PDA) medium with 200 µg/mL hygromycin B.

2.2. Genetic stability test

Transformants were cultivated in a non-restrictive solid medium (PDA without hygromycin B) for three or four days and transferred to a new unrestrictive medium for ten consecutive times. The 11th generation was then cultivated in PDA with 200 µg/mL hygromycin B and the surviving strains were considered genetically stable.

2.3. Determination of enzymatic indexes

2.3.1 Inductive conditions

Mutants and parental strains were cultivated in minimal solid medium containing four different carbon sources (carboxymethylcellulose - cmc, pectin, xylan, or starch) in order to evaluate endoglucanase, polygalacturonase, pectin lyase, xylanase, and amylase production. After incubation, culture media were stained with Congo red (for cmc containing medium) or iodine solution (for the remaining carbon sources), revealing the degradation halo. Colony and halo diameter were measured with a digital caliper rule and enzymatic indexes (EI) were determined (Enzymatic Index = degradation halo diameter / colony diameter).

2.3.2 Repressive conditions

Enzymatic indexes determination under repressive conditions, i.e., in the presence of glucose, were performed at the sugar concentration that completely inhibits CFAM-422 degradation halo formation, which was determined after its cultivation in all four carbon sources and in gradual glucose concentrations (0.05 %, 0.1 %, 0.5 % and 1.0 %).

3. RESULTS AND DISCUSSION

A total of 107 Δcre1 putative transformants resistant to hygromycin B were obtained from protoplast transformation, of which 29% survived until storage. The 31 remaining strains consist of 25 genetically stable transformants and 6 unstable strains.

All stable transformants exhibited increased enzymatic index compared to parental strains CFAM-422 under inductive conditions. However, when glucose (0.5 %) is present, pectinase production at pH 5.0 (polygalacturonase) slightly decreases and endoglunase production is completely inhibited (Figure 1). Unstable mutants did not show improvement of enzyme production regardless of glucose presence and exhibited similar enzymatic index CFAM-422.

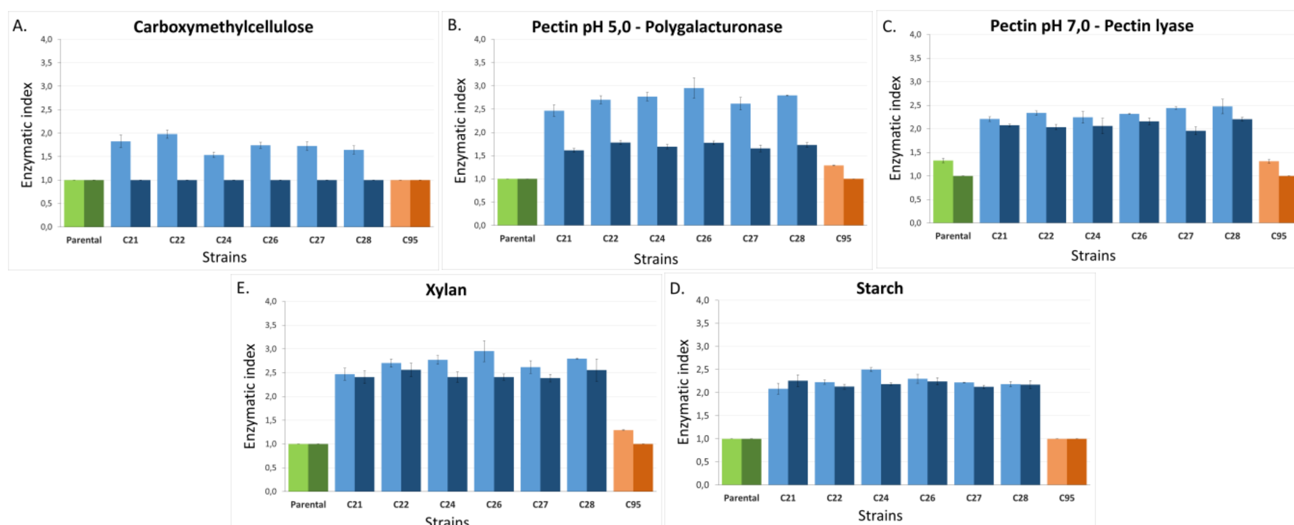

Figure 1. Enzymatic indexes of transformants (blue: genetically stable, orange: unstable) and parental (green) strains. Light colored columns refer to inductive conditions and dark colored to repressive conditions.

4. CONCLUSIONS

Our results indicate that deleting cre1 in T. harzianum CFAM-422 can be useful to cellulase and hemicellulase synthesis with reduced product inhibition, a desired characteristic for commercially applied enzymes. Further analysis regarding biomass hydrolysis are required to evaluate the applicability of Δcre1 mutants for enzymatic bioconversion from plant material.

5. REFERENCES

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