TWO-STEP ENZYMATIC CONVERSION OF ALGAL TRIACYLGLYCERIDES TO HYDROCARBONS

Jennifer Greenstein, Hannah Wapshott, Amy M. Grunden, & Paul T. Hamilton

North Carolina State University

Raleigh, NC, USA

jlgeens@ncsu.edu

Algae can provide the feedstock for biorefining by producing lipids and value-added co-products. To convert the lipids in algae into clean biofuels, industry currently uses a thermochemical deoxygenation process that requires high temperatures, extreme pressure, and an expensive metal catalyst. These processes greatly increase the cost of algae fuel, making it an economically unviable alternative to petroleum. In contrast, in this research, we utilize two-step enzymatic conversion of algal triacylglycerides (TAGs). First, lipase from *Geobacillus* releases free fatty acids (FFAs) from TAGs by hydrolysis, and decarboxylase from *Bacillus methanolicus* next removes the carboxyl group from FFAs. This generates decarboxylated hydrocarbons, which are the ideal substrate for transportation fuel production. The lipase from thermophilic bacterium *Geobacillus kaustophilus* HTA426 was cloned into vector pET-28b and expressed in *Escherichia coli* strain Arctic Express. The enzyme was characterized for feedstock range of carbon chain lengths, activity across pH 4-10 and temperature 35-95°C, methanol tolerance, and performance in aqueous and non-aqueous conditions. Activity assays using chromogenic substrates revealed broad substrate specificity of fatty acids, and the optimal conditions are temperature of 75°C and pH 7. Industry requires robust enzymes, and this lipase maintains activity from 35-75°C and pH 5-9, and it also has tolerance of up to 10% methanol and maintains 40% of its activity in non-aqueous, organic solvent DMSO. A cytochrome P450 fatty acid decarboxylating enzyme from *Jeotgallicocus* sp. (OleTJE) has already been characterized, but the enzyme is only active at mesophilic temperatures. A structural homolog of this enzyme (MGA3) was found in the thermophile *Bacillus methanolicus* BMMGA3 and belongs in the same cytochrome P450 fatty acid peroxygenase family. Structural analysis, however, shows that this enzyme may favor a hydroxylation reaction due to the presence of a Glutamine residue proximal to the heme (position 85) in the active site instead of the Histidine residue found in OleTJE. Using targeted PCR mutagenesis, a point mutation changed the codon for Glutamine (CAG) at amino acid position 85 in MGA3 into a codon for Histidine (CAT). From there, the mutated enzyme was expressed in *E. coli* BL-21 (DE3) using IPTG induced expression, purified using affinity chromatography, and biochemically characterized. The affinity of the enzyme for fatty acid substrate was tested using a tetramthylbenzidine (TMB) assay, and reaction products were confirmed using GC/FID. This two-step enzymatic bioconversion demonstrates potential for developing an economically viable algae to biofuel production platform.