

43. Characterization of the Laminarinases from *Vibrio breoganii* 1C10

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Project Goals: This project will harvest ‘biomass to biofuel’ pathways from algae- associated bacteria, and develop these as reusable genetic parts. Marine algae hold great promise for biofuel production and have advantages over terrestrial biomass and freshwater algae. Despite this potential, little effort has been made to date to harness the enzymatic machinery that bacteria use to convert marine algal carbohydrates into bioenergy substrates. Our project capitalizes on this unexplored opportunity via three distinct activities: bioprospecting for novel algal polysaccharide-degrading genes, functional screening for enzymes with desired biochemical properties, and repackaging pathways in reusable genetic modules.

Brown seaweeds are an attractive source of feedstocks for biofuel production, since they have advantages over terrestrial feedstocks. Brown seaweeds have higher growth rates than terrestrial plants, and they lack crystalline cellulose and lignin. Additionally, brown seaweeds do not impinge on arable land, thus negating the conflict between food and fuel. One of the primary components of brown seaweeds is laminarin. Laminarin is a polysaccharide consisting of β -1,3 and β -1,6 linked glucose. The enzymes that can degrade these linkages are called glycoside hydrolases (GHs). More specifically, the β - 1,3 linkage is degraded by enzymes belonging to seven GH families: GH3, GH5, GH16, GH17, GH55, GH64, and GH81. β -1,6 degrading GHs are remain unknown.

We are investigating the mechanism of laminarin metabolism within marine *Vibrio* sp. Firstly, we have found that laminarinases are induced by growth on laminarin. We next sought to clone, purify, and characterize the four laminarinases within *Vibrio breoganii* 1C10. We found that these enzymes are most active between pH 6.5 and 8.0, 20°C and 25°C, and 50 and 400 mM NaCl. We then determined the enzyme kinetics for each enzyme. We found that each enzyme had a V_{max} between 0.148 and 0.92 $\mu\text{M s}^{-1}$, K_M between 3.4 and 6.0 mM laminarin, and a turnover number between 0.69 and 6.1 s^{-1} .

These results now allow for metabolic engineering of microorganisms that degrade laminarin as their sole carbon source.

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