96. Characterization of Algal Polysaccharide Degrading Enzymatic Machinery and its Refactoring via DNA Assembler for Biofuels Production

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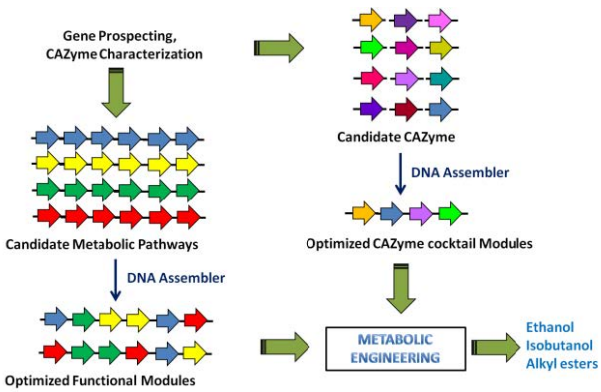
Project Goals: We will employ new methods like DNA Assembler and COMPACTER developed by the Zhao group for rapidly assembling and optimizing complex multi-gene pathways for degrading complex algal polysaccharides using candidate genes from our collection of marine bacteria. A key advantage of this approach is that our designs will not be limited to the metabolic capabilities of a single organism. Rather, our methodology enables us to combine diverse metabolic functionalities from multiple bacteria in a single host. In particular, we will be able to assemble different combinations of genes in parallel from a large and diverse library of marine bacteria. In addition, we will use this approach to rapidly optimize these pathways by altering their promoters, ribosome binding sites, codon bias, and organization within operons for expression in the industrial bacterium *E. coli*. This will enable us to couple our designs with designs previously developed in *E. coli* for production of ethanol, isobutanol, and alkyl esters.

Algal polysaccharides constitute an important carbon and energy resource for marine organisms and have been considered as cost-competitive biomass for the production of biodiesel, bioethanol, and biohydrogen. It is restricted by the availability of tractable microorganisms that can metabolize alginate polysaccharides. More efforts are required to harness the enzymatic machinery that bacteria use to convert marine algal polysaccharides into bioenergy substrate. Here, we present the characterization of three oligoalginate lyases (Oals) and four alginate lyases (Alys) from *Vibrio splendidus* that catalyze depolymerization of alginate into monomer substrates which later converted to biofuels. OalA (PL-15) and OalB, OalC (PL-17) from *V. splendidus*, act on alginate polysaccharides and oligosaccharides release monosaccharides from the substrate terminus. OalA was purified as an active soluble form using MBP-tag affinity chromatography. Protein refolding of OalB and OalC using the flash dilution method resulted in refolding of the protein into its native structure and regaining full biological activity. Exotype OalA had activities toward both poly β-D-mannuronate (polyM) and poly α-L-guluronate (polyG), indicating that it is a bifunctional alginate lyase. The variation in the kinetic parameters for the OalA reaction as a function of substrate length suggests that the enzyme is well adapted to process the oligomers that are imported into the cell. The turnover number was inversely proportional to degree of polymerization of substrates. OalA showed highest activity towards the trimeric substrate, while OalB and OalC preferred dimers among various oligomers. Saccharification of



alginate by OalA produced high concentration of monosaccharides, a substrate for biofuels. Alys belonging to the PL-5 and PL-7 families acted on alginate polymers endolytically and in some cases exolytically to produce oligosaccharides and monomers. AlyA (PL-5) and AlyB (PL-7) specifically depolymerized heteropolymer poly MG in alginate molecules into oligomers. AlyC and AlyD belong to family PL-14 are specific for polyM, and polyG, respectively. Oals and Alys can be used as biocatalysts for saccharification of alginate since they can efficiently degrade alginate and alginate oligomers into alginate monosaccharides. The detection, quantification, and structure determination of the alginate products were carried out using TLC, HPLC, ESIMS or NMR. The characterized algal polysaccharide degradation enzymatic machinery and the redesigned alginate degradation cluster by addition of novel alginate lyases and removal of redundant enzymes are refactored using the DNA assembler method to create recombinant bacterial and yeast strains capable of producing biofuels and chemicals.

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