ABSTRACT

The continuous depletion of fossil fuels has been a major concern in the present era along with the environmental issues like greenhouse effect and global warming. The raising harmful effects of atmospheric greenhouse gases (CO and methane) has enforced upon finding alternate and renewable energy as a substitute for fossil fuels. Biofuels such as biobutanol, bioethanol can recompense the need for petroleum fuels and could be considered as an eco-friendly solution. The butanol and ethanol produced through ABE fermentation has gained importance in the biofuel industry. *Clostridium acetobutylicum* DSM 792 has been characterized as a native Acetone-Butanol-Ethanol (ABE) fermenting bacterium. Strain engineering has led to enhanced solvent production in *C. acetobutylicum* previously. But, further genetic manipulations are required in order to understand the complexity of ABE pathway, improve the butanol/ethanol ratio, and eliminate by-product formation. In this study, the ABE pathway in *C. acetobutylicum* DSM 792 was modified and four recombinant strains (PTAKO, PTBKO, DSM 792(pPDC1), and DSM 792(pPDC2)) were constructed and individually tested for the solvent production. The phosphotransacetylase (*pta*) gene involved in acetate formation was inactivated in the mutant PTAKO and the phosphotransbutyrylase (*ptb*) gene involved in the butyrate formation was inactivated in the mutant PTBKO. The Clostron mutagenesis method was used to construct the mutant strains PTAKO and PTBKO. Pyruvate decarboxylase gene from *Zymomonas mobilis* was cloned in pIMP1 plasmid and expressed in *C. acetobutylicum* DSM 792 under the control of two different promoters. A heterologous constitutive promoter Pferr was used in the strain DSM 792(pPDC1) and a native acidogenic promoter Ppta was used in the strain DSM 792(pPDC2).

In batch fermentation experiments the strain DSM 792(pPDC1) produced total ABE of 22.64 mM and the strain DSM 792(pPDC2) produced total ABE of 120.2 mM under pH uncontrolled conditions. DSM 792(pPDC1) produced 82.9% higher ABE than wildtype. The DSM 792(pPDC2) strain produced 171% higher ABE concentration compared to the wild type. The alcohol to acetone ratio (BE/A) was 2.28, 2.77, and 5.26 in wild type, DSM 792(pPDC1), DSM 792(pPDC2). This suggests that re-routing of pyruvate by over expression of *pdc* pathway could play a role in generation of more reducing equivalents towards ethanol and butanol compared to the *pfor* pathway. The strains PTAKO and PTBKO produced 66.7 mM and 20.2 mM total ABE. The BE/A ratio was 2.78 in the strain PTAKO. Acetate was still produced by the
strain PTAKO while the strain PTBKO did not produce butyrate and acetone. This suggests that reduction in butyrate production indirectly reduces acetone formation which is a major by-product in ABE mixture. Of the four strains the highest butanol (76 mM) and ethanol (25 mM) was produced by the strain DSM 792(pPDC2) under pH uncontrolled batch fermentation studies.