

Using Genetic and Bioreactor Engineering to Produce Oleaginous Bacteria

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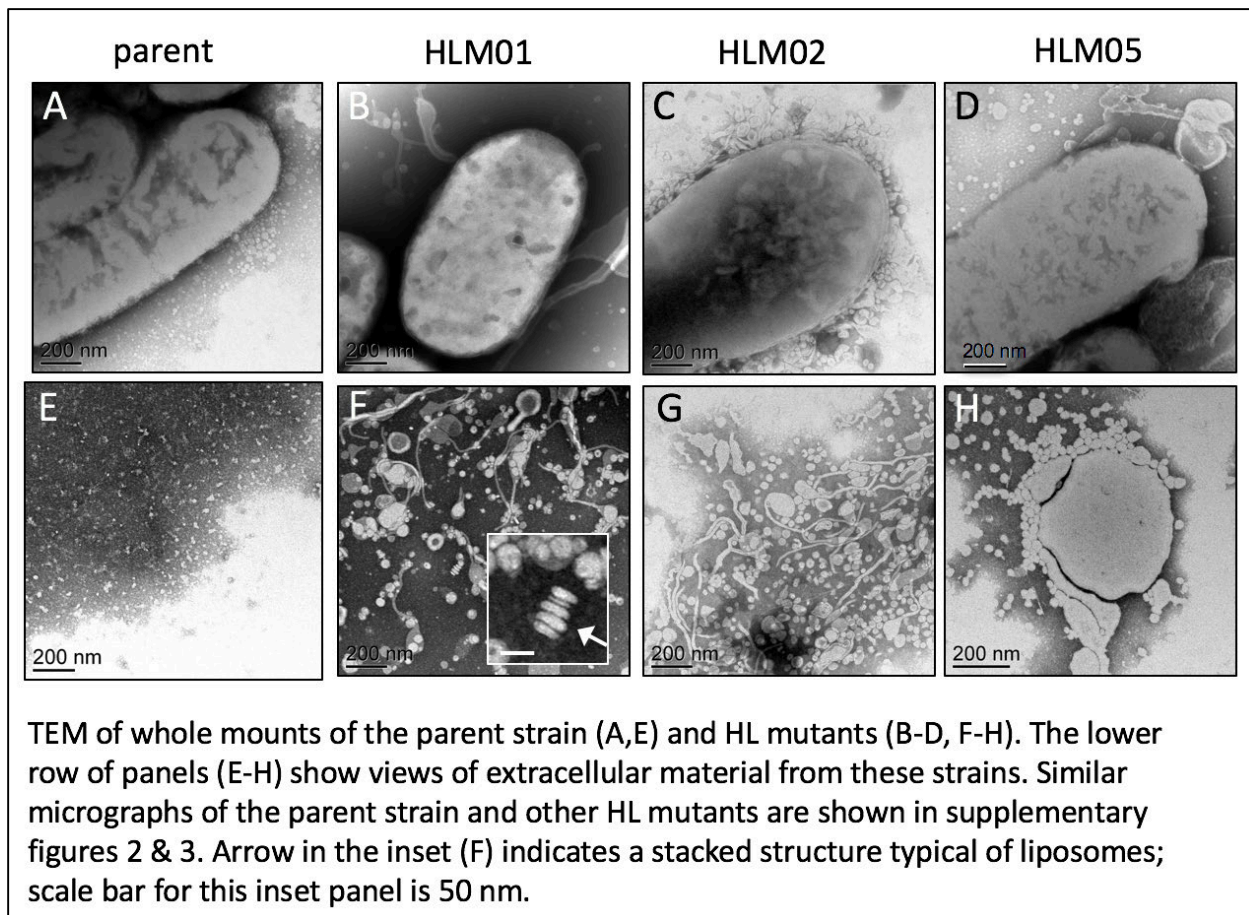
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Project Goals: In order to generate knowledge needed to produce next generation biofuels or fuel precursors, the GLBRC identified a need to increase microbial synthesis of anabolic compounds under low O₂/anaerobic conditions. Our studies address this key research priority for GLBRC by focusing on the synthesis of lipids and fatty acids under low O₂/anaerobic conditions in *R. sphaeroides*, a facultative microbe that has several novel features relevant to production of advanced biofuel precursors. Our strategy is to use the insight we gain to develop approaches to improve the yield of fatty acids or other hydrocarbons in this or possibly other microbes being studied in GLBRC. Thus, we predict that the new information gained from this project will increase our ability to modify bacteria and possibly other cells to advance a systems biology understanding for biofuel and bio-based products.

Lipids from microbes offer a promising source of renewable alternatives to petroleum-derived compounds. In particular, oleaginous microbes are of interest because they accumulate a large fraction of their biomass as lipids. In this study, we investigate whether it is possible to exploit the native metabolic and regulatory pathways of non-oleaginous bacteria to increase lipid production to oleaginous levels. We use *Rhodobacter sphaeroides*, a facultative bacterium that has a native ability to increase phospholipid membrane content under low O₂ conditions. We screened a *Rhodobacter sphaeroides* Tn5-mutant library for insertions that increased fatty acid content at high O₂ and identified ten high-lipid (HL) mutants for further characterization. We found that the genetic lesions in these mutants did not disrupt pathways known to impinge on fatty acid accumulation. Instead, we found that these HL mutants exhibited changes in their cell envelopes, including sensitivity to drugs that target this region, changes in shape, and ability to secrete lipids, with two HL mutants accumulating ~60% of their total lipids extracellularly. Analysis of the lipid secretions suggests that inner membrane or periplasmic components, including quinones, could also be secreted by these mutants as value-added coproducts. We used one of the highest lipid secreting strains to grow high-density cultures in a fed-batch bioreactor,

and produced 1.3 g/L fatty acids, with lipid content comparable to that of oleaginous microbes, but having the characteristic that the majority of the lipids were excreted. Thus, by combining a single genetic alteration with bioreactor engineering we have converted *R. sphaeroides* into an oleaginous bacterium. Based on the properties of these HL mutants, we conclude that alterations of the cell envelope can be used as novel approach to increase microbial lipid production and secretion, and may be applicable to other organisms.



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