



ESCHERICHIA COLI WHOLE-CELL BIOSENSOR WITH TODST TWO-COMPONENT

SIGNAL TRANSDUCTION SYSTEM OF PSEUDOMONAS PUTIDA

Eun-Gyeong Lee¹, Doo-Byoung Oh^{1,2}, Ohsuk Kwon^{1,2}

¹Systems and Synthetic Biology Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, 305-806, Korea. ²Biosystems and Bioengineering Program, University of Science and Technology, Daejeon, 305-350, Korea. oskwon@kribb.re.kr

Key words: Two-component signal transduction system, *TodST*, aromatic compounds

Introduction. The ability to respond to a vast array of environmental signals is vital for the growth and survival of all living organisms. The sensing and processing of these signals are carried out by molecular circuits within the cell, which detect, amplify, and integrate these signals into a specific response. In prokaryotes, these molecular circuits are typically organized by protein pairs, sensory kinase and response regulator, that belong to the large family of two-component signal transduction systems. For diversity, specificity, modularity, orthogonality, and universality, two-component signal transduction systems are gaining increasing interests as a useful bioparts to construct artificial genetic regulatory circuits for synthetic biology. In the present study, we investigated the possibility to use a heterologous two-component signal transduction system, *TodST* of *Pseudomonas putida*, to fabricate an *E. coli* biosensor for the detection of aromatic compounds.

Methods. An inducible expression vector containing *todS* sensor kinase and *todT* response regulator genes of the aromatic compounds-sensing *TodST* system of *P. putida* has been constructed. To use as a reporter strain, a transcriptional fusion between the *TodT* regulatable promoter of *P. putida todX* gene and β -galactosidase gene was then integrated into the chromosome of an *E. coli* strain. The *TodST* expression vector was then transformed into the reporter strain. The β -galactosidase activities of the transformants cultured under various conditions were determined.

Results. *E. coli* strain MC4100 $\lambda_{att::\Phi(P_{todX^-}lacZ)/pEXT20-TodST}$ was constructed (Fig. 1). The β -galactosidase activity of this strain was depend on the expression of *TodS* and *TodT* proteins and the addition of aromatic compounds, benzene, toluene, and 4-chlorotoluene, in the culture medium (Fig. 2). In addition the induction levels of β -galactosidase were affected by culture conditions such as media and carbon sources.

Conclusions. We constructed and characterized a recombinant *E. coli* strain, MC4100 $\lambda_{att::\Phi(P_{todX^-}lacZ)/pEXT20-TodST}$, for the detection of aromatic compounds by employing the *P. putida* *TodST* two-

component signal transduction system. Our results suggest that the *TodST* system can be successfully employed as a backbone to construct a biosensor in a heterologous host.

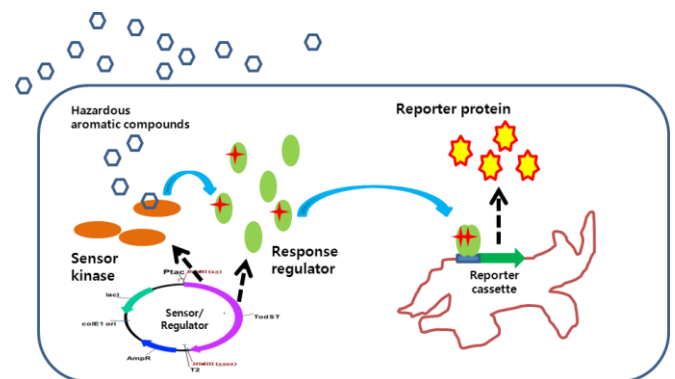


Fig. 1. *E. coli* whole-cell biosensor with *TodST* two-component system for detection of aromatic compounds.

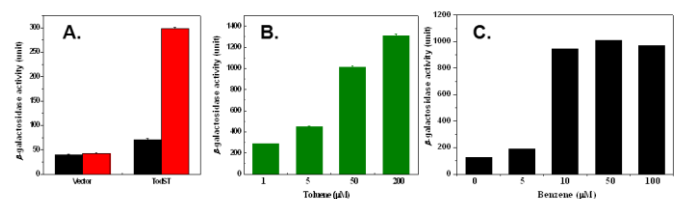


Fig. 2. Induction of reporter gene expression of *E. coli* MC4100 $\lambda_{att::\Phi(P_{todX^-}lacZ)/pEXT20-TodST}$ strain by aromatic compounds.

Acknowledgements. This work was supported by the Intelligent Synthetic Biology Global Frontier Program, and the Next-Generation BioGreen 21 Program.

References.

- Ninfa AJ. (2010). *Curr Op Microbiol.* 13:240-245.
- Behzadian F, Barjeste H, Hosseinkhani S, Zarei AR. (2011). *Curr Microbiol.* 62(2):690-696.
- Lacal J, Busch A, Guazzaroni ME, Krell T, Ramos JL. (2006). *Proc Natl Acad Sci U S A.* 103(21): 8191-8196.
- Busch A, Lacal J, Silva-Jimenez H, Krell T, Ramos JL. (2010). *J Bacteriol.* 192(16): 4246-4250.
- Kim MN, Park HH, Lim WK, Shin HJ. (2005). *J Microbiol Methods.* 60(2): 235-245.