Overproduction of O-Acetylhomoserine sulfhydrylase (*Ca*Met15p) native and His-tag versions.

Objectives: Identification of optimal conditions for O-acetylhomoserine sulfhydrylase from *C. albicans* (*Ca*Met15p) overproduction in *E. coli* cells in a Isopropyl-β-D-thiogalactoside (IPTG) inducible Tabor-Studier system.

Methods: Expression plasmids: pLATE11+*MET15*, pLATE52+*MET15NH* and pET101/D-TOPO+*MET15CH* encoding respectfully, *C. albicans* wild type O-acetylhomoserine sulfhydrylase (*Ca*Met15p), N-terminus His-tagged (*Ca*Met15NHp) and C-terminus His-tagged O-acetylhomoserine sulfhydrylase (*Ca*Met15CHp) were used for *E. coli* cells transformation. Due to optimization of proteins overproduction in Tabor-Studier system we used several chemically competent *E. coli* cells (BL21 Star (DE3); Rosetta (DE3) pLysS, BL21 (DE3) pLysS; Rosetta (DE3) pLysS; Rosetta (DE3) pLacI, Origami 2 (DE3) pLysS and ER2566) as well as optical densities of cell cultures (OD_{600} 0.5-1), different concentrations of iductor IPTG (final 0.05 - 2 mM) and different incubation temp. and time period (37 °C or 30 °C for 6 h or 18 h). Prior to SDS-PAGE analysis 1 mL of cell culture was centrifuged for 1 minute 4000 rpm. Cell pellet was suspended in 100 µL 1% SDS and boiled for 10 minutes. Next, 25 µL of 4x SDS-PAGE sample loading buffer was added, and the mixtures were boiled for 10 minutes. The prepared samples were loaded on the gel and SDS-PAGE electrophoresis was conducted in 5% stacking gel and 10% resolving gel for 180 V cm^{-1}, according to Laemmli method.

Results: The best overproduction was performed with the use of *E. coli* ER2566 for plasmids pLATE11+*MET15* and pLATE52+*MET15NH;* and *E. coli* BL21 (DE3) pLysS for pET101/D-TOPO+*MET15CH* plasmid. Optimal conditions assumed overnight expression cells growth at 37°C in LB medium supplemented with ampicillin. 10 mL of starter culture was added to 800 mL of LB medium supplemented with ampicillin and incubated at 30 °C up to optical density (OD_{600}) = 1.0. Induction was made with IPTG of final concentration 1 mM. Cells were cultured for 24 h at 15°C.