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Bacteria serve up a tasty solution to the global plastic problem

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Rattling around on a cold, damp Edinburgh street, a plastic water bottle is a stark reminder of one of the greatest environmental crises facing our planet. In little under 100 years a growing tsunami of plastic waste has contaminated not just our streets but nearly every corner of the natural world – from Mount Everest to the deepest oceans. But this plastic bottle provided the inspiration that could yet turn the tide. Diverted from landfill to the laboratory the bottle soon grabbed the attention of the world's media by undergoing a remarkable transformation from plastic into vanillin – the main component of vanilla and one of the most in-demand spices in the world. This seemingly impossible act of alchemy was made possible by harnessing the metabolic power of bacteria. Its success has enormous implications. Not only could it meet our insatiable appetite for this rare flavouring, but it could radically change the way we tackle another addiction – the endless stream of single-use and disposable plastics that have become part of everyday life. Yet this only scratches the surface of the potential of this approach. By coaxing microbes to behave as eco-friendly factories that produce useful materials, we could tackle many other global challenges.

In less than 100 years plastic has transformed modern life. From food packaging to medical equipment, it's hard to imagine a world without it. Strong, versatile and durable, it is no surprise that plastic production hit exponential growth. From a modest 2.3m tonnes in 1950 to an eye-watering 448m tonnes in 2015, its production rate expanded faster than any other material on earth. The vast mountains of plastic waste we generate every year now equal the weight of the entire human population.

Half of this plastic is used just once – often in minutes or hours – and then thrown away. But food wrappers and plastic bottles can take hundreds of years to decompose and those that do not end up in landfill go on to wreak havoc in the environment. Every year 8m tonnes of plastic waste escapes into oceans, carried by rivers that act as giant super highways for waste from our cities.

The effects are devastating. Every year millions of animals are killed by plastic waste, often by starvation or entanglement. Cluttering coastlines and swirling in vast ocean currents plastic is also weathered by sunlight, wind and wave action and broken into increasingly smaller particles. These microplastics are a more sinister threat. Invisible to the human eye, they are swallowed by fish and other sea life eventually ending up on our dinner plate.

There is little escape on land – plastic waste frequently clogs sewers and waterways providing the perfect breeding ground for mosquitoes and other pests. Plastic bags in particular can boost transmission of diseases such as malaria. Over time this plastic is also eventually weathered and broken down. Microplastics contaminate the land, providing another pathway into the food chain when they are consumed by farm animals. They have also now found their way into the majority of the world's tap water.

If that isn't bad enough, plastics' environmental impact starts long before it is discarded. The process of producing and refining its chemical building blocks – which are derived from dwindling supplies of fossil fuels, oil, coal and natural gas – consumes vast amounts of energy and emits greenhouse gases. The fact that plastic is dramatically lower in weight than other packaging materials and therefore reduces energy costs and emissions during transport does not offset this.

In 2015, the USA generated the equivalent of the annual carbon dioxide emissions of 45m passenger vehicles from the production of ethylene – the main component of polyethylene plastic. If our relentless appetite for plastic continues to grow it will consume 20% of the world's oil supplies by 2050.

Plastic waste recycling

Sadly recycling is no panacea to the plastic crisis. Only 9% of the plastic waste ever produced has been recycled. Despite the backing of a more environmentally conscious public, plastic recycling trails far behind other materials such as paper, metal and glass. Whilst recycling rates are increasing, several major stumbling blocks continue to hinder its viability. A mish mash of various plastic types, mixed with colours and other additives, first need to be painstakingly sorted and cleaned. This is expensive and it's just the start of a downward spiral in the economics which leads to low-value plastics.

Even those plastics deemed fit for recycling quickly become unusable. When plastic is melted and remoulded the high temperatures degrade its polymer chains. This lower grade plastic has limited uses and each subsequent recycling round quickly renders it unusable. To add to the headache the recycling process itself is energy intensive, requiring temperatures up to 300°C, further adding to plastics' carbon footprint.

To make matters worse, after just one round of recycling the new lower quality plastics have lost 95% of their economic value. Whilst more advanced technologies exist to preserve plastic quality they are also energy hungry and expensive. Although plastic recycling has an environmental benefit it's no surprise that the economic and technical hurdles mean there is little incentive to contribute to recycling. It simply extends the timeline, delaying rather than preventing plastics eventual disposal as waste.

Nature's solution – landfill bacteria

But amongst the mountains of plastic waste piling up in landfill sites there is still hope. Life finds a way! In 2012 researchers in Japan discovered a bacterium which thrives on the surface of the common plastic, polyethylene terephthalate (PET). It slowly chews its way through PET using it as a carbon source to fuel its growth.

The bacteria, named *Ideonella sakaiensis* after the city it was found in, makes an enzyme, known as PETase which breaks PET into the intermediate mono(2-hydroxyethyl) terephthalic acid, or MHET. Another enzyme, MHETase, then breaks down MHET into PET's basic building blocks, terephthalic acid (TA) and ethylene glycol.

Not only have bacteria evolved this surprising ability – less than 100 years after plastic started being discarded on mass – but they can operate efficiently at only 30°C. This offers the intriguing possibility that bacteria could not only devour our plastic waste, but reduce fossil fuel consumption and emissions by regenerating the starting materials under everyday conditions.

It turns out that *I. sakaiensis* was not an anomaly in the natural world. Researchers from across the world are studying plastic-eating microbes, and between them hundreds of strains of microbes have been identified which can grow on plastic waste. Researchers also found an enzyme, similar to PETase, known as leaf-branch compost cutinase (LCC) in a compost heap. It evolved to break down the waxy protective coating on plant leaves. But LCC is also able to cut the bonds between PET's building blocks TA and ethylene glycol. It has a surprising advantage over its landfill cousin PETase. By operating at 65°C – the temperature at which PET starts to melt – the enzyme could more easily break it down. After refining the enzyme's activity researchers found that it even worked when introduced into a mix of plastics. Because the enzyme was able to restore the building blocks in their original form, the second-generation PET was just as strong and durable as conventional plastic.

Whilst the ingenuity of nature is remarkable it offers an even more exciting possibility. If bacteria can convert plastics into their starting materials what else could we coax them to do? Recycling plastic in an infinite loop will not solve the plastic crisis. What if we could find other uses for the building blocks?

The ancient partnership between mankind and microbe evolved over centuries to produce many staples of our modern diet. Harnessing natural fermentation processes made it possible to make bread and beer, but microbes' metabolism offers a far richer world of possibility. Advances in synthetic biology are now allowing us to explore a myriad of opportunities to reprogramme microbes, equipping them with entirely new capabilities.

From petrol to vanillin

Breaking down genomes not only allows us to better understand how living systems work, we can now re-use or redesign genetic parts for new and useful purposes. Enzymes have already been used to convert PET's building block TA into vanillic acid – the precursor to vanillin, the main flavour and aroma component of vanilla. This offered an interesting opportunity. Could we use synthetic biology to coax bacteria to take it one step further to make vanillin?

Vanillin makes a tantalizing target; global demand has long exceeded the supply of its natural source – vanilla beans. Today synthetic production of this popular ingredient has largely eclipsed expensive natural vanilla extract. The ice cream and chocolate industries consume three quarters of synthetic vanillin production but it is also in high demand for use in perfumes, cleaning products and as a chemical intermediate in the production of pharmaceuticals, cosmetics and other chemicals.

By 2018 global demand had soared to over 37,000 tonnes – 1.5 times the weight of the Statue of Liberty. But synthetic vanillin production suffers many of the same problems as plastic production as it comes from the same source – petrol. Around 85% of synthetic vanillin is produced via a two-step process from the petrochemical raw material guaiacol. Like plastic its production relies on high temperatures and spiralling greenhouse gas emissions.

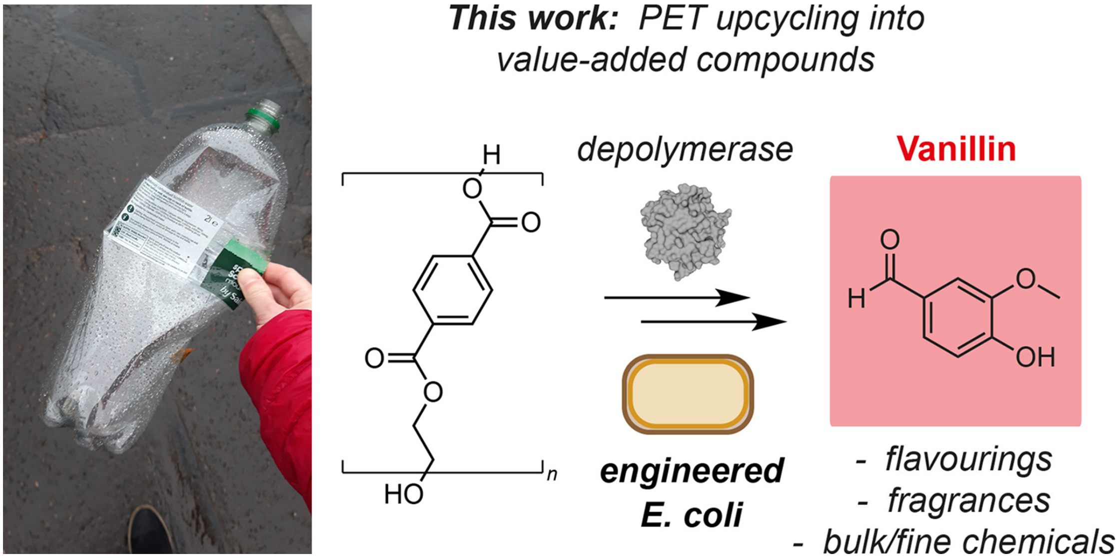

Figure 1. The plastic bottle used in this study (left); The concept of upcycling PET into vanillin using engineered *E. coli* (right). Figure adapted from Sadler et al. [*Green Chem*. (2021) **23**, 4665–4672].

Engineering *Escherichia coli*

If we could turn plastic waste into useful compounds such as vanillin we could cut out the use of fossil fuels entirely and up-cycle our way out of the plastic waste crisis (Figure 1). The initial step was simple enough. The LCC enzyme is already used to produce second-generation plastics – exhibiting high PET to TA degradation activity. Our starting material was less conventional. Lab-grade PET is expensive so a chance encounter with a discarded plastic water bottle on the streets near our laboratory at the University of Edinburgh provided a more economical starting point – as well as a small-scale demonstration of tackling the real-world problem.

Inspired by *Ideonella sakaiensis* we chose *E. coli* as our host bacteria to catalyse the conversion of TA to vanillin. *E. coli* had already been previously engineered to convert glucose to vanillin, so we designed a new four-step enzymatic process that could achieve this using TA as the starting point. The first two enzymes terephthalate 1,2-dioxygenase (TPADO), and dihydroxy-3,5-cyclohexadiene-1,4-dicarboxylic acid dehydrogenase (DCDDH) completed the conversion into an intermediary known as protocatechuate (PC). From that point the final conversion into vanillin could be completed via two further enzymatic steps involving carboxylic acid reductase (CAR) and catechol *O*-methyltransferase (COMT) (Figure 2). The enzymes in this pathway were assembled onto two plasmids – circular pieces of DNA – and inserted into the bacteria.

Whilst the chemistry proved relatively simple, using bacteria to catalyse the reaction posed some challenges. To get TA into bacterial cells they were treated with a chemical to increase their cell membrane permeability. These leaky membranes meant that even under carefully controlled conditions high cell densities were needed to compensate for those that didn't survive. Bacteria have also evolved to attempt to spit out foreign DNA so to prevent them from expelling the plasmids a gene encoding antimicrobial resistance was also integrated. By including antibiotics in their growth media any bacterial cells that spat out their plasmids were killed.

Vanillin also proved toxic to *E. coli* in high concentrations so a reagent known as oleyl alcohol was used to remove vanillin as it was being produced, limiting its toxicity to bacterial cells. There was also a delicate balancing act to ensure the quantities of each enzyme was optimized to ensure a good flow through all the steps in the conversion process. The final methylation step, mediated by the COMT enzyme, proved to be the main bottleneck and stops working after 24 hours. This however was enough time to allow 79% of TA to be converted into vanillin. Reducing the reaction temperature from 30°C to 22°C also had a dramatic effect, with a 5-fold improvement in vanillin yields.

This one-pot process has several advantages that boost its environmental credentials. Not only can it be carried out at room temperature, it does not require lots of expensive chemicals or generate toxic waste and greenhouse gas emissions. Further work will focus on improving yields, particularly by experimenting with

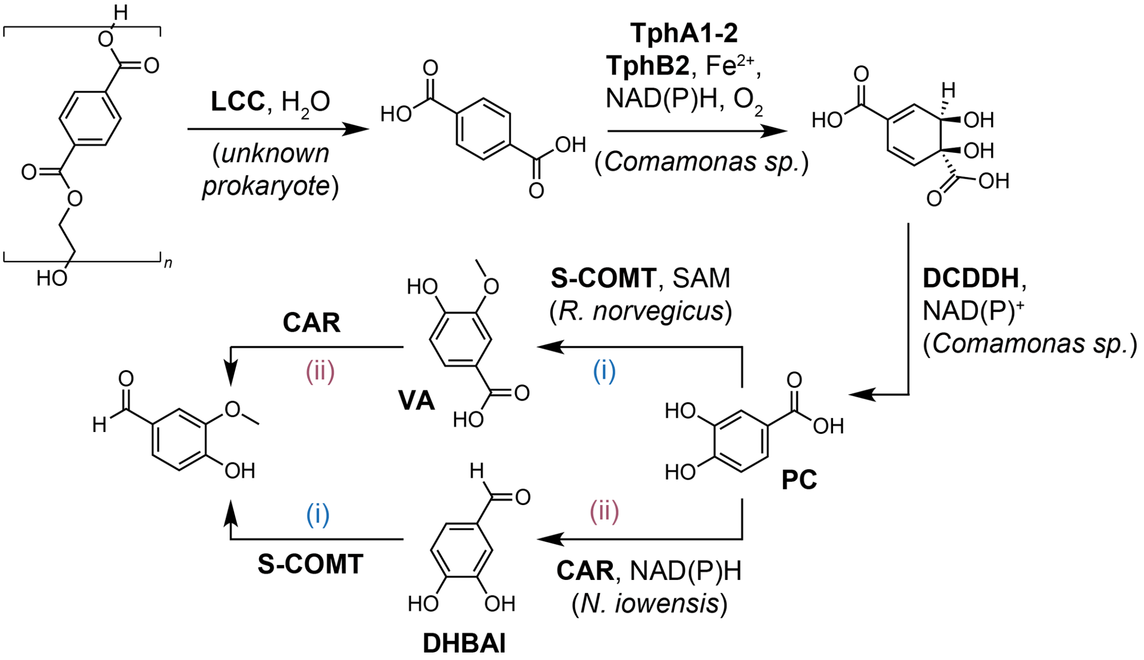

Figure 2. The enzymatic route to vanillin from PET. For clarity, functional groups are represented in their unionised states. Figure extracted from Sadler et al. (*Green Chem*. **2021**, *23*, 4665–4672).

different microbes or enzymes to tackle the bottleneck in the final stage of the conversion process. Checks on the purity of vanillin for food safety as well as potential carbon cost savings could further increase its appeal to industry.

The power and potential of this approach goes far beyond vanillin. Instead of viewing waste, such as plastic, as part of a downward spiral destined eventually for landfill, we could see it as the starting point. By engineering microbes to unlock the potential of other carbon-rich sources of waste, such as those from food, drink and agriculture industries, we could find more sustainable ways of producing other valuable chemicals and materials. ■

Further Reading

- The original research article: Sadler, J.C. and Wallace, S. (2021) Microbial synthesis of vanillin from waste poly(ethylene terephthalate). *Green Chem*. **23**, 4665–4672. DOI: 10.1039/d1gc00931a
- A recent review from some key players in the field describing challenges and future perspectives in the field of plastic upcycling: Ellis, L.D., Rorrer, N.A., Sullivan, K.P. et al. (2021) Chemical and biological catalysis for plastics recycling and upcycling. *Nat. Catal*. **4**, 539–556. DOI: 10.1038/s41929-021-00648-4
- The Ellen McArthur Foundation report: The New Plastics Economy – Catalysing action (https://emf.thirdlight.com/link/ftg1sxxb19tm-zgd49o/@/preview/1?o)
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