

## 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  $\alpha$ - L-guluronate (G) and  $\beta$ -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  $\beta$ -1,3 and  $\beta$ -1,6 linked glucose. The enzymes that can degrade these linkages are called glycoside hydrolases (GHs). More specifically, the  $\beta$ -1,3 linkage is degraded by enzymes belonging to seven GH families: GH3, GH5, GH16, GH17, GH55, GH64, and GH81.  $\beta$ -1,6 degrading GHs are remain unknown.

We are investigating the mechanism of alginate metabolism within marine *Vibrio sp.* We previously characterized the alginate lyases in *V. splendidus* 12B01 [1]. A recent study investigated the ability of different marine *Vibrionaceae* bacteria to degrade alginate [2]. They identified significantly variability within two closely related *Vibrio splendidus* strains, 12B01 and 13B01. In particular, they found that *V. splendidus* 13B01 has significantly higher secreted alginate lyase activity than *V. splendidus* 12B01. To determine the source of this variability, we characterized the six alginate lyases in *V. splendidus* 13B01 using a combination of genomics, proteomics biochemical, and functional screening. These experiments revealed that a single alginate lyase PL7G, unique to *V. splendidus* 13B01, is critical for rapid extracellular alginate degradation.

We are also investigating the mechanism of laminarin degradation in *Vibrio breoganii* 1C10. This bacterium contains four putative laminarinases: LamA, LamB, LamC, and LamD. We cloned, purified, and enzymatically characterized these laminarinase. We also determined the specificity and endolytic/exolytic activity using NMR and MALDI-TOF spectrometry analysis. The transglycosylation ability of these laminarinases and the extent of the hydrolysis were also examined. These results now allow for metabolic engineering of microorganisms that degrade laminarin as their sole carbon source.

1. Badur, A.H., S.S. Jagtap, G. Yalamanchili, J.K. Lee, H. Zhao, and C.V. Rao, *Alginate Lyases from Alginate-Degrading Vibrio splendidus 12B01 Are Endolytic*. Appl Environ Microbiol, 2015.
2. Hehemann, J.H., P. Arevalo, M.S. Datta, X. Yu, C.H. Corzett, A. Henschel, S.P. Preheim, S. Timberlake, E.J. Alm, and M.F. Polz, *Adaptive radiation by waves of gene transfer leads to fine-scale resource partitioning in marine microbes*. Nat Commun, 2016. 7: p. 12860.

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