

BioTech Research & Innovation Hack

2021

# ERA CoBioTech Funded Projects at A Glance: MILIMO

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Microbial conversion of lignin to monomers for bio-based plastics using synthetic biology





### **Project coordinator:**

Timothy Bugg University of Warwick, (United Kingdom)

### Consortium

University of Stuttgart, (Germany)

Spanish National Research Council (CSIC), (Spain)

Institut National de la Recherche Agronomique (INRA), (France)

nova-Institut GmbH, (Germany)

Biome Bioplastics Ltd, (United Kingdom)

### **Project duration:**

01 March 2020 - 1 April 2023

**Total budget:** 975.000 €

## MILIMO

Microbial conversion of lignin to monomers for bio-based plastics

Researchers in the MILIMO project are using synthetic biology tools to engineer Rhodococcus jostii RHA1 and Pseudomonas putida KT2440 to convert the biopolymer lignin into pyridine-dicarboxylic acids, which are monomers for the production of polyester bioplastics.

### Production of new bio-based plastics

Plastic production from petrochemicals and plastic waste are causing global environmental impact, which was recognised by EU in 2018 establishing a goal of all plastic packaging and >50% of plastic residues being recyclable by 2030. Bio-based and biodegradable plastics are an important part of the solution to this societal problem. Companies such as Biome Bioplastics (Biome), which is a partner of this project, see a rising enquiry level for compostable/bio-based packaging. In 2015, Biome Bioplastics and the University of Warwick first developed a biocatalytic route for conversion of the bioploymer lignin, found in lignocellulose, into pyridine-dicarboxylic acids (PDCAs), using engineered Rhodococcus jostii RHA1. The PDCA products can then be converted into a new bioplastic with similar properties to PBAT (Ecoflex), but which is derived 100% from renewable sources. The aim of this project is to use metabolic engineering and synthetic biology approaches to improve the yield of PDCA bioproducts, and to develop a process for bioconversion of lignin or lignocellulose into PDCAs, that could be commercialised. This would lead to a new commercial bioplastic that could be applied to a range of applications.

### Technologies used in the MILIMO project

The MILIMO project brings together researchers with complementary expertise to tackle the project objectives. The groups of Professor Tim Bugg (University of Warwick, UK) and Dr Eduardo Diaz (CIB, Madrid, Spain) have expertise in the metabolic engineering of Rhodococcus jostii RHA1 and Pseudomonas putida KT2440, the two host organisms to be studied in the project, using genetic modification and synthetic biology approaches. The group of Professor Stéphanie Baumberger (INRA Versailles, France) have expertise in lignin characterisation, and her group will use these analytical methods to study the effect on the high molecular weight substrate used for bioconversion. The group of Prof Ralf Takors (University of Stuttgart, Germany) have expertise in scale-up of bioconversions, and biochemical engineering, they will develop efficient methods for product extraction and optimise the bioprocess to produce PDCA products. The inductrial partner Biome Bioplastics Ltd (UK) have expertise in the synthesis and formulation of new polyester bioplastics, they will be the end user of the new technology developed in the project. Nova Institute have experience in sustainability and life cycle analysis, they will apply these methods to exavluate the environmental and social impact of the bioprocess developed in the project.

### **Current results**

The MILIMO project has already made good progress towards the project objectives, in spite of the COVID-19 pandemic. At Warwick, overexpression of lignin oxidation genes and accessory genes for lignin breakdown in the Rhodococcus jostii production strain has been tested, and 2-2.5 fold improvement in PDCA yield has been observed. A novel oxidase & type 1 protein export system has been identified by researchers at CIB in Pseudomonas putida, that is overexpressed in the presence of lignin, and its molecular properties are being studied, in collaboration with INRA Versailles. Synthetic biology is being used at CIB to engineer specialist P. putida strains capable of degrading specific types in lignin. A two-stage process has been successfully developed at Stuttgart for downstream processing of PDCA from aqueous solution, and bioprocess development is underway. Biome Bioplastics Ltd have established a new market for their biodegradable bioplastics, forming a partnership with Suregreen Ltd to manufacture biodegradable tree shelters.



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No [722361] The MILIMO project has so far published two journal publications.

- "Metabolic engineering of Rhodococcus jostii RHA1 for production of pyridine-dicarboxylic acids from lignin" E.M. Spence, L. Calvo-Bado, P. Mines, and T.D.H. Bugg, Microbial Cell Factories, 20, article 15 (2021). This paper describes the metabolic engineering of Rhodococcus jostii RHA1 to produce PDCA products via insertion of ligAB genes to generate PDCAs onto the chromosome, gene deletion of the competing beta-ketoadipate pathway, and overexpression of lignin-oxidising dyp2 gene.
- 2. "Assessment strategy for bacterial lignin depolymerization: Kraft lignin and synthetic lignin bioconversion with Pseudomonas putida." E. Rouches, H. Gómez-Alvarez, A. Majira, Z. Martín-Moldes, J. Nogales, E. Díaz, T.D.H. Bugg, S. Baumberger. Bioresource Technology Reports, 15, article 100742 (2021). This paper describes the development of bioanalytical methods to study lignin breakdown by Pseudomonas putida KT2440, studying both the high and low molecular weight lignin fractions.

A conference poster describing the new downstream processing method has been presented at the Himmelfahrtstagung on Bioprocess Engineering in May 2021. Three further conference presentations have been made by TDHB and ED, mentioning work in the project.



Figure 1: MILIMO Partners MILIMO partners meeting in Paris in 2017

#### Project website :

https://warwick.ac.uk/fac/sci/chemistry/research/bugg/bugggroup/research/eracobiotech/



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