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Repetitive domestication to enhance butanol tolerance and production in *Clostridium acetobutylicum* through artificial simulation of bio-evolution

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HIGHLIGHTS

▶ A novel approach was developed on the basis of dynamics of evolution and selection.
▶ A butanol-tolerant strain with increased butanol tolerance was obtained.
▶ Artificial simulation of bio-evolution (ASBE) was employed in biotechnology.
▶ The efficiency of ASBE was evaluated.
▶ The relationship between butanol tolerance and ABE production was investigated.

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ABSTRACT

To improve butanol tolerance and production in *Clostridium acetobutylicum*, a novel approach was developed in this study, which was called artificial simulation of bio-evolution (ASBE) based on the evolutionary dynamics and natural selection. Through repetitive evolutionary domestications, a butanol-tolerant strain *C. acetobutylicum* T64 was obtained, which could withstand 4% (v/v) (compared to 2% of the wild-type) butanol and was accompanied by the increase of butanol production from 12.2 g/L to 15.3 g/L using corn meal as substrate. Fermentation was also carried out to investigate the relationship between butanol tolerance and ABE production, suggesting that enhancing butanol tolerance could increase butanol production but unlikely improve total ABE production. These results also indicated that the ASBE would be an available and feasible method used in biotechnology for enhancement of butanol tolerance and production.

1. Introduction

Diminishing supplies of fossil fuels along with growing concerns about environmental issues, the need for a clean yet environmentally safe source of energy which is both renewable and cheap (Zhou and Thomson, 2009) as well as research into the production of biofuels as alternatives for the current traditional energy sources has increased (Dwidar et al., 2012). Compared with other biofuels, butanol has been considered as a promising advanced biofuel for its higher energy density and lower vapor pressure, for being less corrosive and having less water solubility (Green, 2011; Jang et al., 2012). Despite its great potential, butanol still suffers from some drawbacks that limit its scale production in industry (Dwidar et al., 2012; Ellis et al., 2012). The main problem facing bio-butanol is the high cost of production, which makes it unable to compete with other energy sources, especially crude oil (Dwidar et al., 2012; Jang et al., 2012), though additional issues are involved, such as high cost of recovery and energy security (Zhou and Thomson, 2009). A complete review has recently focused on these topics and issues, and summarized challenges in butanol production. The greatest share contributing to these problems, however, is the butanol toxicity to its producer which seriously results in a low production from fermentation by the microorganisms (Hu and Wood, 2010; Rude and Schirmer, 2009; Zheng et al., 2009).

Therefore, various research groups are attempting to develop solvent-tolerant strains to reduce the limitation from product itself and improve butanol production in fermentation industry. Butanol tolerance is a complex phenotype involving multiple loci (Kevin and Smith, 2011; Papoutsakis, 2008), making the engineering of strains with enhanced tolerance to this solvent difficult. Hence, it is difficult to enhance butanol tolerance via gene engineering (Dunlop et al., 2011). Currently, efforts for enhancing butanol tolerance have mainly focused on the enrichment of mutants by serial

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