The Ecology of Macroalgal Polysaccharide Utilization: *Verrucomicrobia* Isolates Initiate Fucoidan and Carrageenan Degradation Cascades

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Project Goals: 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.

Marine macroalgae are vital players in the global carbon cycle, and polysaccharides represent a significant output of their primary production. Identifying the microbes and metabolic pathways responsible for degrading these sugars is not only crucial to understanding marine carbon flow, but also offers potential for biofuel production using seaweed feedstocks. Enrichment cultures from coastal waters yielded novel *Verrucomicrobia* isolates capable of degrading the sulfated polysaccharides fucoidan and carrageenan. These fucose- and galactose-based polysaccharides commonly found in brown or red seaweeds, respectively, are often recalcitrant to microbial degradation and require specialized enzymes to degrade. Strains capable of initiating complex breakdown cascades of these sulfated polysaccharides were sequenced, revealing Polysaccharide Utilization Loci (PULs) enriched with numerous and diverse Carbohydrate-Active Enzymes (CAZymes) and sulfatases. Transcriptional analyses revealed specific PULs induced by each macroalgal substrate: one carrageenan-specific PUL (encoding GH16, GH39, GH43 GH82, sulfatases and a TonB-dependant receptor homologue) and three fucoidan-specific PULs (encoding GH29, GH107, sulfatases, hypothetical CAZymes and putative transporters) were identified. CAZymes with potentially novel substrate specificity, particularly among the poorly characterized fucosidases, are the subject of ongoing enzymatic characterization. Culture- and plate-based assays also indicate specific combinations of isolates appear to complement one another and yield greater overall biomass accumulation, suggesting engineered organisms or communities with a full repertoire of enzymatic capabilities may facilitate the efficient conversion of algal biomass.