Exiguobacterium degradation of polystyrene

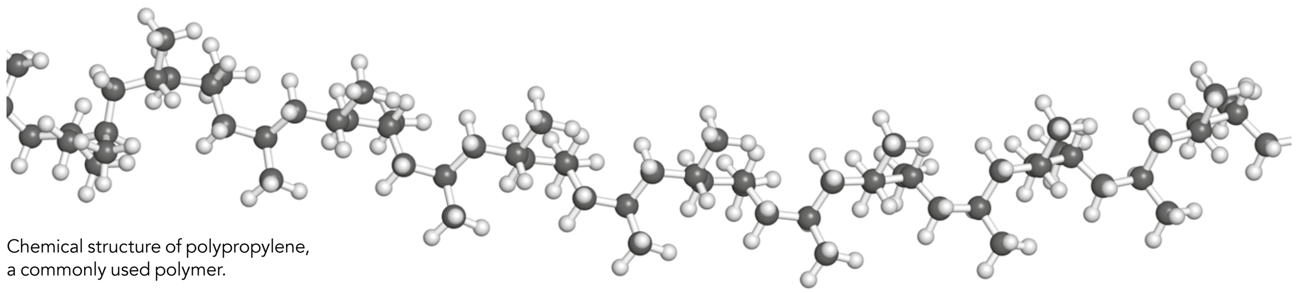
Enlisting bacteria in the war against plastic

Polystyrene is a chemically stable recalcitrant plastic that pollutes many natural environments. Unfortunately, few mechanisms can break down this man-made polymer into its naturally occurring components. Research into the polystyrene-digesting capabilities of the bacteria genus Exiguobacterium *sp RIT 594 by Dr André Hudson and his team at the Rochester Institute of Technology in New York, USA, has uncovered the specific ring-cleaving mechanism which the bacterium uses to degrade the plastic. Bioinformatics, anaerobic culture analysis, and Fourier-transform infrared spectroscopy provided evidence of aromatic ring attack as the primary mechanism by which* Exiguobacterium *sp RIT 594 breaks down polystyrene.*

Plastics are ubiquitous in our modern lifestyles, and their widespread use has made them an ever-present blight on the natural environment. Despite having many valuable applications, from medicine to food packaging, plastic materials don't readily break down once discarded. That's because plastics are made up of synthetic chemical structures called polymers. Polymers are large molecules consisting of a repeating chain of smaller chemical units called monomers. These monomers are often made up of carbon and hydrogen chains but can include other atoms, such as sulphur, nitrogen, or oxygen.

Many polymers exist naturally as essential components of living organisms. For example, DNA is a long polymer chain of nucleotide base pairs. The naturally occurring polymers are easily broken down in the environment because enzymes have evolved specifically to do so. Plastic, on the other hand, is such a new material in the scope of evolutionary history, few mechanisms exist or have been identified that can degrade it similarly to that of other naturally occurring polymers.

As the build-up of plastic in water bodies and the terrestrial environment became one of our most pressing issues, researchers began to search for organisms with the capability to break down these materials. Dr André Hudson and his team at the Rochester Institute of Technology, USA, have been studying a genus of bacteria known as *Exiguobacterium*. Members of this genus are known for their ability to degrade polystyrene in natural ecosystems. Studying the biochemical pathways that allow *Exiguobacterium* to do this will help researchers understand the mechanisms behind plastic degradation and apply them to new technologies aimed at degrading plastic.



Chemical structure of polypropylene, a commonly used polymer.

Polystyrene is a particularly difficult compound to break down due to its ring structure, and few microorganisms are known to degrade it. The polystyrene polymer is made up of styrene monomers, and while these monomers are naturally synthesised in small quantities, polystyrene is a man-made substance which remains relatively chemically inert and resistant to many of the typical natural biodegrading mechanisms. For this reason, *Exiguobacterium* species strain RIT 594 is a vital study focus, as its mechanism for degrading polystyrene is currently not well understood.

GENETIC AND SPECTROSCOPY ANALYSIS

Hudson's team analysed the genome of *Exiguobacterium* sp RIT 594 to determine how it degrades the plastic and whether it can degrade the polymer. Many plastics already break down in the environment when exposed to sunlight or other weathering factors, but this process is slow and can potentially take hundreds of years. The breakdown of larger plastics forms the infamous 'nanoplastics' which have permeated every corner of the planet. These tiny particles of the original synthetic, non-biodegradable plastic polymer are now made small enough to leach into food and enter the human bloodstream. In contrast, a successful bacterial biodegradation mechanism will fully degrade polystyrene.

Based on the genome sequence and gene annotation of *Exiguobacterium* sp RIT 594, Hudson's research group found two potential enzymes – specialised proteins which increase the rate of a chemical reaction – that could be responsible for breaking down different parts of the polystyrene polymer. One of these enzymes was a hydrolase (enzymes that use water to break chemical bonds); the other was an aromatic ring-cleaving dioxygenase (enzymes that incorporate both atoms of dioxygen, O_2, into a substrate). The team hypothesised that the bacterium first degrades polystyrene by attacking the polymer's aromatic ring – the carbon and hydrogen atoms bonded together in a ring structure. The hydrolase was thought to be involved in later steps of the breakdown pathway.

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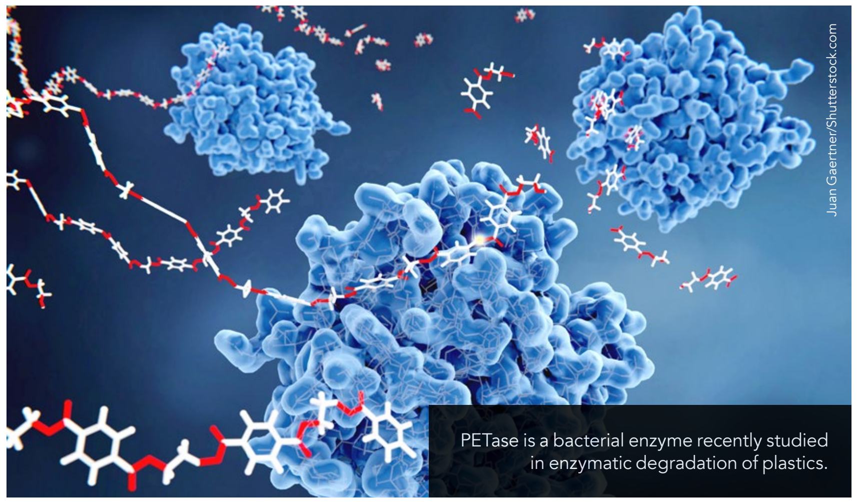
In addition to simply depriving the bacterium of oxygen, the team used Fourier-transform infrared spectroscopy to observe the by-products the bacterium produced when it degraded polystyrene. This technique measures the infrared spectrum of a sample to determine its exact chemical composition. The spectroscopy analysis revealed an increase in carboxyl and hydroxyl groups when the plastic was exposed to a culture of *Exiguobacterium* sp RIT 594, with the appearance of specific compounds that seem to be breakdown products of the original styrene ring. These breakdown products appeared only when the bacteria was cultured in an environment with oxygen, suggesting that aromatic ring attack is an aerobic process.

AEROBIC DEGRADATION

To build a more complete picture of the plastic degradation metabolic pathway, the team investigated whether oxygen was necessary for the bacterium to degrade the plastic. If the polystyrene was being broken down by a dioxygenase and hydrolase pathway, it would have to occur in the presence of oxygen, also known as an aerobic pathway. To test this, Hudson's team submerged the bacterial cultures in an oxygen-free medium for 28 days along with polystyrene and discovered that



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PETase is a bacterial enzyme recently studied in enzymatic degradation of plastics.

the plastic incubated with the bacterium was no more degraded than the plastic submerged without the bacterium. This result ruled out any anaerobic pathways that the bacterium could employ.

The team also noticed distinct patterns of bacterial growth, which appeared to depend on the surface properties of the polystyrene. Thicker biofilms developed after 14 days, within which nanometer-sized polymer particles within the biofilm and on the cell surface were distributed. Styrofoam, or expanded polystyrene, is a form of polystyrene that contains small air bubbles used in products like coffee cups. For *Exiguobacterium* sp 594 to successfully degrade a piece of styrofoam, the surface would have to be accessible to the bacterium.

Styrofoam was not as readily attacked by the bacterium, possibly due to its waxy coating that prevented the bacterium from reaching the polystyrene. When the researchers dissolved the waxy layer in toluene and evaporated this organic solvent, the bacterium was able to degrade the exposed polystyrene. Earlier microscopic analysis showed that the bacterium was first attracted to rough patches or microscopic defects on the plastic surface where it formed a biofilm. These biofilms would then expand to the remaining plastic surface.

THE DEGRADATION MECHANISM

The evidence from the genetic, bacterial culture, and spectroscopy studies of polystyrene revealed a complete picture of the pathway used by *Exiguobacterium* RIT sp 594 to degrade polystyrene plastics. Hudson's team reported the first evidence of this novel oxygen-dependent enzyme used by the soil bacterium. Using genomic analysis, they showed that the microbe had the genes encoding an aromatic ring-cleaving enzyme, as well as a hydroxylase. The incubation studies proved that microbial breakdown of polystyrene needs oxygen, and spectroscopic analysis revealed the biotransformation of styrene monomers into 2-hydroxypenta-2,4-dienoate and acrylic acid, products formed by cleaving specific bonds of the polystyrene polymer. With this interdisciplinary approach, researchers were able to hypothesise the complete metabolic pathway by which *Exiguobacterium* strain RIT 594 degrades polystyrene.

Research toward understanding naturally occurring biochemical degradation pathways will help to solve the plastic crisis.

The evidence from the genetic, anaerobic, and spectroscopy studies revealed a complete picture of this polystyrene degradation pathway.

This relatively short biochemical pathway is a newly discovered route for polystyrene degradation by *Exiguobacterium* strain RIT 594. More research is needed to determine the identity of the dioxygenase and hydrolase enzymes and to ascertain if other species of the genus *Exiguobacterium* are also capable of the same pathway. Polystyrene is one of the most pervasive environmental contaminants, but research toward understanding naturally occurring biochemical degradation pathways and the organisms which use them will form part of the solution to plastic pollution in the environment.

Behind the Research

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Research Objectives

Dr André Hudson and his team uncover the mechanism by which the bacteria genus *Exiguobacterium* degrades polystyrene.

Detail

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Bio
Dr André O Hudson is currently Interim Dean of the College of Science at the Rochester Institute of Technology and served as Head of the Thomas H Gosnell School of Life Sciences from 2017 to 2022. He joined the faculty at the Rochester Institute of Technology in 2008 as an assistant professor in the Thomas H Gosnell School of Life Sciences after a two-year post-doctoral fellowship at Rutgers University.

Funding
- The College of Science at the Rochester Institute of Technology (RIT)

Collaborators
- Dr Anutthaman Parthasarathy, (RIT)
- Dr Renata Rezende Miranda (RIT)
- Dr Christy Tyler (RIT)
- Dr Nathan Eddingsaas (RIT)
- Jonathan Chu (Undergraduate RIT student)
- Ian Freezman (Undergraduate RIT student)

References

Parthasarathy, A, et al, (2022) Polystyrene degradation by *Exiguobacterium* sp. RIT 594: preliminary evidence for a pathway containing an atypical oxygenase. *Microorganisms*, 10(8), 1619. doi.org/10.3390/microorganisms10081619

Personal Response

What inspired you to conduct this research?

" I was inspired to do this research because of my fantastic peer and scholar colleagues working in various aspects of plastic science, namely Drs Tyler, Eddingsaas, and Parthasarathy. Plastic pollution is and will continue to be a global problem that will need to be addressed and rectified, and we have a role to play as scientists, especially as it relates to training, mentoring, and inspiring the next generation of scholars and scientists to develop the workforce to tackle this very important issue. "