PROTEIN PURIFICATION

AIM: To purify histidine-tagged protein prolidase and irisin from Rosetta and E.coli BL21(DE3) strain.

Date: 5/08/22
Friday

Preparation of stock solutions and buffers

All solutions were prepared and stored at room temperature. The recipe and procedure to prepare buffers and stock is given in the protocols tab.(insert link)

Date: 8/08/22
Monday

Test expression and purification of prolidase

100 mL of 2xYT media was prepared and kept for autoclaving in the morning. After autoclaving, the media kept cooling down.
100 mg/mL ampicillin and 34 mg/mL chloramphenicol added. O.D. reaches between 0.4 to 0.6, induction with 1mM IPTG.
Kept overnight at 18 degrees celsius in a shaking incubator

Date: 9/08/22
Tuesday

After induction is over, cell lysis is done using lysis buffer and sonication. Resin equilibration, binding, washing and elution done as per the procedure described in detail in the protocols section.

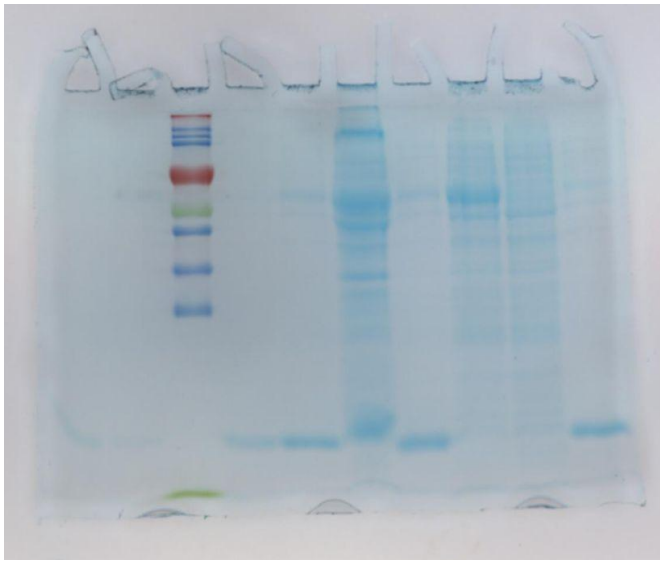
Date: 10/08/22
Wednesday

SDS-PAGE

To confirm the expression and purity of protein, we ran SDS gel with all the flow through, elute fractions and controls.

Date: 11/08/22
Thursday

Result for test purification of prolidase



Got a band for prolidase (mol. Wt 54kDa) between 51-70 kDa
Can proceed for bulk expression

Date: 12/08/22
Friday
Preparation of 2L of 2xYT media and antibiotic solution (Bulk culture)

Recipe:
Tryptone = 32 g
Yeast extract = 20 g
NaCl = 10 g
Rest - add distilled water to fill up to the 2L mark
Keep it for autoclave

100 mg/mL ampicillin recipe:
For 2mL, weigh 200 mg of ampicillin salt. Add 2 mL of Milli Q water. Store it at -20 degree celsius.
34 mg/mL chloramphenicol recipe:
For 2mL, weigh 68 mg of chloramphenicol. Add 2mL of Molecular Biology Grade Ethanol. Store it at -20 degree celsius.

Date: 13/08/22
Saturday
IPTG Induction

2:30 am

- Inoculation from amp+chloram prolidase agar plates. Set up a 7 mL primary culture overnight at 37-degree celsius, 200 rpm shaking incubator.
- Ampicillin added - 7uL (from 100 mg/mL stock)

Chloramphenicol - 7 uL (from 34 mg/mL stock)
- OD of primary culture before inoculation = 1.188
- Ampicillin in 2L expression media - 2 mL (from 100 mg/mL stock)
  Chloramphenicol - 2 mL (from 34 mg/mL stock)
- Add 7mL of the primary medium in secondary media. Keep in a 37-degree celsius incubator at 200 rpm. (time = 5:30 pm)
- OD 600 at 10:45 pm = 0.214
- OD 600 at 12:00 am = 0.566
- Control taken before adding IPTG = 6mL
- Total volume of secondary culture left = (2000-10) mL
- Stock of IPTG = 0.5M, 10 mL
- 1:00 am : Volume of IPTG added in expression culture = 4mL.
- Kept both the control and 5L flask in ice for 10 minutes.
- Centrifuge control - 4,000 rpm for 15 minutes at 4 deg celsius, stored at -20 deg celsius
- Expression culture was kept overnight at 18 degrees Celsius, 200 rpm shaking incubator.

Date: 14/08/22
Sunday
Centrifugation and snap-freezing
After induction got over, centrifuge 2L culture at 4,000 rpm at 4 degrees Celsius for 15 minutes. Did snap freezing and stored the pellet at -80 degree celsius.

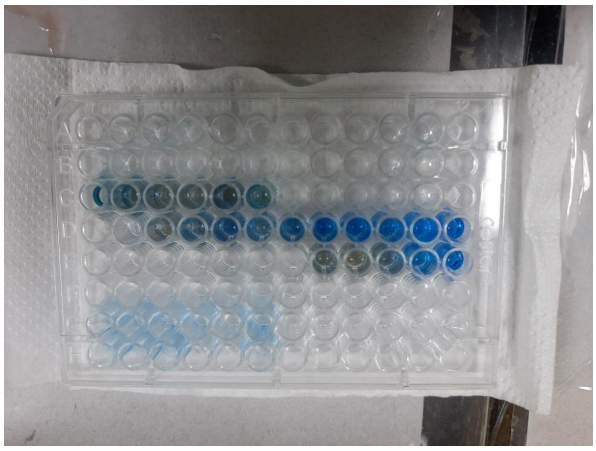
Date: 15/08/22
Monday
Started the experiment with cell lysis, sonication, centrifugation and binding steps. While incubating the lysis supernatant with Ni-NTA resin, our sample leaked due to the loosening of the falcon cap. The next day, we started afresh.

Date: 16/08/22
Tuesday
2:30 am - set up a primary culture in 7 mL of 2xYT media
Ampicillin - 7 ul from stock
Chloramphenicol -7 uL from stock

Date: 17/08/22
Wednesday
Morning - kept 2L 2xYT medium for autoclaving
Cooled it down by afternoon
15 mL of this taken as control, added 2mL of amp and chl in expression media
Inoculation of primary into secondary kept at 37 deg shaking incubator
OD 600 at
9:30 pm - 0.120
11:30 pm - 0.25

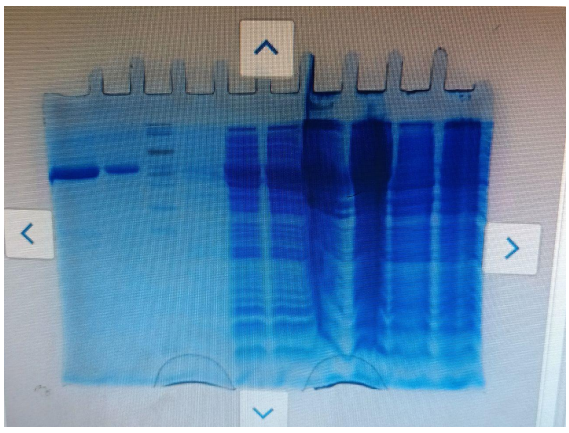
1:30 am - 0.68
Control - 1mL before induction
1mM IPTG added (4mL from 0.5 M IPTG stock)
Overnight 18 deg shaking incubator

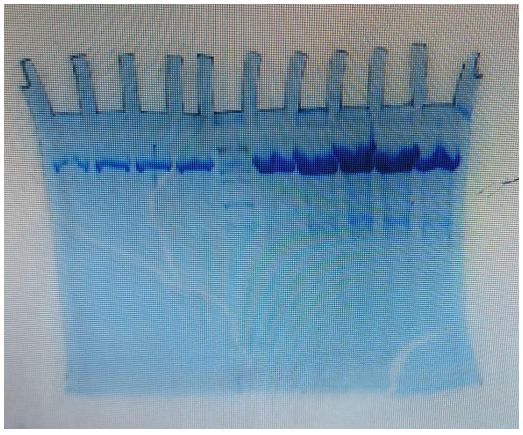
Date: 18/08/22
Thursday
Did pellet down, lysis (using sonication), centrifugation, binding with resin and column purification using wash and elution buffers with varying concentrations of elution buffer.



Bradford test gave the preliminary confirmation for the presence of protein in wash and elute fractions.

Date: 19/08/22
Friday
Ran 15% SDS-PAGE to confirm the purity.





obtained protein in washes 1, 2 and elutes 1 to 9
Did nanodrop to find the concentration of protein in all the fractions.
The maximum concentration of protein was found in elute 4 i.e. 4.989 mg/mL

Date: 20/08/22
Saturday

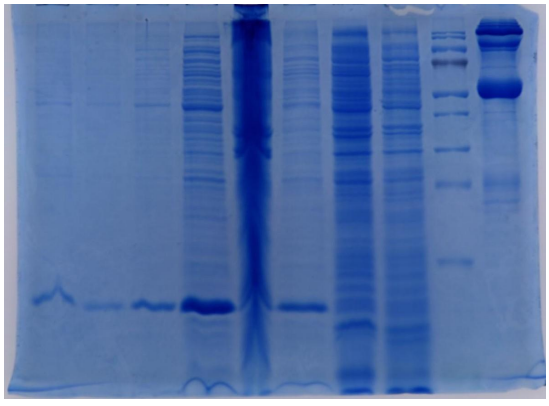
All samples were stored at 4 degrees Celsius.
Date: 21/08/22
Sunday
Pooled down all the washes and elutes. Concentrated it using a 10 kDa concentrator. Did desalting using PD10 column. Aliquots were made, snap-froze and stored at -80 degree celsius. (detailed protocol given in protocols section)

Date: 23/08/22
Tuesday
Test expression and purification of irisin
Inoculation
Inoculation from transformed BL21(DE3) cells into 20 mL of LB media containing 20 uL of ampicillin (100 mg/mL stock) - 11:00 pm
Incubation at 37 degrees Celsius, 200 rpm shaking incubator

Date: 24/08/22
Wednesday
Induction
7:10 am-Induction done with 0.5 mM IPTG when O.D. reaches between 0.4 to 0.6. Kept for 12 hours at 18 degrees Celsius.
7:30 pm- pellet down (centrifugation at 4,000 rpm at 4 degrees celsius for 15 minutes) and lysis (composition given in protocols)
Sonication- 2 minutes, 5 seconds on and 10 seconds off, 1 cycle
Centrifugation at 14,000 rpm at 4 degrees for 20 minutes.
Supernatant collected and pellet stored
Equilibrate 150 uL of Ni-NTA resin in a 2 mL Eppendorf (procedure given in protocols)
Binding, washing and elution were done following the same procedure for prolidase

Date: 25/08/22
Thursday
SDS-PAGE
Ran the SDS-PAGE with different samples obtained during purification steps

RESULT:

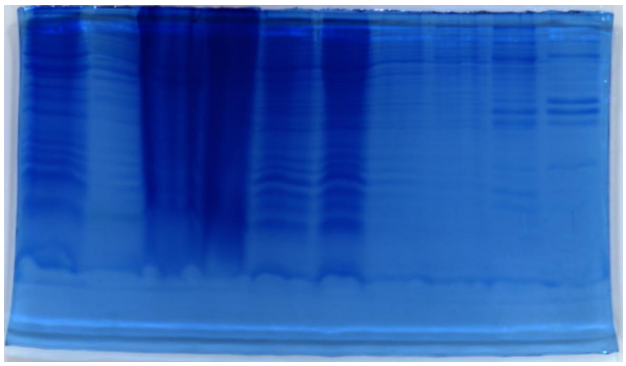


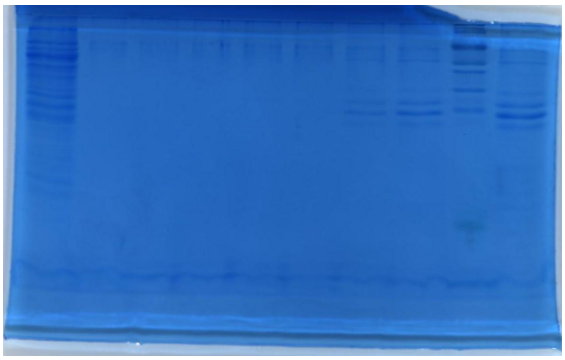
Got a band for irisin between 10-26 kDa range as expected. Can proceed for the 2L bulk culture

Date: 26/08/22 to 25/09/22

Repeated the bulk experiment 3 times, but unfortunately, we were getting multiple bands. Decided to drop the irisin and go for SELEX only with the purified prolidase.

SDS-PAGE on 5/09/22:





Lanes from left to right:

1. uninduced cell pellet
2. Ladder
3. induced cell pellet
4. lysis cell pellet
5. lysis supernatant
6. flow through after binding
7. wash 0
8. wash 1
9. elute 1

Lanes from right to left:

1. Elute 2
2. Ladder
3. Elute 3
4. Elute 4
5. Elute 5
6. Elute 6
7. Elute 7
8. Elute 8
9. Elute 9