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Opinion

Engineering Microorganism to Improve Butanol Tolerance

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Abstract

World is intense of alternative energy and as the fossil fuel reserves are near to be diminished, various alternative biofuels have been proposed to replace the petroleum, such as ethanol, biodiesel and butanol. Among them, the butanol is considered as the most valuable due to high energy content comparatively. The butanol is produced typically by *clostridium* species. Nevertheless, the problem with *clostridium* species is difficulty in manipulation, and proper handling. As an alternative, *Escherichia coli* is used frequently in bio-industries, being easy to manipulate and handle. Therefore, a clostridial butanol producing pathway was introduced into *Escherichia coli* for butanol production. However, the problem with *E. coli* based butanol production is the toxicity to butanol, a hydrophobic solvent, disrupts cellular metabolic processes. Here, we discuss the current progress and strategies to increase butanol tolerance in *Escherichia coli*.

Keywords: *Engineering Microorganisms; Bioenergy; Butanol Tolerance; Phenotype Engineering; Metabolic Engineering; Synthetic Biology*

The world is in enormous pressure due to the depletion of fossil fuel reserves. Therefore, special attention has been made for the production of alternative biofuels. Among biofuels, butanol is considered as an ideal replacement for petroleum, as it possesses special physical and chemical properties such as low viscosity, high energy content and low hygroscopicity [1]. Butanol can be blended with gasoline in any proportion. Traditionally, the butanol is produced with acetone-butanol-ethanol fermentation by *Clostridium* species [2]. Due to complex genetic system, it is hard to engineer the *Clostridium* species for efficient production of butanol. The alternative to clostridial based butanol production, the butanol synthesis pathway was introduced in to *Escherichia coli*, which is easy to handle and have the property to grow abruptly in aerobic conditions. However, the problem in butanol production using *E. coli* is the high toxicity and growth retardation by butanol. The focus of research is to enhance the tolerance of *E. coli* to butanol. Various strategies are applied to achieve these objectives, such as, the use of artificial transcription factors and controlling membrane related functions of an engineered cell [3]. The butanol toxicity is therefore, one of the major barriers for commercial production of butanol. Clostridial cellular metabolism ceases in the presence of 20 g/l or more solvents. The hydrophobic solvent butanol is more toxic than other solvents as it disrupts the membrane fluidity of the cell. It was found out that destabilization of membrane disrupts the important cellular functions like transport processes, glucose uptake and membrane bound ATPase activity. Different strategies are used to obtain the butanol tolerance. One approach is down regulation of *gldA* gene which encodes glycerol dehydrogenase. The natural system for solvent tolerance used by bacteria is efflux pump, these pumps export toxic compounds across the inner and outer membrane of the cell envelope in a single energy-coupled step. The ABC efflux pump of pseudomonas extrudes solvents and several antibiotics, while the DEF of the same species having 70% similarity with ABC extrudes only toluene [4]. The clostridium genes coding for enzymes involved in butanol production pathways have been reconstructed and introduced in *E. coli* and *S. cerevicae*. Although the engineered microorganism synthesizes butanol but the yield is poor. Furthermore,

similar to naturally butanol producing clostridium strains, *L. delbrueckii* and *L. brevis* were found to tolerate and grow in up to 3% butanol [4]. In *L. acidophilus* decarboxylase system of amino acid ornithine the ornithine decarxylase and amino acid permease, G- aminobutyrate antiporter and transcriptional regulator for amino acid Gad R was inactivated by insertional inactivation, improving the acid tolerance [4]. The over expression strategy for butanol tolerance was shown in the strain transformed with pACYC-1869 showed increased tolerance to butanol and prolonged metabolic activity, the CAC1869 clone shares homology with the xenobiotic- responsive element (XRE) family of regulatory protein [5]. Lee., *et al*. enhanced the tolerance of *E. coli* to butanol by developing a technique of artificial transcription factor engineering. The zinc finger DNA binding domains was linked with cyclic AMP receptor protein, and successfully overexpress and knock-out genes globally, as a result, the *E. coli* could tolerate 1.5% v/v butanol [3].

The advances in synthetic biology could provide solutions to many unanswered questions. Gene editing technology, CRISPR-Cas9 system has already solved the long lasting problems by editing the desired sites on a DNA, precisely. A robust butanol producer with a high tolerance activity could be a valuable asset for bio-industrial economy and solution for bioenergy.

Conflict of Interest

No conflict of interest exists.

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