

53. Engineering a *Bacillus subtilis* Strain Capable of Utilizing Marine Macroalgae 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 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 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 important 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. However, there are few studies to unveil and exploit this enzymatic machinery. *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 of *V. splendidus* to engineer a model gram-positive bacterium *Bacillus subtilis* capable of utilizing alginate to produce ethanol. 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 was heterologously expressed in *B. subtilis* that was previously engineered to produce ethanol. The growth of the engineered *B. subtilis* was limited when using alginate as the sole carbon source. It is well known that heterologous pathway expression is challenging and it can be hampered by differences in regulatory elements. To circumvent this drawback the biosynthetic pathway was refactored using a collection of heterologous constitutive promoters, ribosomal binding sites, and terminators that are functional in *Bacillus* sp. Additionally, it was further optimized by addition of novel alginate lyases and removal of redundant enzymes. This microbial platform can be further engineered to create recombinant *B. subtilis* capable of using macroalgal polysaccharides to produce a variety of biofuels and chemicals.

References:

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