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PECTINASE ENZYME FROM THE SOIL MICRO ORGANISM

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Abstract:

Soil sample collected from the field of papaya, Coimbatore, Tamil Nadu and serial dilution was done up to 10^{-6} in order to quantify the number of bacteria's in the solution. Then spread plating of the colonies formed in the10^{-4}, 10^{-5} and 10^{-6} plates was done on nutrient media and incubated for 48 hrs. After 48hrs, 14 individual colonies were formed on the nutrient media of the plate 10^{-6} and these colonies were sub cultured on pectinase selective media by streak plate method, in order to obtain pure culture of individual micro organisms and incubated for 24 hours. After 24 hrs growth over the streaks were obtained on pectinase selective media. Then iodine test was done in order to differentiate the microorganism capable of producing pectinase. Out of the 14 colonies, 4 colonies were capable of producing pectinase.

Further biochemical assay was done in order to find out the enzyme activity of pectinase as different species of bacteria possess different biochemical activity.

Keywords: Soil sample, Serial Dilution, Nutrient Media, Colonies, Starch Media, Amylase.

1. INTRODUCTION:

Enzyme production has been widely grown in the past centuries. Micro organisms play a important role in the production of enzymes. But animals and plants also act as good producers of enzymes. Traditionally enzyme production relied on the natural host but nowadays due to the advancement of genetic engineering, the enzyme producing capacity of the natural host can be improved.

Pectin is a complex polysaccharide. It provides firmness and structure to the plant tissues. Pectin is made up of α-D-galacturonic acid. Pectin is classified into two types .They are homogalacturonans and rahmgalacturonans. It provides strength to the cell wall when the pectin is bound with the cellulose of the cell wall. [1]

Pectinase is produced by many organisms such as bacteria, fungi, protozoan ,plants, yeast etc.The pectinase produced by micro organisms are involved in various process such as phytopathologic

process, in the symbiosis of plant and microbe and in the decomposition of dead plant material, thereby plays a very important role in natural carbon cycle.[2]

Pectinase enzyme has wide applications in industries .They are leading enzymes of biotech sector. Pectinase are widely used in various industries such as wine industry, in paper industry for bleaching of papers, in the recycling of waste paper, in the extraction of oil etc. [3]

The most common industrial application of pectinase is the clarification of fruit juice. A mixture of Pectinase and amylases is used to clarify fruit juices [4]. It decreases filtration time up to 50% (Blanco et al., 1999).One of the major problem faced by the fruit juice industry is the cloudiness ,which is mostly due to the presence of pectin [5] .And this problem can be sorted out by use of pectinase ,as pectinase is capable of degrading pectin.

Pectinase is used in textile industry for scouring of cotton fibers. This process of scouring increases the water absorbing character of the cotton fibers. Initially chemicals were used for the process of scouring but it is replaced by enzymes which is ecofriendly. [6]

Pectinase enzyme plays a very important role in the degumming and retting process of plant fibers. Usually plant fibers consist of gum after decortications which require a degumming process for textile purpose. This degumming process is performed by pectinase enzymes by degrading the pectin content in the gum of the plant fiber. [7]

Pectinase is utilized as animal feed supplement which helps in the reduction of feed viscosity which directly increases the absorption of nutrients that liberates from fibers by hydrolysis process and it also reduces animal faeces (Hoondal et al., 2000).

A very highly pure preparation of virus is required for the physical, chemical and biological studies and this is enhanced by the use of pectinase enzyme in obtaining pure virus from phloem. [8]

It is also used for other purpose like treatment of waste water containing pectin, extraction of DNA and oil extraction. [8]

Pectinase can be obtained from many sources such as plants, animals and micro-organisms .But bulk production can be made from microorganisms.

2. MATERIALS AND METHODS:

2.1. SOIL SAMPLE COLLECTION:

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

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2.2. ISOLATION OF MICROORGANISM:

1g of soil sample is mixed with 9ml of saline water and serial dilution was done up to 10^-6 and incubated for a period of 48 hrs at 37℃ and spread plating was done on nutrient media of composition peptone-1g, beef extract-1g, sodium chloride- 0.5g and agar-1.5g (HIMEDIA).After incubating colonies were formed on plate and CFU was calculated using the formula

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

2.3. SUBCULTURING:

14 colonies were formed on the selected in random and sub cultured on pectinase selective media consisting of pectin 1g, ammonium sulphan 0.3%, KH2PO4 -0.2%, K2HPO4 -0.3% and agar-1.5% and 100ml distilled water and incubated for a period of 48 hrs at 37℃.

3. TEST FOR IDENTIFICATION OF PECTINASE:

3.1. IODINE TEST:

Colonies were formed on the selective media plates after 48hrs and iodine solution was flooded over the plates and after 10 m, the iodine solution was drained and clear zones were formed around the streaks of 4 colonies out of 14 colonies.

4. BIOCHEMICAL ASSAY:

4.1. FOR PECTINASE:

The 4 colonies that formed clear zone were then inoculated in pectinase selective broth and incubated for a period of 48 hrs at 37℃ and the supernatant was separated by centrifuging and used for biochemical assay.

5. RESULTS AND DISCUSSION:

Pectinase is used for the breakdown of the pectin. It is present in apples and bananas and they help not only in digestion but also in anti-aging .They are present in plants, animals and microorganisms. They are mostly used as thickeners in jams, pickles and other preserved foods. Pectinase is also used for extraction of oil and in waste water treatment plant for the purification of water containing pectin

14 colonies were sub cultured on pectinase selective media and by iodine test it was found that 4 colonies were capable of producing pectinase. Then these colonies were inoculated on pectinase selective broth and the supernatant was separated by centrifugation and used for biochemical assay.

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COLONY NUMBER	OBSERVANCE OF IODINE TEST	INFERENCE
1	Zone of clearance	Presence of pectinase
2	No Zone of clearance	Absence of pectinase
3	No Zone of clearance	Absence of pectinase
4	Zone of clearance	Presence of pectinase
5	No Zone of clearance	Absence of pectinase
6	No Zone of clearance	Absence of pectinase
7	No Zone of clearance	Absence of pectinase
8	Zone of clearance	Presence of pectinase
9	No Zone of clearance	Absence of pectinase
10	No Zone of clearance	Absence of pectinase
11	No Zone of clearance	Absence of pectinase
12	No Zone of clearance	Absence of pectinase
13	No Zone of clearance	Absence of pectinase
14	Zone of clearance	Presence of pectinase

Table 1: Results of iodine test.

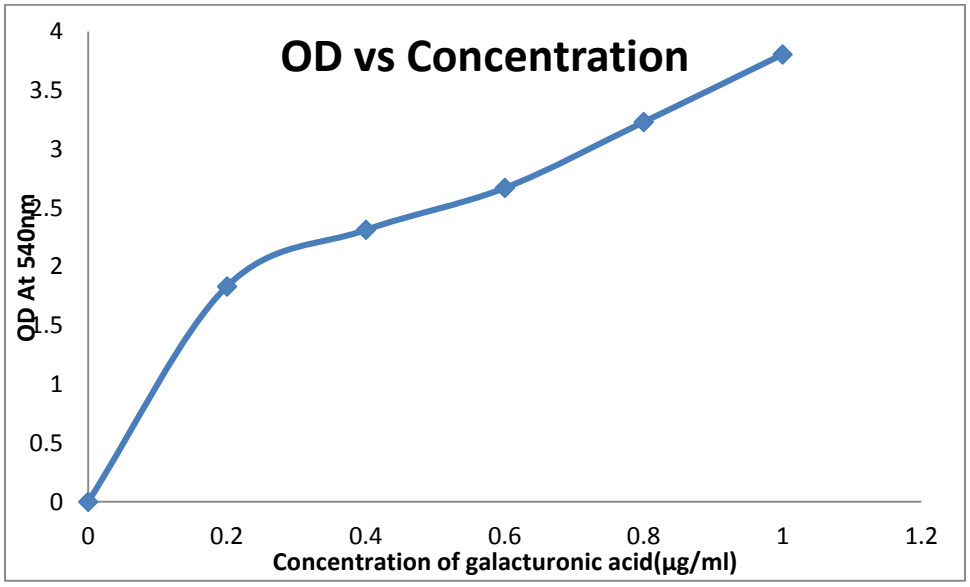

Fig: 1Pectinase assay galacturonic acid standard.

COLONY NUMBER	PECTINASE ACTIVITY in (U/ml)
1	26.6
4	13.47
8	32
14	29

Table 2: Pectinase assay

6. CONCLUSION:

In industrial sector , pectinase is really an upcoming enzyme , as these enzymes has wide application in industries such as food industries, animal feed industries, textile industries , oil industries and in the purification of waste water containing pectin. Pectinase is a naturally produced biochemical; therefore it is very ecofriendly to the environment. Due to its wide applications it has replaced many chemicals, which are difficult to be treated.

Certain micro organisms present in soil sample of papaya field , Coimbatore, Tamil Nadu are capable of producing pectinase enzyme. Out of those several micro organisms colony number 8 is capable of producing pectinase of high activity of 32U/ml .Further study has to be made to find out the micro organism that have high enzyme activity. Then fermentation has to be carried out for the bulk production of pectinase enzyme, thereby providing us wide applications in various industries.

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