WEB326 - COMT expression

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25th November, 2014

1 Day 1

- 500 mL over night culture (+ 200 $\frac{\mu g}{ml}$ Amp) of **ZYP-5052** (autoinduction medium) inoculated with toothpick of COMT (culture that stood at 4°C for 2 weeks)

2 Day 2

- measured $OD^{600} = \sim 2.6$ at 10.00
- measured $OD^{600} = 6.88 \rightarrow$ at 1300
- harvested cells by centrifugation (10.000 x g, 4°C , 3 min)

!! cells grew slowly → probably due to old pre-culture....

3 Purification from AI-medium expression

- resuspended pellet (∼3 g) in 60 mL **Lysis Buffer** (50 mM Tris/HCl, 0.5 M NaCl, 10% (v/v) glycerol, 10 mM Imidazole, 1% Tween 20)
- added a spatula tip of HEWL and incubated for 15 min at RT while inverting
- lysed cells by sonication (3 times for 30 s at 70% amplitude, 1s-1s on-off-cylce)
- added DNase buffer and 200μl DNase I and incubated on ice for 15 min
- removed debris by centrifugation (20 min, 10.000 x g, 4°C)

3.1 ÄKTA

- filtered through a 0.45 μm filter and injected onto 1 mL HiTrap Talon FF column on ÄKTA

! buffers, lysate and also the collection tubes were cooled

- column was equilibrated with **buffer A** (50 mM Tris/HCl, 500 mM NaCl, 10% (v/v) glycerol, pH 7.4)
- after sample injection the column was washed with 8 CV of buffer A, 8 CV of 5% buffer B and 8 CV of 10% buffer B

- eluted with 8 CV **buffer B** (50 mM Tris/HCl, 500 mM NaCl, 10% (v/v) glycerol, 300 mM imidazole, pH 7.4)

!!! only a very small protein peak elutes at 100% B (Fig. **??**) → SDS-Gel to clarify what went wrong in purification