Optimisation of Bio-petrol Production from Lignocellulosic Hydrolysate using *Escherichia Coli*

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Introduction

Several decades of fossil fuels overconsumption has participated in fuelling the current global climate crisis through the release of massive amounts of greenhouse gases into the atmosphere.[1] To tackle this issue, interest for biofuels as a greener alternative to fossil fuels has been increasing in the recent years[2]. Among the currently existing types of biomass, the most abundant and cheapest one is **lignocellulosic biomass.**[3–5] Thus, it can potentially become a viable feedstock for a sustainable large-scale production of biofuels[3] such as **bio-petrol**. Typically made of 60-70% glucose and 30-40% xylose[3], lignocellulosic hydrolysate is a plant material that has been hydrolysed through a pre-treatment process (usually steam explosion followed by dilution in acid),[2] while petrol (or gasoline) is a homogeneous mixture of C_3-C_{12} hydrocarbons including aromatics, branched-paraffins, cycloparaffins and olefins. This poster presents a genuine work aiming at optimising the yield of bio-petrol produced from lignocellulosic biomass using the relevant literature. This optimisation focuses on the modification of the metabolic pathways of a bacteria known as ***Escherichia coli* (*E. coli*)** as well as the enzymatic engineering of an enzyme called **thioesterase (TesA)**, both selected for their propensity to foster the synthesis of short- and longer-chain alkanes (C_3-C_9) as those present in conventional petrol.[3]

Metabolic Engineering

FAS pathway

The **Fatty Acid Synthesis (FAS)** generates longer-chain alkanes: heptane (C_7) and nonane (C_9). [1]

- NADPH cofactor dependent.[1]
- Greater carbon flux.[1]
- High net ATP. [1]
- The RARE *E. coli* strain is used to accumulate aromatic aldehydes whereby the aldehyde reductase gene is deleted, preventing endogenous conversions to fatty alcohol.[1]

ROB pathway

Reverse-β-oxidation (ROB) generates shorter chain alkanes: propane (C_3), butane (C_4) and pentane (C_5).[1]

- Uses universal CoA molecule, therefore has increased transferability to host.[1]
- ↑ carbon efficiency and ↓ net ATP consumption compared to FAS, therefore exhibits overall increased energy efficiency.[1]

Plasmids are used to introduce genes encoding 7 modules used to generate C_3-C_9 alkanes:

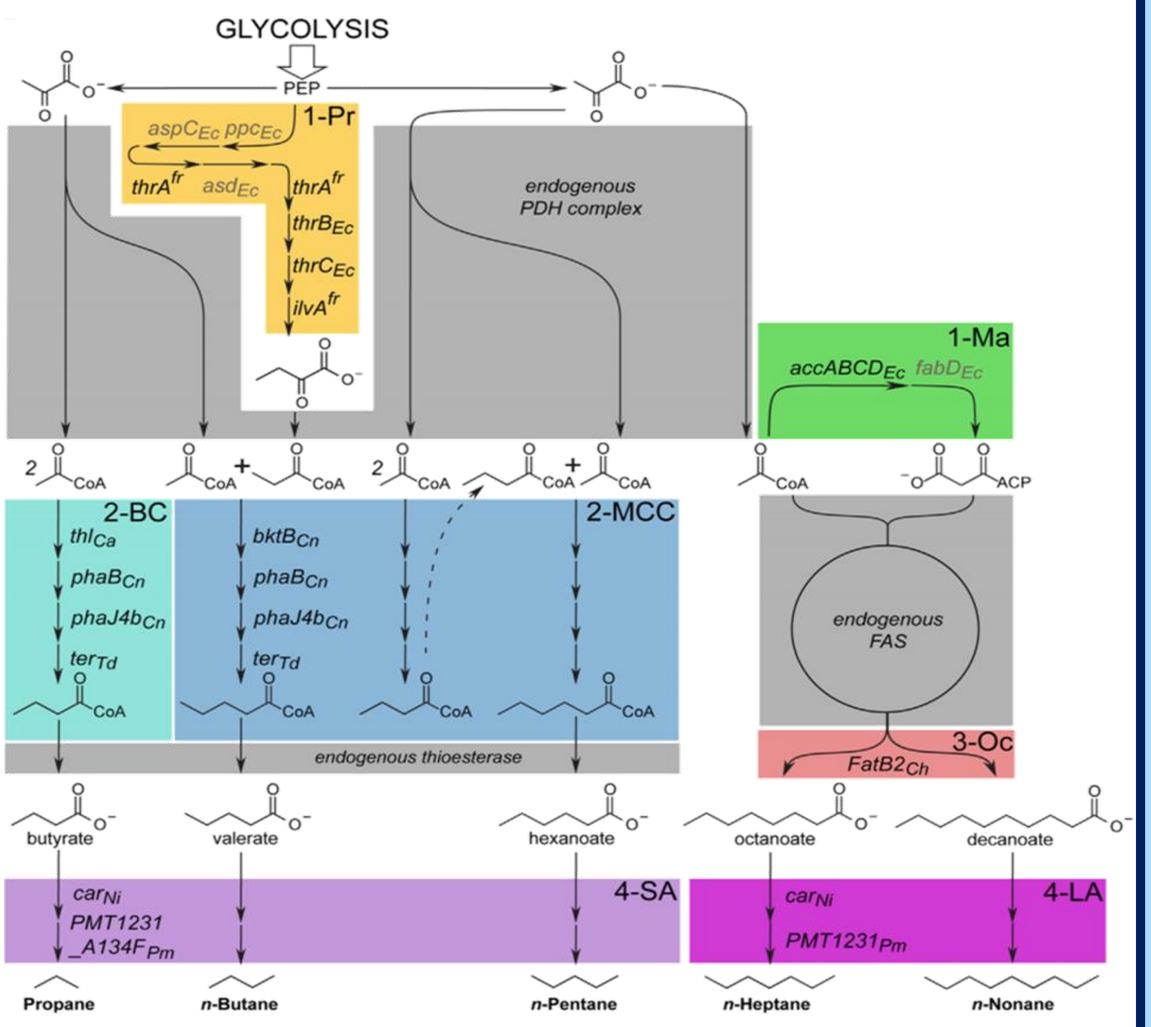


Figure 1: Pathway design for synthesis of petrol in engineered E. coli. Genes $aspC_{Ec}ppc_{Ec}$ and $fabD_{Ec}$ are native ones while all others are overexpressed genes [3].

- ***Module 1- Ma (Malonyl-ACP)***: Overexpression of an artificial operon containing genes encoding the E. coli acetyl-CoA carboxylase complex.[3]
- ***Module 3-Oc (Octanoate)***: Expression of thioesterase $FatB2m2_{Ch}$ through transformation of the E. coli strain MG1655ΔfadD.[3]
- ***Module 4-LA (Long Alkanes)***: Expression of Car_{Ni} from Nocardia iowensis with AD_{Pm}.[3]
- ***Module 2-MCC (Medium-Chain-CoA)***: Module used for hexanoate synthesis.[3]
- ***Module 4-SA (Short Alkanes)***: Expression of Car_{Ni} from Nocardia iowensis with AD_A134F_{Pm}.[3]
- ***Module 1-Pr (Propionate)***: Module used for pentane synthesis.[3]
- ***Module 2-BC (Butyrl-CoA):*** Modified version of module 2-MCC obtaining by substituting $BktB_{Cn}$ Cupriavidus necator with ThlCa from Clostridium acetobutylicum.[3]

Enzyme Engineering

FAS pathway

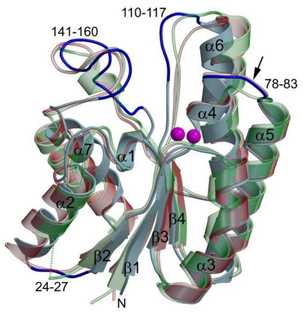


Figure 2: Thioesterase enzyme. [6]

Although TesA thioesterase preferentially hydrolyses long-chain FFAs, our engineered Thioesterase has a substrate specificity for short FFA, achieved by genetic modification of the active site. Furthermore, the preferred long-chain FFA pathway was reverted via the upregulation of *FadD* gene, by the replacement of the native promoter to strong *trc* promoter, increasing the transfer of CoA on C_8 and C_{10} molecules.[3]

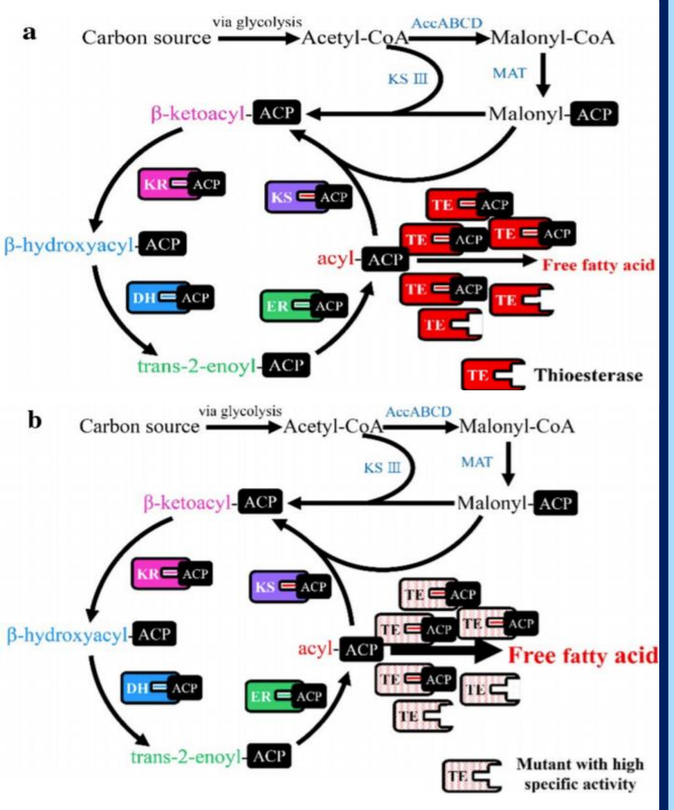


Figure 3: Schematic diagram of FFAs synthesis in E. coli. (a) limited synthesis of FFAs from a non-modified thioesterase due to stoichiometric protein–protein interactions; (b) higher FFAs generation as a catalytic active thioesterase is devised.[7]

Thioesterase enzymes contain a duplication of two 4HBT-like domains. TesA encoded proteins hydrolyse short-chain FFAS when point mutated (leucine to proline) at site 109 on the amino acid chain. The resultant change in the switch loop movement increases FFA yield and allows for controlling the chain length.[1] Additionally, a mutant thioesterase ($TesA^{R64C}$) which enables an increased (x2) production of FFAs was obtained from the following procedures:[7]

- Error-prone PCR mutagenesis of TesA followed by High-Through-put Screening (HTS).
- Catalytically active mutant thioesterases from the 'TesA mutant library are cloned into the pBbB6c-gfp vector.

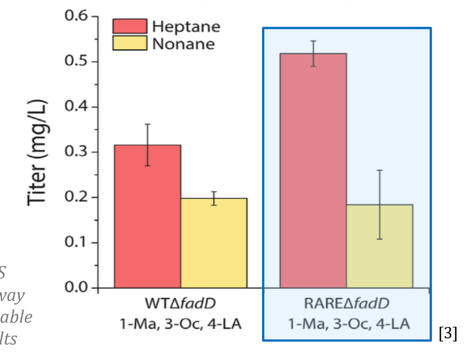
⇒ **Results**

found mutant thioesterase ($TesA^{R64C}$) performed best: substitution of arginine with cysteine at position 64.[7]

Expression of a module combination (1-Ma + 3-Oc + 4-LA) in the E. coli strain *RARE ΔfadD* as host[3]

⇓

Synthesis of relatively high amounts of **Heptane** (**0.52 ± 0.03 mg/L**)[3] and **Nonane** (**Roughly 0.2 mg/L**)[3]



FAS pathway achievable results

Expression of a module combination (2-MCC + 4-SA) in *RARE ΔendA ΔrecA*[3]

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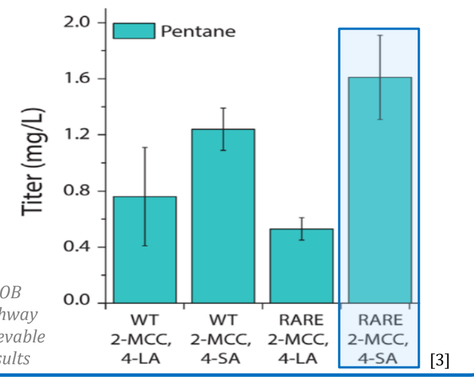
Synthesis of relatively high amounts of **Pentane** (**1.6 ± 0.3 mg/L**)[3]



ROB pathway achievable results

ROB pathway

Expression of module combination (2-BC + 4-SA)in the E. coli strain *RARE ΔendA ΔrecA* as host

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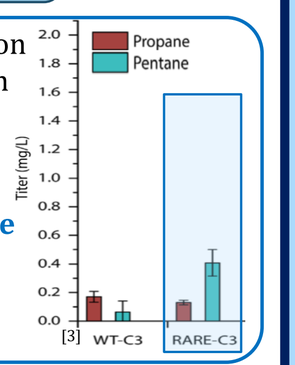
Synthesis of **Propane** (**0.13 ± 0.02 mg/L**) and **Pentane** (**0.41 ± 0.09 mg/L**)

+ additional by-products Butyraldehyde & Butanol



Expression of a module combination (1-Pr + 2-MCC + 4-SA) in the host *RARE ΔendA ΔrecA* [3]

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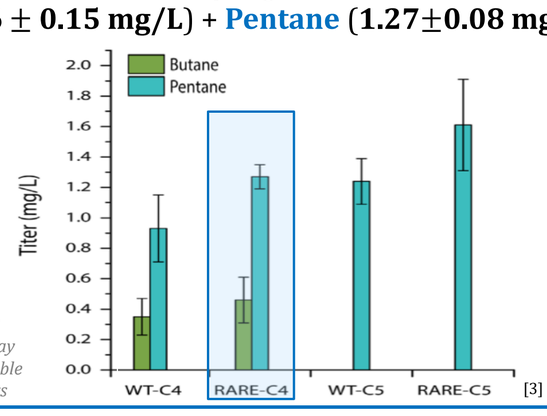
Synthesis of relatively high amounts [3] of **Butane** (**0.46 ± 0.15 mg/L**) + **Pentane** (**1.27±0.08 mg/L**)



ROB pathway achievable results

Conclusion & Potential Improvements

The use of FAS and ROB pathways enabled the synthesis of ≈ 4.59 ± 0.67 mg/L SCAs: propane, n-butane, n-pentane, n-heptane and n-nonane.[3] As these alkanes represent respectively 0.1%, 3.7%, 7.8%, 2% and 0.7% of conventional petrol, it can be concluded that the combination of these two pathways resulted in the production of 14.3% of petrol components, with the ROB pathway offering the highest potential for bio-petrol production (10.5% SCAs produced against 3.8% for FAS).[3] As 30-40% of the lignocellulosic material consists of xylose, a potential improvement for this process includes the use of both xylose and glucose as a carbon source for increased energy efficiency and alkane titer.[6] An additional improvement includes the production of a greater variety of compounds in petrol such as isopentane. As isopentane forms 9.7% of petrol composition, it would be beneficial to introduce the production of this alkane to the system.[1] This could be achieved via the FAS pathway.[1]

References

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