A Study of Creation of Tailor-Made Synthetic Properties Pseudomonas Putida in Bacterial Host

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Abstract

Growing environmental concerns have rekindled interest in the manufacture of sustainable (bio) materials that can replace oil-derived items. Polyhydroxyalkanoates (PHAs) are isotactic polymers that serve as dynamic reservoirs of carbon and reducing equivalents in the central metabolism of producer bacteria. PHAs continue to be a popular starting point for thermoplastic and elastomeric materials that are renewable, biodegradable, biocompatible, and flexible. Pseudomonas species have long been recognised to create high-quality biopolymers, particularly medium-chain-length PHAs. Synthetic biology and metabolic engineering approaches have exploded in popularity in recent years, making it possible to tap into the untapped potential of Pseudomonas cell factories for the synthesis of custom PHAs. An overview of the metabolic and regulatory circuits that control PHA accumulation in Pseudomonas putida is provided in this article, as well as methods to the biosynthesis of new polymers (e.g., PHAs with non-biological chemical components in their structures). The potential for innovative PHAs to disrupt present and future market segments is now closer than ever. The analysis concludes by identifying barriers to widespread acceptance of bio-based PHAs, as well as ideas for programmable polymer biosynthesis in modified P. putida strains using alternate substrates.

Keywords: *Metabolism; Bacteria; Polyhydroxyalkanoates; Properties.*

I. INTRODUCTION

Growing global environmental concerns urgently call for smart alternatives to the use of oil-derived commodities to reduce our dependency on limited fossil resources—while limiting pollution and CO_2 emissions. Among the countless oil-derived commodities we rely on, plastics are in the spotlight as highly recalcitrant polymers produced at a high scale and extensively used for a wide range of consumer and industrial applications. In 2017, global plastic production reached≈350 million tons. The massive production of oil-derived plastics and inappropriate waste management strategies during the last decades inevitably led to significant plastic pollution of lands and oceans. Plastic wastes reach≈5–13 million tons per year, mostly accumulating in marine environments and causing severe environmental damage. Furthermore, plastic production has a direct impact on greenhouse gas emissions and thus con-tributes to global warming and climate change.

Bio based plastics have arisen as an alternative to traditional plastics as they present similar properties and offer additional advantages over petrochemical materials, e.g., reduced carbon footprint and the broader availability of waste management options. Bio based plastics are suited for a range of end-of-life alternatives, including reuse, mechanical and organic recycling, and energy recovery. These materials comprise “drop-in” polymers (chemically identical to their petrochemical counter parts, but derived from biomass and often non-biodegradable), and compostable and/or biodegradable plastics such as poly-hydroxyalkanoates (PHAs), polylactic acid, and starch blends.

Bacterial cell factories for PHAs production have been extensively reported in the literature over the last three decades. Among them, Pseudomonas species constitute an excellent model to study and manipulate PHA biosynthesis from a wide variety of substrates. Pseudomonas putida, for instance, is a natural PHA producer considered as a model bacterium for biodegradation studies and a production platform, mainly because of its high tolerance to solvents and oxidative stress conditions.[12]Such properties, together with a plethora of synthetic

biology (SynBio) tools tailored for targeted genetic manipulations developed in the last years and the availability of no less than eight genome-scale metabolic models (GSMMs)for in silico studies, position P. putida as one of the preferred SynBiochassis. Such unique features are of interest for several technical applications, e.g., production of complex molecules, degradation of contaminants, and, importantly, bio synthesis of tailor-made biopolymers. SinceP. Putida exhibits a remark-able capability to breakdown aromatic compounds, many of them being recalcitrant pollutants of their own, this host offers unique opportunities for the up cycling of contaminants into value-added polymers.

II. GENERAL ASPECTS OF BACTERIAL METABOLISM OF PHA AND PHYSICOCHEMICAL PROPERTIES OF THE MATERIALS

PHAs are a group of structurally varied storage polyesters that are collected as carbon and energy reserves by many bacterial species. PHAs have been recognised as a green alternative to currently used plastics generated from petroleum since their discovery in the early 1900s, because some of these biopolymers exhibit physical and thermochemical qualities equivalent to traditional, oil-based materials. PHAs are also biocompatible and may be made in bacterial cell factories using agro industrial waste materials or low-cost substrates. Furthermore, these materials are well known as biodegradable and compostable, a characteristic that offers PHAs as a viable solution to the worldwide plastic waste dilemma. Decades of study have advanced our knowledge of bacterial PHAs' biochemical routes, metabolic control, and ecological relevance.

Bacterial polyester biosynthesis is a response to unbalanced growing conditions characterised by a high supply of carbon and a lack of an inorganic nutrient, such as nitro-gen, oxygen, or phosphorous. PHAs are accumulated by bacteria as intracellular granules. Carbonosomes are insoluble and highly hydrophobic inclusions that contain PHAs and are made up of the polymer itself and an organised layer of granule-associated proteins (GAPs) that perform structural, biosynthetic, catabolic, and regulatory functions. PHA synthases, depolymerases, and a group of low-molecular-weight proteins known as phasins are all GAPs. Other GAPs, such as transcriptional regulators, hydrolases, reductases, or acyl-coenzyme A (CoA) synthetases, can also be found on the granule surface, though their functions are unknown. PHA synthases, on the other hand, are necessary GAPs for polyester biosynthesis. Synthases release one CoA molecule each catalytic cycle to convert (R)-3-hydroxyacyl-CoA [(R)-HA-CoA] units into a polyester chain. PHA depolymerases (yet another form of GAP) can further breakdown the resultant molecule into free (R)-HA monomers to meet metabolic needs. Phasins, on the other hand, are amphipathic proteins found in the interphase between PHA and the cytoplasm, and they perform regulatory and functional functions in cell division, such as controlling the formation, location, quantity, and size of polymer granules, as well as granule segregation.

III. PHA BIOSYNTHESIS IN PSEUDOMONAS SPECIES

Early on, Pseudomonas polymers of the mcl-PHA type were discovered as potential compounds. Although other bacterial species have been found to collect such intracellular inclusions, the focus of this analysis will concentrate on PHAs generated by Pseudomonas, with P. putida KT2440 as the ideal chassis for biopolymer metabolism modification. The most common Pseudomonas species described as PHA makers in the original literature, where it is clear that multiple natural isolates have been identified as viable bioplastic candidates. The species originally identified as P. oleovorans is one such example. The first-case occurrence of mcl-PHA was discovered in the intracellular inclusions of P. oleovorans (later termed P. putidaGPo1) growing on n-octane (i.e., a C8 substrate) as the only carbon source. A co polyester containing 89 percent (mol/mol) 3HO and 11 percent (mol/mol) 3HHx forms these inclusions. Many more studies have now confirmed that the manufacture and accumulation of mcl-PHAs are a common feature of Pseudomonas species belonging to the rRNA homology group I, which includes both fluorescent and non-fluorescent specimens. This feature is utilised as a phylogenetic marker since it is so conserved across species.

P.putidaKT2440, a common soil bacterium, makes mcl-PHA copolymers with monomers ranging from C6 to C14, depending on the substrate. The phagene cluster architecture in this model organism is highly conserved among mcl-PHA producing strains.

IV. PSEUDOMONAS PUTIDA AS AN INDUSTRY BACTERIAL HOST: CREATING TAILOR-MADE SYNTHETIC PROPERTIES

P. putida has established a reputation as a potential biotech microbial chassis. Several research organisations have begun to explore ways to increase its inherent capacity. Improved features of streamlined P. putida strains with shortened genomes include enhanced ATP and NAD (P) H availability, greater growth capabilities, and increased resilience to oxidative stress.

Due to the lack of fermentative pathways and the inability to employ alternate electron acceptors, P. putida is a strictly aerobic bacterium. The performance of P. putida in large bioreactors can be improved by addressing this issue. In strain KT2440, synthetic fermentation pathways and nitrate/nitrite respiration were added, resulting in increased survival in anoxic conditions. In addition, anoxic culture of P. putida in the anodic compartment of a bio electrochemical system (BES) was established, employing redox mediators and an anode as an extracellular electron sink to balance intracellular redox and energy parameters. P. putida's unique field of electro biotechnology is an ideal starting point for high-yield sugar acid generation without the need of oxygen. Furthermore, an intriguing research has succeeded to modify the morphology of P. putida in order to change its lifestyle from planktonic to biofilm-based, allowing it to be more resistant to severe reaction conditions during bio transformations. The whole-cell catalyst in Pseudomonas species may be modified to adopt a spatial layout that considerably simplifies the purification of extracellular products. Furthermore, in some whole-cell bio catalytic techniques, auto display of enzymes might be advantageous: The surface-displayed biocatalyst is easily stable and purified since it is attached to the cell as a matrix, and substrates and products do not need to traverse the membrane barrier.

Using the native P. putida OprF signal peptide, an enhanced auto transporter-based surface display of an esterase and a glucosidase was recently shown using P. putida KT2440. Surface adhesion proteins, exopolysaccharides, fimbriae, the O-antigen side chain, the flagellum, and other envelope-associated components were removed from the parent strain P. putida KT2440 (4.7 percent genome reduction size) to make the cell surface more accessible to the outside media. The derived strain EM371 serves as a platform strain for artificial adhesins, having previously been utilised to successfully display designer protein scaffolds on the surface of P. putida cells, allowing for the creation of artificial cellulosomes. However, under difficult bioprocessing conditions, the degree of surface exposure may need to be tuned further, as EM371's higher cell surface contact area also leads to increased vulnerability to external stressors.

V. CONCLUSION

The extensive primary literature detailing PHA biosynthesis in Pseudomonas species attests to the host's metabolic plasticity, which has allowed it to exploit a wide range of substrates to drive (and change) polymer production throughout time. The use of refractory substances as carbon sources (their decomposition requires severe biochemical transformations) and feeding substrates modified with non-natural chemical groups are two of the most prevalent instances identified in the literature. Many obstacles must yet be overcome in order to attain effective biopolymer synthesis. The relatively low titers of new PHAs generated utilising feeding strategies—due, in part, to the toxicity of substrates or their sluggish and inefficient conversion to PHA precursors—is one of the key challenges to overcome. Furthermore, the integration of changed monomers is still not programmable and is still reliant on complex host regulatory signals (some of which still elude full understanding, let alone engineering). In the near future, smart metabolic engineering and SynBio techniques will be aimed at overcoming this barrier. In relation to this, all chemical groups incorporated into polymers formed by Pseudomonas are invariably visible in the polymer's side chain.

VI. REFERENCES: -

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