PROMICON

A MODEL-DRIVEN APPROACH TO UPCYCLING RECALCITRANT FEEDSTOCKS IN *PSEUDOMONAS PUTIDA* BY DECOUPLING PHA PRODUCTION FROM NUTRIENT LIMITATION

Key message

Pseudomonas putida is a key organism in sustainable biopolymer research due to its ability to produce and degrade polyhydroxyalkanoates (PHA), a biodegradable bioplastic. Its metabolic versatility, stress adaptability, rapid growth, genetic accessibility, and high productivity make strains like *Pseudomonas putida* KT2440 promising for biotechnological, environmental, and industrial applications. Researchers have developed a collection of *Pseudomonas putida* strains that overproduce PHA using growth-coupling methods, utilising plastics and lignin-related compounds.

Background

In the context of the current climate crisis and the emerging circular economy, a major goal is the replacement of crude-oil-based plastics with more sustainable and biodegradable alternatives. PHAs are native products of many bacteria and can be considered as an alternative to fossil-based plastics, with wide applications in medical and material fields. PHAs are stored as intracellular reserve storage granules, and they can play a role as sinks for carbon and reducing equivalents. During the first phase of their production, the bacteria are provided with an abundant supply of nutrients, particularly carbon, to allow them to multiply and accumulate biomass. This phase ensures that a substantial population of bacteria is available for the subsequent production phase. Once the desired cell density is achieved, the second production phase begins by limiting the availability of certain nutrients, particularly nitrogen, while still maintaining sufficient levels of other nutrients necessary for PHA synthesis. While the two-step bioprocess effectively produces PHA, it comes with important challenges including the need for separate growth and production phases, which results in energy-intensive and resource-consuming processes.

Objective

The goal of replacing crude-oil-based plastics with sustainable alternatives, such as Polyhydroxyalkanoates (PHAs), is important in the context of the climate crisis and the circular economy. PHAs, produced by bacteria, offer potential as biodegradable replacements for fossil-based plastics, with applications in medicine and materials. Their production requires a two-step bioprocess which, although effective, is energy-intensive and resource-consuming. In this study, these challenges are addressed through a model-driven approach to reroute carbon flux and remove regulatory constraints using synthetic biology.

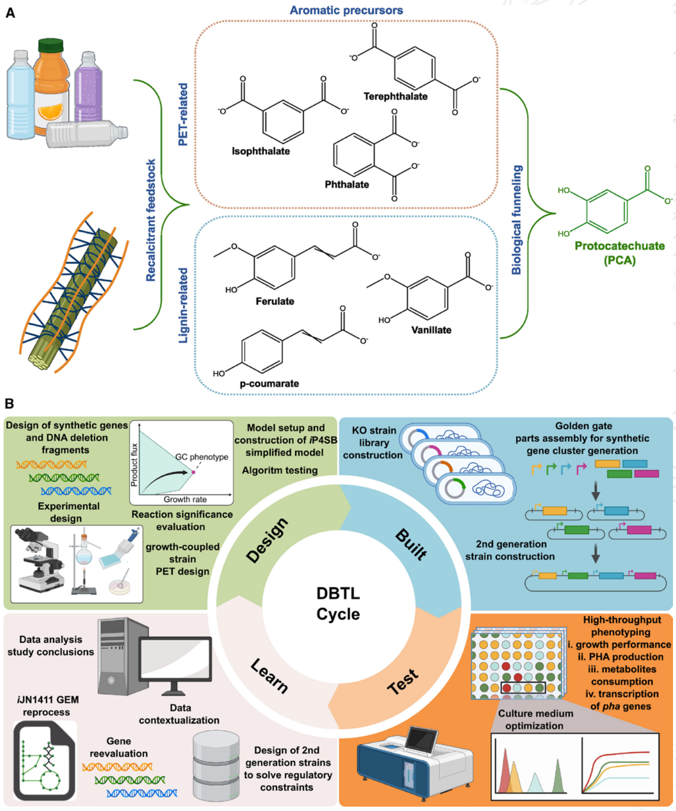

Figure. Workflow used in this study.

Results

The study achieved PHA production during the growth phase, resulting in the production of up to 46% PHA per cell dry weight while maintaining a balanced carbon-to-nitrogen ratio. The strains are also validated under an upcycling scenario using enzymatically hydrolysed polyethylene terephthalate as a feedstock. These findings have the potential to revolutionise PHA production and tackle the global plastic crisis by overcoming the complexities of traditional PHA production bioprocesses.

Source

Manoli, M.-T., Gargantilla-Becerra, Á., del Cerro Sánchez, C., Rivero-Buceta, V., Prieto, M. A., & Nogales, J. (2024). A model-driven approach to upcycling recalcitrant feedstocks in *Pseudomonas putida* by decoupling PHA production from nutrient limitation. *Cell Reports*, 43(4), Article 113979. https://doi.org/10.1016/j.celrep.2024.113979

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