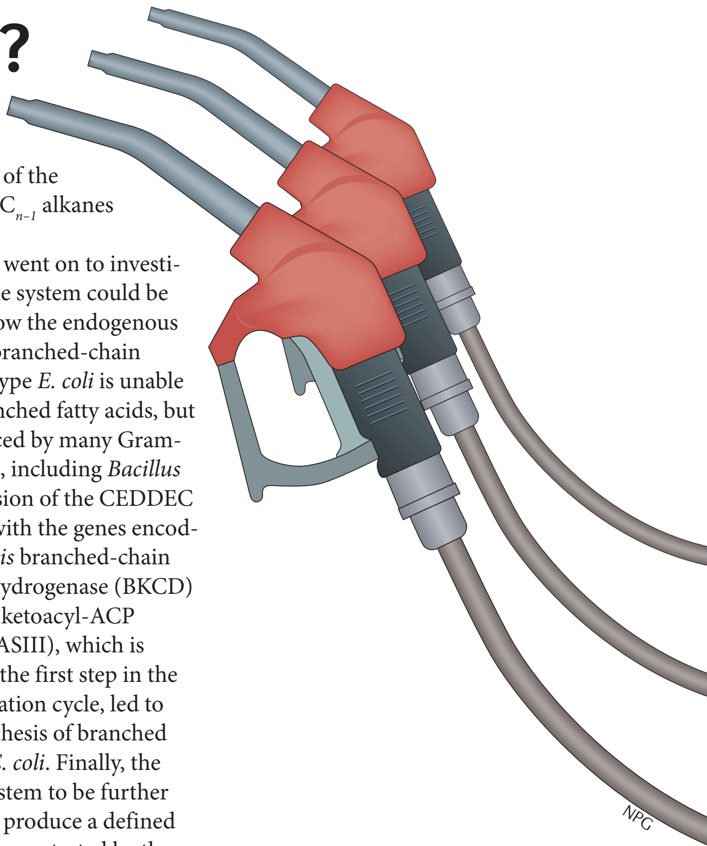
INDUSTRIAL MICROBIOLOGY

Designer biofuels?



With the ever-increasing demand for fuel, there is an urgent need to develop sustainable strategies to reduce our dependence on fossil fuels. The biofuels that are currently available require substantial downstream processing and are not fully compatible with the latest combustion engines. Now, a new paper reports on the use of *Escherichia coli* to produce 'designer' alkanes and alkenes that are chemically and structurally identical to those found in petroleum.

Fossil fuels contain a mixture of *n*-alkanes, *iso*-alkanes and *n*-alkenes of different chain lengths. John Love and colleagues were interested in investigating whether these compounds could be produced in *E. coli*. The *Photorhabdus luminescens* fatty acid reductase (FAR) complex, which is a key component of the luciferase-catalyzed bioluminescence reaction in this organism, comprises LuxC, a fatty acid reductase, LuxE, a fatty acid synthetase, and LuxD, a fatty acid transferase. Expression of *luxCED* produces a fatty aldehyde that can be converted to alkanes and alkenes by a particular aldehyde decarbonylase. The authors therefore constructed a synthetic module (named CEDDEC) comprising *P. luminescens luxCDE* and the aldehyde decarbonylase gene from *Nostoc punctiforme*. Expression of this module in *E. coli* led to the production of branched-chain fatty acids. As this system uses free fatty acids, rather than fatty acid thioesters, the authors could tailor the reaction products by varying the fatty acids that were provided in the medium, with the exogenous supply of fatty acids resulting in the production of the corresponding C_{n-1} alkanes and alkenes.

> the authors could tailor the reaction products by varying the fatty acids that were provided in the medium

The authors went on to investigate whether the system could be modified to allow the endogenous production of branched-chain alkanes. Wild-type *E. coli* is unable to produce branched fatty acids, but they are produced by many Gram-positive species, including *Bacillus subtilis*. Expression of the CEDDEC module along with the genes encoding the *B. subtilis* branched-chain α-keto acid dehydrogenase (BKCD) complex and β-ketoacyl-ACP synthase III (KASIII), which is responsible for the first step in the fatty acid elongation cycle, led to *de novo* biosynthesis of branched *iso*-alkanes in *E. coli*. Finally, the ability of the system to be further manipulated to produce a defined product was demonstrated by the co-expression of the CEDDEC module with a thioesterase-encoding gene, which led to the production of tridecane.

The product yields involved are low, and there is clearly some way to go before this research is ready for industrial production, but it provides important proof-of-principle that industrially relevant fuel molecules can be produced using a synthetic alkane biosynthesis pathway.

Sheilagh Molloy

ORIGINAL RESEARCH PAPER Howard, T. P. *et al.* Synthesis of customized petroleum-replica fuel molecules by targeted modification of free fatty acid pools in *Escherichia coli*. *Proc. Natl Acad. Sci USA* 22 Apr 2013 (doi:10.1073/pnas.1215966110)