A Study of Pseudomonas Putida of Core Carbon and Energy Metabolism in Biotechnology

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Abstract

The Gram-negative, rod-shaped bacteria Pseudomonas putida may be found in a wide range of biological environments. This ubiquity can be attributed to its extremely diverse metabolism, which has been evolved to endure physicochemical stress, as well as its ability to flourish in severe conditions. Because of these qualities, there is a rising interest in using this microorganism in industry, and research in this area has advanced rapidly in recent years. The use of low-cost renewable feedstocks and waste streams to manufacture value-added compounds, as well as constant advances in genetic strain engineering and systems biology knowledge of this bacterium, are also important drivers in this regard. Systems biotechnology, which combines systems metabolic engineering methodologies with unique bioprocess engineering ideas, such as novel reactor designs and renewable feedstocks, is driving this progress.

Keywords: *Pseudomonas putida; metabolic engineering; Biotransformation; feedstock's.*

I. INTRODUCTION

Due to its metabolic adaptability and low nutritional requirements, Pseudomonas putida is a Gram-negative road-shaped bacterium that may be found in a variety of environmental niches. Extensive biochemical investigation of this bacterium has been carried out in recent years, sparked by the pioneering finding of its high capability to breakdown very resistant and inhibitory xenobiotics. Furthermore, P. putida is extremely resistant to harsh environmental conditions such as high temperature, low pH, or the presence of toxins or inhibitory solvents. It's also genetically accessible and develops quickly with little nutritional requirements. Meanwhile, P. putida is effectively employed to produce bio-based polymers and a wide spectrum of chemicals, much beyond its original goal of harmful compound breakdown. The genome-wide pathway modelling and sequencing of its genomic repertoire have opened up new options for further engineering this bacterium into a versatile cell factory for bio-industrial use. As a result, the genetic repertoire and phenotypic behaviour of various species of P. putida vary to some extent, resulting in a wide variety of industrial application possibilities. This review focuses on fundamental elements of P. putida's cellular physiology as well as current advances in systems biology and systems metabolic engineering.

The opportunistic and undemanding feeding capacities of P. putida, as well as its fast development and resilience in the face of oxidative stress and harmful substances, reflect the diversity of its natural habitat. Beginning in the 1960s with the discovery of P. putida's potential for xenobiotic biodegradation, research into the genetics, biochemistry, and physiology of this organism has progressed steadily throughout the previous five decades. This resulted in the decryption of the whole genetic repertoire and the creation of genome-scale metabolic models for in silico simulations and data mapping, among other things. A rising variety of techniques for systems-level profiling, targeted genetic and genomic modifications are also being developed. This growing body of knowledge and technology, along with the bacterium's inherent biochemical capabilities, opens up a world of economic possibilities. Representative members of the species have been discovered as plant growth-promoting bioremediation agents and hosts for industrial bio-manufacturing, including bulk and speciality chemical synthesis, among other things.

II. CARBON CORE METABOLISM OF P. PUTIDA

The core routes of carbon metabolism, which receive carbon from the many converging pathways of substrate use and supply building blocks, cofactors, and energy for the added-value products of interest, are of special relevance for industrial application of P. putida. It's worth noting that P. putida's major catabolic pathways differ in crucial ways from those seen in many other prokaryotic organisms, making its route repertoire and application highly unique (Fig. 1). Fast growth, high biomass output, and low care requirements are all key traits for industrial use.

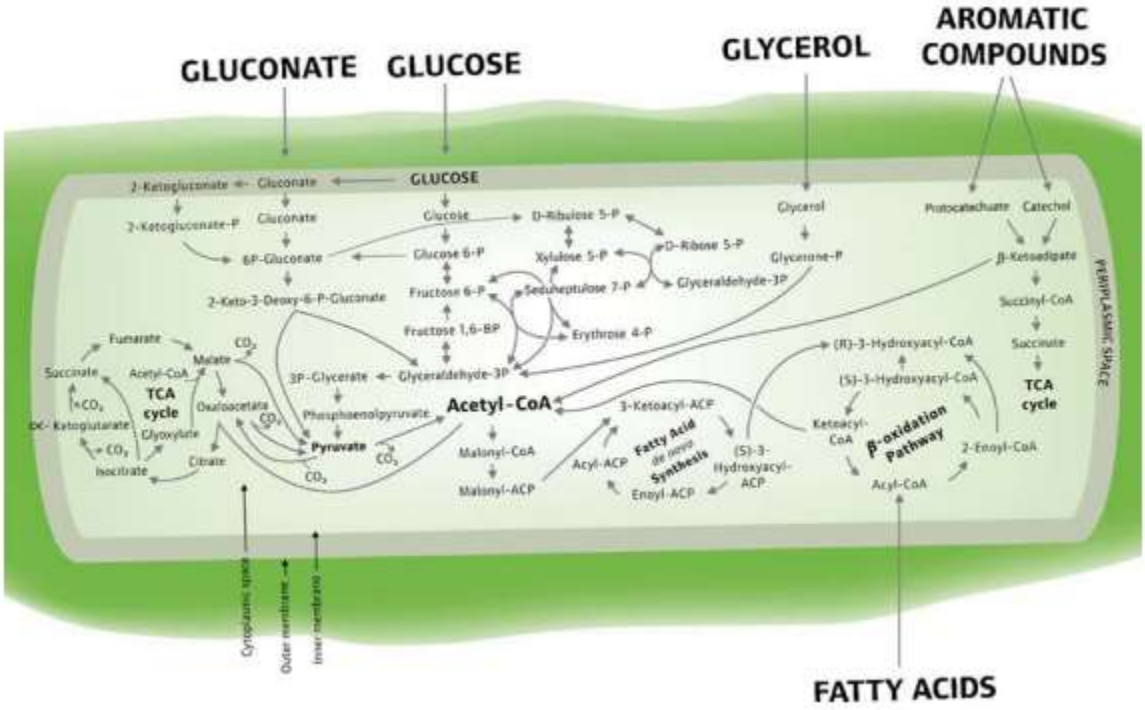

Figure 1: Metabolic pathways in the carbon core metabolism of Pseudomonas putida

Take-up of the substrate unlike many other industrial microbes, such as Escherichia coli, Coryne bacterium glutamicum, or Bacillus subtilis, pseudomonads do not favour glucose as a carbon source. Carbon catabolic suppression prevents glucose absorption in the presence of succinate and other tricarboxylic acid (TCA) cycle intermediates. It's worth noting that several of the methods of substrate absorption differ from those seen in other bacteria. P. putida assimilates glucose via facilitated diffusion via the unique porin OprB, unlike the commonly seen phosphoenolpyruvate-carbohydrate phosphotransferase system (PTS). P. putida is capable of using raw glycerol, a technical by-product of the biodiesel industry, as an industrial substrate. It cannot naturally grow on carbon five sugars like D-xylose or L-arabinose, but it has recently been modified to use these sugars, which are essential elements of lignocellulosic biomass.

III. REGULATION OF CORE CARBON AND ENERGY METABOLISM

P. putida is frequently discovered in polluted areas, indicating that the organism has a remarkable capacity to adapt to harsh circumstances. Its distinctive cyclic core metabolism, which is regulated by redox requirement, is critical for the great endurance seen.

Glucose is either absorbed into the cytoplasm or oxidised in the periplasm after entering the periplasmic region. The later oxidation route produces gluconate (GLN) and then 2- ketogluconate (2KG). Both acids may be carried into the cytoplasm and phosphorylated into 6-phosphogluconate (6PG) and 6-phospho-2-ketogluconate (6PG) respectively (2K6PG). As a result, there are three separate glucose entry mechanisms into core metabolism, all of which converge at the level of 6PG. P. putida uses the oxidation pathways to avoid direct ATP-costly glucose absorption via an ABC transporter (GtsABCD) and to partially uncouple ATP and NADH production. Each oxidation step from periplasmic glucose to GLN and 2KG releases two electrons, which is connected to ATP synthesis via the ATP

synthase. It was recently shown that glucose-grown cells produce an excess of ATP, whereas the oxidation route contributes considerably to the ATP supply. Furthermore, due to the lack of the crucial glycolytic enzyme 6-phosphofructo-1-kinase, P. putida has an incomplete Emden-Meyerhof-Parnas (EMP) pathway (Pfk). The Entner-Doudoroff (ED) route catabolizes the core intermediate 6PG nearly entirely, yielding the two C3 intermediates pyruvate (PYR) and glyceraldehyde-3-P. (G3P). Lower catabolism is used by a large proportion of the former. However, a considerable portion (about 10–20% under balanced development conditions) is recycled back to hexoses via the gluconeogenic EMP route, which is part of the EDEMP cycle, an amphibolic architecture.

Because the NADPH production is linked to the process catalysed by glucose-6-P 1-dehydrogenase (G6PDH), it is heavily influenced by recycling and the proportion of glucose phosphorylated by glucokinase (GLK). In P. putida, the capacity to modulate NADPH production at the expense of ATP is a critical element in oxidative stress endurance. This property is extremely useful in redox-demanding biocatalytic activities, and it has also been demonstrated to be critical for the evolvability of new catabolic pathways in this bacteria. Implementation of a functional linear glycolysis based on the EMP route, which endows cells with fresh tailor-made features, revealed de novo remodelling of the core carbon metabolism of KT2440.

IV. CONCLUSION

P. putida's product range, like that of other industrial microbes, has changed dramatically in recent years. Its high genetic accessibility and natural tolerance appear to be favourable characteristics for overcoming the toxic and harsh circumstances commonly associated with commercial biocatalysis and the de-novo production of frequently artificial compounds. It's worth noting that this adaptable bacteria is used in a number of commercial biotechnology operations. The enzyme repertoire of P. putida is used in the commercial production of chiral chemicals. Given the intriguing portfolio of innovative products becoming available via efficient P. putida cell factories, the application range of P. putida in industrial biotechnology has strong potential to grow and extend in the future. Integration of P. putida into existing or already developing pipelines of using renewable feedstocks or industrial wastes for sustainable bio-production, as depicted, might be an interesting future development. P. putida can degrade and metabolise a wide range of chemicals, including complex aromatics, thanks to its extensive intrinsic route repertoire. It appears that coupling P. putida to diverse streams of renewable feedstocks is helpful.

For example, lignocellulosic biomass from catalytic pyrolysis containing aromatic compounds might be transformed into added-value products like cis–cis muconate, an ideal precursor for the synthesis of adipic acid, by P. putida, which is capable of using such substrates. P. putida appears to be useful in the development of new chemicals or materials, which is also in the sugar pipeline. P. putida's inherently strong endurance to harsh and hazardous environments, along with flexible genetic alterations, provides a great starting point for converting it into a manufacturing platform for additional, nonnatural compounds that are currently unavailable. To unravel the complexity of the P. putida central and peripheral metabolic pathways and optimise them, experimental and computational systems level methodologies will be required. Synthetic biology will open up a new degree of design flexibility for reshaping metabolism for increased bio-production, such as fine-tuning expression and controlling regulatory networks, or integrating complicated heterologous pathways. Rational strain engineering will be supplemented by fresh ideas or evolutionary engineering, enhancing industrial Pseudomonas strain implementation via novel phenotypes with even greater tolerance to industrial conditions, a promising future for P. putida.

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