## Heterologous Expression of Heat-Shock Protein Improves Butanol Yield in a High-Speedy Growing Clostridium acetobutylicum Mutant

Authors: Min-Shiuan Liou, Yi Shan Yang, Yang-Zhan Huang, Chia-Wen Hsieh

Abstract: A high speed growing and butanol-tolerant Clostridium acetobutylicum HOL1 mutant was screened throughout continuous adaption culture with C. acetobutylicum ATCC 824. The HOL1 strain can grow well in 10 g/L butanol contained CGM medium and can produce about 12.8 g /L butanol during 24 hrs. The C. acetobutylicum HOL1 strain was able to produce 166 mM butanol with 21 mM acetone at pH 4.8, resulting in a butanol selectivity (a molar ratio of butanol to total solvents) of 0.79, which is much higher than that (0.6) of the wild-type strain C. acetobutylicum ATCC 824. The acetate and butyrate accumulation were not observed during fermentation of the HOL1 strain. A hyper-butanol producing C. acetobutylicum HOL1 (pBPHS-3), which was created to overexpress the Bacillus psychrosaccharolyticus originated specific heat-shock protein gene, hspX, from a clostridial phosphotransbutyrylase promoter, was studied for its potential to produce a high titer of butanol. Overexpression of hspX resulted in increased final butanol yield 47% and 30% higher than those of the the ATCC824 and the HOL1 strains, respectively. The remarkable high-speed growth and butanol tolerance of strain HOL1 (pBPHS-3) demonstrates that overexpression of heterogeneous stress protein-encoding gene, hspX, could help C. acetobutylicum to effectively produce a high concentration of butanol.

**Keywords:** Clostridium acetobutylicum, butanol, heat-shock protein, resistance

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