METABOLIC ENGINEERED *NEUROSPORA CRASSA* IS A PROMISING SOURCE FOR BIODIESEL PRODUCTION: A TEAM ALBERTA iGEM PROJECT

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Kayla Baretta, Adam Foster, Yuan Guo, Murray Pelech, Raymond Odsen, Michael Simpson, Helena Zakrzewski, Douglas Ridgway, & Michael Ellison
iGEM, University of Alberta, Edmonton, Alberta

Our current transportation infrastructure is heavily reliant on traditional fossil fuel sources. Developing sustainable alternative fuels is the most important challenge to reversing this trend. In Alberta, besides the production of fossil fuels, there is an abundance of cellulosic biomass from the forestry and agricultural industries. This presents the opportunity to develop a biofuel production process utilizing waste biomass, including grass clippings and excess agricultural straw, to lower energy costs and reduce carbon emissions.

To address this challenge, we propose to metabolically engineer the filamentous fungus, *Neurospora crassa*, a natural cellulose metabolizer, to produce high levels of fatty acids. In turn, the fatty acids will be chemically modified to fatty acid methyl esters (FAMEs), more commonly known as biodiesel. FAMEs have been commercially tested as substitutes for traditional fossil diesel in all modern engines.

To increase the fatty acid content of *Neurospora crassa*, we will be attempting a simultaneous knockout of fadD1 gene and an insertion of a codon optimized TesA' gene, a thioesterase from *E. coli* by homologous recombination. The fadD1 knockout will impair fatty acid degradation through β-oxidation. Furthermore, TesA' cleaves fatty acyl chains from ACP domain of the fatty acid synthase (FAS) complex reducing the inhibition of the FAS complex. .

The chemical conversion of fatty acids to FAMEs is through acid catalyzed esterification using MeOH and HCl. In this process, the collected *N. crassa* mass can be directly added to the reaction vessel without the prior extraction of the fatty acids.

Figure 1. The genetic construct used to transform N. crassa. It consists of four pieces. The 5' upstream region and 3' downstream region around the fadD1 are points for homologous recombination. The synthetic tesA' gene encodes for the thioesterase to reduce inhibition of the FAS complex. The HygB gene encodes for resistance to hygromycin B allowing for selection of transformed cells.