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*Optimizing Algae Biofuels Artificial Selection & Nitrogen Stress as Methods to Induce Lipid Synthesis*

Algae biofuel is a promising renewable alternative to fossil fuel, but microalgal oil yields must increase for this option to become feasible. This study investigates nitrogen stress and artificial selection as methods to increase lipid synthesis via molecular changes in lipid metabolism.

Incremental nitrogen deficiency was implemented on three microalgal strains. Lipid analytical techniques using gas chromatography/mass spectrometry were refined and applied for lipid profiling. Hemacytometry and flow cytometry measured growth.

Concurrently, artificial selection was investigated as a method to heighten the activity or expression of acetyl-CoA carboxylase (ACCase), an enzyme active in lipid synthesis, by application of the ACCase-inhibiting herbicide sethoxydim to select for tolerant cell lines. This novel approach to increasing algal oil yields required considerable development, including sethoxydim characterization in liquid culture. ACCase immunoblotting, a spectrophotometric ACCase enzymatic assay, and lipid and growth analyses were conducted on selected cell lines to determine changes in lipid metabolism.

Results indicate heightened lipid synthesis and decreased growth with increasing nitrogen deprivation. Developed lipid analytical protocols show success, and NMR spectroscopy indicates efficient esterification. Cellular protein extractions were successful, although immunoblotting with non-algal antibodies requires further refinement. Tests with the ACCase activity assay are underway. Work on sethoxydim characterization has honed artificial selection techniques.

Importantly, artificial selection results demonstrate significant increases in lipid accumulation in sethoxydim-selected cultures, indicating success in selecting for algal cell lines with increased ACCase and lipid production. If such cell lines can be sustained, artificial selection could prove a novel method for increasing microalgal oil yields.