221. Production of anteiso-branched fatty acids in *Escherichia coli*, next generation biofuels with improved cold-flow properties

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Abstract
A major disadvantage of fuels derived from biological sources is their undesirable physical properties such as high cloud and pour points and high viscosity. Here we report the development of an *Escherichia coli* strain that efficiently produces anteiso-branched fatty acids, which can be converted into downstream products with lower cloud and pour points compared to less heterogeneous mixtures produced via the native metabolism of the cell. This was achieved through the deletion of *metA, tdh, ilvB*, and *ilvN* and the overexpression of *thrABC* from *E. coli*, *ilvA* from *Corynebacterium glutamicum*, *ilvGMCD* from *Salmonella typhimurium*, as well as *bFabH2* and the *bkd* operon from *Bacillus subtilis*, which together promote the synthesis of the 2-methylbutyryl-CoA and use this metabolite to prime fatty acid synthesis. When these genetic manipulations are coupled with those that promote free fatty acid synthesis and accumulation, 22% of the free fatty acids produced in the engineered *E.coli* cells were anteiso-branched. This work addresses a serious limitation that must be overcome in order to produce renewable biodiesel and oleochemicals that perform as well as their petroleum-based counterparts.

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