

Growing bacteria with CMC as carbon source

Goal

Quantification of the cellulolytic complex of recombinant bacteria.

Testing if engineered *E.coli* can grow with carboxymethyl cellulose (CMC) as carbon source.

We expect cellulose-bearing bacteria to grow successfully with CMC as carbon source.

Tested cellulase

1,4-endoglucanase (Endo5a) from *Paenibacillus* sp. ICGEB2008E1 , DH5 recombinant bacteria.

General protocol

Repeats of variable CMC concentrations in medium, in all repeats the enzyme concentration is constant and measurement of bacterial growth rates will be performed using absorbance measurements of optical density (OD) at a wavelength of 600 nm in time.

Controls

- Negative control: grow bacteria on medium that does not contains carbon source (no CMC) - we want to make sure that the bacteria could grow only with the carbon source available.
- Positive control: bacteria with rich medium (LB- Lysogeny Broth) – we want to make sure that the bacteria could grow when it is given the materials it requires.

Protocol

Part 1-paring substrate concentrations:

1. Prepare solutions of CMC diluted with DDW (Double Distilled Water)
 - a. Prepare 1-4 tubes tagged with 0%, 0.25%, 0.5%, 1%
 - b. Prepare solutions of CMC diluted with DDW according to $c_1V_1 = c_2V_2$

Part 2-growing bacteria with LB Broth:

2. Take 4 new 50 mL falcon tubes
3. Add 10 ml LB Broth + 10 μ L AMP (ampicillin) +1 colony
4. Insert into a 50 mL falcon tube and incubate overnight at 37°C (shake)

Part 3-refresh:

5. Take new tube and add 10mL LB Broth + 150 μ L from the sample
6. Incubate without shaking at 30°C for 2-3 hours
7. introduced into culture medium in each tube solutions with a concentration range between 0 and 1.0% CMC

Part 4-results

8. Set the Spectrophotometer for OD-600nm
9. Blank -1 mL LB Broth
10. Add 1mL sample each time
11. Measure OD

Resources

1. Chen, H. H., Chen, L. C., Huang, H. C., & Lin, S. B. (2011). **In situ modification of bacterial cellulose nanostructure by adding CMC during the growth of *Gluconacetobacter xylinus*.** *Cellulose*, 18(6), 1573-1583.
<https://link.springer.com/article/10.1007/s10570-011-9594-z>
2. Cheng, K. C., Catchmark, J. M., & Demirci, A. (2011). **Effects of CMC addition on bacterial cellulose production in a biofilm reactor and its paper sheets analysis.** *Biomacromolecules*, 12(3), 730-736.
https://pubs.acs.org/doi/abs/10.1021/bm101363t?casa_token=GWsuGJMbVRsAAAAA%3AF-W6cRiC2c8Oc-n8L4PU8aOtGCUzQPtgw7isArPnBGqF3Rwx0wYVNsREeuOierhrlZpTtaafsc-md_iBQg&