Abstract

The toxicity of styrene is the most important obstacle facing its production biologically in *E. coli* using the tools of synthetic biology. One potential solution to counter this toxicity involves manipulating a membrane transporter in *E. coli* to export the chemical outside the cell membrane.

In this project, SrpABC and AcrABTolC were used as candidate solvent pumps to investigate styrene resistance in *E. coli*. The solvent resistant pump, SrpABC, is a membrane protein that transports several organic solvents such as styrene and toluene out of the cell in *Pseudomonas putida* S12. Another important efflux system originally found in *E. coli* is the AcrABTolC system which expels a wide range of substrates from antibiotics to chemical solvents.

SrpABC genes were expressed in *E. coli* MG1655 (DE3), which was modified as part of this project by the incorporation of the λDE3 lysogen into a parental *E. coli* MG1655. Confirmation has been obtained regarding the overexpression of recombinant SrpABC in that strain by conducting RT–PCR analysis and an improvement of the detection of SrpA protein expression by western blotting has also been done. Subsequently, the other efflux system, AcrABTolC, has been overexpressed in *E. coli* BW25113 and confirmed by SDS PAGE and western blot techniques.

Tests of tolerance towards styrene and other organic solvents were conducted in the exponential phase of liquid cultures. The results showed that overexpressed SrpABC in *E. coli* confers more tolerance towards styrene than the AcrAB pump in *E. coli* C43 (DE3) Δ*acr*AB. Besides, the cells were tolerant towards cyclohexane, cyclohexene, 1, 3-cyclohexadiene and ethylcyclohexane at concentrations 25, 10, 10 and 100 mM, respectively.

The effect of the SrpABC was tested on the bio-production of styrene proceeded in the same strain. Genes encoding PAL2 and FDC1 were cloned into pACYCDuet-1 and pETDuet-1 plasmids which were compatible with the efflux pump systems. The strain bearing the SrpABC pump (*E. coli* MG1655 (DE3)-pETDuet_pal-fdc_pACYCDuet_SrpABC) produced 8.05 mmoles styrene per 1L culture styrene using glucose (12 g /L) and L-phenylalanine (2 g / L) in a bioreactor, 30 % higher than the strain without the pump (*E. coli* MG1655 (DE3)-pETDuet_pal-fdc); 6.2 mmoles/ 1L culture. It was concluded that the SrpABC pump could improve styrene production.