The Neotia University

Metabolism Practical

Integrated M.Sc. Biotechnology

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CONTENT

Practical 1: Isolation of starch degrading microorganism from soil

Objectives: To isolate different starch degrading microorganisms from soil and characterization of their features.

Principle: Among different types of enzymes obtained from microbial sources, amylases aew the most widely used in industries as well as on commercial basis. Amylase is an enzyme that catalyses the breakdown of starch into sugars and plays a pivotal role in a variety of areas like use as digestives, for the production of ethanol and high fructose corn syrup, detergents, modified starches, hydrolysis of oil fluids and proper recycling. Depending upon mode of action, they are divided into types such as α-amylase acts randomly on 1,4 linkages without any attack on 1, 6-linkages. Whereas, β-amylase acts alternatively on 1,4-linkages without any attack on 1, 6-linkages. In addition to that gluco amylase acts sequentially on each α-1,4-linkage and 1,6-linkage. However, microbial production of amylase is more beneficial than other sources because its economical productionrate is high and can be engineered to obtain enzymes of desired characteristics. Many fungi as well as bacteria are capable of producing α-amylase. They are *A. aureus, A. niger, B-subtilis, B. coagulase* etc. In the laboratory, it is tested by performing the starch test to determine the absence or presence of starch in the medium by using iodine solution as an indicator. Starch in the presence of iodine, produces a dark blue colour in the medium and yellow zone around a colony in an otherwise blue medium indicates amylolytic activity. In this experiment amylase producing microorganisms will be isolated from soil and some of its features will be characterized.

Materials required

- Starch agar medium
- Nutrient agar medium
- Czapekdox agar medium
- Distilled water
- Diluted HCl OR NaOH (pH adjustment)
- Test tubes
- pH paper
- Tip box
- Pipettes
- Tissue paper
- Cotton Petri dishes
- Paranitrophenyl phosphate
- Test tubes
- Soil sample
- Gram staining kit
- iodine
- Lacto phenol cotton blue
- Alcohol
- Glass spreader
- Petri dishes

Procedure:

Day 1: Preparation of starch agar, nutrient agar Czapekdox agar media

Day 2: Collection of soil sample and preparation of several dilution and isolation of macroorganisms in different media through spread plate, slanting and pour plate method

Day 3: Detection of amylase producing microorganisms by iodine test

Day 4: Characterization of the features of starch degrading organisms by Gram staining procedure and study their colony morphology.

Observation:

Result:

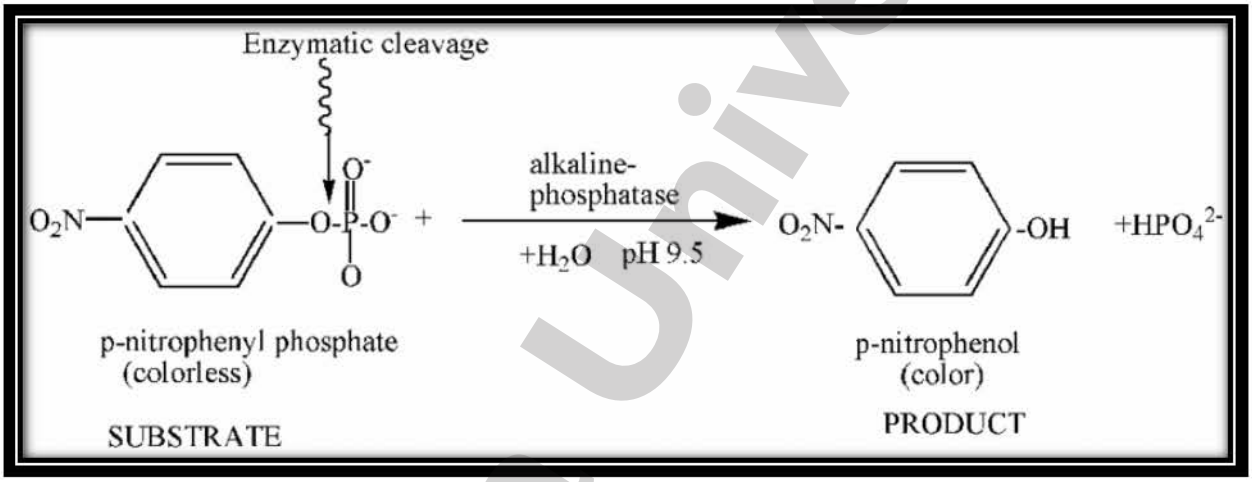
Conclusion: Based on the result obtained

Safety precaution: Students should wear lab coat and maintain hygeine during practical

Practical 2: Isolation of alkaline phosphatase producing microorganisms from soil

Objectives: Isolation of Alkaline phosphatase producing microorganisms from soil and study their characteristics

Principle: Alkaline phosphatase enzyme can produce para-nitrophenyl phenol from para-nitrophenyl phosphate.



Alkaline phosphatase is an enzyme that is seen in the human body. Its role in our metabolism is somewhat unclear as of now though. It is known to have a structure of a zinc metallo-enzyme with two identical subunits and serine at the centre of the molecule. It is responsible for the removal of phosphate groups from molecules in the body. It is found in the placenta and in the intestines for humans. It also plays a large role in bone resorption, as well as human calcification. In bone resorption, it alkaline phosphatase retards the rate of deposition of calcium and phosphate. Although it is not clear yet, alkaline phosphatase may also play a role in DNA synthesis and growth. A possible role in the development of our genetic material makes alkaline phosphatase a very relevant enzyme. It has also been found in bacteria and many other mammals. Scientists hope to get a better understanding of the enzyme in the near future and develop ways to possibly control and discover its actual

function soon. Recently, alkaline phosphatase has become a part of the medical field. Its serum content is helpful to people suffering from skeletal disorders.

Materials Required

- Soil sample
- Pikovskaya media
- Enriched media
- Glass pipettes
- Conical flask
- Glass beaker
- Test tubes
- 70% alcohol
- Gram staining kit
- Para-nitrophenyl phosphate
- Glycine-NaOH buffer

Procedure:

Day 1: Preparation of Pikovskaya and Enriched media

Day 2: Soil sample collection from university premises and inoculation of soil sample in different media

Day 3: Isolation of Alkaline phosphatase producing organisms and study their colony morphology and Gram character.

Observation:

Result:

Discussion: Based on the result obtained

Safety precaution: Students should wear lab coat and maintain hygeine during practical

Practical 3: Isolation of microorganisms from curd

Objectives: Isolation of lactic acid producing microorganisms from curd and study their different features.

Principle: Curd is a dairy product by coagulating milk in a process called curdling. This coagulation can be caused by adding rennet or any edible acidic substances like lemon juice, vinegar or by microorganisms and allowing them to sit. The increases acidity causes the milk protein casein to tangle into solid masses or curd. Microorganisms play an important role in food industry. Many genera of microorganisms are present in curd. The microorganisms that are isolated from curd include *Streptococcus lactis, Lactobacillus lactis, Lactobacillus coagulans* etc. Strains of these lactic acid bacteria are very useful and commonly used as probiotics. In this experiment microorganisms will be isolated from curd and their morphology as well as catalase producing activity will be studied. The ability of isolated microorganisms to produce curd will also be tested.

Materials required

- Curd
- YPD media
- Nutrient media
- Milk
- Petri dishes
- Distilled water
- Alcohol (70%)
- Conical flasks
- Tip box
- Pipettes
- Test tubes
- Hydrogen peroxide

Procedure:

Day 1: Preparation of MRS agar, nutrient agar YPD agar media

Day 2: Inoculationn of soil microorganisms from curd in different media by spreading and pour plate methods.

Day 3: Characterization of the features of organisms of curd by Gram staining procedure and study their colony morphology as well as catalase activity

Day 4: To study the ability of isolated microorganisms to produce curd by inoculating them in milk.

Observation:

Result:

Conclusion: Based on the result obtained

Safety precaution: Students should wear lab coat and maintain hygeine during practical

Practical 4: Production of alcohol (ethanol) by fermentation

Objectives: Production of alcohol (ethanol) by fermentation using yeast

Principle: Fermentation is a metabolic process that converts sugars to amino acids, gases or alcohol. It occurs in yeast and bacteria and also in oxygen-starved muscle cells as in the case of lactic acid. Alcoholic fermentation is a biological process which converts sugars as glucose, fructose and sucrose into cellular energy, producing ethanol and carbon di oxide. Yeast typically function under aerobic conditions, but can also functions under anaerobic conditions. When no oxygen is available, alcohol fermentation occurs in the cytosol of yeast cells. The basic equation for alcohol fermentation shows that yeast starts with glucose and finishes with carbon di oxide and ethanol. The process of alcohol fermentation can be divided into two parts:

1. At first yeast breaks down glucose to form 2-pyruvate molecules. This is known as glycolysis.
2. In the second part the 2-pyruvate molecules are convertd into 2 molecules of carbon di oxide, 2 molecules of ethanol. This part is known as fermentation.

The main purpose of alcoholic fermentation is to produce ATP, the energy currency for cells under anaerobic condition. The conversion of glucose to pyruvate creates a net total 2 ATP molecules. Before pyruvate can be converted to ethanol, it is first converted to a acetyldehyde. This releases carbon di oxide. Next, acetaldehyde is converted into ethanol. This conversion by itself does not create any more ATP for yeast.

Materials required

- Molasses media
- Glucose media
- Sucrose media
- YPD media
- Conical flasks
- Alcohol
- Yeast culture
- Balloons

Procedure:

Day 1: Preparation of YPD broth, Molasses media, Glucose and Sucrose media

Day 2: Inoculation of yeast cells into mdifferent media and observations will be taken after 48 hr.

Observation:

Result:

Conclusion: Based on the result obtained

Safety precaution: Students should wear lab coat and maintain hygeine during practical