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Mini Review Open Access

Microbial Production of Short Chain Alkanes: A Future Biofuel

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Short Review

For many decades we have been depending on fossil resources to produce liquid fuels such as gasoline, diesel, kerosene, etc. It is estimated that oil reserves mined at current rates will last only about 40 years before running dry. Increasing concerns about the global petroleum supply, environmental issues including global warming and climate change have focused attention on the need to develop alternative methods to produce fuels. Therefore, it is of paramount importance that the scientific community comes up with novel ways to obtain fuels and chemicals that are both sustainable and eco-friendly. Production of short-chain alkanes required for gasoline using *Escherichia coli* bacterium satisfies both sustainability and eco-friendliness crirerias [1].

Gasoline is a petroleum-derived product. Mainly it is used as a fuel for transportation. It is a mixture of hydrocarbons, blending agents and additives. The hydrocarbons, called alkanes, consist only of carbon and hydrogen atoms. Gasoline has a combination of straight-chain and branched-chain alkanes (hydrocarbons) consisted of 4-12 carbon atoms linked by direct carbon-carbon bonds.

Previously, there has been success in the past at metabolically engineering *Escherichia coli* to produce 13-17 atom long-chain hydrocarbons, which is suitable for replace diesel [2]. However, the first report on the microbial production of short-chain alkanes was released by a team at the Korea Advanced Institute of Science and Technology (KAIST). They have developed a novel strategy for microbial gasoline production through the metabolic engineering of *Escherichia coli*. The team engineered platform *Escherichia coli* strains that are capable of producing short-chain alkanes via the fatty acyl-ACP to free fatty acid to fatty acyl-CoA pathway [1].

In the beginning, in order to maximize the production of short-chain alkanes in vivo, the production of free fatty acids needed to be maximized. Therefore genetic modifications have been made to *E. coli* genome to increase the formation of short-chain fatty acids suitable for subsequent conversion to short-chain alkanes. The fadD gene of *E. coli* was deleted to prevent conversion of free fatty acids (both short-chain and long-chain) to fatty acyl-CoA. And fadE gene was deleted from the *E. coli* to prevent the beta-oxidation that degrades the fatty acyl-CoA generated in vivo. The FabH enzyme promotes the initiation of fatty acid biosynthesis. But the unsaturated fatty acyl-ACPs inhibit the activity of FabH enzyme. Therefore the fadR gene was deleted to prevent the up regulation of the fabA and fabB genes responsible for unsaturated fatty acid biosynthesis [3], which in turn enhances the initiation of fatty acid biosynthesis. After the deletion of genes, expression vectors have been introduced into the *E. coli* bacteria in order to produce short chain alkanes from those free fatty acids. Four enzyme activities are introduced into the *E. coli* using two expression vectors called pTrcAcR'TesA which contains tesA and acr genes that are responsible for producing modified thioesterase and fatty acyl-CoA reductase respectively and pTacCer1fadD which contain CER1 and fadD genes that are responsible for producing fatty aldehyde decarbonylase and fatty acyl-CoA respectively.

Once a glucose molecule enters into the genetically modified *E. coli* it undergoes glycolysis and forms pyruvic acid. Then it gets converted into acetyl-CoA under the presence of pyruvate dehydrogenase enzyme [4]. The acetyl-CoA is converted into fatty acyl-ACP in the presence of Acetyl CoA:ACP transacylase. In this strategy, acetyl-CoA is used directly for fatty acid chain elongation, allowing for improved carbon and energy efficiency compared to the fatty acid biosynthesis pathway which requires activation of acetyl-CoA to malonyl-CoA. To produce biofuels with an even-numbered carbon chain, the fatty acyl-ACP is cleaved by a modified thioesterase [5] releasing the corresponding free fatty acid. The thioesterase is yet another key target for metabolic engineering. Free fatty acids get converted into fatty acyl-CoA under the presence of fatty acyl-CoA synthetase which is derived from fadD gene. Fatty acyl-CoAs were reduced to fatty aldehydes and then to short-chain alkanes which are required to produce gasoline using fatty acyl-CoA reductase and fatty aldehyde decarbonylase respectively.

Free fatty acids are converted into their corresponding short chain alkanes (petrol) by introducing a new synthetic route and optimizing culture conditions. The final engineered strain produced upto 580.8 mg/l of short chain alkanes consisting of nonane (327.8 mg/l), dodecane (136.5 mg/l), tridecane (64.8 mg/l), 2-methyl-dodecane (42.8 mg/l) and tetradecane (8.9 mg/l), together with small amounts of other hydrocarbons [1].

Pumping out petrol with bioengineered microbes is an excellent new starter platform for the sustainable production of biofuels.

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