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Strain Engineering Furfural Resistance in E. coli for Biofuels Applications

Sustainable production of biofuels will require the inclusion of cellulosic biofuels that incorporate ethanologenic Escherichia coli that produce alcohols from carbon sources. A limitation to the efficient production of biofuels from cellulosic biomass is the creation of growth-inhibitory compounds, such as furfural, during the chemical pretreatment of the biomass. To design a strain of E. coli resistant to furfural of industrially relevant concentration (0.96 g/L), clones resistant to furfural of 1.0 g/L concentration were isolated. A parent strain was selected from those clones and the individual genes were molecularly cloned into separate plasmids through polymerase chain reactions (PCR), ligations, and chemical transformations. After confirming the presence of the individual genes through third-party sequencing and DNA electrophoresis gels, growth curve analyses and minimum inhibitory concentration (MIC) tests were performed to determine which gene confers furfural resistance. Using the MIC and growth curve, the groSL gene was determined to confer furfural resistance. To confirm the presence of the groSL proteins, SDS-PAGE gel electrophoresis analysis of the designed strain was performed. The groSL designed strain grew for 48 hours at 1.20 g/L furfural concentration. A curve was fit to each of the growth curves and the area underneath the curve was used in a p-test. The p-test indicated the groSL designed strain grows significantly differently from control. A designed strain resistant to furfural for at least 48 hours was isolated, suggesting the designed strain could be used to improve the efficiency of cellulosic biofuels production via increased resistance to growth-inhibitory compounds.