Biomimetic strategy for constructing *Clostridium thermocellum* cellulosomal operons in *Bacillus subtilis*

Jui-Jen Chang

Department of Medical Research, China Medical University Hospital, China Medical University, No. 91 Hsueh-Shih Road, Taichung 402, Taiwan

Email: lancecjj@gmail.com

Background: Enzymatic conversion of lignocellulosic biomass into soluble sugars is a major bottleneck in the plant biomass utilization. Several anaerobic organisms cope these issues via multiple- enzyme complex system so called 'cellulosome'. Hence, we proposed a "biomimic operon" concept for making an artificial cellulosome which can be used as a promising tool for the expression of cellulosomal enzymes in *Bacillus subtilis*.

Results: According to the proteomic analysis of *Clostridium thermocellum* ATCC27405 induced by Avicel or cellobi- ose, we selected eight highly expressed cellulosomal genes including a scaffoldin protein gene (*cipA*), a cell- surface anchor gene (*sdbA*), two exoglucanase genes (*celK* and *celS*), two endoglucanase genes (*celA* and *celR*), and two xylanase genes (*xynC* and *xynZ*). Arranging these eight genes in two different orders, we constructed two different polycistronic operons using the ordered gene assembly in *Bacillus* method. This is the first study to express the whole CipA along with cellulolytic enzymes in *B. subtilis*. Each operon was successfully expressed in *B. subtilis* RM125, and the protein complex assembly, cellulose- binding ability, thermostability, and cellulolytic activity were demonstrated. The operon with a higher xylanase activity showed greater saccharification on complex cellulosic substrates such as Napier grass than the other operon.

Conclusions: In this study, a strategy for constructing an efficient cellulosome system was developed and two differ- ent artificial cellulosomal operons were constructed. Both operons could efficiently express the cellulosomal enzymes and exhibited cellulose saccharification. This strategy can be applied to different industries with cellulose- containing materials, such as papermaking, biofuel, agricultural compost, mushroom cultivation, and waste processing industries.

Keywords: Cellulosome, *Bacillus subtilis*, *Clostridium thermocellum*, Biomimetic strategy, Biomimetic operon