Engineered for tolerance, bacteria pump out higher quantity of renewable gasoline

November 5 2014

Aindrila Mukhopadhyay and Heather Jansen engineer *E. coli* to produce biogasoline at JBEI. Credit: Berkeley Lab - Roy Kaltschmidt

An international team of bioengineers has boosted the ability of bacteria to produce isopentenol, a compound with desirable gasoline properties. The finding, published in *mBio*, the online open-access journal of the American Society for Microbiology, is a significant step toward developing a bacterial strain that can yield industrial quantities of renewable bio-gasoline.

The metabolic engineering steps to produce short-chain alcohol solvents like isopentenol in the laboratory bacteria Escherichia coli have been worked on extensively by many research groups, explains Aindrila Mukhopadhyay, director of host engineering at the Joint BioEnergy Institute in Emeryville, California and senior author on the study.

"Biofuels are one tool in the array of alternative energy solutions that can be used in our infrastructure immediately," she says. Sustainably produced fuel compounds can be added directly into gasoline blends used today to offset reliance on fossil fuels and also lower the net carbon emissions from vehicles.

"But the solvent-like compounds inhibit microbial growth and that was an aspect that we realized would come up sooner rather than later," says Mukhopadhyay, who holds a joint appointment at Lawrence Berkeley National Laboratory. "We wanted to look at that aspect with a systems biology approach—could we engineer bacteria to also tolerate the solvent it is producing?"

Improving tolerance is key to moving production toward levels that are industrially relevant. Industrial production requires a robust strain that can stably produce for longer periods of time and withstand the accumulation of the solvent-like biofuel.

To address this challenge, the team, which also included researchers from Nanyang Technological University in Singapore, National University of Singapore, and the University of California, Berkeley, treated a non-producing E. coli strain with isopentenol by adding it to the culture. As the bacteria responded to the solvent-stressor, the team measured which genes were shifted up or down by looking at messenger RNA transcripts across the entire genome.

They chose 40 genes that the bacteria cranked up in response to isopentenol—presumably because their actions helped mitigate the toxicity in some way. Next, they overexpressed each one in a bacterial strain actively producing isopentenol to see which ones might improve the strain's growth.

Of the eight genes that rescued growth, two stood out as promising—MetR, a biosynthesis regulator, that improved isopentenol production by 55%, and MdlB, a transporter, that improved production by 12%. If the researchers bumped up the levels of the MdlB transporter protein inside the cells even further, they saw production improve by as much as 60% over the original strain.

"Finding a transporter really appealed to us because it has the potential to export the final solvent product out of the cell," says Mukhopadhyay. "And in this case, once enough alcohol gets outside the cell, it might phase separate and not even be accessible to the organism anymore." In other words, the biofuel would separate away to sit atop the watery broth the bacteria live in.

As an added bonus, the MdlB protein is a good candidate for directed evolution experiments that could improve the performance and specificity of the transporter for shuttling isopentenol out as quickly as possible. Combining a more efficient transporter with other genes that improved tolerance might produce a strain that can generate bio-gasoline for the gas pump in the near future.

More information: The article can be found online at mbio.asm.org/content/5/6/e01932-14

Provided by American Society for Microbiology