MICROBES WITH AN APPETITE FOR PLASTIC: EXPLORING THE ENZYMATIC POTENTIAL OF CONTAMINATED SITES

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INTRODUCTION

The search for effective plastic waste management methods has been the focus of recent intense scientific research. One of the most promising approaches is enzymatic degradation of plastic and the retrieval of monomers for their upcycling (1,2).

A large number of potential plastic-degrading enzymes have been identified from associated microbes. The aim of this study is to identify microbial strains that have been exposed to heavily polluted sites with enzymatic potential to utilize polyethylene terephthalate (PET) and polyurethane (PU) as a sole source of carbon and energy.

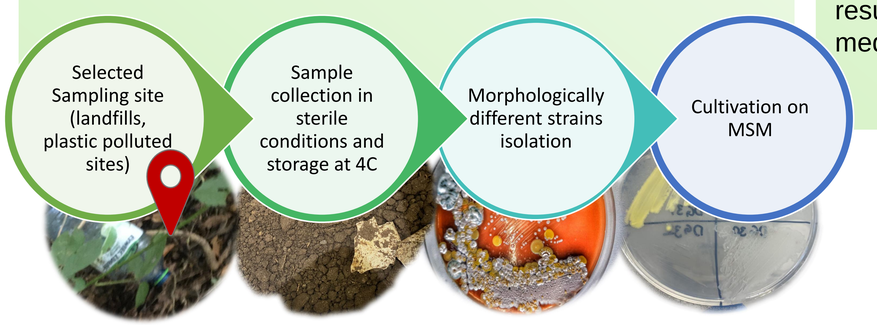

Figure 1. Microbial isolation process

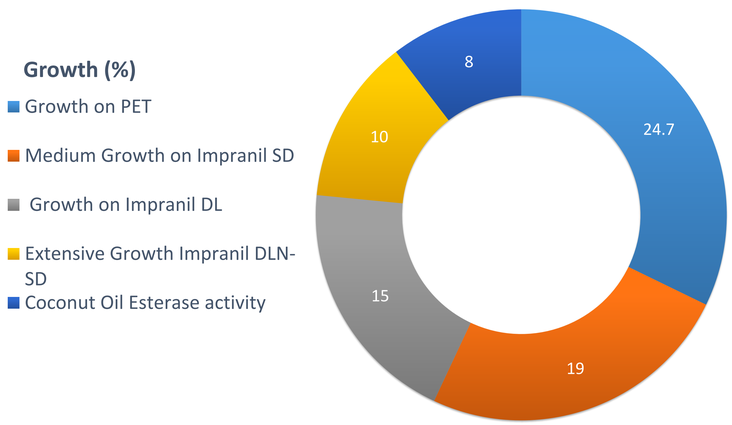

Figure 2. Overview of screening of the novel isolates for the potential to biodegrade plastic substrates

METHODS

Soil samples were obtained from landfills and other plastic polluted sites. Microbial isolation was carried out using three different growth media: LA (10 g/l tryptone, 5 g/l yeast extract, 10 g/l NaCl, 15 g/l agar), SAB (40 g/l glucose, 10 g/l peptone, 15 g/l agar) and ISP2 (4 g/l yeast extract,10 g/l malt extract powder, 4 g/l glucose, 20 g/l agar). The ability to use plastic related substrates as a sole carbon source was tested using a Mineral Salt Medium (MSM) (15 g/l agar, 9 g/l $Na_2HPO_4 \times 12H_2O$, 1.5 g/l KH_2PO_4, 1 g/l NH_4Cl, 0.2 g/l $MgSO_4 \times 7H_2O$, 0.2 g/l $CaCl_2 \times 2H_2O$ and carbon source 3-10 g/l). Esterase activity screen was conducted using coconut oil as a substrate. Cultures were incubated for 14 days at 30°C. Visible growth on the plates was considered as a positive result. Selected isolates were cultivated in liquid MSM medium using PET and PU as a sole carbon source.

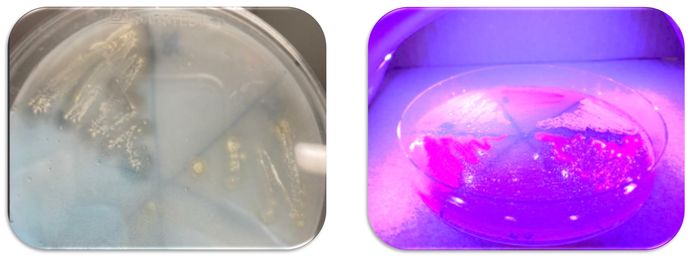

Figure 3. Isolate growing on Impranil showing halo zone (left); Esterase activity detection under UV light, using coconut oil plates (right)

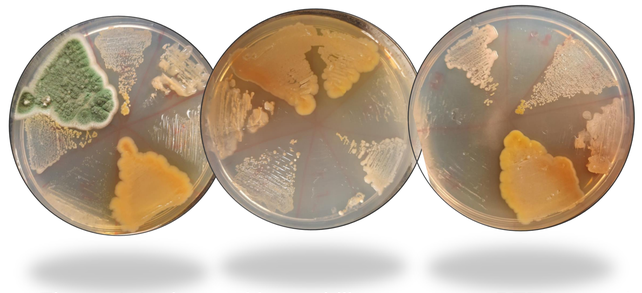

Figure 4. Isolates with an ability to grow on PET as a sole carbon source in liquid culture

RESULTS

Visible growth on the plates was considered as a positive result. A total of 98 morphologically distinct cultures were isolated and screened for esterase activity (8%). 24.7% of tested isolates showed an ability to grow on PET. 19% isolates showed medium growth on Impranil, while 10% showed extensive growth in contrast with the control plates (Figure 2).

Certain strains showed a visible halo in medium with Impranil substrate (Figure 3). These isolates were tested in MSM liquid culture with polyurethane as a sole carbon source and the growth rate was estimated. Experiment in liquid culture was conducted with strains that showed ability to grow on PET substrate as well (Figure 4). Results are presented in Figure 5.

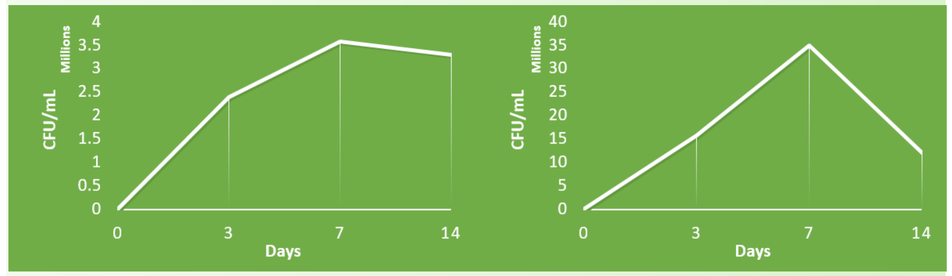

Figure 5. Growth rate of isolate 53 cultivated in liquid MSM with PU (left) and isolate 48 cultivated in liquid MSM with PET (right) as a sole carbon source

CONCLUSIONS

Proving its potential to metabolize these substrates, strains proved to be able to adapt to oil-based polymer carbon sources which indicates of the targeted enzymes such as polyesterases and polyurethanases that enables them to adapt in these substrates. This could be especially seen in those strains that formed a visible halo in the polyurethane based media as Impranil DLN-SD Screening assay.

References:
1: DOI 10.1186/s40643-017-0145-9
2:
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