SORTASE A-ASSISTED METABOLIC ENZYME LIGATION IN ESCHERICHIA COLI

Takuya Matsumoto, Graduate School of Science, Technology and Innovation, Kobe University, t_matsu@rabbit.kobe-u.ac.jp
Tsutomu Tanaka, Department of Chemical Science and Engineering, Graduate School of Engineering, Kobe University
Akihiko Kondo, Graduate School of Science, Technology and Innovation, Kobe University

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We demonstrated the metabolic enzyme ligation by sortase A-mediated ligation (sortagging) for the redirection of metabolic flux thorough metabolic channeling. Staphylococcal sortase A (SrtA) is utilized for the ligation of metabolic enzymes. SrtA is transpeptidase, which recognizes Leu-Pro-Xaa-Thr-Gly sequences (LP tag) and cleaves between Thr and Gly, and subsequently links amino group of oligoglycine (G tag) thorough a native peptide bond. Sortagging enables to conjugate protein with other molecules in a site-specific manner. Minimal modifications of protein with short peptide tags; LP tag and G tag are only required for site-specific ligation. Hence, sortagging has been utilized for preparing a variety of bioconjugation not only in vitro but also in vivo.[1] In current study, we hypothesize that SrtA-mediated metabolic enzyme ligation in cytoplasm of *Escherichia coli* facilitates processing metabolic intermediate, and redirects metabolic fluxes to desired pathway. As proof of concept, we constructed acetate producing *E. coli* with engineered endogenous metabolic pathway, which redirect central metabolic fluxes to acetate producing flux by the induction of chemical additives (Figure 1). The expression of SrtA was controlled by Lac operating promoter, metabolic channeling was videlicet occurred by the addition of IPTG. Acetyl-CoA was chosen as the intermediate model because acetyl-CoA is one of the most important central metabolic intermediates, which is converted to alcohols, fatty acids, and mevalonate derivatives. In this study, we tested covalent linking of pyruvate-formate lyase and phosphate acetyltransferase by sortase A-mediated ligation and evaluated the production of acetate. The time point of addition of IPTG was not critical for facilitating metabolic enzyme ligation, and acetate production increased upon expression of sortase A. These results show that sortase A-mediated enzyme ligation enhances an acetate-producing flux in *E. coli*. We have validated that sortase A-mediated enzyme ligation offers a metabolic channeling approach to redirect a central flux to a desired flux.[2]

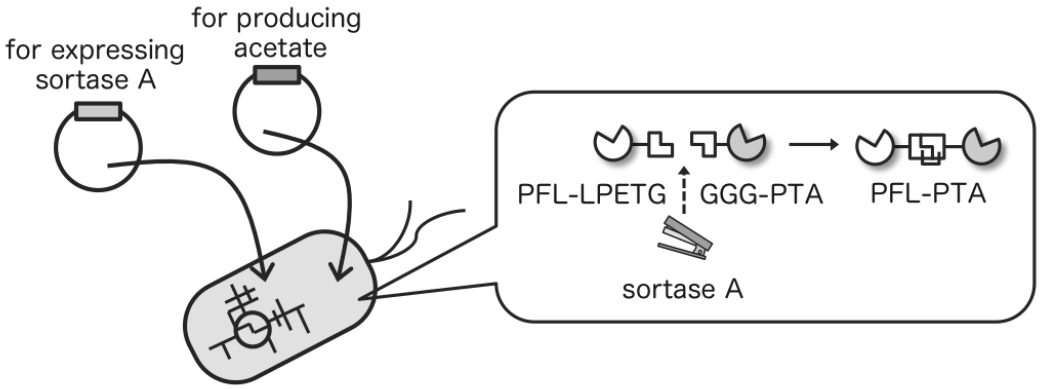

Figure 1. Sortase A-mediated metabolic enzyme ligation for enhancing PFL-PTA flux

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2. Matsumoto, T., Furuta, K., Tanaka, T. and Kondo, A. (2016). Sortase A-Mediated Metabolic Enzyme Ligation in Escherichia coli. ACS Synthetic Biology, 5, 1284-1289.