Bacterial BUILDERS

Frances Arnold is directing bacteria to build molecules never before assembled in nature.

by Emily Velasco

Chemistry is on the verge of a bacterial revolution. Fast-forward into the future, and you might find that the polyester in a Hawaiian shirt, the rubber in a new set of tires, or even Grandma's blood pressure medication was made in much the same way beer is brewed: by harnessing the powers of the tiny unicellular organisms that are ubiquitous in our world.

Sound far-fetched? The yeast that have been used to make beer for hundreds of thousands of years, as well as some even smaller bacteria, are already being co-opted to create materials like spider silk, biodegradable plastics, vitamins, insulin, and human growth hormone. Scientists perform such transformations by pulling the genetic material out of another organism—a spider, for example—and inserting it, with a few edits and additions, into a microorganism. When the modified organisms return to their normal microbial business, they will also churn out what their new DNA tells them.

While effective, the method works only if a living creature already exists with DNA encoded for the desired product and if the DNA can be appropriately modified to be read by the microbes. But what are scientists to do if they want bacteria to make something that is not already a natural, organic, preexisting compound?

They give evolution a little nudge.

One way to do that is through a process familiar to breeders of all sorts of domesticated animals. When ancient humans wanted wolves as hunting companions, for instance, they picked the friendliest wolf cubs and kept breeding them and their friendliest offspring until they arrived at man's best friend, in all its different forms.

The difference is that today when researchers want new breeds of bacteria, they can deliberately tweak the genes directly instead of just waiting for useful mutations to arrive by accident. It is a process known as directed evolution, first developed by Caltech chemical engineer Frances Arnold in the early 1990s. In the years since, she and her colleagues have created genes that encode not just better versions of enzymes that already exist but genes that compel the organisms to perform a variety of chemical feats never before seen in nature.

"You mutate a gene, see which progeny have the properties that you want, pick the one or ones that you like, and repeat the process," says Arnold. "You keep doing it over and over again until they do what you want them to. The great thing about bacteria is that you can do this very quickly because bacteria reproduce rapidly. A generation can be as short as a few days, sometimes even shorter."

In 2016, Arnold and her group announced that they had used directed evolution to create enzymes that enable bacteria to bond silicon atoms to carbon atoms. Though silicon-carbon bonds are common in synthetic materials (think silicone caulk), such bonds in organic materials had previously only been created by chemists.

"No living organism is known to put silicon-carbon bonds together, even though silicon is so abundant, all around us, in rocks and all over the beach," says Jennifer Kan, a postdoctoral scholar in Arnold's lab and the lead author on the paper describing the advance.

To create this new capability, they started with a gene from a bacterium that lives in Icelandic hot springs; Kan discovered that this bacterium already produced a protein that could bond silicon to carbon under certain laboratory conditions, albeit not particularly well. Their new enzyme—a common protein called cytochrome c—was then shaped by the researchers through directed evolution to produce an efficient enzyme dedicated to making new silicon-carbon bonds. Within three generations, they had a bacterial enzyme that was 15 times more efficient at combining silicon and carbon than the very best chemist-created catalysts for the same reaction.

"This iron-based, genetically encoded catalyst is nontoxic, cheaper, and easier to modify compared to other catalysts used in chemical synthesis," says Kan. "The new reaction can also be done at room temperature and in water."

A year later, Arnold's team announced another creation: bacteria that could form carbon-boron bonds. This, too, was a first for a living creature; no organism in its native state is known to make bonds between boron and carbon. They started with the same gene for cytochrome c from the Icelandic bacteria they had used before but pushed it in a different direction. This time, the enzyme they created was 400 times more efficient than comparable human-made catalysts. The group's paper describing the boron-carbon bonds was published in October of last year in the journal *Nature*.

"We have given life a whole new building block that it did not have before," says Arnold. "This is just the beginning. We've opened a new space for biology to explore, a space that includes useful products invented by humans."

Her group's discoveries and creations piled up. Arnold and her colleagues have evolved bacterial enzymes that can create amino acids that do not exist in nature—amino acids that may become the basis of future pharmaceuticals. They have evolved bacteria that create a red fluorescent protein that can be used for imaging other cells under a microscope. And, in April of this year, they announced that they had evolved an enzyme that enables a common strain of *Escherichia coli* to make tiny, energy-packed carbon rings that are rarely found in nature.

These rings, called bicyclobutanes, contain four carbon atoms arranged to form two triangles that share a side. Bicyclobutanes are difficult to make because the bonds between the carbon atoms are bent at angles that put them under a great deal of strain; bending these bonds away from their natural angle takes energy. It is that strain that makes bicyclobutanes valuable, however, since the bent bonds pack energy that can drive chemical reactions, making bicyclobutanes useful precursors to various types of materials, pharmaceuticals, and agrochemicals.

"I have to say we are flabbergasted at the sorts of things that we can make and how inventive evolution can be," Arnold says. She points out that, even without human help, bacteria are adept at developing biological solutions to chemistry problems, like breaking down antibiotics so they can thrive where humans do not want them to grow. It seems reasonable, then, to use those powers to create manufacturing processes that are both more environmentally friendly and potentially less expensive than traditional chemical processes.

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In recognition of "her discoveries that launched the field of 'directed evolution,'" Arnold was awarded the Millennium Technology Prize in 2016. The prize, worth 1 million euros, is considered one of the most prestigious awards for technological innovations.

"The big question is how I best use biology's inventive capabilities to create new chemistry that would be useful not to a bacterium but to me," Arnold says. "Can I use these remarkable evolutionary mechanisms to innovate in real time and program microorganisms to do what human chemists struggle with?"

Today, the answer, it seems, is yes. As the technology Arnold invented 25 years ago has matured, it has become more accessible: high school students are conducting experiments in which they direct new genes to evolve, for instance, and amateur biologists across the country have organized themselves under a banner called DIYBio that conducts, among other things, directed evolution experiments. One DIYBio group in the San Francisco Bay Area used directed evolution to breed bacteria that can produce sunblock.

Arnold predicts that in the decades to come directed evolution will tackle problems of growing complexity, such as evolving multiple genes at once, and will be aided by emerging technologies like machine learning and ever-faster methods of test-tube evolution. Her goal, however, will remain the same.

"We would like to be able to program sustainable, self-replicating microorganisms to make catalysts for the cost of the sugar to feed them," Arnold says. "We would like for them to produce many of the materials, chemicals, pharmaceuticals, and even fuels we use in our daily lives and do so cleanly and efficiently, using renewable resources."

Frances Arnold is the Linus Pauling Professor of Chemical Engineering, Bioengineering and Biochemistry and director of the Donna and Benjamin M. Rosen Bioengineering Center. Her work on directed evolution is supported by the National Science Foundation, the National Institutes of Health, the Jacobs Institute for Molecular Engineering for Medicine, the Army Research Office, and the Rothenberg Innovation Institute.

Frances Arnold pioneered directed evolution and has been a driving force for its development. The technology is increasingly being used to create synthetic materials using biological means.