Biochemical and applied studies of aminoalcohol dehydrogenase from *Rhodococcus erythropolis*

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Ephedrine, which is used as anti-inflammatory or anti-asthmatic pharmaceutical agent, carries two asymmetric carbon atoms, so that there are four stereoisomers (Fig.1). In these four isomers, only *d*-pseudoephedrine (dPE) or *l*-ephedrine shows such activities. Therefore, stereospecific synthetic method of these compounds is required. Recently, we found that an aminoalcohol dehydrogenase (AADH) from *Rhodococcus erythropolis* MAK154 catalyzes the stereoselective reduction of (*S*)-1-phenyl-1-keto-2-methylaminopropane ((*S*)-MAK) to dPE. We constructed *E. coli* transformant harboring genes encoding AADH and GDH (Fig. 2). Using this recombinant *E. coli* cells as catalyst, 4 wt% (200 mM) of (*RS*)-MAK was converted to 3.6 wt% (178 mM) of dPE with molar reaction yield of 89.1% and optical purity of >99% under the mild alkali conditions that allow spontaneous racemization of substrate, (*RS*)-MAK. However, the concentration of dPE production stopped at about 4%, because AADH was inhibited by the dPE produced in the reaction mixture. We screened for enzyme variants, prepared by a 'directed evolution method', showing activity in the presence of high concentration of dPE. Random mutagenesis was performed on an *aadh* gene by error-prone PCR to construct a mutant library. The mutant library was screened with color detectable high-throughput screening method. Two mutant enzymes showed higher ability of dPE tolerance than wild-type enzyme. Both of these two enzymes had one amino acid substitution in different position, and then the third mutant enzyme containing both of these amino acid substitutions was constructed. Kinetic parameter analyses of each enzymes suggested that these amino acid substitutions influence to enzyme activity and enzyme-substrate binding affinity, respectively.

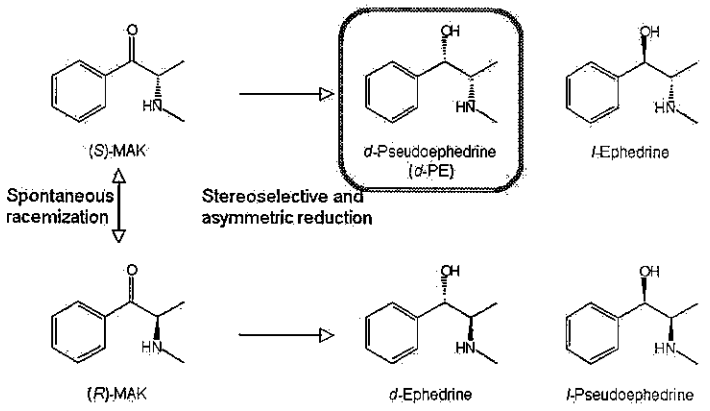

Fig. 1. Stereoisomers of ephedrine

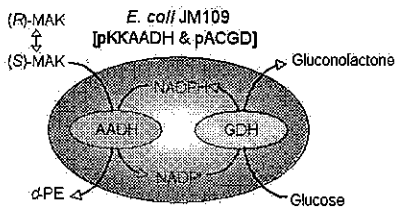

Fig. 2. AADH and GDH co-expressing *E. coli* transformant