AZCL-amylose - version june 2019 – Rosanne Hertzberger

This method is currently under development at REBLAB and not finished yet.

Testing the presence of amylose (α1-4 linked glucose polymer) degrading activity in a solution by mixing with labeled amylose (AZCL-amylose, Sigma-Aldrich, nr 05404).

Some issues with this assay are 1) solubility of the substrate. Previously, we used sonication and long shaking with NaOH. Here we instead focus on preventing pellet-forming by the insoluble substrate by adding 0.5% w/v xanthan gum (Megazyme recommendation, see https://www.megazyme.com/docs/default-source/analytical-applications-downloads/screening-for-polysaccharide-endo-hydrolases-using-insoluble-dyed-polysaccharide.pdf?sfvrsn=2).

The insoluble substrate seems to be broken down without problem by amylase. I have used the plate reader for continuous reading while incubating at 37 degrees.

I would like to include AZCL-pullulan assay as an additional substrate in this assay, however I have not found enzymes that can break it down.

Requirements

-2.0 mg/mL of "AZCL-amylose" (Megazyme, S9765) in "amylase buffer" (100 mM sodium acetate, 5 mM $CaCl_2$) with 5 mg/mL xanthan gum (Sigma-Aldrich).

To prepare: add xantan-gom to buffer, vortex, heat in waterbath to 60°C and vortex thoroughly. Then add the azcl-amylose, vortex again. Visual check all particles are evenly distributed. They should not pellet even after days.

-Transparent flat bottom polystyrene 96-wells plate (Greiner).

-optional: 10 mg/mL chloramphenicol solution dissolved in ethanol (1000x). I add this to the amylose assay to prevent growth because of the long incubation time and the fact that many of my samples have cells in them.

-plate reader that can incubate at 37°C and shake.

ASSAY

-pipet 10 uL enzyme solution in the flat bottom polystyrene 96-wells plate. I either use culture supernatants by removing the cells through spinning 20 minutes at 4°C and maximum speed (4754 rcf in our case). Or I use the pellet that I wash with PBS. Or human specimen. Use saliva (10x and 100x diluted in amylase buffer) or a known amylase quantity (*Bacillus licheniformis*, Sigma-Aldrich A3403) as a control and an appropriate negative control (either amylase buffer or growth medium or the medium used to wash the cells which is normally PBS).

-pipet 190 uL AZCL-amylose solution into the plate (Greiner ref 651101). Make sure the AZCL-amylose granules are well distributed and no pellet is formed. Pipet slowly and make sure the pipet tip is filled and volume is equal in all wells. The substrate has high viscosity because of the xanthan gum and amylose granules may block the tip.

-seal the 96 well plate with a 96 well seal and parafilm around the edges to prevent evaporation.

-Measure liberation of the blue label using a plate reader overnight, every ten minutes. Before each measurement, introduce a 5 second shaking step.

-measure the slope of the first hours as a measure of amylase activity.