

## Functional expression system of bacterial P450s in Escherichia coli

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Electron transfer from NAD(P)H is required for a cytochrome P450 to exert its catalytic This electron transfer is performed via (a) redox function as monooxygenase. partner protein(s), and classified in 4 classes based on the difference in their electron-transfer manners (Fig. 1). Some bacteria such as actinomycetes and cyanobacteria contain P450s that belong to Class I, whose catalytic activities are hardly displayed in heterologous hosts or in vitro reactions, because of difficulties to find the corresponding redox partner proteins. A self-sufficient P450 (called P450RhF) that belongs to Class IV (Fig. 1) was found in actionomycete Rhodococcus P450RhF included the C-terminal redox partner domain sp. NCIMB 9784. (reductase domain) consisting of a ferredoxin reductase that requires flavin mononucleotide (FMN) as the coenzyme, and a ferredoxin [iron-sulfur protein (FeS)], by way of a linker sequence. Using the P450RhF reductase domain and linker region, we constructed a vector pRED for functionally expressing various bacterial P450 A pRED bioconversion system of a variety of genes in Escherichia coli. low-molecule substrates has been constructed using approximately 150 types of bacterial P450s we possess. In this presentation, we also show stabilities of several recombinant P450 proteins fused to the P450RhF reductase domain during their storage. In addition, another bioconversion system is presented with E. coli cells carrying P450BM3 (F87V) that belongs to Class III (Fig. 1), which was N-terminally fused to an archeal peptidyl-prolyl cis-trans isomerase (PPlase).



Fig. 1. Class of P450s

Fig. 2. Structure of vector pRED