FINAL REPORT: SDS-PAGE

ELECTIVE SUBJECT: MOLECULAR BIOLOGY

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METHODOLOGY

- Sample used where previously mixed with Sample Buffer 2X and bromophenol blue 1:1. They were putted to water bath at 80 ºC for 3 minutes.
- Two gels were put into the vertical electrophoresis chamber with running Buffer (Glycine+SDS) and 5uL of HMW marker (High Molecular Weight –Pharmacia-). 10uL for each E.coli protein extract sample.
- In the first gel different samples of periplasmic fractions were added with pET 21d Non-recombinant and recombinant to: CueO 1,4; CueOdD2; CueO∆D2.
- In the second gel samples of periplasmic and supernatant fractions were submitted to different expression times of the CueOdD2 clone with 0.4mM IPTG at 0h, 1h, 3h, 6h and 24h.
- Gels were run at 100V and 20mA for 2 hours.
- After completion of the run, the gels were stained with a 0.25% Coomassie Blue solution, 10% acetic acid and 50% methanol for one hour and then a discoloration with 7% acetic acid and 5% methanol overnight.
- UV gels were observed and photographed for analysis.

RESULTS AND DISCUSSION

In Figure 1. gels revealed under UV are shown.

Observing the migration of the marker in both gels, the five bands corresponding to the weights: 669, 440, 232, 140, 67 kDa could be identified. However, a higher migration and lower intensity of the bands were observed in gel A.

In lanes 2 and 5 no bands corresponding to periplasm of E. coli or of the plasmids were found with the different clones of CueO.

In lane 3, three bands of approximately 440, 232 and 140kDa were observed, but there was no evidence of CueO 1.4 that would correspond to a weight of 55kDa.

In lane 4, two bands of 600 and 140kDa were observed, and no presence of CueOdD2 corresponding to 35kDa was observed.

Performing a search of the proteins corresponding to these molecular weights in the databases EcoProDB, UniProtKB and EchoBASE, it was found that the maximum weight is 150kDa for proteins present in *E.coli.* Therefore 140kDa band may be attributed to the proteins, PutA, RpoB, FtsK or HerpA.

However, higher weight bands were evident in the gel, and since no proteins of a molecular weight greater than 150kDa were found in the databases and the migration front did not reach the entire gel, it would indicate a problem in the running with the migration time or the voltage.

Thus, a comparison of the molecular weight between the marker and the samples of gel A could not be done.

In gel B, the same marker was used and would not serve as a reference for sample weights. However, we can observe some proteins that were expressed at different times in *E.coli* in the periplasm and supernatant.

Lanes 2-5 correspond to extracts of *E.coli* with the clone CueOdD2 of periplasmic fractions and lanes 6-10 of supernatant.

For the periplasmic fractions it is evident how the proteins from time 0h (Lane 2) to time 24h (Lane 5) were changing in their presence and intensity. However in lane 3 and 5 there was no presence of proteins. This is a result inconsistent with what was expected because as time goes by, the amount of protein present in the periplasm should change progressively as evidenced in the second band of lane 4 (Fig.1, gel B, white box). This could have been the result of the absence of sample in the lane at the moment of loading it.

In the samples of supernatant fractions corresponding to lanes 7 and 9, no results were observed. In lane 6, it is possible to visualize a band of low intensity (yellow arrow) and is progressively increasing in lanes 8 and 10. Likewise, other proteins that change in intensity over time (green and purple arrows) are present.
It should be noted that the highest intensity of the bands was presented in lane 10 (24h); This is expected since this time would be the result of the accumulation of proteins expelled to the medium by the cell.

By comparing the amount of proteins observed in the periplasm and the supernatant, the presence and quantity of proteins in the periplasm decreased over time while in the supernatant it was progressively increased.

The explanation for this could be that the observed proteins are produced inside the cell are therefore expelled from the cell and found in the supernatant (Koster et al, 2000). In addition, if these proteins have catalytic activity they would be expected to have peaks of activity that vary over time because of their function and the substrate they use.

Since the enzymatic activity depends on the medium in which the cell is found, the expected results agree with the observed, being different between the fractions and also within the times of the fractions (Thanassi et al, 2000).

Finally, the presence of any of the CueO variants in *E. coli* could not be determined by having no molecular weight reference points for less than 55kDa.

BIBLIOGRAPHY

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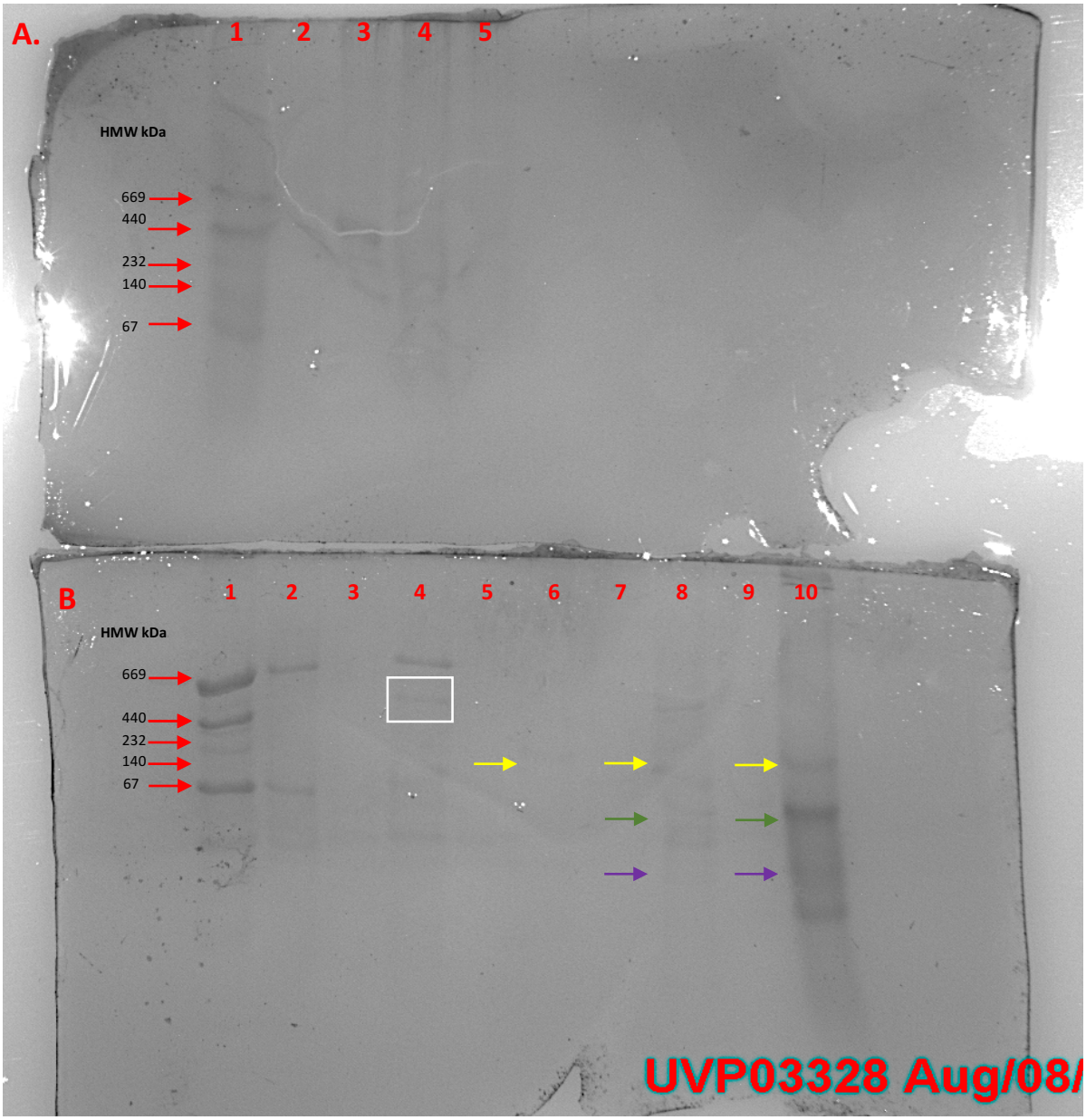

Fig. 1. SDS-PAGE Gels 9% ⇔ 0,8mm

A) Extracts of *E.coli* periplasmic fractions, lanes correspond to:

1- HW Marker
2- PET 21d NR
3- CueO 1,4
4- CueOdD2
5- CueOΔ

1- B) Extracts of *E.coli* with clone CueOdD2 of periplasmic fractions (FP) and supernatant (FS):

2- HW Marker
3- 0h FP
4- 1h FP
5- 3h FP
6- 24h FP
7- 0h SP
8- 1h SP
9- 3h SP
10- 6h SP
11- 24h SP

Note: Yellow, green and purple arrows indicate the change in intensity of some FS proteins.