In Search of the Plastic Potato

Scientists in the emerging field of biopolymer engineering are aiming to produce bacteria and, eventually, food crops that are genetically tailored to yield a whole new breed of plastics

EVERYONE KNOWS THAT THE TOMATOES at the supermarket tend to taste like plastic, but just wait. Some crops of the future may really be plastic.

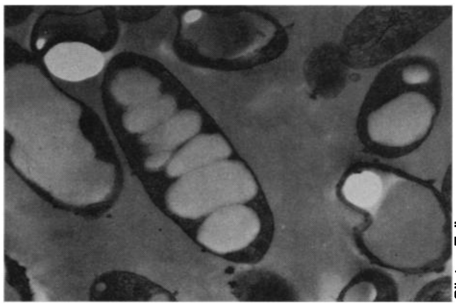
Researchers are now experimenting with ways to grow plastics instead of manufacturing them. Already, one chemical company is using vats of bacteria to produce a polymer that can be processed into a polypropylene-like plastic. And several research teams are trying to modify the genetic structures of bacteria to create new products, including synthetic rubbers and unusual types of plastics.

Some scientists even dream of putting genes for plastic production into food crops. In this fantasy, the farmers of tomorrow will raise polyesters right alongside potatoes.

Sound absurd? Millions of dollars are now being invested worldwide—with the promise of hundreds of millions to come—into the study of biologically produced plastics. The impetus: a mixture of environmental, commercial, and scientific factors. These "biopolymers" are by their nature biodegradable, and they could eventually become a renewable source of plastics not dependent on the supply of petroleum. Although biopolymers are relatively expensive now, they have the potential of becoming cheaper than the synthetic polymers used in conventional plastics, especially if the dreams of plastic corn and plastic potatoes come true.

But to the researchers engaged in this plastics revolution, the greatest appeal of biopolymers lies in the scientific arena, where biologically produced polymers promise to open up a whole new area of study. With the enzymes of nature at their disposal, chemists say they will be able to exert an unprecedented amount of control over the structures of the materials they build, allowing them to custom design strange new types of plastics, rubber-like substances, and perhaps some materials unlike any ever seen before.

The current explosion of interest in biopolymers was ignited last year when researchers at James Madison University in Virginia announced they had cloned the polymer-making genes from a bacterium that naturally produces a plastic-like polymer. Since then, the number of journal articles on the subject has jumped dramatically, and Massachusetts Institute of Technology biochemist Anthony Sinskey predicts the field is "going to be filled with ooh and ahh people in the near future." In Japan, the government has announced a $200-million research program in biopolymers, and in Europe and the United States both industrial and university labs are getting into the act.


Clinton Fuller

Miniature plastics factory. *The large oval objects in the* Rhodobacter sphaeroides *bacteria are granules of polymers (magnification ×20,000).*

The natural first step down this path was actually taken several years ago when Imperial Chemical Industries of England, with little fanfare, began exploiting a common bacterium that produces a form of polyester naturally. At a small pilot plant in Billingham, England, ICI grows vats of *Alcaligenes eutrophus*, a bacterium that turns out a polymer called polyhydroxybutyrate (PHB). PHB plays the same role in the bacterium that fat does in humans or starch in plants. A single molecule of PHB consists of thousands of hydroxybutyrate molecules linked end to end, and in bulk, PHB is a stiff, brittle plastic that can be used for plastic soft-drink bottles.

It is possible to grow colonies of *A. eutrophus* that are more than 80% polymer by dry weight. The bacteria are grown in a glucose solution, but deprived of nitrogen, which the bacteria interpret as a sign that rough times are ahead. Much as bears add on an extra layer of fat before hibernation, the bacteria then devote most of their resources to making PHB.

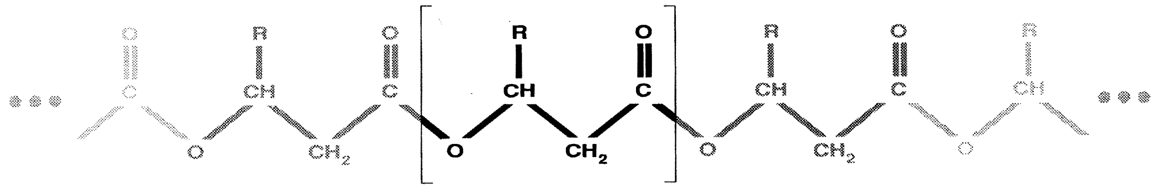
Unfortunately, it is not such a simple matter to harvest the polymer from the bacteria, says Thomas Galvin, new ventures manager for ICI Americas. The PHB is stored inside the cell walls in granules, each of which consists of thousands of individual polymer chains. In order to recover the polymer, the cell walls must be broken apart and the PHB separated from the cell debris, all without damaging the granules. And after this is done, it is tricky to process the polymer into a plastic, since PHB's melting temperature (170°C) is nearly as high as the temperature at which it decomposes.

ICI gets around the difficulty by the clever ploy of convincing the bacteria to make a different, more workable polymer. When the bacteria are grown on a mixture of glucose and organic acids instead of just glucose, Galvin says, they end up making a copolymer—a chain of two different types of molecules. In particular, the bacteria add 5 to 20% hydroxyvalerate molecules into the chain of hydroxybutyrate molecules, and the resulting polymer, called PHB-V, is stronger, more flexible, and has a lower melting temperature than PHB.

PHB-V has properties very similar to polypropylene and can be used for plastic films and bags. Its advantage over normal plastics is that it is biodegradable—since it is produced by bacteria as an energy source, it can also be completely broken down by bacteria into water and carbon dioxide. Potential uses for it include not only plastic products that will break down in the environment but also medical implants, such as sutures or drug delivery devices, that will gradually degrade in the body.

ICI's pilot plant is now turning out about 50 tons of PHB-V a year, Galvin says, and is being expanded to 500 tons. That expansion will halve the current price of $15 a pound, he says, still far from competitive with commodity plastics, which sell for around 50 cents a pound, but in the right ballpark for specialty markets, such as medical implants. Galvin says the company calculates that a 10,000-ton plant could produce PHB-V that would sell for $2 a pound and be profitable.

As ICI is making plans to produce more PHB-V, research is under way at a number of institutions on the next stage in developing biopolymers: altering the bacteria's genetic structure to improve the production of



Biopolymer blueprint. *The general structure for PHAs. R is an alkyl group; if R is methyl (CH_3), the result is PHB.*

the plastic and to make new kinds of polymers.

In 1987, Douglas Dennis at James Madison University in Virginia succeeded in cloning the genes in *A. eutrophus* that tell the bacterium how to make PHB. Dennis then transferred those genes into another bacterium, *Escherichia coli*, and showed that the modified bacteria produce PHB, something normal *E. coli* do not do.

The importance of this step, Dennis says, is that now all of the tools developed for working on *E. coli*—which is the standard laboratory bacterium—can be brought to bear on the PHB genes. "Now that it's in *E. coli* we can do a lot of genetic manipulation," he says.

For example, Dennis notes that one *E. coli* mutant has cells that are ten times larger than normal. Putting the PHB genes into this form of *E. coli* might produce a bacterium that makes ten times as much polymer per cell, he suggests, which could make the polymer much easier to process.

Ironically, Dennis did not realize the potential commercial value of his work at first. He and his co-workers had cloned the PHB genes because they were interested in the scientific question of which mechanisms turn PHB production on and off, and they published their results before patenting them. Fortunately, the United States allows patent applications up to a year after a discovery has been publicized. But "in Europe it's gone," Dennis says.

In Austria, Werner Lubbitz of the University of Vienna is using Dennis's cloned genes to develop commercial production of PHB and PHB-V from *E. coli*. Working with the Biotechnologische Forschung, a biotechnology consortium founded by several companies, Lubbitz inserted Dennis's PHB genes into a strain of *E. coli* that bursts when heated to a certain temperature. In this way, Lubbitz says, he can avoid exposing the bacteria to a solvent or some other damaging treatment in order to get them to release the polymer granules. With his new strain of bacteria, a worker can grow the cells at 28°C until they have filled up with PHB and then raise the temperature to 42°C. Ten minutes later, the *E. coli* split open and spit out the polymer—no solvent, no heat or acid, nothing to harm the polymer or make its recovery more difficult. "That is a major breakthrough," Lubbitz says. With such a genetically modified bacterium, it should make it possible to produce the polymer more cheaply than ICI can, he says.

There are still a few problems with the genetically engineered bacteria, however. So far, *E. coli* does not produce PHB-V the same way that *A. eutrophus* does when fed the same diet of sugars and organic acids. Nor does *E. coli* commit 90% of its dry body

Redesigning Nature's Plastics Factories

At the Massachusetts Institute of Technology, Anthony Sinskey and ChoKyun Rha are studying the mechanics of how bacteria manufacture their energy-storing polymers. "We focused on understanding the pathway of assembling at first PHB and now, more generally, PHAs," Sinskey says. His team wants to understand the role of the different enzymes that direct the production of the polymers, with the goal of eventually controlling that production.

PHB production in *A. eutrophus* is carried out by three enzymes, Sinskey says. The first joins two molecules of acetyl-CoA, a basic metabolic molecule found in all organisms, to form acetoacetyl-CoA. A second enzyme modifies the composition of this molecule slightly, and a third enzyme links these modified molecules together to make the polymer. The third enzyme is apparently the key to explaining why *A. eutrophus* makes different polymers when fed on different substrates, but since no one has identified the enzyme's structure, the process remains a mystery for now.

Rha, who describes herself as a molecular engineer, is interested in the structure and design of bacteria-produced polymers. Rha's goal is to discover how the structure of a biopolymer determines its properties and to learn how to devise new polymers that will have certain desired characteristics.

"What's so exciting about biopolymers," Rha says, "is that even if you take the simplest glucose molecule, there are eight different ways to link those glucose molecules together." If the glucose units are strung together in one way, it will produce a very flexible molecule; a different linkage will result in a very strong, rigid polymer. Thus, even biopolymers constructed from a single building block can exhibit a whole range of properties depending on how they are put together. And, she adds, "When we start to add functional groups to these monomers, there is a whole new dimension."

Rha has a list of things she would like to try with biopolymers. By adding certain side groups to the long backbone of the polymer, for instance, it should be possible to put either a positive or negative charge on the molecules. Then the electrostatic attraction between the positively and negatively charged polymers could be used to bind them together to make hollow capsules that could be deployed, for example, in drug-delivery systems for implantation in the body.

With bacteria to do the work, it should also be feasible to build polymers with a row of side groups running along either side of the central chain—"like centipedes," Rha says. These rows of side groups should prevent the molecule from balling up, which would make the resulting polymer very stiff, she suggests. "We cannot produce such complicated molecules with any specificity using normal chemistry," Rha says. "The true advantage of biopolymer engineering is that we can specify such complicated designs. Biological systems can make it exactly so."

Sinskey says there are several ways to control the type of polymers produced by a bacterium. One is to vary the feedstock, which is straightforward and is being done in a number of places. Others involve changing the enzymes—the types of enzyme, their specificity, or when they are expressed—in order to alter the production process inside the bacteria. This is more difficult, but Sinskey has taken the first step. He has recently modified a single enzyme in one of the plastic-producing bacteria, he says, but he has not yet had time to see what the altered bacteria will produce.

■ R.P.

weight to the polymer; 70% is the norm, Lubbitz says.

This hasn't dampened interest in his technique from investors, though. Lubbitz says a group of venture capitalists from Austria, Germany, and the United States has expressed interest in building a plastics plant that will use the modified *E. coli* to produce PHB-V, and several chemical companies have said they would consider buying the plastic from such a plant.

In the United States, there are at least two full-scale biopolymer engineering programs under way. Both groups, one at MIT and the other at the University of Massachusetts, are treating the field as a blend of molecular biology/biochemistry and polymer chemistry. The biologists fiddle with the bacteria, and the chemists analyze the polymers that result. In the long run, they hope to be able to design new polymers and then program the bacteria to produce them.

At the University of Massachusetts, Robert Lenz and Clinton Fuller are studying the range of polymers produced by various bacteria. They point out that PHB is only one of a number of types of polymers naturally produced in different bacterial species. The general class of polymers used by bacteria for energy storage is the polyhydroxyalkanoates, or PHAs. The PHAs differ from one another by only a single group of atoms in the base molecule, but their properties vary widely.

So far, Lenz says, he and Fuller have raised five or six species of bacteria on different feedstocks to see what comes out, not only when they are given their normal food but also when they are fed organic molecules they would not see in nature. "You feed the bacteria different stuff, and they either die or make a new energy storage material," Lenz explains.

As an example, he says that by using an aliphatic acid with an aromatic group as the sole feedstock for one bacteria, they have made a polymer containing that aromatic group. "It is unlike any found in nature," he says.

Fuller and Lenz have been working intensely on a bacterium that lives on hydrocarbons—octane, nonane, and decane—and produces an elastic polymer with properties similar to rubber. Because of its biodegradability, the elastomer might be handy in grafts of blood vessels, Lenz suggests. He and Fuller have also studied a photosynthetic bacteria, *Rhodospirillum rubrum*, that uses energy from sunlight to produce a new class of polyesters.

Fuller, a biochemist, says the chemical skills of bacteria are a constant source of amazement for Lenz, who is a polymer chemist. "Bob comes to me and says, 'Clint, your bugs can do overnight what it took me 10 years to do.' "

Bacteria can produce polymers faster, with high molecular weight, purer, and cheaper than can chemists, Lenz says. "If we had to make PHB chemically, instead of $2 a pound it would be out of sight—at least $100 a pound," he says.

The Office of Naval Research has supplied the funding for much of the biopolymer research in the United States. Michael Marron, manager of ONR's molecular biology program, says he sees several reasons to synthesize polymers biologically rather than chemically. With bacteria, you can "tailor things at the molecular level," making polymers of a purity and specificity that are impossible with normal chemical means. He also suspects that in the long run, biopolymers could be cheaper than polymers made from hydrocarbons.

"One of the ultimate strategic considerations is the dependence on petroleum," he adds. If polymers could be grown in food crops, it would give the United States control over its plastic supply, "but that's really a pipe dream at this time."

Illustration by Y. Rook.

Nonetheless, it is a dream that a number of people share. "It would be wonderful to put this stuff into corn because corn starch is so cheap," says Chris Somerville, a biochemist at Michigan State University's Plant Research Laboratory. Somerville is putting the PHB genes from *A. eutrophus* into several different plants in an effort to get them to produce the polymer.

The ideal accomplishment would be to modify plants such as corn or potatoes so that instead of producing starch they produce plastic. There are some huge hurdles, of course. Inserting the genes for the various polymer-making enzymes into the plants is a relatively simple problem, Somerville says, but regulating those enzymes once they are in the plants is not. And once the plant is producing PHB, it will be necessary to turn off the starch production so that the plant devotes all its energy to making the polymer. "This will be the most challenging part," he says, "because we can't simply add a new enzyme. We will have to block the activity of existing enzymes." And plant cells will probably present more of an obstacle than bacterial cells to getting the polymer out.

Even with these problems overcome, one more obstacle would remain to the creation of plastic-producing corn. "The plants can't metabolize the PHB, so the seeds wouldn't grow," Somerville points out. Somerville foresees several possible solutions. One would involve crossing two non-polymer-producing lines of corn, each of which has some of the enzymes needed to make the PHB, to get a non-fertile, polymer-making hybrid. Another might be to put enzymes into the corn that would allow the seeds to break down the PHB into something they could use to grow.

However, Somerville says he will probably avoid this problem altogether and try potatoes or turnips first. In these plants the tubers or roots serve only as storage organs and are not essential to reproduction. It should be possible, Somerville says, to modify the potato so that the tuber manufactures polymer in place of starch, but the rest of the potato's functioning would be unchanged. The result would be a potato that looks very much like the ones in the grocery store but that is not too tasty. "The joke in the lab is that we're making plastic potatoes to go with the plastic tomatoes in the supermarket," Somerville says.

At least one agricultural company has recognized the potential of turning farms into plastics factories. Pioneer Hybrid, a major seed producer in the Midwest, has given Sinskey's group a $25,000 grant. "I see it as a long-range project, but we want to be in on the bottom floor," says Barry Martin, a research specialist at Pioneer. Martin acknowledges the difficulties involved in altering corn to produce polymers—"We're thinking 10 years before we even see a glimmer in this thing"—but he is also aware of how revolutionary it would be to be able to produce plastics as cheaply as corn starch, say about 10 cents a pound.

Give the American farmer the chance at a cash crop like this, and he might very well change "amber waves of grain" into "amber waves of plastic." ■ **ROBERT POOL**