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AMYLASE ENZYME FROM THE SOIL MICRO ORGANISM.

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ABSTRACT **- Soil sample was collected from the field of Papaya Coimbatore, Tamil Nadu and serial dilution was done up to 10^{-6} in order to reduce the concentration of micro organisms. Then spread plating was done on nutrient agar containing nitrogen source, which increase the productivity of cells, phosphate source, which play the role of regulations, ions for the stimulation of production of enzyme produced by micro organisms and incubated. After 24 hrs of incubation, all together 13 different colonies were formed in the plates10^{-4}, 10^{-5} and 10^{-6} and these colonies were sub cultured on starch plates, which is amylase specific media , to obtain individual micro organisms by streak plating method and incubated for another 24hrs. After 24 hrs starch iodine test was done, as amylase is capable of degrading starch. Out of the 13 colonies sub cultured 6 colonies were capable of producing amylase.**

Further biochemical assay was done in order to find out the enzyme activity of amylase, as amylase produced by different micro organisms may vary.

KEYWORDS**-Soil sample, serial dilution, nutrient media, starch media, starch test, amylase.**

1. INTRODUCTION:

Enzymes are macro molecules that act as biological catalysts. For a period of long time the use of chemical catalysts has been in existence. The disadvantages of using catalyst include certain conditions like high temperature, pressure. These disadvantages were overcome by the use of enzymes. Enzymes work at milder conditions when compared to that required by chemical catalysts for operation. Enzymes are more specific and they do not require any high specific conditions for their reactions to begin. Among the various enzymes that are being used α-Amylase has high demand due to its role of starch hydrolysis. (4)

Amylase, are the enzymes that are employed for the breakdown of the starch. Amylase can be obtained from many sources such as plant cell, animal cells, and micro organisms. But usually the production of amylase is usually done with micro organism, as it aid us in bulk production. Moreover the multiplication of micro organism is very rapid. (1)

Amylase is used mostly in food, beverage, textile and pharmaceutical industry. Amylase enzyme is used in detergents as amylase is capable of removing stains from the cloths. It is used in paper and textile industry to obtain the quality and it is very much useful in ripening the fruits. (6)

A mixture of Pectinase and amylases is used to clarify fruit juices (5). It decreases filtration time up to 50% (Blanco et al., 1999).

Initially chemicals were used for the breakdown of the starch .But it was changed in 1811 by the discovery of amylase enzyme by the Kirchhoff. In later 1930, Ohlsson classified the starch digestive enzymes in malt as a- and b-amylases according to the enzyme reaction. Amylases can be classified into two types. They are endoamylases and exoamylases. Endoamylases catalyze hydrolysis in the interior of the starch molecule. Exoamylases hydrolyze from the non-reducing end, successively resulting in short end products. Today large number of enzymes are discovered which are capable of hydrolyzing starch. Therefore for the complete hydrolysis of the starch, a combined action of various enzymes is required. (7)

2. MATERIALS AND METHODS:

2.1. SAMPLE COLLECTION:

Soil sample was collected from the field of papaya Coimbatore, Tamil Nadu using a sterile spatula into a sterile plastic cover.

2.2. ISOLATION OF MICROORGANISM:

One gram of soil sample was weighed and mixed in 9ml saline water. Serial dilution up to 10^{-6} dilution was done and spread plated on nutrient agar of composition peptone 1g, beef extract-1g, sodium chloride –0.5g, agar-1.5g and distilled water – 100ml (Himedia technical data). After an incubation period of 2 days at 37˚C, colonies were formed on plates 10^{-6}, 10^{-5} and 10^{-4}.Then CFU were calculated using the formula,

No. of colonies * dilution factor/ weight of sample.

2.3. SUB CULTURING:

Colonies were selected in random from the plates 10^{-4}, 10^{-5}, 10^{-6} and sub cultured by streak plate method ,in amylase selective media of composition : beef extract – 0.3g, starch soluble – 1g, peptone – 1g, agar – 1.2g and distilled water – 100 ml by streak plating method(HIMEDIA technical data). After an incubation period of 24 hours at 37˚C colonies were obtained**.**

3. TEST FOR THE IDENTIFICATION OF AMYLASE ENZYME:

3.1. STARCH IODINE TEST FOR AMYLASE:

An iodine solution using potassium iodide and iodine crystals was prepared and flooded over the starch plates, in which the growth over the streaks were formed and allowed to stand for about 30 seconds .After 30 seconds the iodine solution over the plates were drained and noticed .Clear zones around the streaks were formed in some streaks, due to starch hydrolysis indicating the presence of amylase. (2)

4. BIOCHEMICAL ASSAY:

4.1. FOR AMYLASE:

The streaks that indicated the presence of amylase were sub cultured in starch broth of composition beef extract 0.3g, peptone 1g, starch 1g, distilled water 100ml. After an incubation period for about 48 hours at 37℃, turbidity was obtained. Supernatant was separated by centrifugation .This supernatant is used for the biochemical assay of amylase.

5. RESULTS AND DISCUSSION:

5.1. FOR AMYLASE:

Amylase is used mostly in beverage industry, pharmaceutical industry and textile industry. It is mostly used for reducing the viscosity of the liquids. The soil sample was serially diluted and spread plate technique was done on the nutrient media and incubated for a period of 24hrs at 37℃. After 24-48 hrs of incubation at 37˚C, colonies were formed.

13 colonies were formed clearly and sub cultured on starch media and 6 colonies were found to form clear zones in starch iodine test indicating the presence of amylase. Biochemical assay for those 6 colonies that showed clear zone in starch iodine test was done by using, it's supernatant after centrifuging.

COLONY NUMBER	OBSERVANANCE OF STARCH IODINE TEST	INFERENCE
1	Absence of zone of clearance	Absence of amylase
2	Zone of clearance	Presence of amylase
3	Zone of clearance	Presence of amylase
4	Absence of zone of clearance	Absence of amylase
5	Absence of zone of clearance	Absence of amylase
6	Absence of zone of clearance	Absence of amylase
7	Absence of zone of clearance	Absence of amylase
8	Zone of clearance	Presence of amylase
9	Zone of clearance	Presence of amylase
10	Absence of zone of clearance	Absence of amylase
11	Zone of clearance	Presence of amylase
12	Absence of zone of clearance	Absence of amylase
13	Zone of clearance	Presence of amylase

Table 1: Results of starch iodine test.

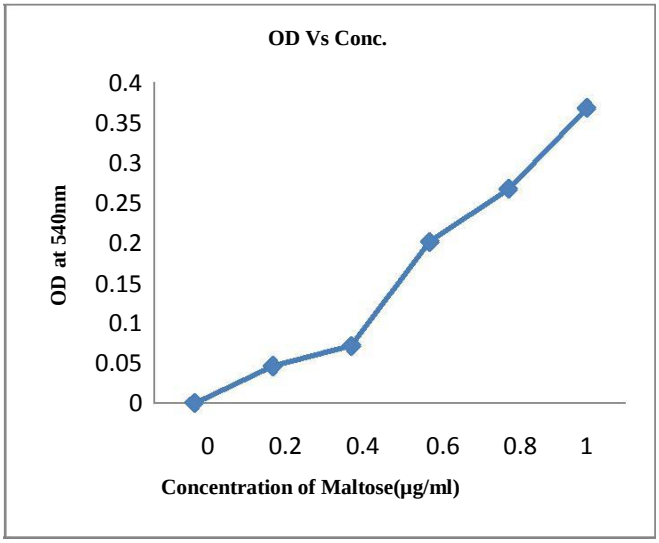

Fig: 1Amylase assay maltose standard.

Colony number	Amylase activity in U/ml
2	38.45
3	33.09
8	59.28
9	56.898
11	42.61
13	52.613

Table: 2 Amylase assay result

6. CONCLUSION:

Several micro organisms obtained from the soil sample of papaya field are capable of producing amylase. Out those several micro organisms, colony number 8 is capable of producing amylase with high activity of 59.28U/ml.

This work has been taken up on the view to identify and isolate amylase producing micro organisms from the soil sample of papaya field. Further work should be done on searching a micro organism that is capable of producing high amount of amylase. By bulk production of the amylase, by fermentation technology, provide us wide applications on food, beverage and pharmaceutical industry.

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