Abstract

Increasing global concerns about limited reserves of conventional fuel sources has led to exploration of alternative feedstock for the renewable production of fuels and chemicals worldwide. Lignocellulosic feedstock is a fascinating alternative which mainly constitutes of cellulose, hemicellulose and lignin. Efficient and cost-effective methods are essential for breakdown of cellulosic materials into sugars that can be fed to microbes for bioconversion. Present work aims at development of Bacillus subtilis strains for heterologous expression extracellular export of Endoxylanases for and extracellular depolymerization of hemicellulose. Different combinations of signal peptides with genes were verified for optimal functionality. Three signal peptides namely YwmC, SacC and AmyE; two xylanases from Trichoderma resei and Neosartorya fischeri (Nf); were selected based on compatible functionality with respect to pH and temperature conditions. The recombinant and wild type strains of *B. subtilis* WB800N were screened for extracellular hydrolysis of the substrates and comparative analysis was performed on the basis of DNSA assay. All the recombinants demonstrated increase Xylan depolymerization over wild type. The engineered strain with YwmC-Xy(Nf) displayed 2 fold increase in activity compared to SacC and AmyE signal peptides. In situ depolymerization of Xylan showed 15 g/L of xylose yield which is 4 folds higher than published works. B. subtilis: E. coli Coculture process was developed to break down xylan and produce succinate in a single pot achieving a succinate titer of 3.7 g/L. This approach can be further explored for effective degradation of complex polymers like cellulose and hemicellulose to obtain substrates for production of wide variety of fermentation by products.

Keywords: Xylan; Consolidated bioprocessing; Co culture; *Bacillus subtilis*; Succinate