

Harnessing a P450 fatty acid decarboxylase from *Macrococcus caseolyticus* for microbial biosynthesis of odd chain terminal alkenes

Background

- Alkenes (or olefins) are industrially important platform chemicals used to manufacture polymers, lubricants, surfactants, and coatings, and can be used as advanced biofuels.
- To date, studies of fatty acid (FA) decarboxylases are mainly focused on OleT_{JE} derived from *Jeotgalicoccus* sp. with substrate specificity towards C12-C14 FAs.

Approach

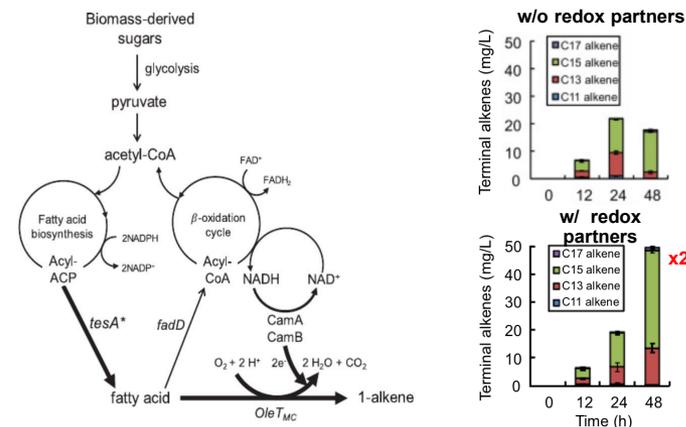
- Used bioinformatics to discover novel FA decarboxylases.
- Demonstrated alkene biosynthesis *in vitro* and *in vivo*.
- Applied metabolic engineering strategies to enhance alkene production.
- Developed an *in silico* protein modeling protocol to illuminate substrate specificity of FA decarboxylases useful for making designer olefins.

Outcomes and Impacts

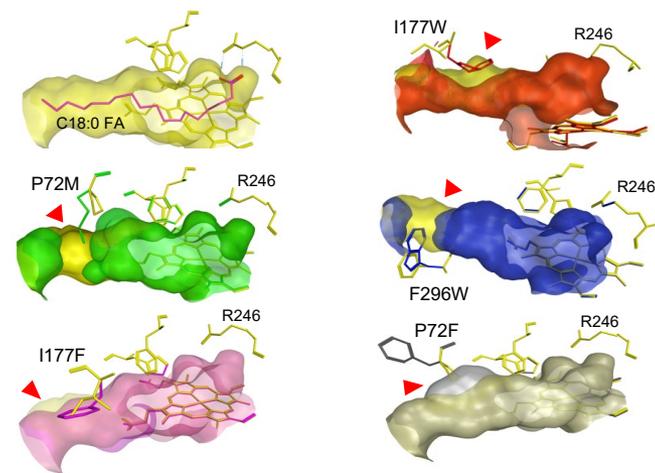
- Identified a novel OleT_{MC} from *Macrococcus caseolyticus* that was successfully expressed and characterized *in vitro*.
- Discovered the H₂O₂-independent activity of OleT_{MC}, providing an advantage for the microbial production of terminal alkenes from lignocellulosic biomass.
- Demonstrated alkene biosynthesis directly from glucose in a recombinant *E. coli*.
- Alleviated electron transfer limitation in OleT_{MC} critical for improved alkene production.
- Illuminated the potential underlying mechanism responsible for substrate specificity of OleT_{MC} towards C16-C18 FA moieties and provided protein engineering strategies to alternate substrate specificities.

Significance

- This study provides a better understanding of novel functions of FA decarboxylases and helps lay out a foundation for future engineering of OleT_{MC} to be used for production of designer olefins for biofuels and biochemicals applications.



De novo biosynthesis of terminal alkenes in engineered *E. coli* strains



Overlay of the potential FA binding pocket of the wildtype OleT_{MC} (in yellow) and the OleT_{MC} variants including I177W (in red); P72M (in green); F296W (in blue); I177F (in purple); P72F (in gray). Filled red triangles point to the distinctive features in the FA binding pockets of the OleT_{MC} variants as compared to the wildtype.