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BOOK OF ABSTRACTS

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L22: **A Systems Biology Approach for Enhancing the Synthesis of Functionalyzed Polymers in *Pseudomonas putida***

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The environmental challenge associated with conventional plastics has created a need for alternative solutions, placing bio-based polymers like polyhydroxyalkanoates (PHAs) in a promising position. These polyesters are produced under nutritional stress conditions by several microorganisms including the model bacterium *Pseudomonas putida*. Carbon sources for PHA synthesis can be classified into two categories: i) PHA related substrates, primarily fatty acids, which provide high production yields, and ii) non-PHA related substrates, such as sugars, whose conversion into PHA precursors through *de novo* fatty acid synthesis route has been associated with low production yields [1]. Significant efforts have been devoted for the use of carbohydrates as carbon sources within a circular economy framework. To address this challenge, systems biology approaches, like genome-scale metabolic models, offer valuable insights into the metabolic features of PHA cycle in *P. putida* KT2440 [2]. These approaches enable the prediction of the key reaction to be deleted in order to redirect the metabolic routes of interest, and represent powerful tools for optimizing and enhancing the utilization of non-PHA related carbon sources in a sustainable manner.

In order to construct an optimized PHA producer *P. putida* KT2440 strain when using sugars as feedstock, we employed the *i*JN1411 genome-scale metabolic model to predict those genes to be deleted for eliminating potential competing pathways and redirecting carbon flux from acetyl-CoA towards PHA biosynthesis [3], and those genes to be added for using sucrose as carbon source. We obtained the engineered MT9 strain which was tested to produce PHA from sucrose. Additionally, the synthesis of the antimicrobial biopolymer PHACOS was explored by co-feeding the chemical precursor 6-acetylthiohexanoic acid (6-ATH) and glucose or sucrose [4].

The results revealed that MT9 cultures grown with sucrose as carbon sources achieved a PHA accumulation of 35% per cell dry weight (CDW) compared to 22% for the WT strain under the same conditions. Moreover, the addition of 6-ATH significantly increased the PHA content, both with sucrose and glucose, to approximately 70% of CDW, revealing the presence of 90% of functionalized 4- and 6- carbons monomers, and providing further evidence for the production of PHACOS.

References

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