

Butanol-tolerant mechanism of *Escherichia coli* mutants and construction of high-resistant strains

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Butanol is important biofuel and chemicals in industry. Development of butanol tolerant strains and identification of functional butanol-tolerant genes is essential for high yield of bio-butanol production due to the toxic of butanol. *Escherichia coli* BW25113 was subjected for the first time to error-prone PCR based whole genome shuffling. The resulting mutants BW1847 and BW1857 were found to tolerate 2% (v/v) butanol and short chain alcohols, including ethanol, isobutanol, and 1-pentanol. The mutants exhibited good stability under butanol stress, indicating that they are potential host strains for the construction of butanol pathways. Genome resequencing and PCR confirmation revealed that BW1847 and BW1857 had nine and seven single nucleotide polymorphisms, respectively, and a common 14 kb deletion. Functional identification of the SNPs and deleted genes demonstrated that the mutations of *acrB* and *rob* gene and the deletion of *TqsA* increased the tolerance of the two mutants to butanol. The *rob* gene encodes a transcriptional regulator factor. Comparative transcriptome analysis of revealed that there are 285 differentially expressed genes (DEGs) between *rob* mutant strain and control under butanol stress, indicating that they are regulated by Rob to resist stress. Some functional genes significantly up-regulated or down-regulated by Rob, were also proved to be able to improve butanol tolerance. The key genes of butanol tolerance in the two mutants were thus identified by comparative functional genomic analysis. Some reported tolerant genes and those revealed by this study were integrated into *E.coli* BW25113 step by step, and high-resistant strains were finally obtained. In conclusion, stable *E. coli* mutants with enhanced butanol tolerance were obtained, and the key tolerant mechanism in the two mutants was clarified. High-butanol-tolerant

strains were further constructed by using reverse metabolic engineering strategies.

CV

Dr. Ma is from Tianjin University. She received Ph.D. degree from Nankai University in Dec 2006. He joined "Biomass conversion Lab, R&D Center for Petrochemical Technology, Tianjin University" as an assistant professor in Mar 2007, and then she becomes an associate professor and the head of biomass conversion Lab in 2012. She worked in industrial biotechnology lab of Institute of Chemical & Engineering Sciences (ICES) in Singapore as a visiting scholar for one month in 2012. Her research interest focuses on anti-stress mechanism of industrial microorganism, synthetic biology, biofuel and biocatalysis. He has published papers in journals including *Biotechnol Biofuels*, *Applied Microbiol Biotechnol*, *J Biotechnol* and *BioEnergy Research* etc. She also is a reviewer for journals like *Appl Biochem and Biotech*, *Biochem Eng* etc.

