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Isolation and identification of pectinase producing microbial strains from rotting fruits and soil

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ABSTRACT
The present investigation was carried for identification of pectinolytic bacteria and determination of their pectinolytic activity. The isolation was made from soil sample and rotten orange fruit. The growth characteristics of isolates were studied on LA agar medium. Bacillus cereus produced Milky white colonies and staphylococcus aureus produced cream yellow colonies. The isolates were found Gram's positive rod shaped and positive for biochemical test viz. catalase, Indole production and starch hydrolysis. Different growth parameters were carried out for pectinase production in which the maximum growth was observed at 72 hrs. of incubation, the optimum temperature for pectinase was found at 37°C, where the maximum pectinase production was observed at pH 8. Bacterial strains Showed similar pectinolytic activity clear zone but Bacillus cereus showed the highest potential of pectinase production with clear zone of 12 mm.
Keywords: pectinase, pectinolytic activity, Indole production

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INTRODUCTION

Pectinase constitutes a complex enzymatic system responsible for the degradation of pectic substances [2]. Pectinases are group of enzymes that attack pectin and depolymerize it by hydrolysis and trans-elimination as well as by de-esterification reactions, which hydrolyses the ester bond between carboxyl and methyl groups of pectin [3].

Pectins form the major components of the middle lamella and primary plant cell wall [4]. There are four main types of pectic substances protopectin, pectic acid, pectinic acid and pectin. Pectins are the soluble polymeric materials containing pectinic acids as the major component. They can form insoluble protopectin with other structural polysaccharides and proteins located in the cell wall. There are basically three types of pectic enzymes: de-esterifying enzymes (pectinesterases), depolymerizing enzymes (hydrolases and lyases), and protopectinases. They can be further classified as: endo-liquefying or -depolymerizing enzymes or exo-saccharifying enzymes [Kashyap et. al.,2000]. Pectic enzymes contribute to the degradation of pectin by various mechanisms. Elimination of pectic substances is an essential step in many foods processing and wine industries. These enzymes are mainly synthesized by plants and microorganisms [Naidu G. and Panda T.,1998]. Microbes synthesize polygalacturonases, polymethylgalacturonases, pectin lyases and pectin esterases. The biotechnological potential of pectinolytic enzymes from microorganisms has drawn a great deal of attention for use as biocatalysts in a variety of industrial processes [10].

These pectinases have wide applications in fruit juice industry and wine industry. In fruit juice industry, it is used for clarification, where reduction in viscosity is caused which ultimately leads to formation of clear juice. They increase the yield of juices by enzymatic liquefaction of pulps these pectinases also help in formation of pulpy products by macerating the organized tissue into suspension of intact cells. In wine industry, pectinases are mainly used for decreasing astringency by solubilizing anthocyanins without leaching out procyadin polyphenols, and pectinases also increase pigmentation by extracting more anthocyanins [11]. Many plant pathogenic bacteria and fungi are known to produce pectinolytic enzymes useful for invading host tissues. Moreover, these enzymes are essential in the decay of dead plant material by pathogenic microorganisms and thus assist in recycling carbon compounds in the biosphere [6].

Pectinases are produced during the natural ripening process of some fruit and act in combination with cellulose. A large number of microbial strains have been studied for the production of pectinase. The main sources for the pectinolytic complex enzymes are yeast, filamentous fungi and bacteria a large number of which the most relevant ones are Bacillus spp. The pectinase production in yeast has received less attention due to less yield obtained in comparison to bacteria [1-3]. A range of bacterial and fungal strains produce a variety of pectinolytic enzymes [9]. The selection of high yield pectinase producing strain is difficult, however studies revealed that bacterial strains usually have been shown to produce more yield of pectinolytic enzymes than those of fungal ones.

Today, the enzymes are commonly used in many industrial applications, and the demand for more stable, highly active and specific enzymes is growing rapidly. Pectinases have extensive commercial importance for various industrial food applications like in fruit juice industries in order to improve fruit juice yield and clarity. The use of liquefying enzymes for mash treatment results in improvement of juice flow, as maceration of tea leaves, processing of cotton fabric leading to a shorter press-time without the necessity for pressing aids. At the same time pectin is broken down into such an extent that the viscosity of mash is reduced as viscosity relates to molecular weight on another hand pectinase also has wide importance in pharmaceutical industries to improve nutritional value of food.

The main importance of these study to developed a sustainable technique for isolation and identification of pectinase producing microorganisms to fulfil the need of pectinase for mankind's.

MATERIAL AND METHODS

Collection of Sample

Soil sample were collected from the farm of citrus orchard. Rotten fruits were collected from weekly market of Loni.

Isolation

Pectinolytic bacteria were isolated from collected soil samples and rotten juice by serial dilution method and spread on Luria agar plates. Serial dilution was done by taking one gram of soil in 100 ml distilled water in a flask. 1 ml suspension from flask was taken into test tube containing 9 ml of sterile distilled water and 1 ml from one to another, dilutions were made up to 10 -5, 0.1 ml of sample spread on petri plates from last two dilutions and these plates were incubated at 37° C for 24-48 hours. After incubation mixed cultures were obtained which were purified by streaking on Luria agar plates.

Morphological and biochemical Characterization of bacterial strains –

Identification of strains was done on the basis of colonial morphology (Size, shape, edges and colour of colonies), staining (Gram's staining) and biochemical testing viz. catalase test, starch hydrolysis, Indole production.

Study of growth parameters of isolates showing maximum pectinase production

Bacterial isolates were subjected to different culture conditions to derive some of the optimum growth conditions for pectinase production. Growth parameters such as growth curve, effect of Temperature and effect of pH were studied in order to detect the optimum parameters by growing cultures in Lauria Broth.

A) Growth Curve:

In order to detect the growth curve, 100 ml Lauria broth was prepared and autoclaved. Loopful of culture was inoculated in it and incubated in shaker for 24 hours. After incubation optical density was taken at 600 nm everyday till the decline phase did not reach and checked the optimum growth.

B) Effect of Temperature:

The most potent bacterial isolates were allowed to grow in the Lauria broth having pH 7 at 37°C on continuous shaking (150 rpm) for starter culture. After 24 hrs, 100 µL bacterial culture was incubated in 100 ml conical flask containing 20 ml L-broth supplemented with 2% pecticsubstrate.and incubated for 24 hrs. at 10, 20, 30, 37 and 40°C on continuous shaking (150 rpm). After incubation O.D. was taken at 600nm to check optimum growth.

C) Effect of pH:

The effect of pH for pectinase production was determined by inoculating and incubating the bacterial culture in the Lauria broth having different pH. The experiment was carried out individually at various pH i.e. 5, 6, 7, 8, 9 and 10. The optical density was checked after 24 hours at 600 nm.

Screening of bacterial isolates for pectinolytic activity –

Bacterial cultures were incubated at 37°C as starter culture in 20 ml LB (Luria broth) having pH 7 [6]. After 24 h the broth was centrifuged and supernatant was obtained. The resultant supernatant was used for the screening of bacterial isolates for pectinase activity by well plate method. Petriplates containing autoclaved modified MS medium [6] supplemented with 2% pectin were prepared. After solidification of the medium, three wells of 8 mm in diameter were cut in the agar with the help of corkborer. Each well

was filled with 25 µL of cell supernatant and incubated for 24 hrs.at 37°C.After incubating for 24 hrs., plates were observed for pectinase activity by flooding them with iodine solution.

1) The enzyme activity was observed by measuring the diameter of clear zone around the well in millimeter.

RESULTS AND DISCUSSION

Staphylococcus aureus and Bacillus cereus were isolated from rotten orange and soil respectively by serial dilution and plating technique method using LA agar medium and Lauria broth medium. Bacteria were relatively identified based on colony, morphological and biochemical characters as per bergy, s manual of determinative bacteriology. Observations were recorded on the basis of morphological and biochemical test of isolates also studied the pectinolytic activity of isolates.

Isolation of Bacteria-

Two isolates were isolated from the collected sample. These isolates were purified by frequently restreaking them on Lauria agar plate. Cream yellow colonies identical as *Staphylococcus sp.* and milky white colonies identical to *Bacillus sp.* were selected for further study. These isolates were maintained on slants. [2].

Morphological and Biochemical characteristics of isolates-

The isolates were studied with respect to their colony, colour, shape and gram's staining reaction. The *Bacillus cereus* produced circular Colonies on Lauria agar medium and *Staphylococcusaureus* produced oval Colonies on Lauria agar medium. Isolates were subjected to the biochemical test for their identification, some of the test were performed for comparing the characteristics depicted in burgeys manual of systemic bacteriology. It is spherical shaped, gram positive, circular, cream pale, yellow colonies on Lauria agar plate [7]. Biochemical test viz catalase, starch hydrolysis, Indol production confirms the bacterial pathogens

Table 1-Morphological and Biochemical characteristics of isolates.

Characteristics	Isolates	
	Staphylococcus aureus	*Bacillus cereus*
Morphological and cultural characteristics of isolates		
Shape	Oval	Circular
Colour	Cream Yellow	Milky white
Gram's character	Grams Positive Cocci shaped	Gram's positive Rod shaped
Biochemical characteristics of isolates		
Catalase Test	Positive	Positive
Indol test	Positive	Positive
Starch hydrolysis	Positive	Positive

A) Growth parameters of different isolates-

Different growth parameters were carried out in which the maximum growth was observed at 72 hours, the optimum temperature for pectinase production was found at 37ºC, where by the maximum pectinase production was observed at pH 8.

Similar sort of result was observed by G.A. Aaisha *et al*. [1] who reported that different growth parameters were carried out in which the maximum growth was observed at 72 hrs the optimum temp. for pectinase produce was found at 37ºC where by the maximum pectinase production was observed at pH 8 for *Bacilllus firmus*. This is in agreement with study of Anna *et. al.* [2] who reported that *Bacillus firmus* is able to produce high percent of pectinase.

Table 2- O.D.at 600nm of different incubation period at 37°C.

Bacteria	24 hrs.	48 hrs.	**72 hrs.**	96 hrs.
Staphylococcus aureus	0.926	1.621	**1.858**	1.215
Bacilllus cereus	0.904	1.128	**1.920**	1.009

Effect of temperature-

Table 3- O. D. at 600 nm at different temperature levels

Bacteria	10°C	20°C	30°C	**37°C**	40°C
Staphylococcus aureus	0.928	1.327	1.629	**1.824**	1.121
Bacilllus cereus	1.294	1.691	1.899	**1.941**	1.378

Effect of pH-

Table 4- O. D. at 600 nm at different pH level

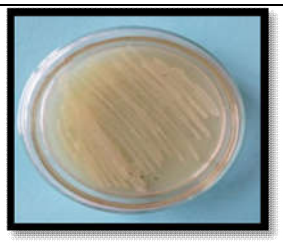
Bacteria	5	6	7	**8**	9	10
Staphylococcus aureus	0.240	0.928	1.625	**1.628**	0.710	0.552
Bacillus cereus	0.811	0.904	1.122	**1.183**	0.898	0.705

Screening of bacterial isolates for pectinolytic activity-

Bacterial isolates were subjected to preliminary screening by well plate method on modified MS medium [6-9] supplemented with 2 % pectin. Two isolates i.e. *Staphylococcus aureus* and *Bacillus cereus* were selected for pectinolytic activity on the basis of zone size ranging from 8 mm to 12 mm after flooded the plate with iodine solution.Bacterial strains Showed similar pectinolytic activity clear zone but *Bacillus cereus* showed the highest potential of pectinase production with clear zone of 12 mm

Table 5: Size of zone in mm by bacterial isolates in well plate method

Sr no	Isolates	Zone size in mm
1.	*Staphlococcusaureus*	8
2.	*Bacillus cereus*	12

Isolates of pectinase producing bacteria		**Screening of bacterial isolates**	
			
Staphyllococcus aureus	***Bacillus cereus***	***Staphyllococcus aureus***	***Bacillus cereus***

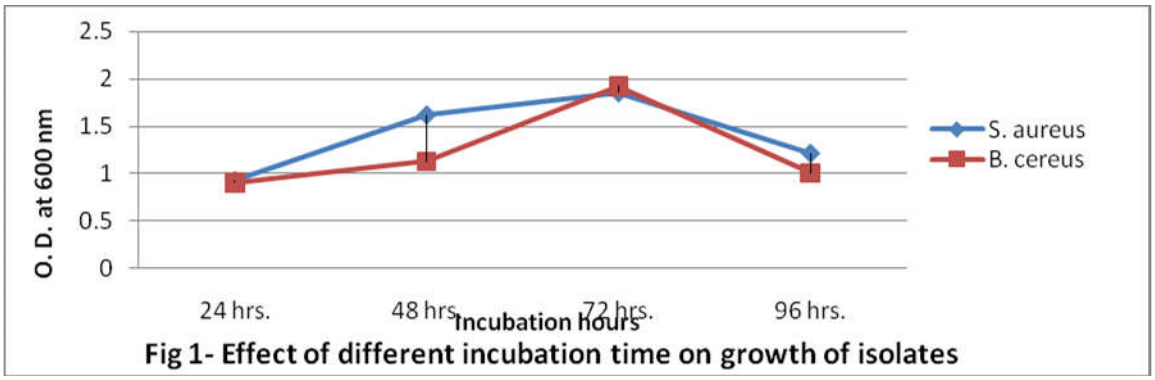


Fig 1- Effect of different incubation time on growth of isolates

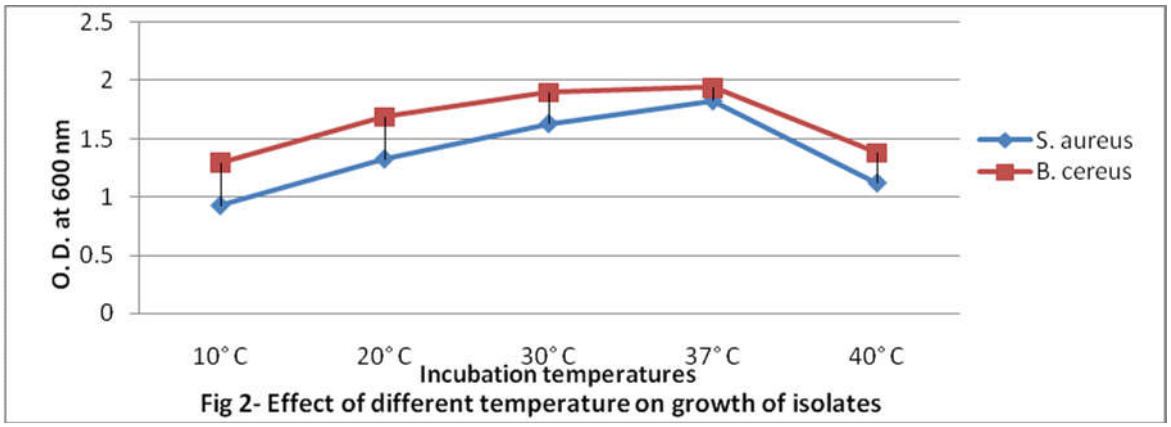


Fig 2- Effect of different temperature on growth of isolates

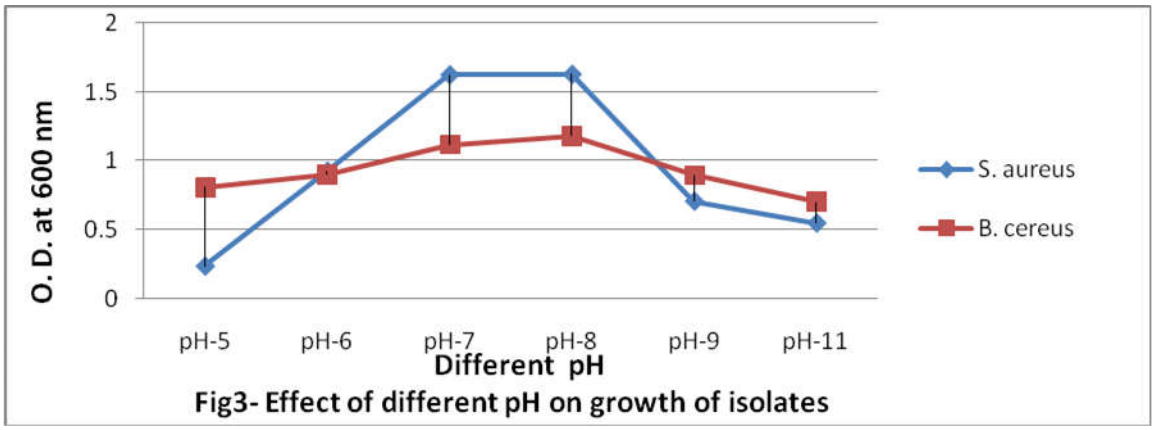


Fig3- Effect of different pH on growth of isolates

CONCLUSION

The Pectinolytic microorganism *Bacillus cereus* and *Staphylococcus aureus* were isolated from soil and rotten orange respectively. and identified, purified culture which maintained on Lauria Agar (LA) slants. Isolates were Gram's Positive, rod shaped and produced yellow, cream white colour colonies also exhibited positive reaction to indole production, catalase and starch hydrolysis. Different growth parameters were carried out in which the maximum growth was observed at 72 hrs. of incubation, the optimum temperature for pectinase was found at 37°C, where the maximum pectinase production was observed at pH 8. Bacterial strains Showed similar pectinolytic activity clear zone but *Bacillus cereus* showed the highest potential of pectinase production with clear zone of 12 mm.

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