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> ONE MONTH, ONE PROTEIN <

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ramifications

Vivienne Baillie Gerritsen

There is much talk about plastic these days. And with reason. Besides depending on fossil fuels, plastic is infesting every nook and cranny of our planet because there is simply too much of it. Since the invention of bakelite in 1907, human dependency on the astonishingly varied properties of plastics has, understandably, never ceased to grow. Up popped the polyester polyethylene terephthalate, or PET, in the 1940s and an inventor's idea to use it to bottle soft drinks in the 1970s – which marked the beginning of a catastrophe. Today, we are desperate to find ways of recycling plastics and degrading them in eco-friendly ways. We have already discovered bacteria that are happy to eat PET for dinner – although not fast or efficiently enough for our liking. Lately, scientists came across a similar process that occurs in Nature when certain fungi, such as *Aspergillus oryzae*, invade plants. Plant cells in contact with the air are protected not only by a film of wax but also by cutin polyesters which are similar in structure to PET. Fungi have to degrade the cutin polyesters in order to reach the plants' flesh. They do this with the help of an intriguing little protein known as hydrophobin which, it turns out, can also be used to stimulate the degradation of PET.

"10 Green Bottles" by Alison Deegan

Courtesy of the artist

PET – a synthetic polymer consisting of ($C_{10}H_8O_4$) units – was discovered in the 1940s in the hunt for new man-made fibres following the revolutionary discovery of nylon. Natural polymers such as resin, rubber and cellulose have been used throughout history by humans but, as the industrial revolution kicked in, scientists began to look for materials whose properties were less limited. Bakelite, the first fully synthetic plastic, had already emerged in the 1920s and was used for all sorts of electrical and household appliances, automobile parts and even jewellery.

However, when PET made its appearance with its stash of superior physical and chemical properties, bakelite could not compete. PET became widely used in fibres for clothing, in containers for foods and liquids and in all kinds of manufacturing thanks to its thermoforming characteristics. In the 1980s, production technology had reached such a level of sophistication that PET bottles fast became the primary material for beverage packaging. The polymer is light, durable and can be blow-moulded into shape. Almost half a century later, though, we do not know how to deal with all the PET we are producing.

Hydrophobins may turn out to be part of the solution. Hydrophobins are small proteins, all of which carry eight conserved cysteine residues and four disulfide bonds. They are found exclusively in filamentous fungi, notably in *Aspergillus oryzae* – which sports 50 different kinds. Secreted onto the surface of fungal hyphae, hydrophobins self-assemble into rodlike formations – or rodlets – to form a hydrophobic protective sheath which has its role in fungal growth, development and dispersal. Hydrophobins also seem to contribute to host infection, and it is this particular trait that turned out to be, in a meandering kind of way, comparable to what is involved in PET degradation.

The outer tissues of plants – and insects for that matter – are covered with a protective hydrophobic sheath, the cuticle which, among other things, prevents dehydration while acting as a barrier to bacterial or fungal infection. Cuticles are composed of various waxes and polyesters known as cutins. One characteristic of hyphal hydrophobins is their capacity to adhere to hydrophobic surfaces such as those formed by cutins. Hydrophobin adhesion is thought to stimulate the fungal expression of cutinases, enzymes that are able to degrade cutin. This would then create vulnerable areas and points of entry for fungal infection.

Surprisingly, how scientists currently understand the mode of action of hydrophobins on cuticles was discovered while studying the interaction of *A.oryzae* with a biodegradable plastic known as polybutylene succinate-coadipate, or PBSA. The team found that one hydrophobin, called rolA, adheres specifically to the hydrophobic PBSA surface. This particular adhesion causes a conformational change in rolA which goes on to stimulate the expression of a fungal cutinase, cutL1. RolA and cutL1 have an affinity for one another. Even more so, it seems, if rolA has adhered to a hydrophobic surface. Once the two are paired, the cutinase then proceeds to degrade the plastic whose carbon atoms are used as a source of energy for fungal growth. In a way, *A.oryzae* doesn't really mind where it gets its food from – synthetic plastic or plants – as long as it gets it. And this form of fungal indifference can be put to use by scientists. It seems, too, that as long as rolA is not bound to cutL1, the hydrophobin can move laterally on the plastic surface. Lateral mobility ceases, however, the moment the two are paired, which probably is just a way of giving cutL1 a better chance to degrade the plastic.

This was observed with PBSA. Could rolA and cutL1 behave the same way with the synthetic plastic PET? The answer is yes. A team of scientists presented rolA to some PET while adding a pinch of an enzyme they knew could degrade PET: an enzyme of bacterial origin, coined PETase. Like with PBSA, hydrophobin rolA adhered to the hydrophobic plastic PET surface. As expected, adhesion caused a conformational change in rolA which increased the degradative action of PETase.

This is very good news. The degradation of PET via PETase is not only environment friendly but occurs at relatively low temperatures, i.e. 30°C. PET plastic recycling by any current method requires high temperatures and therefore consumes a lot of energy – besides generating other, perhaps even toxic, substances and just a different kind of pollution. The rolA/PETase system still remains slow, however. Its mechanism needs to be better understood so that it can be fine-tuned to speed up the reaction. Especially as more than half of the world's beverages continue to be stored in PET bottles not to mention many fibres that continue to be made of polyester – and there is little sign that production will decrease.

Cross-references to UniProt

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Cutinase I, *Aspergillus oryzae* (strain ATCC 42149 / RIB 40) (Yellow koji mold) : P52956

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