Directed evolution of highly active and coincidently thermostable tyrosine phenol-lyase

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Highly active and thermostable enzymes have been long, storied wish for the development of biocatalytic synthesis. Though tyrosine phenol-lyase (TPL) is very useful for the synthesis of tyrosine derivatives, the catalytic turnover is generally slow and resultantly a lot of enzyme is required to get high productivity. The TPL originated from *Symbiobacterium toebii* was attempted to improve the catalytic rate by use of directed evolution technology, while keeping its original thermal stability. Genetic variants of the enzyme was prepared by error-prone PCR, cloned in constitutive expression vector pHCEIIb, and subjected to a wellplate-based screening method. After an extensive screening, seven variants superior in activity and thermostability were defined and subjected to subsequent staggered extension PCR to recombine the beneficial mutations obtained from error-prone PCR. From the second library two variants were finally acquired as highly active and coincidently thermostable TPLs. The activity level was close to *C. freundii* enzyme even at 37℃ and they were extremely active and stable at 65℃ where the wild-type enzyme is inactivated within 30 min.

[This project was supported by Cleaner Production Program 10007946 of NCPC]