Waste colonies yield bacterium with 3 enzymes that may break down polyester

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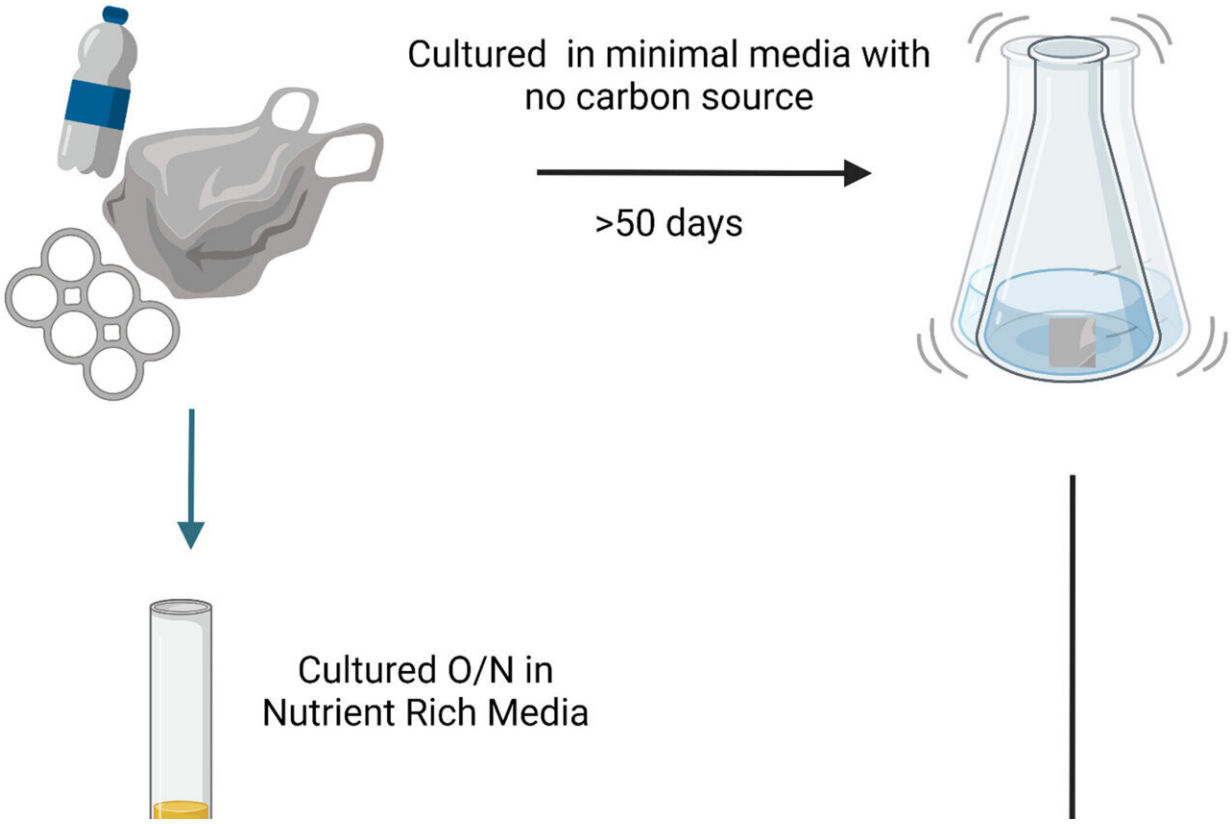

Experimental design. Waste plastic was collected from the coast and brought to the lab before being sectioned and added to nutrient rich media for growth overnight and cultures were then spotted onto PCL agar plates. Another section was put in high salt M9 minimal media with no carbon source and left to incubate for 53 days before plastic-degrading activity was assessed. Credit: *Environmental Microbiology* (2023). DOI: 10.1111/1462-2920.16466

Scientists have enriched expanded polystyrene waste from a beach in Ireland to isolate a bacterium shown to contain three enzymes that could break down polyester.

The team, from Brunel University London, is studying microorganisms that can degrade plastic, in the hope the microorganisms or their plastic-degrading enzymes can be used to manage the growing plastic waste problem.

Their research paper "Enrichment of native plastic-associated biofilm communities to enhance polyester degrading activity," has been published in the journal *Environmental Microbiology*.

"If microorganisms can degrade plastic that cannot be recycled, this will reduce the amount of plastic that is incinerated and landfilled," said corresponding author Dr. Ronan McCarthy.

"Many of the known plastic-degrading microorganisms and enzymes have naturally low efficiency, so we need to select for organisms that have higher efficiency or engineer the enzymes to work better. We found that native plastic waste communities can be enriched for communities that have better degradation activity through our enrichment experiment.

"This method can be applied to any waste plastic and the enrichment experiment conditions adapted to optimize isolation of bacteria that are appropriate for industrial batch culture. We also identified three putative enzymes that could be involved in polyester degradation."

The team collected native bacterial communities from environmental waste plastic and then conducted an enrichment experiment to find communities that had improved plastic degrading abilities after only having the plastic waste as a carbon source.

They observed a change in community composition, and identified a strain of Pseudomonas stutzeri that had three putative enzymes that could have a role in polyester degradation.

"Plastic waste is an increasing worldwide problem with limited environmentally friendly or sustainable solutions to deal with the vast quantities," Dr. McCarthy said.

"While recycling can offer a second life for some plastics, not all plastic types are easily recycled and plastic can only be recycled a few times. Only 9% of all plastic waste has been recycled.

"Plastic that cannot be recycled is incinerated or landfilled, but a more environmentally friendly solution could be to use microorganisms to degrade the plastic, and the breakdown products can then be used in different industries or even to make new plastic.

"We wanted to find new bacteria that could degrade plastic, specifically we wanted to collect them from environmental waste plastic to improve the chances of them being able to degrade it.

"We not only wanted to isolate the native community from the waste plastic, but we wanted to improve it, because natural plastic-degrading activity tends to be too slow and inefficient for application in industry."

The aim was to isolate and characterize native communities of bacteria from waste plastic. In the lab, the team found that expanded polystyrene promoted most bacteria to form biofilm communities on it.

This could be because expanded polystyrene is full of air holes and floats in the ocean, allowing lots of bacteria to attach to it and be protected from the elements.

"Since we wanted to collect native plastic-associated communities, we sought out expanded polystyrene waste to increase the number of bacteria present. We collected expanded polystyrene waste from a beach in Ireland and took it back to the lab in London," Dr. McCarthy said.

"We set up an enrichment experiment where we split each piece of expanded polystyrene in two. One half was grown overnight in a rich broth to collect the originally isolated community of bacteria. The second piece was incubated for nearly two months in a broth without any carbon source for the bacteria to grow, except for the plastic waste itself, to enrich the community of bacteria for those species that are able to survive and grow with only the plastic waste for energy."

After the enrichment experiment, the researchers collected the enriched community from the broth and tested its plastic degradation abilities. They initially compared how the original and enriched communities could degrade polycaprolactone (PCL), which is a model for polyester degradation.

Seven of the enriched communities were able to degrade PCL and were able to do so better than the original community—in most cases the original community showed no sign of being able to degrade PCL.

When comparing one particular set, PS13, the original community weakly degraded PCL, but when tested at an individual colony level, only 4.8% of colonies could degrade PCL after three days, whereas the enriched community strongly degraded PCL, and 94.7% of colonies could degrade PCL after three days.

"We looked into PS13 further—community sequencing found that the original community was quite diverse, whereas the enriched community was predominantly Pseudomonas stutzeri," Dr. McCarthy said.

"We isolated a strong PCL degrading colony from each of the seven PCL-degrading enriched communities. Six of these were found to be Pseudomonas stutzeri as well—while all different strains with varying phenotypes, they are closely related.

"The seventh was a Bacillus species, which are quite well known plastic-degraders, unlike Pseudomonas stutzeri. We found that the PS13 Pseudomonas stutzeri was able to grow using PCL as a sole carbon source, and we performed whole genome sequencing on this strain.

"The sequencing revealed that this strain contained three putative enzymes with similar sequences to known polyester and PET-degrading enzymes. Particularly, PsP1 was a very strong match to PmC, indicating that this could be a novel polyester-degrading enzyme."

Dr. McCarthy said it was surprising to find that so many of the enriched communities were dominant in Pseudomonas stutzeri.

"The presence of these polyester degrading enzymes has not been described in Pseudomonas stutzeri previously. It is also relatively rare to have a PETase and an MHETase enzyme in the one bacteria," he said.

Future studies could focus on carrying out the same enrichment experiment on different plastic waste samples to identify new species of bacteria that can degrade different types of plastic.

"It offers a promising way to enrich for species that can thrive on plastic waste. The more bacteria we find that are capable of degrading more diverse plastic, the better options we have for industrial applications of microorganisms to degrade plastic waste," Dr. McCarthy said.

"The three putative enzymes we found will be investigated further to confirm if they are active against polyester. If they are, they could be

added to the repertoire of known plastic-degrading enzymes, which could be engineered to have even better activity."

More information: Sophie A. Howard et al, Enrichment of native plastic-associated biofilm communities to enhance polyester degrading activity, *Environmental Microbiology* (2023). DOI: 10.1111/1462-2920.16466

Provided by Brunel University