ONE-POT SYNTHESIS OF AMINO-ALCOHOL USING A DE NOVO TRANSKETOLASE:TRANSAMINASE PATHWAY IN PICHIA PASTORIS STRAIN GS115

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*Pichia pastoris (P. pastoris)* is an attractive industrial host cell due to its ability to grow up to 60% wet cell weight (WCW) by volume, a far higher level of biomass than the typical values reached by *Escherichia coli (E. coli)* and *Saccharomyces cerevisiae*. This thesis seeks to explore how the genetic tractability and high cell densities characteristic of *P. pastoris* can be exploited to intensify whole-cell biocatalysis.

Chiral amino alcohols such as 2-amino-1,3,4-butanetriol (ABT) are key building blocks of small molecule pharmaceuticals and have previously been produced by whole-cell biocatalysis using cells engineered to overexpress a *de novo* enzyme pathway consisting of transketolase and transaminase.

Within this work, native and foreign *P. pastoris* transaminases were characterized with respect to their biocatalytic potential. Genomic data mining was performed to explore the GS115 strain genome, allowing the selection of three putative Class III transaminase genes and the construction of overexpressor strains PpTAm107, PpTAm677 and PpTAm410. The well-studied ω-transaminase CV2025 from *Chromobacterium violaceum* was also successfully engineered to generate two strains; PpTAmCV708 for single expression of CV2025, and PpTAm-TK16 strain for CV2025 co-expression alongside a native transketolase previously characterized for L-erythulose production.

The rapid growth and high biomass characteristics of *P. pastoris* were successfully exploited for production of ABT by whole-cell biocatalysis. At high cell density, the best performance for the *de novo* pathway was obtained with the engineered PpTAm-TK16 strain, which tolerated high concentrations of substrate to achieve STY 0.57 g L⁻¹ h⁻¹ of ABT, 40-fold higher than levels previously achieved with *E. coli* for the same reaction.