Deciphering the diversity of microbial 1-alkene producers and engineering of the production conditions

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INTRODUCTION

Terminal olefins (1-alkenes) are important platform chemicals and can be applied as drop-in compatible hydrocarbons [1,2]. Currently, 1-alkenes are almost exclusively derived from petroleum. Only a few microbial biosynthetic routes have been reported. *Jeotgalicoccus* sp. ATCC 8456 is known to produce 1-alkenes using $OleT_{JE}$, a fatty acid decarboxylase. UndA and UndB are recently identified non-heme iron oxidases/decarboxylases converting medium-chain fatty acids into terminal alkenes [1] (***Figure 1***). Their pathways are highly conserved in three main genera: *Burkholderia, Pseudomonas, and Myxococcus*; However, there is still limited knowledge about microbial 1-alkene synthesis and diversity of $OleT_{JE}$, UndA and UndB homologue enzymes in other microorganisms, especially in bacteria [3,4].

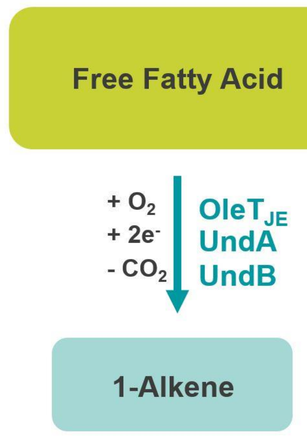

Figure 1: *Production of 1-alkenes by decarboxylation of free fatty acids [5].*

CHARACTERIZATION OF *JEOTGALICOCCUS* SP. ATCC 8456

To gain a better insight into the microbial 1-alkene synthesis we sequenced the genome of *Jeotgalicoccus* sp. ATCC 8456, annotated it and employed the genomic information for data mining. Whole genome sequencing allowed reconstruction of the fatty acid biosynthesis pathway. This is of interest because fatty acids are the substrates of $OleT_{JE}$.

FATTY ACID FEEDING

Additionally, we looked into the conditions leading to 1-alkene production *in vivo*. The production of certain 1-alkenes was successfully influenced by feeding fatty acids with a certain chain length as precursors for the reaction (***Figure 2***).

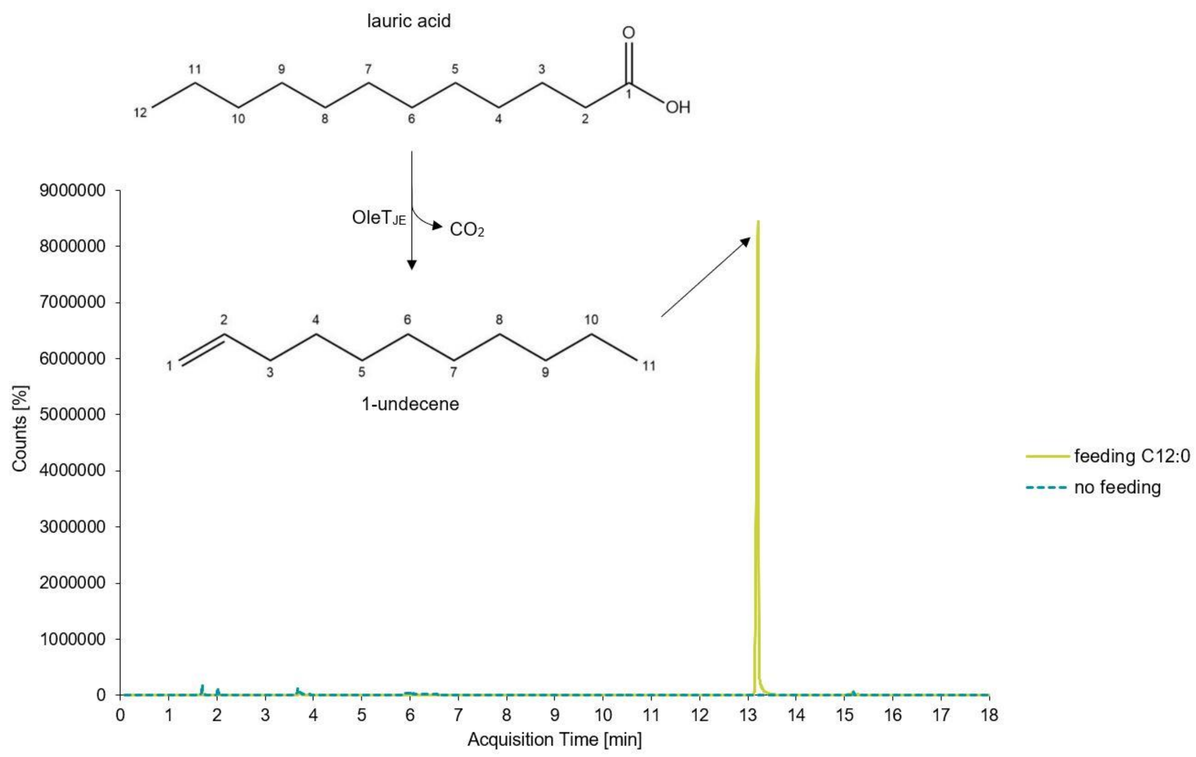

Figure 2: *Feeding lauric acid led to a 1000 fold increase of 1-undecene in Jeotgalicoccus sp. ATCC 8456 measured by GC-MS.*

PCR-BASED SCREENING FOR $OleT_{JE}$, UndA AND UndB HOMOLOGUE ENZYMES

In our culture collection we identified other 1-alkene producers belonging to the genera of *Pseudomonas* sp. and *Bacillus* sp. Based on bioinformatic analysis, degenerated PCR-primers were designed binding to conserved regions of $OleT_{JE}$, UndA and UndB genes. These primers allow the PCR-based screening of genomes and metagenomic libraries to identify new enzymes for hydrocarbon synthesis. We are currently screening 9000 clones of a metagenomic library from a *Sphagnum magellanicum* microbiome and successfully amplified sequences using degenerated UndA and UndB primers (***Figure 3***).

UndB Fwd.	WSC ATG TAY TTY CGC AAG CA
UndB Rev.	CDS YTC BAY RTC GCC GTA

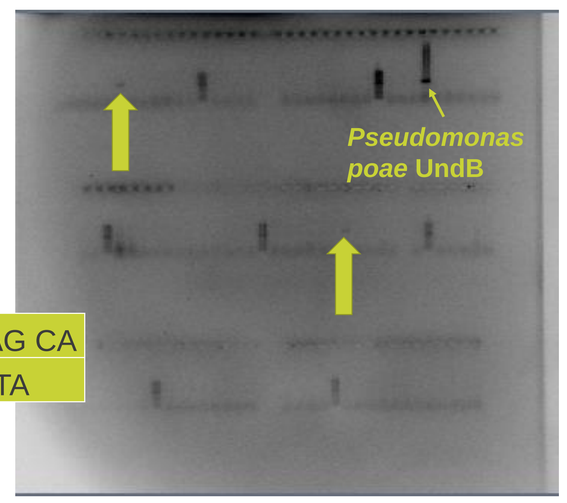

Figure 3: *PCR based screening for identification of positive hits using degenerated UndB Primers.*

CONCLUSION

Whole-genome sequencing of *Jeotgalicoccus* sp. ATCC 8456 showed that the bacterium is equipped with an entire pathway for fatty acid biosynthesis. Feeding of fatty acids as precursors of 1-alkene biosynthesis in growing *Jeotgalicoccus* cultures has the potential to induce and enhance the production of the corresponding and targeted 1-alkenes. To shed light onto the diversity of 1-alkene producing environmental bacteria, we established a PCR-based screening strategy using degenerated primers and managed to identify specific PCR products in a metagenomic clone library from a *Sphagnum magellanicum* microbiome.

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