Starch – iodine assay to detect starch degrading enzymes – Rosanne Hertzberger – October 2017, update February 2019

Testing the presence of starch degrading activity in a solution by mixing with starch and measuring residual starch after a certain time period. Starch consists of α1-4 linked amylose and α1-4 and α1-6 amylopectin. Enzymes that can break down starch are: glycogen debranching enzyme, amyloglucosidase, amylase, pullulanase.

The assay has been working well for a positive/negative result (no rates so far) on starch degrading enzymes in supernatants and pellets of lactobacilli which are suspected to be pullulanases. The enzyme activity has been very slow hence the long incubation time. This also meant I have been using chloramphenicol and have not included stepts to stop the enzymatic reaction. You will have to adjust the assay in case you require rates and in case your activity is fast.

Requirements

-10 mg/mL of “soluble starch” (Sigma S9765) in “amylase buffer” (100 mM sodium acetate, 5 mM $CaCl_2$)

-Iodine stock solution: 0.2 gram I_2 and 2.0 gram KI in 100 mL H_2O (store at room temperature in the dark)

-Iodine working solution: dilute iodine stock solution 100x in 0.05 M HCl (~300 uL per sample, to prepare: 2.1mL HCl in 500 mL water)

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

Assay

-150 uL starch solution into flat-bottom transparent polystyrene 96-wells plate (Greiner ref 655191),

-add 50 uL enzyme solution, mix by pipetting. 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 two times with PBS.

-as a positive control, use either saliva (100x diluted) or amylase (*Bacillus licheniformis*, Sigma-Aldrich A3403). As a negative control use amylase buffer.

-seal airtight using a sterile covering adhesive film (VWR) and parafilm around the edges.

-incubate for 24h at 37°C .(if needed in case assay is developed later: store at 4 degrees, short centrifuge step to remove condensed liquid from the seal)

-let plate adjust to room temperature, remove seal and parafilm

-transfer 10 uL to a new flatbottom transparent 96 well plate, add 290 uL of the working iodine solution.

-make a calibration curve by diluting starch in water, I use 1%, 0,9%, 0,8% etc.

-measure the uncovered plate at optical density 600 nm.