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Evolution of native plastic associated biofilm communities to enhance polyester degrading activity

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Introduction

Plastic waste is an increasing worldwide problem urgently requiring a solution. Whilst recycling rates are increasing globally, only 9% of all plastic waste has been recycled, and with the cost and limited downstream uses of recycled plastic, an alternative is needed. Some microorganisms have been found to biodegrade certain plastics; this natural process could be used to our benefit to work as a solution for the plastic waste problem. The presence of potential plastic-degrading genes has been found to be positively correlated with the presence of plastic pollution, so the plastic-degrading ability of microorganisms has likely evolved from exposure to vast amounts of plastic pollution. We hypothesised that native plastic-associated biofilms would therefore be more likely to possess plastic-degrading genes, so sought out plastic waste as a source of plastic-degrading bacteria. Since the natural degradation is often low, due to the alternative carbon sources available in the environment, we wanted to promote the evolution of these plastic-associated bacteria by limiting their carbon source to only plastic waste.

Experimental

We performed a long-term *in vitro* evolution experiment on native plastic associated biofilm communities to improve their plastic-degrading ability. We found that bacteria formed most

biofilm on expanded polystyrene (EPS) per gram, so we collected EPS waste from the coast of Ireland to characterise these native communities. The EPS pieces were split in two, one half was added to nutrient rich media and grown overnight to collect the originally isolated plastic-associated community. The other half was added to M9 minimal media with no carbon source other than the EPS and was grown for two months to evolve the bacteria under carbon starvation. We then collected the evolved community and compared its ability to degrade plastic to the original communities. We used polycaprolactone (PCL), which is a model polyester to screen for polyesterase ability.

Results and Discussion

We demonstrated that the original EPS attached communities had limited polyester-degrading activity. However, after the evolution experiment, seven of the evolved bacterial communities had increased polyester degrading activity. 16S metagenomic analysis of one of the evolved communities found that *Pseudomonas stutzeri* was dominant. An isolate of this *P. stutzeri* was subsequently tested for PCL degradation and was found to possess the ability to not only degrade PCL but use it as a sole carbon source. Whole genome sequencing of this *P. stutzeri* isolate identified two putative polyesterases and one putative MHETase that could be responsible for the degradation.

Conclusions

This study indicates that waste plastic-associated biofilms are a source for bacteria that have plastic-degrading potential, and that this potential can be augmented through selective pressure and further *in vitro* evolution experiments, resulting in biodegradative communities that are better than nature. This is an important step to developing these plastic-degrading bacteria as a biotechnological tool against the plastic waste problem, and we identified three potential novel polyesterase enzymes that could be used as part of the solution. Future work will aim to characterize these enzymes and repeat the evolution experiment on more sources of plastic waste.

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Biography:

Sophie Howard completed her PhD from Imperial College London in 2020 on the Type VI secretion system of *Pseudomonas aeruginosa*. She has worked as a postdoctoral research fellow

for 3 years at Brunel University London on bacterial plastic biodegradation. She is co-author on 7 peer reviewed papers and currently has 2 papers under review on biodegradation.