AZCL-amylose - version june 2021 – Rosanne Hertzberger

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

Previous issues with this assay were solubility of the substrate. We were using 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).

Make sure to order the 'fine' AZCL-amylose, and not the regular AZCL-amylose, to facilitate pipetting with regular tips.

The insoluble substrate is degraded by amylase. we have used the plate reader for continuous reading while incubating at 37 degrees.

Requirements

-2.0 mg/mL of "fine AZCL-amylose" (Megazyme) 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). We add this to the amylose assay to prevent growth because of the long incubation time and the fact that many of our 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. we either use culture supernatants by removing the cells through spinning 20 minutes at 4°C and maximum speed (4754 rcf in our case). Or we use the pellet that we wash with PBS. As controls/calibration curve, use saliva (100x - 1000x diluted in amylase buffer) or a known amylase quantity (*Aspergillus oryzae*, Sigma-Aldrich) 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. 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 release of the blue dye using a plate reader overnight, every ten minutes at OD600 at 37°C.

-We have calculated the rate by finding the maximum R^2 over a time course of 6 hours (36 measurements) and taken the slope as a measure of amylase activity.

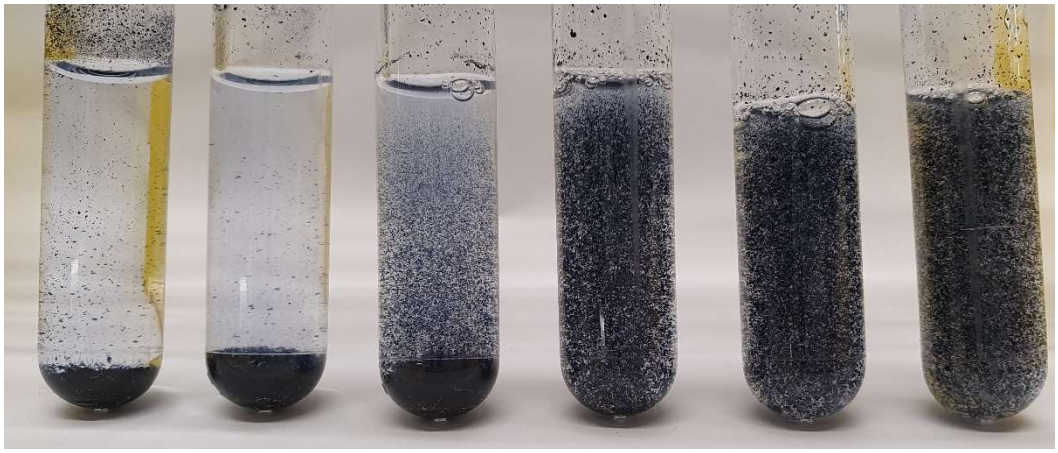

Figure 1 pelleting and dispersion of 2mg/mL azcl-amylose granules at different x-gom concentrations (0-5 mg/mL) in amylase buffer (pH5,5) after 7 days.

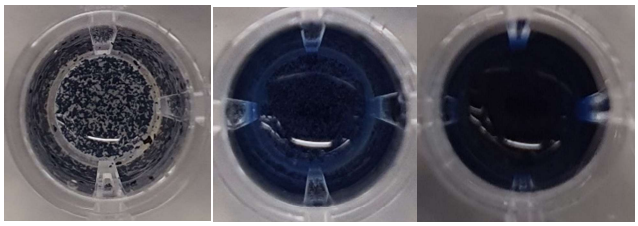

Figure 2: azcl-amylose at different stages of digestion with human saliva.