

Characterization of the Alginate Lyases and Laminarinases from *Vibrio sp.*

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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. Two of the primary components of brown seaweeds are alginate and laminarin. Alginate is a copolymer consisting of 1,4 linked epimers α - L-guluronate (G) and β -D-mannuronate (M). The local structure of alginate can take one of three forms: short stretches of polyguluronate (polyG), short stretches of polymannuronate (polyM), or alternating sequences of guluronate and mannuronate. The enzymes that can degrade the linkages within alginate are called alginate lyases. Alginate lyases are classified based on their specific dyad G-G (EC 4.2.2.11), M-M (EC 4.2.2.3), and M-G/G-M bonds that they cleave. Additionally, alginate lyases are classified based on whether they have exolytic or endolytic cleavage. 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 alginate and laminarin metabolism within marine *Vibrio sp.* To this end, we next cloned, purified, and characterized the alginate lyases within *Vibrio splendidus* 12B01 and 13B01 and *Vibrio breoganii* 1C10. We found that these enzymes are most active between pH 7.5 and 8.5, 20°C and 25°C, and 250 and

1000 mM NaCl. We then determined the enzyme kinetics for each enzyme. We found that each enzyme had a V_{\max} between 0.090 and 1.7 $\mu\text{M s}^{-1}$, K_M between 22 and 300 μM laminarin, and a turnover number between 0.19 and 4.9 s^{-1} . We also determined the dyad specificity of each lyase; we found G-M, G-G, M-M, and M-G specificity. Between the three organisms, we have characterized 18 alginate lyases, which allows for a broad sampling of how *Vibrio sp.* degrade alginate. We have begun to elucidate synergies between lyases. Since a single organism contains multiple lyases, we want to find how alginate lyases can be used simultaneously to degrade alginate to completion and with faster kinetics.

We are also 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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