**Introduction**

**Medium chain fatty acid**

Fatty acid and its derivatives are crucial in food, fragrance, health care and many other industries. In particular, medium chain methyl ketone has been found additional uses as precursor for advanced biofuels and promising ingredients in pharmaceutical industry. However, the majority of fatty acids present in nature are classified as long-chain, leaving medium chain fatty acid in short supply. Therefore, metabolic engineering is useful in generating medium-chain length fatty acid and its derivatives. [1]

**Yarrowia lipolytica**

The oleaginous organism, Yarrowia lipolytica, is of great potential in production of fatty acid. It has many natural advantages over many other microbes. For example, the oleaginous organism is capable of dedicating most of carbon flux to the lipid accumulation. Moreover, compared to more robust microbial platform such as E.coli and S. cerevisiae, Y. lipolytica can naturally generate a large flux to cytosolic Actyl-CoA, which is a key building block for all oleochemicals. Therefore, in this project, Y. lipolytica is selected as the cell platform for production of medium chain fatty acids. [1]

**Type I fatty acid synthase.**

Then, FAS genes and PhaG gene can be assembled via recombinant DNA technique. First, replacing MPT gene with PhaG gene to remove such channel, enabling medium chain intermediate circulating out from the FA elongation cycle. Next, inserting PhaG gene in adjacent to ACP gene demonstrated in either second or third design, ensuring efficient medium chain intermediate substrate transfer. A schematic design of engineered type I fatty acid synthase is shown in Figure.5.

**Methods**

**Pathway design of medium chain fatty acid synthesis**

In order to overcome FAS structural constrain, we propose to redirect carbon flux from fatty acid elongation cycle to medium chain fatty acid intermediate before it becomes long-chain end product. Herein, we propose to incorporate a foreign 3-hydroxyacyl ACP-CoA transacylase (PhaG) enzyme from Pseudomonas putida in fatty acid synthase to relieve such constrain. The 3-hydroxyacyl ACP-CoA transacylase (PhaG) is an ideal heterogenous enzyme candidate, recently being identified by Hoffmann et al in Pseudomonas putida PhaG is found to have the capability to divert R+/-3-Hydroxyacyl-ACP, an ACP thioester intermediate in FA biosynthesis cycle, to medium chain methyl ketone precursor: R+/-3-Hydroxyacyl-CoA. [4]. With the inserted PhaG enzyme, the synthetic FAS is anticipated to direct carbon-containing substrate towards medium chain fatty acid derivatives synthesis. A complete designed pathway is shown in Figure.4.

**Design construct**

The key strategy to maximize a particular chain length oleochemical production is to alter Type I fatty acid synthase (FAS) in Y. lipolytica. As shown in Figure.3., wild type FAS consists two subunits, in total eight enzyme domain. The unique structure of MPT enzyme domain, which only allows C16 or longer chain length fatty acid intermediate passing through its channel, explains why wild type Y. lipolytica rarely produce medium chain fatty acid derivative. Secondly, ACP enzyme has been shown to play a central role in substrate binding shuttling substrates into the elongation cycle [3].

**Preliminary Results**

**Preliminary modeling results.**

A constraint-based E.coli metabolic model (COBRA) using python programing language was used to analyze flux balances and test the feasibility of PhaG pathway design. The results are shown in the Figure.6. Figure 6 demonstrates that the PhaG pathway design was theoretically feasible.

**Preliminary experimental results**

Y. Lipolytica was grown on YDB selective agar overnight. Single yeast colonies will be selected into YDB media at 15 mL test tube at 30 °C for two days to amplify the cells. The methyl ketone products was extracted by FAME method [4] and analyzed by GC-FID to quantify the titers and composition of methyl ketone in the samples. The result showed Y. lipolytica tends to produce long chain fatty acids as expected.

**Ongoing work plan**

Clone PhaG genes from Pseudomonas putida to reconstruct type I FAS and test methyl ketone production

**Acknowledgements**

This research was supported by Professor, Brian Pfleger and Dr. Qiang Yan in Department of Chemical and Biological Engineering. I show great gratitude to both of them for providing precious insights and valuable suggestions into my research.

**References**