Practical 03: Primary Screening of Extracellular Enzyme (Amylase) Producing Microbes

Amylases are starch hydrolysing enzymes. Starch is composed of amylose and amylopectin. Amylose is a long unbranched chain of D-Glucose bonded by α-1, 4 glycosidic linkages. It is not positively soluble in water but forms hydrated residues giving blue colour with iodine. Amylopectin is a highly branched structure consisting chains D-Glucose bounded by α-1, 4 and α-1, 6 glycosidic linkages.

There are two types of amylase.
(i) α- amylase and
(ii) β amylase

- α - amylase, which is also known as glucan 4–glucan hydrolase, hydrolyze α-1, 4 linkage at random to yield mixture of glucose and maltose.

- β -amylase, which is also known as glucan-maltose hydrolase gives away successive maltose units from non reducing end to yield glucose.

Examples of some amylase producing microorganisms are....
- *Aspergillus oryzae*
- *Aspergillus niger*
- *Mucor species*
- *Rhizopus species*
- *Bacillus subtilis*
- *Bacillus amyloliquiefaciens*

The isolation and screening of amylase producers can be done using nutrient agar medium supplemented with starch as a sole carbon source. The production of amylase is indicated by hydrolysis of starch which shows zone of hydrolysis of starch on addition of iodine. Thus amylase production can be confirmed by adding iodine because by doing so starch free zone appears clear against non-hydrolysed starch showing blue zone.

**Requirements**
- Soil sample
- Dilution blank
- Sterile pipettes
- Glass spreader
- Sterile nutrient agar plates with 5% starch
- Water bath at 80°C
Procedure

- Take fertile soil, sieve it and allow to dry for 24 to 48 hrs.
- Take approximately 0.1 gram of dried soil sample and suspend it in the test tube consisting 10 ml of sterile distilled water.
- Mix it well and vigorously shake the tube for 5 to 10 minutes and then allow it to stand for 10 minutes. This will result in the settle down of coarse soil particles giving supernatant consisting suspended microbes.
- Place the tube in water bath at 80°C for 10 min. (Many species of Bacilli produce amylase and being a spore former they can survive at high temperature. Hence for its selective isolation heat treatment is given to selectively kill vegetative cells of other microorganisms.)
- Now, prepare various dilutions from this supernatant.
- Spread 0.1ml of inoculum from each dilutions on the nutrient agar plate supplemented with starch as a sole carbon source.
- Incubate the plate at 37°C for 24 to 48 hours.
- Select the plates showing isolate colonies and flood it with iodine to check starch hydrolysis around the colony.
- The replica of plate must be taken prior to addition of iodine solution.
- Mark the amylase producing colonies, write colony characteristics and perform Gram staining.
- After obtaining pure culture, confirm the amylase producing activity of the selected isolate.

References

- Bacteriological Techniques By F.J. Baker
- Introduction to Microbial Techniques By Gunasekaran