A Novel Enzymatic Biodigester Pretreatment using Synthetic Yeast

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ABSTRACT

Lignin, a complex organic polymer, is a major roadblock to the efficiency of biofuel conversion as it both physically blocks carbohydrate substrates and poisons biomass degrading enzymes, even if broken down to monomer units. A pretreatment process is often applied to separate the lignin from biomass prior to biofuel conversion. However, contemporary methods of pretreatment require large amounts of energy, which may be economically uncompelling or unfeasible. Taking inspiration from several genes that have been isolated from termites and fungi which translate to enzymes that degrade lignin, we want to establish a novel "enzymatic pretreatment" system where microbes secrete these enzymes to degrade lignocellulosic biomass. We incorporated the following genes into yeast vectors: laccase, lignin peroxidase, and aldo-keto-reductase from Reticulitermes flavipes; versatile peroxidase from Colletotrichum fioriniae PJ7; manganese peroxidase from Heterobasidion irregulare TC 32-1; and tyrosinase from Agaricus bisporus. These vectors code for fusion proteins with yeast secretion tags at the end of each enzyme gene, fluorescent protein tags at the beginning, as well as standardized restriction sites for synthetic biology manipulation. Furthermore, we designed an additional vector to contain our genetically modified yeast using an oxygen-repressed killswitch. We expect that transformants with our construct will be able to secrete said enzymes and contribute to lignin degradation if added to a biomass slurry. Future studies may focus on constructing a prototype bioreactor system and optimizing which combination of enzymes lead to the most efficient biofuel production.

KEYWORDS

Synthetic biology, yeast, cellulose, biodigester, biofuel, energy, biotechnology, iGEM, lignin