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Efficient extracellular secretion of an endoglucanase and a β-glucosidase in *E. coli*

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Escherichia coli is considered one of the most appropriate hosts for the production of recombinant proteins. However, its usage is undermined by its inability to efficiently secrete proteins into the extracellular medium. We selected two cellulolytic enzymes with potential biofuel applications, β-1,4-endoglucanase (Endo5A) and β-1,4-glucosidase (Gluc1C), and determined the genetic and environmental parameters for their optimal secretion into culture medium. Endo5A and Gluc1C were fused with the hyperosmotically inducible periplasmic protein of *E. coli*, OsmY, and their activities in the extracellular, periplasmic and cytoplasmic fractions were monitored. Most of the endoglucanase activity (0.15 μmol min^{-1} ml^{-1}) and β-glucosidase activity (2.2 μmol min^{-1} ml^{-1}) in the extracellular fraction was observed at 16 h post-induction. To reduce the overall cost, we expressed Endo5A and Gluc1C together either via a synthetic operon or through a bifunctional chimeric protein. Both systems efficiently secreted the enzymes, as evident from the functional activities and protein profiles on SDS–PAGE gels. The enzymes secreted via a synthetic operon showed higher activities (0.14 μmol min^{-1} ml^{-1} for endo-glucanase and 2.4 μmol min^{-1} ml^{-1} for β-glucosidase) as compared to the activities shown by the bifunctional chimera (0.075 μmol min^{-1} ml^{-1} for endoglucanase and 2.0 μmol min^{-1} ml^{-1} for b-glucosidase). The cellulase secretion system developed here has potential for use in the production of lignocellulosic biofuels.

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