

# HARNESSING MICROBES IN THE BATTLE AGAINST MICROPLASTICS

With seas and soils increasingly contaminated by tiny pieces of plastic, **JAPANESE RESEARCHERS HAVE BEEN EXPLORING BIODEGRADABLE SOLUTIONS.**

Since the 1980s, awareness has been growing among scientists about the environmental problems of landfill waste, widespread microplastics pollution, and climate change caused by the use of fossil fuels.

In the 1990s, Japanese researchers began to investigate whether micro-organisms could be used to make biodegradable plastics as a potential solution for these issues. They discovered that bacteria produce a diverse range of biopolymers as intracellular carbon and energy storage materials to help them survive in hostile environments.

There has long been an interest in how these biopolymers could be scaled up and used in industrial and medical applications. The

potential in this bacteria-led approach prompted chemical manufacturer Kaneka to change its focus in the 1990s from petrochemical plastics to biobased and biodegradable plastics, says biologist, Shunsuke Sato, based at Kaneka's CO<sub>2</sub> Innovation Laboratory in Takasago, Hyogo.

## BACTERIAL PRODUCTION

That strategic shift led to the development of a biodegradable, bacteria-derived bioplastic called Green Planet, made from vegetable oils, which is widely in use around Japan, and abroad in products including straws, cutlery and food packaging.

The development of Kaneka's bioplastic started with the challenge of how

to make a biopolymer that was strong, flexible and biodegradable. The bacteria-produced biopolymers from those early days of research, around 30 years ago, were made of polyhydroxybutyrate or PHB, a biopolymer with high crystallinity which makes it inflexible and brittle.

Polyhydroxybutyrate is a homopolymer, which means it consists of a chain of one type of monomer; in this case, the monomer 3-hydroxybutyrate. Polyhydroxybutyrate is the most common polymer produced by a variety of bacteria including *Cupriavidus necator*, which occurs naturally in soil and water.

This led Kaneka researchers to search for polymers with improved material

properties that could replace conventional plastics, and in 1991, they discovered poly(3-hydroxybutyrate-co-3-hydroxyhexanoate), or PHBH. "PHBH is more flexible and has a lower melting temperature than PHB, so it is a more suitable choice to replace plastics like polystyrene, polyethylene and polypropylene," Sato says.

PHBH is produced naturally by a number of different bacteria in the genus *Aeromonas*, which are found in the environment. Kaneka researchers first isolated *Aeromonas caviae* FA440 from the soil at Kaneka's Takasago manufacturing site.

## SYNTHESIS CHALLENGE

In their wild form, *Aeromonas* bacteria make relatively small

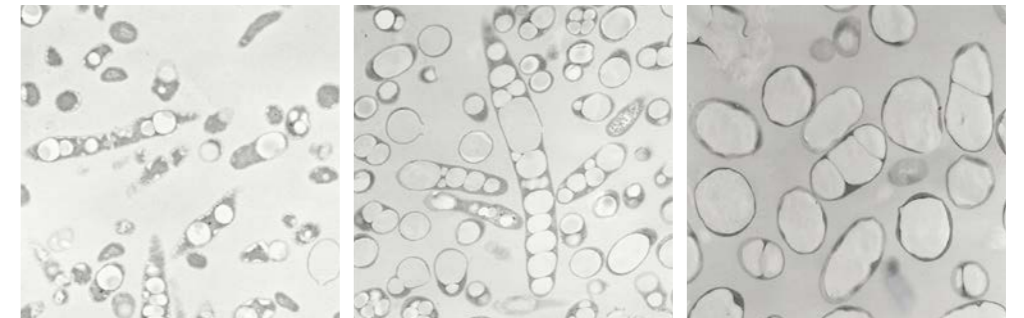
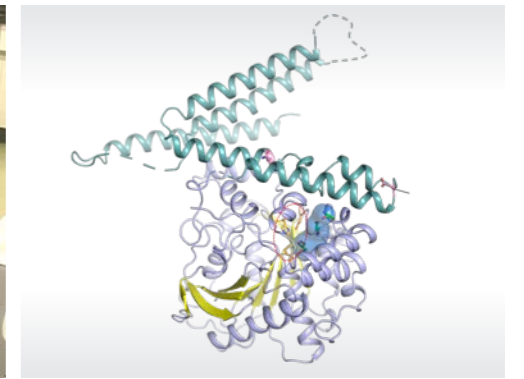
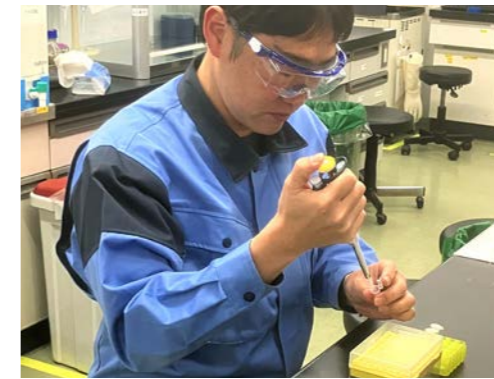
amounts of PHBH, which would make it difficult to produce the large quantities of bioplastic needed to commercialize the product. In contrast, *C. necator* produces high volumes of PHB<sup>1</sup>.

Sato and colleagues decided to see if they could engineer *C. necator* to produce PHBH. They identified the critical enzyme that *Aeromonas* uses to produce PHBH, and engineered *C. necator* to carry the gene for the PHBH-producing enzyme, called polyhydroxyalkanoate synthase through collaborative research with Toshiaki Fukui, a microbiologist from the Institute of Science Tokyo's Yokohama campus.

But, even after it had been engineered to include the genetic instructions, *C. necator* were still only able to synthesize PHBH, which contains small amounts of 3HH monomers. To meet their goal, Sato and colleagues first had to understand all the steps of the synthesis pathway for PHBH, and in particular the production of the 3-hydroxyhexanoyl-CoA that was the critical part of PHBH.

That began with vegetable oil, which is a raw material for biopolymer synthesis. Vegetable oil is mainly made up of oleic acid, a fatty acid that consists of 18 carbon atoms, 34 hydrogen atoms and 2 oxygen atoms. When oleic acid is oxidized, it is gradually broken down into smaller fatty acids with 16, 14, 12, 10, 8 and finally 'caproic acid' with 6 carbon atoms. Sato and colleagues then worked out that the hydration of C<sub>6</sub> intermediate, 2-hexenoyl-CoA, is the key reaction for 3-hydroxyhexanoyl-CoA production.

"The 3HH fraction of the biopolymer is very important to regulate its material properties," Sato says. "If the 3HH fraction is higher, PHBH becomes more flexible due to the prevention of crystallization."



▲ Shunsuke Sato at Kaneka's CO<sub>2</sub> Innovation Laboratory in Hyogo, Japan (top left). An illustration of the three-dimensional structure of PHA synthase, the enzyme that makes PHBH (top right). The accumulation of PHBH within bacterial cells as the hydrogen-oxidizing bacteria proliferate (bottom left to right).

Now the research team understood the metabolic pathway for 3HH-CoA, and they had characterized the 3D structure of the enzyme responsible for polymerizing PHBH by working with structural biologists, Toshio Hakoshima, and Chek Min Fey from Nara Institute of Science and Technology, in Ikoma, Japan<sup>2</sup>. It was time to bring all this knowledge together to use *C. necator*, to generate industrial quantities of PHBH.

They achieved this in 2011, and now PHBH is a biopolymer with a wide range of applications, from shopping bags and drinking straws used by major chains across Japan.

Sato's team is now working to make the engineered *C. necator* even more efficient at producing the biopolymer, for example by altering the structure of the polyhydroxyalkanoate synthase enzyme to try to increase its efficiency<sup>3</sup>.

## DEEP SEA EXPLORATION

Unlike non-biodegradable plastics, which can persist in the environment as microplastics for decades or far longer, PHBH can more rapidly biodegrade into carbon dioxide and water through the action of PHBH-degrading bacteria in the soil and water<sup>4</sup>.

"If the soil is very high in bacterial activity, warmth, nutrients and moisture it can happen in several months, but the rate of biodegradation basically depends on the microbial activity in the natural environment," says Sato.

Another advantage of PHBH is that it will biodegrade in marine and the deep-sea environments that are very cold, and have low concentrations of microorganisms. Sato's research team collaborated with the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) to look at how the PHBH bioplastic might break down even in

the high-pressure deep-sea environment. They discovered that deep-sea microorganisms can degrade PHBH in the deep-sea environment, high-pressure and cold water temperature.

Sato says this work has the potential to significantly contribute to reducing the accumulation of microplastics that is threatening to overwhelm the natural environment. ■

## REFERENCES

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Researchers are working to develop bioplastics that can degrade in marine and deep-sea environments.

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