

High production of glutathione by in vitro enzymatic cascade after thermostability enhancement

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Abstract

The cell free system has been paid more attention due to its potential of facilitating more efficient catalysis of multistep reactions. In this study, an efficient enzymatic cascade of GSH production was developed through the evolution of bifunctional glutathione synthetase (GshF), coupled with polyphosphate kinase (PPK). First, the stability and activity of GshF were enhanced by loop interchange and site-directed mutagenesis. As a result, the GshF half-value period increased 163.3-fold, and its activity raised 18 %. PPK from *Jhaorihella thermophile* (PPKJT) was characterized and used to regenerate ATP in the GSH synthesis, with hexametaphosphate (PolyP(6)) as the phosphate donor. After the process optimization, 99.9 mM GSH and 7.6 mM oxidized glutathione (GSSG) were produced within 2 h. The molar yield was 95.9 mol/mol based on the amino acid added, while the productivities of GSH achieved 49.95 mM/h, which was the highest yield and productivity ever reported about GSH synthesis.

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