ERA CoBioTech

BioTech Research & Innovation Hack

2021

ERA CoBioTech Funded Projects at A Glance: MIPLACE

Microbial Integration of Plastics in the Circular Economy

PART OF
EUROPEAN BIOTECH WEEK
INNOVATION IS IN OUR GENES

MIPLACE

Microbial Intergration of Plastics in the Circular Economy

The technology in development will use polyethylene terephthalate (PET) and polyurethane (PU) plastic waste as feedstock for microbial transformations resulting in the production more sustainable Bio-PU (PU that contains monomers from biological origin) and contributing to a circular economy.

A circular economy for PET and PU plastic waste

Plastics are extremely successful materials with many properties that make them essential in our everyday lives. They are lightweight, waterproof, easy and affordable to make. However, they are difficult to degrade and this has led to unacceptable levels of plastic pollution which is a danger to health and the environment.

Two commonly used plastics are polyethylene terephthalate (PET), widely used in the beverage industry, and polyurethane (PU) found in many products including insulation panels, furniture and footwear. Plastics can be recycled by mechanical and chemical methods but the main goal of the MIPLACE project is to develop an efficient bio-based process that uses PET and PU plastic waste as feedstock to produce molecules that are of value and of industrial interest. Specifically, PET and PU plastic waste undergo microbial transformation into monomers that can be used for the synthesis of Bio-PU (PU that contains monomers from biological origin) which leads to more sustainable and environmentally friendly construction and insulation material. In addition, Bio-PU is recyclable which completes a fully circular strategy for the sustainable production of this important material.

Microbial transformation of PET and PU into value-added materials not only creates a circular economy for this type of plastic waste but also generates an economic incentive to increase the end-of-life plastic collection in favour of a bio-based economy, keeps plastic waste out of the environment and reduces the use of fossil fuels.

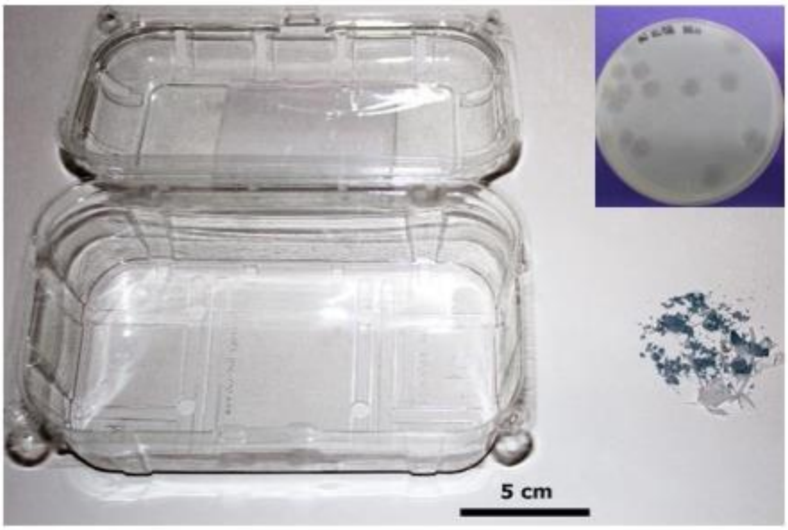

Figure 1: Biological degradation of a PET tray and screening of enzymes active against PU.

The screening of microorganisms from relevant environments results in the identification of enzymes able to degrade post-consumer PET. The picture shows the result of 24 hours of hydrolysis of a plastic tray with a newly discovered enzyme in MIPLACE. The inset shows bacterial strains generating clearing halos due to the degradation of impranil, a liquid formulation of PU.

The MIPLACE approach for tackling plastic waste

MIPLACE is comprised of five partners from across Europe. The partners have complementary expertise and work synergistically towards a common goal. This expertise includes PET enzymatic degradation, microbial community engineering, metabolic engineering, computational understanding of complex microbial communities and PU synthesis.

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Consortium

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Project duration:

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Total budget: 1.7 M€

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Our experimental approach is based on the following main tasks that run in parallel: 1) the identification of enzymes or natural microorganisms able to degrade PET and PU plastic waste; 2) engineering microorganisms for the biodegradation of plastics and the assimilation of the resulting monomers and biosynthesis of molecules of interest and 3) the chemical synthesis of novel polymers using the monomers obtained biologically in Tasks 1 and 2. PET plastic is difficult to degrade enzymatically but researchers have explored different ways to improve its hydrolysis. For example, by using chemical and physical pretreatments on the polymer and by conducting hydrolysis at higher temperatures (although most organisms and enzymes cannot tolerate high temperatures). Screening of environments polluted with different types of PU has identified microorganisms that can be cultured using PU as a growth substrate. Enzymes involved in plastic degradation have been transferred to strains of biotechnological interest. This allows the use of the products resulting from degradation to be used as substrates for bacterial growth and their conversion into other molecules of interest. We are investigating the synthesis of environmentally friendly and biodegradable Bio-PU which is made from a combination of building blocks from chemical and biological origin. Towards the end of MIPLACE several of the building blocks for Bio-PU synthesis will be sourced from our sustainable microbial processes. The physicochemical properties and biodegradability of new PU formulations is being investigated.

Current results

MIPLACE has achieved results in all areas of its research. Advances have been made in the understanding of physical and chemical pre-treatment methods for enhancing the enzymatic hydrolysis of PET. Solvents have been identified that significantly help to increase PET degradation by reducing its crystallinity. Alternatively, it is possible to conduct the hydrolysis at high temperatures (65°C), which makes the polymer more accessible to enzymatic attack. This has the caveat that most organisms and enzymes cannot tolerate the high temperatures. However, different synthetic strains, including some thermophiles such as *Geobacillus* which can grow at temperatures of 60-70°C and at which enzymatic activity against PET is maximal, are being engineered. Screening efforts have also resulted in several new enzymes able to hydrolyse PET including one, PHL7, that outperforms others reported previously by exhibiting greater thermostability and activity at 65°C compared to others such as leaf-branch compost cutinase (LCC), currently considered the gold standard in the field. Screenings in environments polluted with different types of PU (such as construction boards and foams) have been conducted and have identified microorganisms that can be cultured using PU as a growth substrate and display activities that promote its degradation. We have confirmed that newly engineered strains are able to produce the enzymes required for degradation and can grow using the monomers of PET – terephthalic acid (TA) and ethylene glycol (EG) – as their main feedstock. These strains are being manipulated further and currently we have demonstrated that they can be used for the production of hydroxyalkanoic acids (HAAs) which can be used for the synthesis of novel polymers. Several formulations of PU have been generated, the components of which were from commercial or chemical origin. However, some of these components are expected to be obtained from microorganisms towards the end of the project.

Five scientific papers have been published in peer-reviewed journals.
"Out of the Abyss: Genome and Metagenome Mining Reveals Unexpected Environmental Distribution of Abyssomicins". https://www.frontiersin.org/articles/10.3389/fmicb.2020.00645/full . This paper describes a meta-analysis of published genomes and metagenomes for the identification of novel enzymatic activities of interest.

"A quantitative method for proteome reallocation using minimal regulatory interventions" https://www.nature.com/articles/s41589-020-0593-y . This work reports a new method to optimise bacterial strains for the increased expression of recombinant proteins and pathways.

"Loss of a pyoverdine secondary receptor in Pseudomonas aeruginosa results in a fitter strain suitable for population invasion" https://www.nature.com/articles/s41396-020-00853-2 . This work explores the evolutionary stability of microbial populations and describes a method for stable invasion with an organism of interest based on the exploitation of interactions via public goods in the community.

"Genome analysis of the metabolically versatile *Pseudomonas umsongensis* GO16: the genetic basis for PET monomer upcycling into polyhydroxyalkanoates" https://sfamjournals.onlinelibrary.wiley.com/doi/10.1111/1751-7915.13712 . The paper describes the characterisation of a bacterial organism able to feed on PET monomers and produce environmentally friendly bioplastics.

"Low Carbon Footprint Recycling of Post-Consumer PET Plastic with a Metagenomic Polyester Hydrolase" https://chemistry-europe.onlinelibrary.wiley.com/doi/10.1002/cssc.202101062 . The work describes de identification and characterisation of a new set of enzymes able to hydrolyse PET including one twice as fast and stable as the best one known to date.

Project website: www.miplacebio.com
Twitter: @miplacebio

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