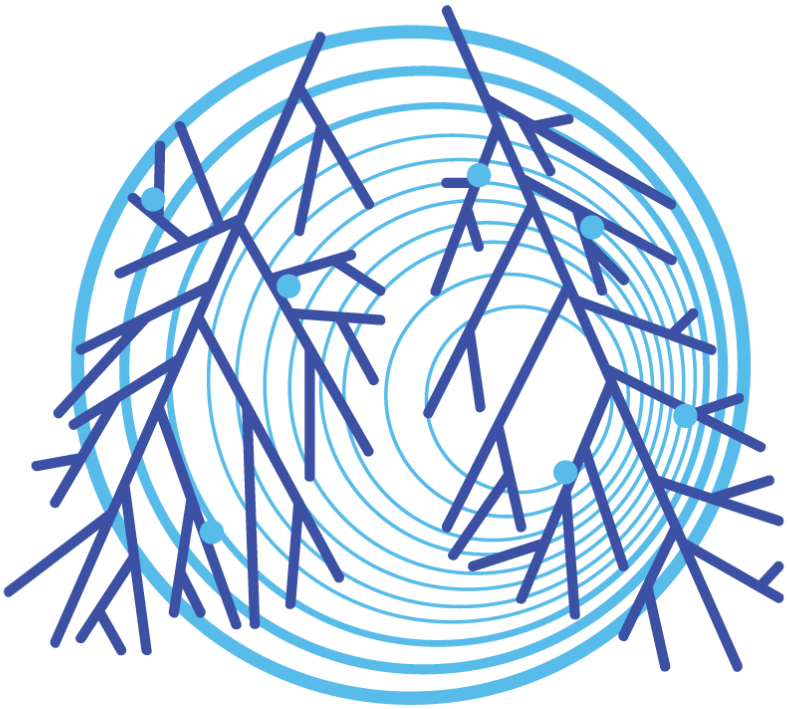

PROLUNG

SENSING

LAB BOOK 11

iGEM Stockholm

Expressing sialidase with OmpR promoter

Background

Our team have previously shown that the OmpR promoter (BBa_R0082) can sense changes in osmotic pressure caused by sucrose. We have also shown that our biobrick for sialidase (BBa_K2235009) can produce sialidase enzyme that can be purified using IMAC and then analyzed with SDS-PAGE. We have created a new composite biobrick that combines the OmpR promoter (BBa_R0082) and sialidase (BBa_K2235005). This biobrick should be able to express sialidase enzyme at increased levels as the osmotic pressure increases.

We will use both TOP10 and ΔEnvZ cells. ΔEnvZ are cells that do not have the EnvZ system, which the OmpR promoter is dependent upon. Thus, these cells should work as a kind of negative control and not produce sialidase (except from leakage).

Aim

Test if our new biobrick (BBa_K2235007) can sense osmotic pressure changes and activate transcription of the downstream gene sialidase. By cultivating transformed cells in different concentrations of sucrose we hope to be able to see an increase of sialidase produce as the sucrose concentration increases. We will analyze the sialidase expression with SDS-PAGE.

Transformation in TOP10 and ΔEnvZ

The transformation protocol was used with no modifications.

Osmo test

Material
Preparation
8 100 ml flasks
8 250 ml flasks
150 ml LB broth
60 ml 30% sucrose
240 µl chloramphenicol 20 mg/ml
ddH_2O

OD measurement
Cuvettes
OD spectrophotometer

Sonication
8 15 ml falcon tubes

IMAC
8 IMAC columns
Buffer A
Buffer B
20% ethanol

Procedure
We prepared flasks of different concentrations of sucrose in LB (0%, 5%, 10% and 15%, see appendix) for both TOP10 and ΔEnvZ cells (8 flasks in total). To each flask we added a colony of cells and let them incubate on a shaking table (150 rpm and 37℃) overnight.

The next morning we took 1 ml from each flask and diluted it 1:20 in 20 ml of the respective sucrose concentrations. We let them incubate for 15-30 min and then measured the OD. If the OD had reached a value of 0.4 we put the flask in the fridge to stop the growth as much as possible. If the OD had not reached 0.4 we let them incubate for 15-30 min again. When all flasks had reached OD 0.4 we used a sonicator to break open the cells.

The sonication protocol was used with no modifications.

When all samples had been sonicated we centrifuged the falcon tubes for 15 min at 4600 rpm. The pellet that formed was discarded and the supernatant was purified with IMAC.

The IMAC protocol was used with no modifications.

The purified solutions were then analyzed with SDS-PAGE. For a positive control we used sialidase that our team had purified from a previous test for our sialidase biobrick with a T7 promoter (BBa_K2235009)

The SDS-PAGE protocol was used with no modifications.

Results

Expected results

Increased sialidase expression in TOP10 cells when sugar concentrations are increased and no (or very little) expression of sialidase in ΔEnvZ cells at all concentrations.

Obtained results

The obtained results were inconclusive. The gels did not come out very well. We could see some faint bands at the size of sialidase, which would indicate that the cells and our biobrick are able to produce sialidase at least. However we could see no correlation between the increase in sucrose and sialidase expression. The ΔEnvZ cells also seems to have the highest expression of sialidase which they really should not have. Overall there were many parts of the experiment that could be factors of error and we therefore suggest that these results are not to be trusted. If we had more time we would repeat the test.

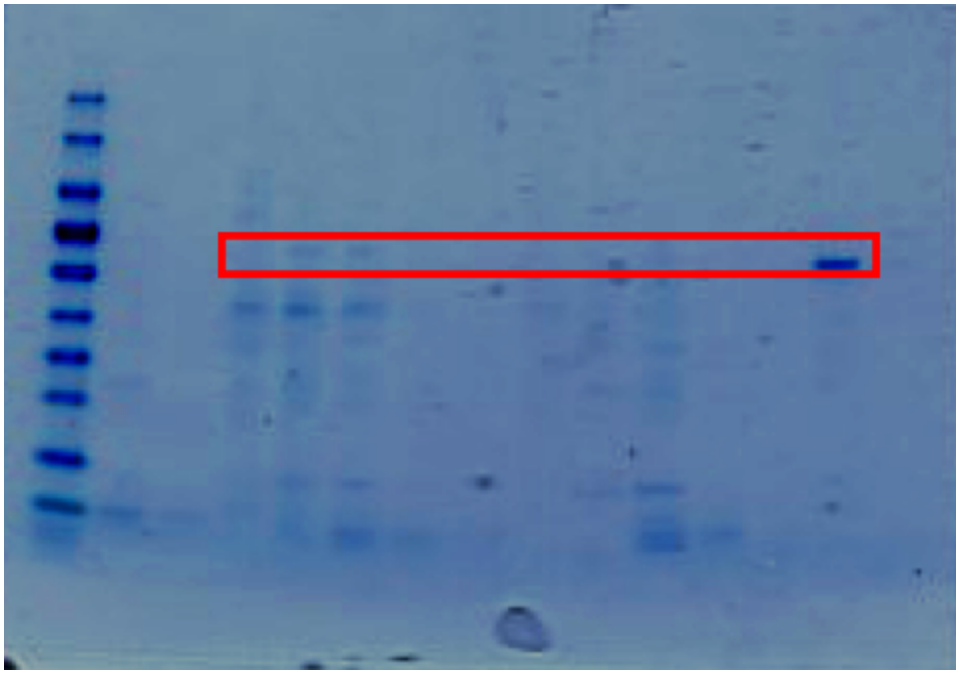

Figure 1. SDS-PAGE gel of sialidase expression. From left to right: ladder, well 2-3: expression in 5% sucrose, well 4-8: expression in 10% sucrose, well 9-14: expression in 15% sucrose, well 15: positive control.

Appendix

0% sucrose (x2)

For 10 ml:
5 ml nutrient broth
5 ml ddH_2O
10 µl chloramphenicol 20 mg/ml

5% sucrose (x2)
For 10 ml:
8.33 ml nutrient broth
1.67 ml 30% sucrose solution
10 µl chloramphenicol 20 mg/ml

10% sucrose (x2)
For 10 ml:
6.67 ml nutrient broth
3.33 ml 30% sucrose solution
10 µl chloramphenicol 20 mg/ml

15% sucrose (x2)
For 10 ml:
5 ml nutrient broth
5 ml 30% sucrose solution
10 µl chloramphenicol 20 mg/ml