Team Number: SBBC162
Project Title: A search for polyester-degrading bacteria to clean up waste
Project Type: Investigation

Hong Kong Student Science Project Competition 2022

To our best knowledge and after thorough literature research, as at 30/06/2022, there are similar works. If there are, the reference links are as below:

Diversity of polyester-degrading bacteria in compost and molecular analysis of a thermoactive esterase from Thermobifida alba AHK119
Agar plate-based screening methods for the identification of polyester hydrolysis by Pseudomonas species - Molitor - 2020 - Microbial Biotechnology - Wiley Online Library
A Bacterium that degrades and assimilates poly(ethylene terephthalate)

I. Background

The UN's sustainable development goal #12 is to 'ensure sustainable consumption and production', which includes the 'Environmentally sound management of hazardous wastes'. Polyester microfibre pollution is dangerous for aquatic life – and eventually for us. We think that using bacteria to digest polyester would be the most environmentally sound way to manage this hazardous waste.
Polyester is composed of benzene molecules linked by ester bonds. Any bacteria able to degrade this must be good at hydrolysing esters and breaking down aromatic compounds.

II. Objectives

The goal of this project is to identify bacteria with the potential to degrade polyester. Our school recently decided to change the school uniform, creating a lot of polyester waste, the main material used for our uniforms. In the interests of sustainability, our team has decided to investigate methods of reducing polyester waste in Hong Kong's landfills using a biological approach (rather than incineration that will produce smoke, toxic fumes and a great deal of planet-warming carbon dioxide).

III. Hypothesis

Since bacteria capable of degrading polyester are likely to be rare, we think that screening a large number of samples will give us the best chance of succeeding. Therefore, we propose to ask each student of a primary school year group (around 200 students in Grade 5) to collect samples for us from different sources. The primary school science teacher also thinks it will be a good idea for students to take part in the search as it will help to introduce them to microbiology and the importance of sustainability. Our screening process will look first for the ability to digest aromatic compounds. We will ask the G5 students to begin the bacterial screening by transferring extracts of their samples to a medium into which we can add toluene as the only carbon source. Bacteria surviving after a 7-10 day incubation will be transferred to a medium containing sodium benzoate as a carbon source and their growth monitored using a plate reader. Those that grow best in sodium benzoate will then be tested with benzyl benzoate (which contains both the ester bond and aromatic groups). Bacteria able to digest all three types of compounds will be streaked several times to obtain pure isolates. We then hope to identify these isolates by DNA sequencing using the Nanopore MinION sequencer in our school's molecular biology lab.

IV. Methodology

We asked a primary school year group to help us collect samples to screen and this will also help to teach the students about sustainability and also about microbes.

Bacterial screening:

- Through three different medium: Toluene, Sodium Benzoate, Benzyl Benzoate

Materials and equipment	
Bacterial screening	Bacterial testing on polyester
• 200 cryotubes • Sterile disposable inoculation loops • Saline (0.9%) • 1.5 mL microcentrifuge tubes • basic growth medium with 10% toluene (*v:v*)	• Basic growth medium • Polyester fibres from old school uniform • Petri dishes with Luria agar • L-shaped spreaders

- Petri dishes with Luria agar
- inoculation loops
- 96-well plate for plate reader (CLARIOstar Plus)
- basic growth medium with sodium benzoate 5% w:v
- Petri dishes containing agar emulsified with benzyl benzoate
- Incubator and shaker-incubator

DNA sequence analysis:

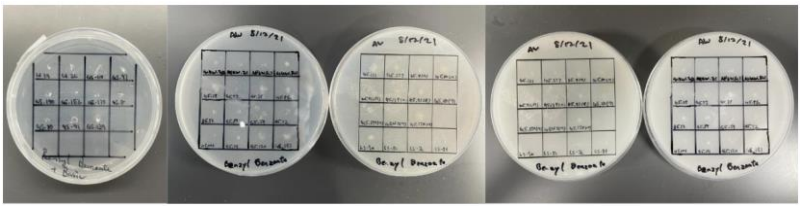
- Isolates that pass the screening will be sequenced using the Oxford Nanopore MinION sequencer in our school's molecular biology laboratory as well as via the Illumina MiSeq platform at Hong Kong Science and Technology Park (HKSTP).
- Genomes will then be analyzed using multiple bioinformatic tools: PATRIC, NCBI BLAST…

Experimental polyester degradation:

- Isolates that passed the screening will also be put into medium with physical polyester fibers, to assess polyester degradation experimentally

V. Results

Bacterial screening: Agar plates prepared from a minimal salts medium containing benzyl benzoate as sole carbon source:



The following is a table of the genome information for the six isolates that passed all methods of screening and analysis:

	AHKW.3h1	**G5.18**	**G5.80**	**G5.97**	**G5.120**	**G5.139**
Species	*Staphylococcus pseudintermedius*	*Klebsiella pneumoniae*	*Pseudomonas citronellolis*	*Pseudomonas sp.*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
Genome size	2.63 Mbp	5.47 Mbp	7.37 Mbp	5.64 Mbp	6.41 Mbp	6.79 Mbp
Source	Dog's paw	Plant pot	Soil (Repulse Bay)	Soil (Telegraph Bay)	Garden soil (Braemar Hill)	Plant pot (West Kowloon)

VI. Conclusion

We have suggested the ability for 6 isolates to degrade polyesters through three main methods of investigation.

1. We showed that our isolates can survive in medium with chemical structures (toluene, sodium benzoate, benzyl benzoate) that are present in polyester as the sole carbon source.
2. We identified enzymes in the DNA sequences of our isolates likely to have the capability to degrade polyester, especially dienelactone hydrolase which breaks the polymer into workable chunks and which is similar in structure to PETase.
3. We showed that our isolates can survive in medium with fine polyester as the sole carbon source.

Through these tests and exploration, we identified 6 isolates that show promising ability to degrade polyester as they are highly capable of breaking down polymers into smaller chains and degrade these small chunks into usable carbon compounds. A limitation that arises from this investigation is the incapability for us to observe physical change in polyester degradation as the yield and rate is rather small and slow. But despite this limitation, growth in the presence of polyester as the only carbon source shows experimentally that these isolates appear able to degrade polyester and further investigation and research can be conducted to improve degradation efficacy. If these isolates can be implemented to an industrial scale, polyester waste and environmental waste can be largely reduced, moving one step closer to achieving the UN's sustainable development goal #12 is to 'ensure sustainable consumption and production'.