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Next generation industrial biotechnology based on extremophilic bacteria

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Industrial biotechnology aims to produce bulk chemicals including polymeric materials and biofuels based on bioprocessing sustainable agriculture products such as starch, fatty acids and/or cellulose. However, traditional bioprocesses require bioreactors made of stainless steel, complicated sterilization, difficult and expensive separation procedures as well as well-trained engineers that are able to conduct bioprocessing under sterile conditions, reducing the competitiveness of the bio-products. Amid the continuous low petroleum price, next generation industrial biotechnology (NGIB) allows bioprocessing to be conducted under unsterile (open) conditions using ceramic, cement or plastic bioreactors in a continuous way, it should be an energy, water and substrate saving technology with convenient operation procedure. NGIB also requires less capital investment and reduces demand on highly trained engineers. The foundation for the simplified NGIB is microorganisms that resist contaminations by other microbes, one of the examples is rapid growing halophilic bacteria inoculated under high salt concentration and alkali pH. They have been engineered to produce multiple products in various scales.

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Introduction

Due to the low petroleum price combined with other new energy sources including solar power, shale gas, wind power and natural gas hydrate, products of traditional industrial biotechnology such as small molecular chemicals, polymeric materials and biofuels cannot compete with those based on petroleum [1,2]. It is therefore very urgent to develop new technology, namely, the 'Next Generation Industrial Biotechnology' or 'NGIB', to overcome the disadvantages of the current industrial biotechnology [3].

The NGIB should avoid the problems of current industrial biotechnology that reduce the competitiveness including heavy consumptions of fresh water and energy (for sterilization, oxygen supply and agitation), frequent microbial contaminations, batch instead of continuous production processes, difficulty to recycle the broth water, difficult and expensive product separation and purification, lengthy process development of one product from one strain (without a platform strain for multiple products), slow growth of production organisms, difficulty to develop fully automatic production processes, a low substrate to product conversion efficiency, and very expensive capital investment on facilities and equipment to fight contamination, among others (Table 1) [4–8].

All the difficulties from the current industrial biotechnology could be attributed to the production microorganisms that are fragile to contaminations by other microbes. For example, common industrial strains, such as *Escherichia coli*, *Bacillus* spp., *Corynebacterium glutamicum*, *Pseudomonas* spp. and yeast, are grown under mild conditions at weak acidic or neutral pH of 5–7 and 30–37 °C supplemented with yeast extract, the mild condition allow most microorganisms in the air, water and soils to grow [9]. Production facilities have to be sealed and completely sterilized to prevent any microbial invasion, this procedure dramatically increases the complexity of the bioprocesses, leading to high production cost. Thus, a platform microorganism with robustness and contamination resistance is very important. Other new properties of the platform organisms could be added via metabolic engineering or synthetic biology approaches [10].

This paper reviews challenges, opportunities and recent progresses on developing NGIB to increase biotechnology competitiveness.

Challenges

Economic and technological challenges

Except labor cost, the production cost of a bio-product consists of upstream and downstream parts: the upstream part contains substrates, including substrate pre-treatments, process energy including sterilization,

Table 1

Next generation industrial biotechnology (NGIB) compared with current industrial biotechnology.

Traditional industrial biotechnology	NGIB	How to achieve?	References
Heavy consumption of fresh water	Less dependent on fresh water	Seawater based or recycling process water	[17••,42,47]
Heavy energy consumption	Reduced energy consumption	Open unsterile process and high O_2 update	[17••,52]
Frequent microbial contaminations	Contamination resistant organisms	Screening robust microorganisms	[10,17••]
Heavy capital investments	Reduced capital investment	Low cost facilities and equipment	[44••]
Narrow microbial growth conditions	Flexible growth conditions	Selection on robust microorganisms	[41]
Batch processes	Continuous processes	Contamination resistant microorganisms	[42]
Hard separation of cells from medium	Reduced separation difficulty	Morphology engineering for large cell sizes	[51••]
Lengthy cell growth processes	Acceleration of cell growth	Synthetic biology to speed up cell growth	[52]
One strain for one product	A platform strain for multiple products	Construction of multiple product synthesis pathways in the platform strain	[44••,45••]
Product either in medium or in cells	Intra-cellular and extracellular products	Engineering co-production of more products	[53•,54•]
Growth on a single carbon source	Growth on mixed carbon sources such as kitchen wastes	Screening or constructing multiple substrate consuming organisms	[17••,28•,42]
Complete automation difficult	AI control automation	Robust organisms allow large control errors	[11]
Low conversion of substrate to product	Convert more substrate to product	Removal or weakening competing pathways	[17••,28•]
Hard to obtain intracellular products	Weakening the cell walls	Engineering cell wall synthesis mechanism	[54•]
Low extracellular product excretions	Increase outer membrane leakages	Weakening the outer membrane structures	[45••]

AI: artificial intelligence.

agitation, aeration, cooling and heating. Although the downstream part requires equipment and energy to separate tiny microbial cells from their growth medium, extract and purify intracellular or extracellular products. Any attempt to reduce energy consumption is beneficial for production cost reduction (Table 1). In addition, most microbial fermentation processes are prone to be contaminated over a long period of culture time, prohibiting the more efficient continuous processes from being widely used. A robust and contamination resistant microorganism is thus important to reduce production cost. This forms the basis for the NGIB to reduce bio-production cost (Table 1).

A robust strain resistant to other microbial contamination is the most important factor for the development of NGIB. The strain should also be able to rapidly grow. It should also have molecular engineering methods and tools for improving performances and for producing multiple products. As a platform production strain, it should have its genome information fully available. A strain with the following properties meets the requirements as a platform strain for the NGIB (Table 2): (1) rapid growth at very low or high temperatures, low or high pH and/or low or high osmotic pressure; (2) rapid growth on some unusual substrates such as long-chain fatty acids, methanol, cellulose, chitin, rubbers or even gaseous substrates H_2, CO_2 or methane; (3) rapid growth in the presence of high concentrated substrates, toxic chemicals such as short-chain length alcohols (C1–C4), short-chain length fatty acids (C1–C6), heavy metals (such as Cu, Zn, Pd, Hg, among others) or toxic compounds such as unsaturated fatty acids or aldehydes; (4) fast growth in the absence of sufficient water. A strain with combinations of any two or three or more features will even be better than a single property as the property combination will provide the platform strain even stronger property to resistant contaminations (Table 2).

Many archaea meet some of the above requirements except that they can only grow slowly. In addition, they are very difficult to be genetically engineered. Therefore, archaea are not suitable platform strains for NGIB. Although some extremophile bacteria, algae or fungi satisfy the above requirements, engineering methods and tools are also available or more easily developed for improved properties, especially for prokaryotic bacteria.

The main challenge is now becoming to identify and develop platform bacterial strains for NGIB with some combinations of the above properties.

Opportunities

Fortunately, some prokaryotes possess some properties that meet some of the above requirements, including acidophiles, alkaliphiles, psychrophiles, thermophiles, xerophiles, methanotrophs, halophiles and some bacteria that can utilize gaseous substrates or cellulose, among others (Table 2). Biocatalysts isolated from these organisms are termed extremozymes that possess extraordinary properties of salt allowance, thermostability, cold adaptivity, among others [11]. A few of these bacteria could be developed as platform strains for NGIB as described below.