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Construction of *Clostridium acetobutylicum* strain with enhanced productionof *n*-butanol

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Aim of the study: develop recombinant stress tolerant strain of *C. acetobutylicum* SGMwith increased butanol production.

Material and Methods: microbiological (condition of cultivation of microorganism optimizing), spectrophotometrical (bacterial culture optical density measuring), genetic (chemical mutagenesis, transformation, electroporation), biochemical (chromatographic analysis of fatty acid composition of cell membrane, chromatographic identification of solvents) and molecular-genetic techniques (DNA extraction, PCR and overlap extension PCR, restriction analysis, molecular cloning).

Results: Chemical mutagenesis was done for improvement of the strain for better n-butanol tolerance and production. Obtained mutant *C. acetobutylicum* 5H produce 20 % more butanol, inherit butanol tolerance (2,5% *w/v*) stably and have their cell membrane lipid composition changed.Genetically engineered plasmid pCB20pg was constructed. pCB20pg contains thiolase promoter and *gro*ESLoperon, which determine starch utilitsation and heat shock proteins synthesis respectively. In order to prevent DNA destruction by restriction enzymes, using induced *C. acetobutylicum* methylase gene-expression system) methyl groups were added. Methylated plasmid was injected into *C. acetobutylicum* 5H.ABE-fermentation of *C. acetobutylicum* SGM (which contains pSOLAMY) resulted in 24.02 g/L of n-butanol (49% more than original strain) in 72 hours (using AMYLEX® 4T and Viscoferm® to reduce the viscosity) in 13% rye media(*w/v*), while total solvent production reaches 37.22 g/L (105% more than original strain).

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Keywords: Butanol, recombinant strain, biofuel, ABE-fermentation.