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SACCHARIFICATION AND FERMENTATION AT HIGH TEMPERATURES WITH ETHALOGENIC *Escherichia coli* DISPLAYING A THERMOSTABLE *BETA*-GLUCOSIDASE

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An autotransporter system (AT) was used to secrete and display, in the outer membrane of the ethanologenic *Escherichia coli* strain MS04 (1) (MG1655 Δ*pflB*, Δ*adhE*, Δ*frdA*, Δ*xylFGH::Km*^{R}*, gatC S184L, ΔmidarpA, Δreg 27.3 kb,* Δ*ldhA*, P*pflB*::*pdc*$_{Zm}$-*adhB*$_{Zm}$), the protein BglC (*β*-glucosidase) from *Thermobifida fusca*. ATs can be used to secrete hydrolytic enzymes for the production of biofuels or chemicals from lignocellulosic residues (2). The *β*-glucosidase BglC from *T. fusca* was selected because catalytic properties are compatible with cell growth and fermentation conditions of *E. coli*, pH 6-7, 37°C and aerobic-anaerobic conditions (3). An expression plasmid (pAg43BglC), based on the AT antigen 43 (Ag43) from *E. coli* and the tunable vector pTrc99A, was developed. The strain MS04 transformed with pAg43BglC was used to hydrolyze and ferment 40 g/L of cellobiose producing 17 g/L of ethanol in 2 days. Most of the *β*-glucosidase activity obtained with the Ag43-BglC system was cell-associated, allowing the recovery of the whole-cell biocatalyst for subsequent simultaneous saccharification and fermentation (SSF) process. Furthermore, knowing that BglC shows its highest activity at 50 °C and retains more than 70% of its activity at pH 6, MS04/pAg43BglC was evaluated at temperatures above 37°C. Therefore *E. coli* MS04/pAg43BglC was used to ferment crystalline cellulose (Avicel) in a SSF process using a commercial cocktail of endo and exo without the addition of commercial *β*-glucosidases, at pH 6 and a relatively high temperature for *E. coli*: 45 °C. Remarkably 22 g/L of ethanol were obtained under SSF conditions. The results shows that Ag43-BglC system can be used in *E. coli* strains without the addition of commercial *β*-glucosidases, reducing the quantities of commercial enzymes needed for the SSF process and that ethanologenic *E. coli* cells are able to ferment sugars at 45 °C during the SSF process using 40 g/L of Avicel (4). Further work is under development to express and secrete endo and exocellulases compatible with *E. coli* growth conditions to reduce or avoid the addition of endo and exo-cellulases and to produce biofuels and chemicals from lignocellulosic residues.

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