Development of active whole-cell biocatalysts for two-component, flavin-diffusible styrene monooxygenase

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Styrene monooxygenase (SMO) can produce chiral (S)-styrene oxide, an important chemical intermediate for several drugs and functional food ingredients, by epoxidation of the side chain of styrene. The enzyme is present in several Pseudomonas species which can grow on styrene as a sole carbon and energy source. SMO consists of two separate enzymes, an NADH-FAD oxidoreductase (StyB) and an FAD-dependent hydroxylase (StyA). The reaction requires the continuous regeneration of NADH by carbon metabolism and thus, the use of SMO-expressing whole cells rather than that of purified SMO enzymes is preferred for catalytic reactions. With Escherichia coli BL21 as a host, three types of recombinants were developed: pET system with the strong promoter T7, pBAD system with the weak arabinose promoter, and pET-chaperon system where various chaperons were co-expressed in pET system. In addition, genetic modifications in carbon metabolic pathways and NADH utilization were conducted with E. coli BL21 host. As a result, we could achieve a highly active whole-cell SMO biocatalyst of 400 U/ g cell which is more than 4 fold higher than the best activity reported so far.