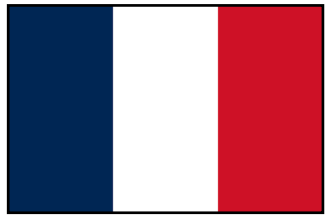
Summary of Results from Group 4 Lactase Downstream Processing

Evreux 23. – 27.03.2026

Group 4 + presentation of group members

Manon Sala
French



Fabian Klingel
German

Rasa Rudzevičienė
Lithuanian

Hana Melcherová
Slovakian

Evaluation Steps of our Experiment

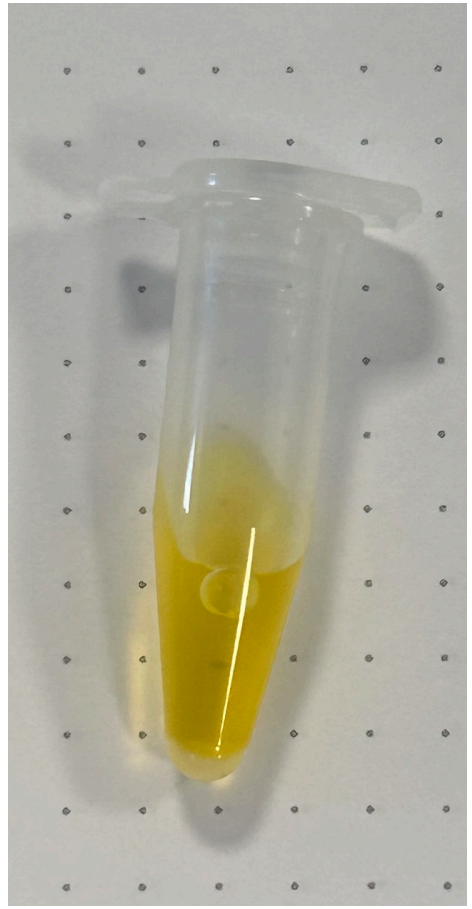
1. **PARTICLE FREE LYSATE – o-NPG-test (enzymatic activity assay)**
2. ANION EXCHANGE CHROMATOGRAPHY (AEXC) – o-NPG + photometer (enzymatic activity assay)
3. SDS – PAGE – gel + calibration line (purity, molecular weight – lactase)
4. RAW MILK TEST – colour change (conc. of glucose)
5. EVALUATION

O-NPG-TEST of 1:10 diluted Particle Free Lysate

Test 50 µL supernatant + 500 µL o-NPG-solution.

- **Observation:** Yellow
- **Evaluation:** β(1→4) bond was broken down
- **Conclusion:** lactase is present.

→ We can start AEXC.



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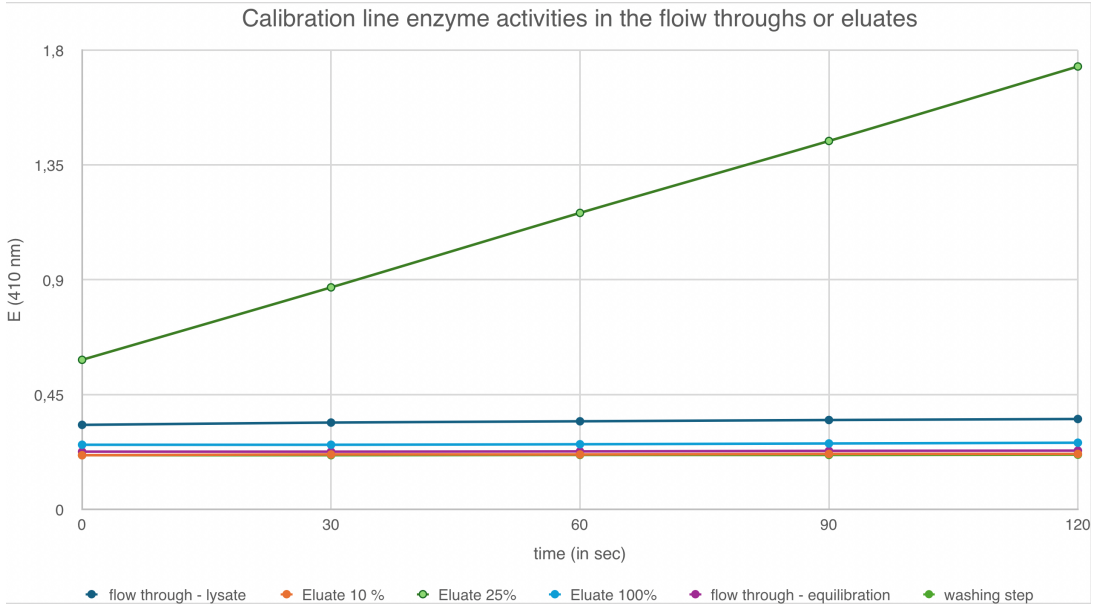
ANION EXCHANGE CHROMATOGRAPHY

O-NPG-test measured by photometer – enzymatic activity measurement

Test 50 µL eluate + 800 µL o-NPG-solution.

- **Observation:** one sample turned Yellow
- **Evaluation:** only one of the samples contains lactase
- **Conclusion:** the highest lactase activity was in eluate 25%



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5. EVALUATION

SDS - PAGE

MARKER

S1: lysate (dilution factor 10)

S2: loading flow through

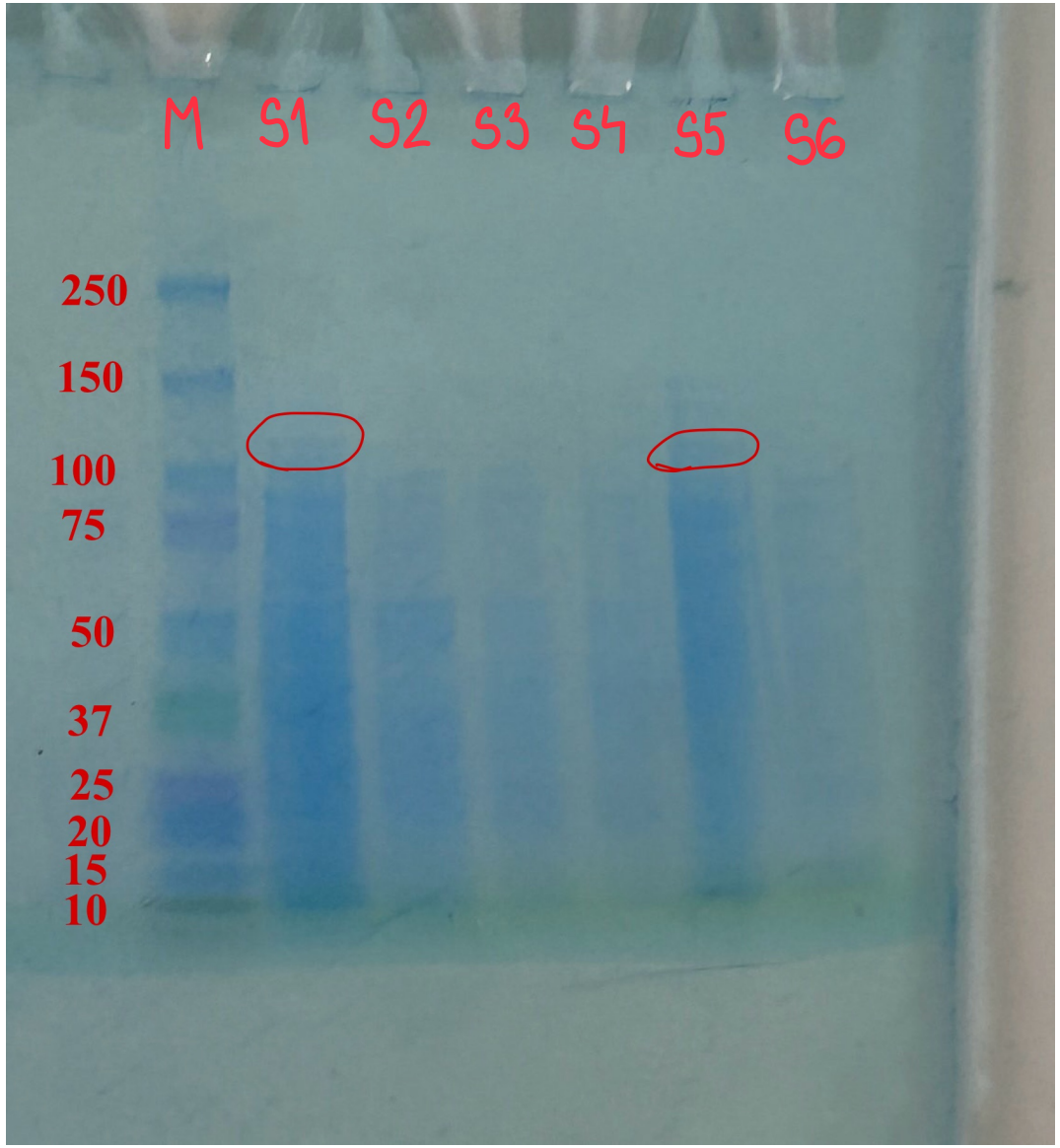
S3: washing step

S4: eluate 10%

S5: eluate 25%

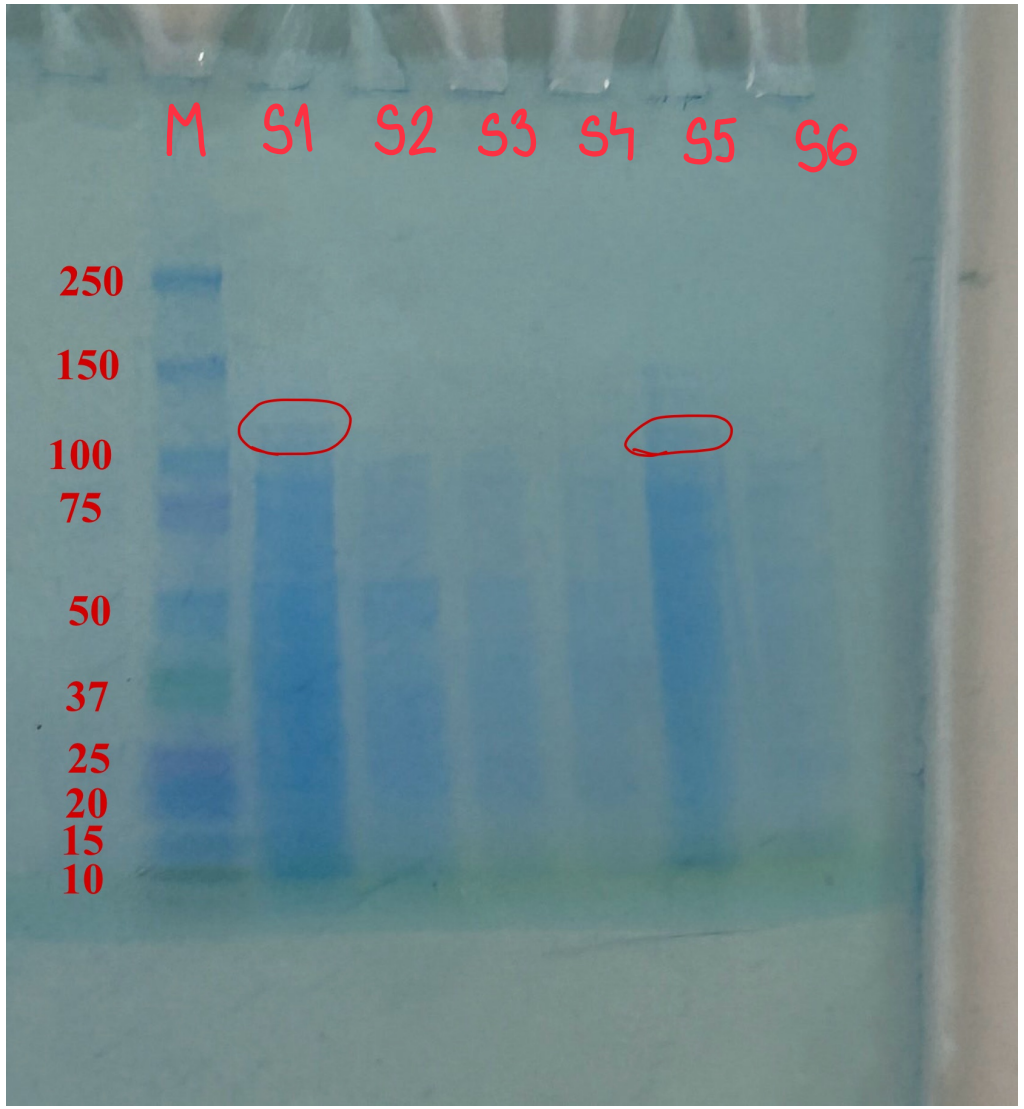
S6: eluate 100%

- Conclusion: by AEXC we reduced the number of proteins in our sample to an uncountable amount of proteins (starting with 3000)



SDS - PAGE

- We identified the lactase subunit (116kDa) in our gel using marker
- Lactase Band is a little bit above 100 marker



Evaluation of the SDS – PAGE

- Identification of molecular weight of lactase subunit with your calibration line

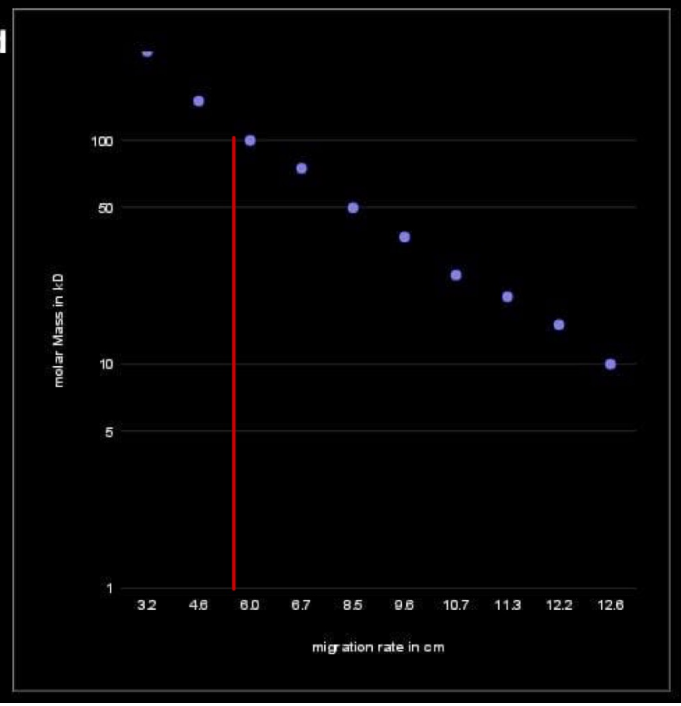
- **Migration rate:** 5,6cm
- **Molecular weight:** 117kDa

Estimation of Molar Mass of Lactase

Precision Plus Protein Standard

migration rate (in cm	molar Mass
3.2	250
4.6	150
6.0	100
6.7	75
8.5	50
9.6	37
10.7	25
11.3	20
12.2	15
12.6	10

migration rate (in cm	molar Mass
5.6	117



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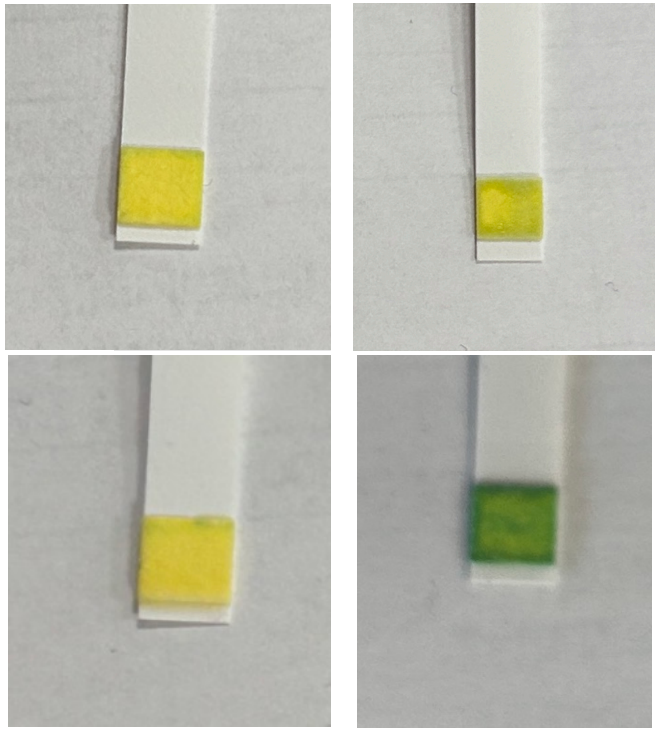
5. EVALUATION

RAW MILK TEST

- **Observation:** barely any colour change after 20 and 40 mins. After 70 mins and applying heat, sample turned green
- **Evaluation:** glucose level of approximately 2.8 mmol/L
- **Conclusion:** to create lactose free milk we would have to change the conditions so the enzyme can work better.



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EVALUATION

1. TASK: SDS-PAGE and AEXC both showed the same results. 25% eluate had the highest enzymatic activity and showed the lactase subunit band.

2. TASK: We would use the 25% eluate, we would have to further purify it by chromatography.

Thank you for your attention!