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Microbial Cell Factories: Biological Agents for Three Carbon Backbone Organic Acid Production Using Agro-Industrial Wastes

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Abstract

At present, the mass production of basic and valuable commodities is dependent on petroleum-based industries, which leads to the depletion of finite natural reserves and accumulation of non- biodegradable and hazardous wastes. Therefore, a major step in the development of a sustainable environment is the shift from dependence on petrochemicals to the use of renewable resources such as agro-industrial wastes. Microbial cell factory is an approach of bioengineering which considers microbial cells as a production unit in which the optimization process largely depends on metabolic engineering. Microorganisms are bio-engineered using evolutionary, enzyme and process engineering tools to reduce the detrimental effects of inhibitory compounds on microbial performances and to effectively utilize the inexpensive renewable carbon sources from agro-industrial wastes to produce various chemicals of interest. Organic acids are valuable compounds with extensive applications, primarily serving as raw materials for various chemical industries. Microorganisms produce these compounds mainly as intermediate products or occasionally as the major products. The use microorganisms to produce such organic acids has been normal and a very simple method to synthesize them in very pure form. In particular, bio-based organic acids with three-carbon backbones, including lactic acid, propionic acid and 3-hydroxypropionic acid have gained significant attention due to their wide application in manufacturing, pharmaceutical and textile industries. Therefore, the use of microbial cell factories would enhance the production of such organic acids using agro-industrial wastes.

Introduction

The intensive economic growth during the past century is accompanied by high energy consumption. Now-a-days fossil fuels (coil, petroleum, and natural gas) continue to serve as primary energy source and fundamental raw materials for manufacturing various chemicals. The fossil fuels were formed and stored for millions of years underground and their extensive use has led to the situation where the vegetation present on Earth cannot treat the carbon dioxide emitted by photosynthesis. As a consequence, the strong emissions of carbon dioxide and greenhouse gases affect and change the climate. One of the ways to manage with this global problem is to close the natural carbon cycle using renewable sources as a platform for biofuels and chemicals production, and thus enabling recycling of the biological sources. A major step in the development of a sustainable, industrial society will be the shift from dependence on oil to the use of renewable resources. In the prospect of environmental sustainability, the utilization of agro-industrial waste residues as feedstock for the production of biofuels and basic synthetic chemicals in biorefineries has gained widespread attention (Prasad *et al*., 2020).

The agro-industrial residues are defined as different types of wastes which are generated from the food and agriculture industry. According to NPMRC (2020) every year, roughly 500 million tonnes of agricultural residue are produced, of which 18.4% (92 million tonnes) is burnt in India. These residues include multiple plant-based materials, such as straws, stems, leaves, stalks, husks, shells, peels, seeds, lint, pulps, stubbles, bagasse brewer's spent grains, spent coffee grounds, and some animal byproducts (Yankov *et al*., 2022).

Microbial Cell Factories

Microbial cell factory is an approach of bioengineering which considers microbial cells as a production facility in which the optimization process largely depends on metabolic engineering. Microbial cell factories are engineered microorganisms designed and optimized to produce chemicals of interest from renewable resources such as nonedible biomass or even carbon dioxide. The term “metabolic engineering” was first officially suggested by Baily in 1991. Microorganisms can theoretically produce all the metabolites present in their metabolic network, but the efficiencies of producing most, if not all, of these chemicals by employing naturally occurring microorganisms are rather low. Through metabolic engineering, a microorganism can be engineered to utilize an inexpensive renewable carbon source as a substrate to produce a chemical of interest, even those non-native to its metabolism. The first generation of metabolic engineering began with the development of molecular tools that enabled the deletion, insertion or replacement of genetic components in the microbial chromosome or through the use of plasmids (Son *et al.*, 2022).

Construction of Microbial Cell Factories

Selection of starting strain

The metabolic engineering process starts with the careful selection of the initial strain for potential modifications. Since it is improbable that a single wild type organism will possess all the necessary traits to produce a wide range of products, the choice of the initial strain considers factors such as its metabolic capacity for the desired product, compatability with the bioprocess, ease of genetic and metabolic manipulation, ability to efficiently use cost-effective raw materials, and others. In silico genome-scale metabolic modelling and simulation proves to be a valuable tool in the process of selecting an appropriate organism (Blin *et al*., 2016).

Removal of negative regulatory circuits

In the process of overproducing certain natural metabolites, a prevalent challenge arises in the form of feedback inhibition and transcriptional attenuation control within the production pathway. These mechanisms are instigated by the buildup of the desired product and can manifest as inhibitory regulations occurring at both the transcriptional level and via allosteric control of pathway enzymes. Addressing these negative regulatory factors is imperative at the initial stages of strain development. When dealing with transcriptional regulation, the approach is relatively straightforward. DNA manipulation techniques enable the relatively straightforward modification of chromosomal transcription regions to incorporate desired alterations. An alternative approach involves the disruption of transcription factors engaged in this regulation. These strategies find application in various scenarios, such as the synthesis of diverse amino acids, were it comes to addressing allosteric feedback inhibition, it presents a greater challenge compared to dealing with transcriptional regulation. The simplest resolution involves seeking out heterologous enzymes that do not possess allosteric regulation. Another viable approach is to employ enzyme engineering to produce mutants that are resistant to feedback inhibition (Gustavsson and Lee, 2016).

Substrate utilization engineering

The production organism should not only have effective and finely optimized production pathways as discussed earlier, but it must also exhibit efficient utilization of the selected cost-effective feedstock. A classic illustration of this challenge is the incapability of *S. cerevisiae* to thrive on xylose. Given that xylose typically constitutes a significant portion, approximately 15-23% of the carbohydrates present in lignocellulose, this issue holds crucial importance for achieving enhanced yields from such feedstock. Consequently, this has been one of the extensively researched areas, with numerous report now available describing the engineering of *S. cerevisiae* to address this issue as reviewed (Laluce *et al.*, 2012).

Engineering cells to tolerate target products and inhibitors present in feedstock

To effectively utilize complex substrates like lignocellulose biomass and accumulate the target products to substantial concentrations, it is essential for industrial strains to exhibit resistance against both the target product itself and the inhibitors found in the feedstock. Nevertheless, developing inhibitors tolerance proves to be one of the most complex phenotypes to engineer, often involving numerous genes that are challenging to predict. Consequently, this phenotype is typically achieved through one of two primary approaches. Alternatively, rational engineering strategy to enhance both productivity and product tolerance is to introduce product efflux pumps that facilitate the efficient export of the desired product (Gustavsson and Lee, 2016).

Genetic stability and strain robustness

Industrial strains must exhibit long-term stability across numerous generations to enable the scaling up of production processes. This presents a significant challenge, as cost constraints and environmental considerations restrict the use of antibiotics at production scale. Consequently, it is more favorable to opt for chromosomal pathway integration over relying on plasmid-based expression if possible (Bassalo *et al.*, 2016).

Organic acids are high-value compounds which are utilized extensively as precursors in the chemical industry, making them a significant component of the global bulk chemicals market. The production of these compounds from microorganisms has been a common practice, and they represent the third largest category of bulk chemicals worldwide. These compounds are typically generated through microbial fermentation of carbohydrates and related substrates, primarily as intermediate products in metabolic pathways, though occasionally they may serve as the primary products (Liew *et al.*, 2016). Organic acids have been used from many years in the food, chemical, agriculture and pharmaceutical industries. Bio-engineered microorganisms could efficiently utilize the inexpensive renewable carbon sources from agro-industrial wastes and helps to produce various chemicals of interest. Therefore, the shift from dependence on petrochemicals to the use of renewable resources such as agro-industrial wastes could be an eco-friendly and sustainable approach.

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