

## **Cellulolytic complex (Endo5a-CBD-AIDA1) characterization**

### Goal

Characterization of the cellulolytic complex Endo5a-CBD-AIDA1 by several means:

- Generate a calibration curve of the cellulolytic complex required for fluorometric reaction signal of 4-methylumbelliflferone (MU).
- Discover the optimal substrate (4-MUC) concentration.
- Discover the effect of temperature on enzyme activity.

We expect to kinetically characterize our cellulolytic complex – discover the optimal substrate concentration, temperature, and substrate concentration for the growth of our recombinant bacteria.

### Tested cellulase

1,4-endoglucanase (Endo5a) from *Paenibacillus* sp. ICGEB2008E1.

### General protocol

Repeat a set of ascending recombinant *E.coli* culture concentrations with reaction buffer containing 4 - methylumbelliferyl β-D-celllobioside (MUC) in excess. Terminate the reaction with stop mix (pH 10) and determine fluorescence with a fluorescence microtiter plate reader.

Later, repeat a set of ascending 4-MUC concentrations with a fixed recombinant *E.coli* culture concentration. Finally, test at a wide temperature range.

### Controls

- **Control A** – negative control – contains only reaction buffer (without recombinant *E.coli*). We expect no reaction, and we want to verify there is no spontaneous reaction of our substrate during the experiment period.
- **Control B** – negative control – contains recombinant *E.coli* culture (without reaction buffer). We expect no reaction since there is no substrate.
- **Control C** – positive control – contains control cellulase (E1) with reaction buffer. We expect a reaction, and we want to verify the experiment conditions are suitable.

### Detailed protocol

#### Part 1- optimal recombinant *E.coli* culture concentration:

1. Use a 96-well microtiter plate (flat-bottomed, polystyrene plate, black).
2. Prepare the reaction buffer containing:
  - a. 50 mM sodium acetate pH 5.5
  - b. 100 mM NaCl
  - c. 0.5 mM 4- methylumbelliferyl β-D-celllobioside (MUC)
3. Load 100 µl reaction buffer in each well.
4. Load 0.1, 0.5, 1, 2, 3, 4, 10 µl of recombinant *E.coli* culture.
5. Prepare control wells:
  - a. Control A: contains only 100 µl reaction buffer.

- b. Control B: Contains 10 µl of recombinant *E.coli* culture + adequate amount of DDW (Double Distilled Water) to reach 100 µl.
- c. Control C (positive control) contains control cellulase (E1) with reaction buffer.
- 6. Cover plates with adhesive lids to prevent evaporation and incubate for 30 min at 65 °C.
- 7. Terminate the reaction by adding 100 µl of stop mix (0.15 M glycine pH 10.0).
- 8. determine fluorescence with a fluorescence microtiter plate reader using excitation and emission wavelengths of 365 nm and 455 nm, respectively.
- 9. Compare fluorescence values to control A (blank, contains reaction buffer).

Part 2 - optimal 4-MUC concentration:

- 10. Prepare reaction buffer with different 4-MUC concentrations: 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1, 2, 5 mM 4-methylumbelliferyl β-D-celllobioside (MUC).
- 11. Repeat the experiment with the optimal recombinant *E.coli* culture concentration found in Part 1.

Part 3 - optimal reaction temperature:

- 12. Repeat the experiment with the optimal cellulose concentration found in Part 1 and optimal 4-MUC concentration found in Part 2.
- 13. Incubate the plates for 30 min at 28 °C, 37 °C, 45 °C, 55 °C, 65 °C, 75 °C.

Resources

1. T. Ziegelhoffer, J.A. Raasch, S. Austin-Phillips, **Dramatic effects of truncation and sub-cellular targeting on the accumulation of recombinant microbial cellulase in tobacco**, Mol. Breed., 8 (2001), pp. 147-158  
<https://link.springer.com/content/pdf/10.1023/A:1013338312948.pdf>
2. Lehmann, C., Sibilla, F., Maugeri, Z., Streit, W. R., de María, P. D., Martinez, R., & Schwaneberg, U. (2012). **Reengineering CelA2 cellulase for hydrolysis in aqueous solutions of deep eutectic solvents and concentrated seawater**. Green Chemistry, 14(10), 2719-2726.  
[https://pubs.rsc.org/en/content/articlehtml/2012/gc/c2gc35790a?casa\\_tok=en=bXySI7kkm7kAAAAA:eaU2GRcouW9JIV7jCc7sBNPWCfVBqUzlsdu-x8nrl9OZ92HA0kDGVZbFJZ\\_U-qOiXJtHxhP2e\\_Nt3QZU](https://pubs.rsc.org/en/content/articlehtml/2012/gc/c2gc35790a?casa_tok=en=bXySI7kkm7kAAAAA:eaU2GRcouW9JIV7jCc7sBNPWCfVBqUzlsdu-x8nrl9OZ92HA0kDGVZbFJZ_U-qOiXJtHxhP2e_Nt3QZU)
3. British Columbia iGEM team 2016 -  
[http://2016.igem.org/Team:British\\_Columbia/Project/S-Layer/Cellulases#Introduction](http://2016.igem.org/Team:British_Columbia/Project/S-Layer/Cellulases#Introduction)