Knock-out Experiment in *Clostridium acetobutylicum* ATCC 824 Using a Replicating Plasmid

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Chromosomal manipulation of solventogenic clostridia using a suicide plasmid has been considered extremely difficult, owing to their low efficiencies both in electroporation and in homologous recombination. In the present study, knock-out using an E. coli-C. acetobutylicum shuttle vector was conducted. After plasmid curing, double crossover mutants of the butyrate kinase gene were found, but all found mutants lost the megaplasmid pSOL1, which contains the genes for solvent production. This was caused by successive subculturing for the knock-out plasmid curing. Further studies are required for developing a conditionally sensitive replicon to make the knockout process fast and secure from the strain degeneration. [This work was supported by the Advanced Biomass R&D Center of Korea (ABC-2011-0028386) through the Global Frontier Research Program of the Ministry of Education, Science and Technology (MEST). Further supports by BioFuelChem, EEWS program of KAIST, and the World Class University program (R32-2008-000-10142-0) of the MEST are appreciated.]