TOWARDS WHOLE-CELL BIOSENSOR DEVELOPMENT FOR MONITORING NATURALLY OCCURRING PHENOLIC ACIDS

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Phenolic acids including hydroxybenzoic and hydroxycinnamic acids are important antioxidants and antimicrobial agents utilized in various industries, including food, pharmaceutical, cosmetics, and chemical[1]. They are usually synthesized chemically or extracted from plant biomass using physicochemical methods. However, these approaches have disadvantages, including the requirement of large amounts of solvent, the low recovery yield, and the consequent high cost that limits widespread use[2, 3]. The use of microbial cell factories based on *Escherichia coli*, *Streptomyces* sp., *Corynebacterium glutamicum*, *Pseudomonas* sp., *Bacillus* sp., *Amycolatopsis* sp., and *Klebsiella pneumonia* has come into focus as a sustainable alternative for phenolic acid production[4]. Inducible gene expression systems composed of chemical molecule-responsive transcription factor (TF) and inducible promoters have come into the focus as a platform for TF-based whole-cell biosensors and can be used as an in *in vivo* analytical tool for extracellular and intracellular metabolite analysis. Previous studies have shown that this type of biosensors is adaptable to the design–build–test–learn cycle and has been successfully applied for high-throughput systems screening to study phenolic acids metabolism and forward engineering[5]. Here, by applying a multi-genome approach, we have identified phenolic acid-inducible gene expression systems composed of TF-inducible promoter pairs responding to different phenolic acids. Subsequently, they were used for the development of whole-cell biosensors based on model bacterial hosts, including *Escherichia coli*, *Cupriavidus necator* and *Pseudomonas putida*. The dynamics and range of the biosensors were evaluated by establishing their response to the primary inducer, while the specificity of biosensors was determined by screening twenty major phenolic acids. To exemplify applicability, we utilize a protocatechuic acid-biosensor to identify enzymes with enhanced activity for conversion of *p*-hydroxybenzoate to protocatechuate.

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