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Synthesis of potential pharmaceutical active ingredients using omega-transaminase

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Transaminases (EC 2.6.1.X) are enzymes which catalyze reversible transfer of amino group from amino acids to α-keto acids by using piridoxal-5'-phosphate as a coenzyme. There is a huge interest for the application of ω-transaminases in industrial production of chiral amines and alkaloids since those compounds are extensively used in pharmaceutical, agricultural, and chemical industries. Application of ω-transaminases in asimetric synthesis of these compounds enables efficient production of biologicaly active amines, due to their catalytic properties for synthesis with a high level of enantioselectivity, supstrate promiscuity (they are capable to aminate keto acids, aldehydes and ketones), high turnover number, no requirement for regeneration of external cofactors, and among other cheaper, simpler and green process of production [1,2].

We are developing biocatalytic route for the synthesis of amino steroids by using ω-transaminase, (R)-selective, ATA-117 enzyme variant from *Arthrobacter* sp [3]. It can be observed that enzyme expression was done in *Echerichia coli* BL21 D3 pLysS (Figure 1), and HPLC analysis of enzyme activity and specificity toward 15 structuraly different steroid compounds was performed. (R)-methylbenzylamine was used as amino group donor and pyridoxal-5'-phosphate as cofactor. Activity of the enzyme was measured in bacterial lysate based on the absorbance of acetophenone, that is formed during the transamination reaction of (R)-methylbenzylamine. Figures 2 and 3 are showing chromatograms of acetophenone standard and products of reaction performed with enzyme expressed in *E. coli* and 16,17-epoxypregnenolone. Reactions were analysed on reversed phase column NucleosilC18. Based on the results, we have selected four steroid compounds for which enzyme showed highest activity and with a potential for biological activity. The next step was optimisation of the reaction conditions with a low cost amino donor isopropylamine, and isolation and characterisation of a pure amino steroid products. Until now we have managed to enzymatically synthesize and purify one amino steroid which should be further analysed by spectral characterization and its biological activity will be determined.

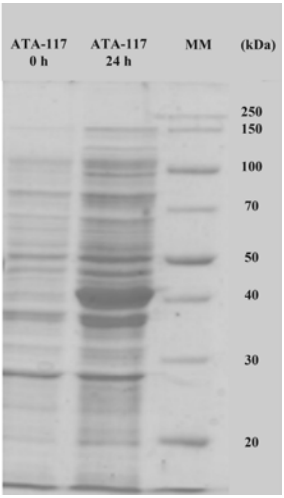

Figure 1. SDS-PAGE of ATA-117 expressed in *E. coli* BL21 DE3 pLysS compared against proteins in bacterial lysat before the induction of enzyme expression.

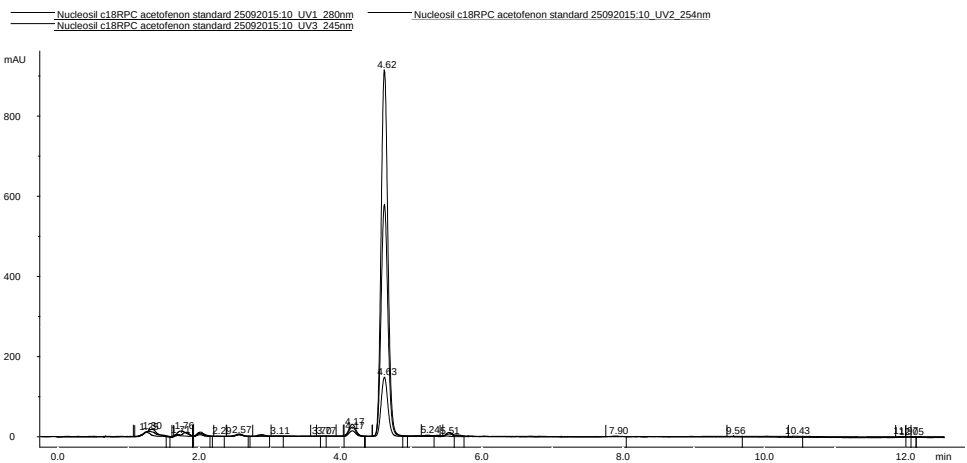

Figure2. Chromatogram of acetophenone (20 mM) in buffer solution (100 mM HEPES, pH 8.0)- retention time 4.62 min.

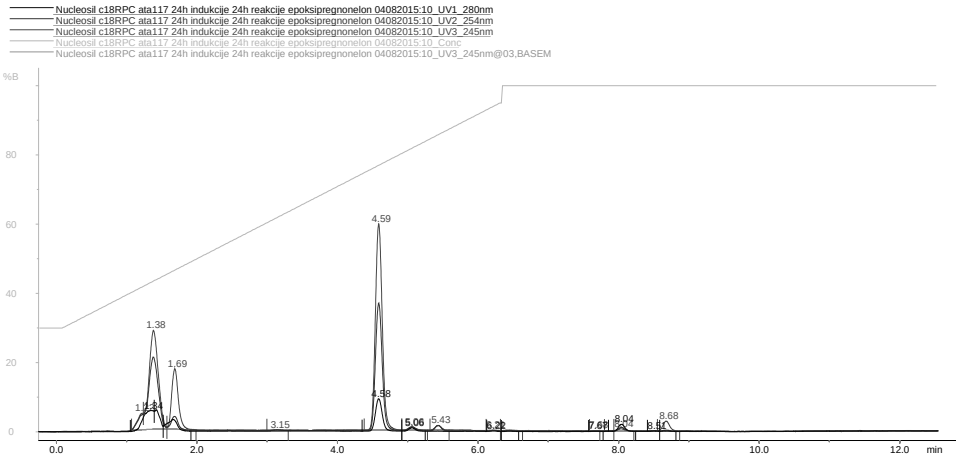

Figure 3. Chromatogram of reaction performed with induced enzyme (24h of reaction) and 16,17-epoxypregnenolone-retention time of acetophenone 4.59 min

Acknowledgements

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