Optimization of Alginate Utilization in Engineered Bacteria for Biofuels Production

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Project Goals: This project is focused on unveiling pathways from algae-associated bacteria and refactoring them to use algal polysaccharide as a feedstock for biofuel production. Algal polysaccharides are considered a promising carbon/energy source and are emerging as an important feedstock for the production of biofuels. 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. This project harnesses the unexplored bacterial polysaccharide-degrading pathways to 1) bioprospect novel algal polysaccharide-degrading genes, 2) characterize enzymes with desired biochemical properties, and 3) repackage pathways in reusable genetic modules. This project will yield a set of functional modules for the producing biofuels from marine macroalgae.

Marine macroalgae is emerging as an attractive feedstock for biofuels production. A number of marine microbes are able to degrade and catabolize efficiently macroalgal polysaccharides by specialized enzymatic pathways which convert these carbohydrates into bioenergy substrates. *Vibrio splendidus* is a marine bacterium capable of degrading and catabolizing alginate (a linear copolymer of two uronic acids: β-D-mannuronate and α-L-guluronate) by specialized enzymatic pathways. In this work, we harnessed the alginate-degrading machinery from *V. splendidus* via heterologous expression in the highly genetically amenable host *Escherichia coli*. The alginate-degrading pathway in *V. splendidus* is clustered in two separated fragments of DNA that contain a set of genes for alginate transport and metabolism. This cluster of ~49 kb was assembled using the DNA assembler method and expressed on a fosmid in *E. coli* ATCC 8739. The resulting strain was able to grow on minimal media with alginate and oligoalginates as the sole carbon sources, albeit at a slow rate. To increase utilization rate of alginate, selected key *V. splendidus* pathway genes and homologs were overexpressed to identify degradation pathway bottlenecks and increase utilization rate. The resulting strain can be used as a basis for further optimization efforts using pathway-scale and genome-scale methods.

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