94. Cloning and Characterization of the Alginate Lyases from *Vibrio splendidus* 12B01 and 13B01

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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 have proven potential as feedstocks for biofuel production, and have several advantages over other marine algae. Alginate is one of the major polysaccharides of brown algae. It is a heterogeneous polymer of two uronic acids, β-D-1,4-mannuronate (M) and its C5 epimer α-L-1,4-guluronate (G). These residues appear as homopolymeric MM and GG blocks or in a heteropolymeric distribution GM. Alginate is degraded by alginate lyases which are abundant in marine bacteria, but the enzymes for initial attack and subsequent catabolism of the building blocks are poorly characterized or unknown.

We have investigated two strains of *Vibrio spendidus* (12B01 and 13B01) for their ability to degrade alginate. We performed a preliminary analysis of the secretome from 12B01 and 13B01 using shotgun proteomics. LC-MS/MS identified 5 putative lyases in the 12B01 secretome and 2 in the 13B01 secretome. We have also cloned, purified, and enzymatically characterized eight alginate lyases from 12B01 and six from 13B01. These enzymes were identified based on sequence homology to known alginate lyases and include the lyases identified by LC-MS/MS.

This project is a part of the Biosystems Design Program supported by the Office of Biological and Environmental Research in the DOE Office of Science.