Designer Microbes Make Green Surfactants

Surfactants enable the stable mixing of oil and water, the stable suspension of solids in liquid, and the stable suspension of gas in liquid. They are the chemical components that give products such as laundry detergent and shampoo their unique cleaning power. Surfactants are used in a broad range of industries, from personal care products to mining.

Surfactants are typically made from petroleum-based feedstock or palm oil. Producing surfactants from petroleum creates greenhouse gases, while producing them from palm oil is linked to deforestation. Another disadvantage of existing surfactant manufacturing processes is that they involve the use of toxic chemicals (*e.g.*, phosgene gas) and generate hazardous byproducts, including 1,4-dioxane and nitrosamines.

Massachusetts-based biotech company Modular Genetics, Inc., which specializes in engineering microorganisms to synthesize useful chemicals from inexpensive, renewable feedstocks, is using a National Science Foundation grant to address these problems. The company has engineered *Bacillus subtilis* strains to convert cellulosic sugar into a surfactant consisting of a fatty acid linked to an amino acid. Its approach uses no oil of any sort (no petroleum or palm oil) or toxic chemicals, and the surfactants do not generate hazardous byproducts.

B. subtilis encodes a modular peptide synthetase enzyme, which naturally produces surfactin — a lipopeptide surfactant consisting of a fatty acid chain of 13–16 carbons linked to a peptide ring of seven amino acids. Each peptide synthetase module is specific for a particular amino acid, and the modules work together to catalyze the linear stepwise assembly of particular peptides, the order of which is determined by the linear order of the enzyme modules.

Each enzyme module contains a condensation domain, an adenylation domain, and a thiolation domain. The adenylation and thiolation domains bind amino acids. Modular Genetics and others have shown that these enzyme modules can be moved around, removed, and replaced to produce different derivatives of surfactin. Condensation domains typically catalyze the joining of one particular amino acid to another specific amino acid. The condensation domain of the first module of the enzyme is special. Rather than catalyzing the joining of one amino acid to another, it catalyzes the linkage of particular fatty acids to the first amino acid of the surfactin peptide (glutamate), via an amide bond.

Instead of shuffling around enzyme modules, Modular Genetics employs synthetic biology tools to create hybrid enzymes. The company has patented its technology for synthesizing acyl amino acid surfactants in *B. subtilis* and used it to make an acyl glycinate surfactant known as FA-Gly. To do this, the company engineered a *Bacillus* strain that expresses fusion proteins composed of a fatty-acid-incorporating (condensation) domain linked to a glycine-incorporating (adenylation and thiolation) domain. The particular fatty-acid-incorporating domain used in this case has a preference for myristic acid, a 14-carbon fatty acid. Other naturally existing domains have different specificities, preferring fatty acids ranging from 6 to 18 carbons. In addition, the amino-acid-incorporating domain can be designed to be specific for amino acids other than glycine.

▲ Modular Genetics has used synthetic biology tools to engineer a *Bacillus subtilis* strain to convert cellulosic sugar via fermentation into an acyl glycinate surfactant, which can be used in personal care products such as shampoo.

About 300 unique naturally occurring amino-acid-incorporating domains are known. The successful NSF-funded feasibility study suggests that Modular Genetics' technology enables custom design and synthesis of a virtually limitless array of surfactants by specifically linking nearly any fatty acid to nearly any amino acid. Significantly, these biodegradable surfactants can be manufactured from cellulosic sugar using the natural process of fermentation. Vast quantities of cellulosic material are generated on a global scale that are either wasted or burned. Modular Genetics has shown that FA-Gly can be produced from sugar derived from rice hulls — a byproduct of rice production.

"Modular's approach of using engineered microorganisms to convert underutilized agricultural material into useful chemical products through microbial fermentation has enormous potential in addressing our future needs," says K. P. Ananthapadmanabhan, Senior Principal Research Scientist at Unilever, which is testing the new surfactant.

Modular Genetics expects to begin selling FA-Gly commercially by the end of 2016.

This technology was funded through the NSF Small Business Innovation Research Program.

This article was prepared by the National Science Foundation in partnership with *CEP*.