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Screening of Pectinase Producing Bacteria , Isolated From Osmanabad Fruit Market Soil

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ABSTRACT-

Pectinases are the enzymes which breakdown pectin polysaccharide present in plant tissues into simpler molecules like sugar and other useful compounds. Investigation of pectinases is a central issue in enzymology research due to their wide applications in Pharmaceutical, food, Agricultural products and Bioremediation processes. Pectinases account for 10% of the total worldwide production of enzymes. The major sources of the pectinase are plant and microorganism. But for both technical and economic aspect microbial source of pectinase has become increasingly important. This study was undertaken to screen out and isolate efficient pectinase producing bacterial strains and to identify bacterial strains; which could be utilized in different pectinase dependent operations. Isolates were screened for pectinase production by using pectinase screening agar medium (PSAM). Six pectinase producing bacterial strains were isolated from Osmanabad soil samples of fruit market.One strain showing maximum zone was selected and this strain was identified .This strain was tested for morphological and biochemical characters and was designated as Staphylococci sp.

Key words:Pectinase, Pectin, Screening, *Staphylococci sp.*

INTRODUCTION-

Pectinase (EC 3.2.1.15) belongs to the class hydrolase which are able to hydrolyse pectin(Fogarty & Kelly 1982). Pectic substances, are structural polysaccharides in the middle lamella and primary cell wall of higher plants. Pectic substances are glycosidic macromolecules with high molecular weight .Pectic substances consists of pectinic acids, protopectins, pectins and pectic acids. The main chain of pectin is partially methyl esterified 1,4 – D-glacturonan. Demethylated pectin is known as pectic acid (pectate) or polygalacturonic acid. Pectinases are produced by many organisms such as bacteria (Horikoshi 1972; Karbassi & Vaughn 1980), yeasts (Gainvors & Belarbi 1993) and Fungi (Aguilar & Huitron 1990). Microbial production of pectinases has been extensively studied (Torres *et al*., 2006) .Among the various pectinase, bacterial extracellular pectinase are the most significant, compared with animal,Plants,viruses and fungal extracellular pectinase. Pectinases are the single class of enzymes which play an important part in the metabolism of almost all organisms (Plants, Animals, Fingi, Bacteria and Viruses). In the world market, pectinases accounts for about 10% of total enzyme production.. Soils microorganisms have recently emerged as a rich source for the production of industrial enzymes so many efforts have been made to isolate pectinase producing microbes from soil.

Applications of pectinases-The largest industrial application of pectinase is in food industries for fruit juice extraction and clarification. Pectins contribute to fruit juice viscosity and turbidity. A mixture of pectinases and amylases is used to clarify fruit juices. It decreases filtration time up to 50%. Treatment of fruit pulps with pectinases also showed an increase in fruit juice volume from banana, grapes and apples. Pectinases are also used in industries for textile processing and bioscouring of cotton fibers, for degumming of plant bast fibers,

retting of plant fibers, in waste water treatment,in coffee and tea fermentation, in paper and pulp industry, for making animal feed,for purification of plant viruses for citrus oil extraction, for improvement of chromaticity and stability of red wines and other foods.

MATERIALS AND METHODS:

Collection of sample:

The soil samples were collected from dump yards of vegetable market regions of Osmanabad (Maharashtra)India. These samples were stored in refrigerator at 4^0 C until further use.

Isolation of bacteria from soil sample:

1 gm soil sample was aseptically inoculated in 100 ml sterile pectin broth flask and this flask was incubated in rotary shaker for 7 days at 37^0C for enrichment.For isolation a loop full of enriched sample was streaked on nutrient agar (Nutrient broth + 2% agar agar). Then plates were incubated at 37^0C for 24 hours. After incubation plates were checked for growth of bacterial colony.

Screening of pectinase producing Bacteria:

Primary screening:

All of the bacterial isolates were tested for production of pectinase enzyme .The media used for primary screening was pectinase screening agar medium(PSAM).The sterile PSAM plates were prepared and all bacterial isolates were spot inoculated on those plates.All plates were incubated at 37^0C for 48 hours. After incubation the strains which are able to utilize pectin as a source of carbon develop colony on PSAM media and were selected as positive cultures.

Secondary screening:

Cultures which were showing positive results in primary screening were then screened for pectinase enzyme activity in secondary screening. The isolated colonies on PSAM were spot inoculated on Mc Beath's medium. The plates were incubated at 37^0C for 48-72 hrs. Following incubation they were observed for the zone of clearance around colonies which indicate pectinase activity. These zones could be observed only after flooding the pre incubated plates with 1% CTAB solution (Cetyltrimethyl ammonium bromide solution) for15 min. Clear zone of hydrolysis shows production of pectinase enzyme.

RESULT AND DISCUSSION-

Screening Of Pectinase Producing Bacteria- Total 32 bacterial strains were isolated from enriched soil sample on sterile nutrient agar plates.

Fig 1: Isolates obtained from soil sample.

Primary Screening- Out of 30 isolates 20 isolates grew on PSAM and therefore gave positive results for the screening.

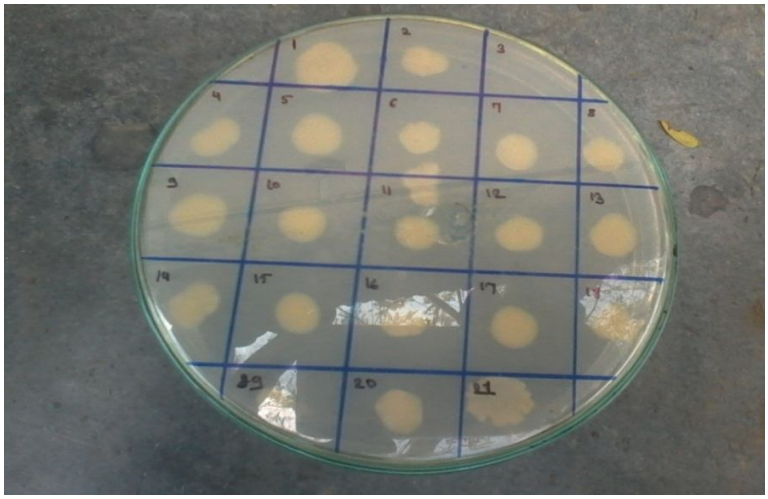

Plate 1: Primary screening of isolates

Secondary screening: Out of 20 positive isolates of primary screening,9 isolates showed good zones around the colonies and therefore selected as potent pectinase producing isolates.

Table1:showing zones(in mm) aound bacterial colonies on Mc Beath's agar plate

Colony no.	B1	B3	B5	B7	B9	B11	B13	B14	B16
Zones(in mm)	3mm	2mm	2mm	2mm	5mm	4mm	3mm	6mm	3mm

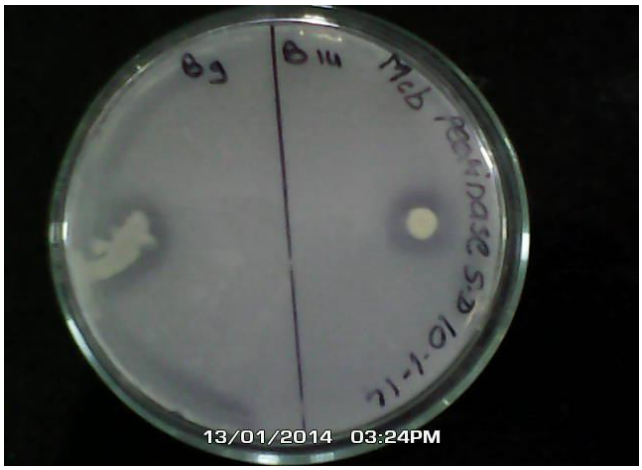

Plate 2: Isolates showing zone around colonies on Mc Beath's agar plate

DISCUSSION-

Pectinase producing microbes are present mainly in soil where plant(vegetable,fruit etc) waste matter are present. Many scientists have been tried to isolate efficient pectinase producing microbe from the soil.In this study,we tried to isolated pectinase producing bacteria from the soils of fruit market dump area and we were successful. Many scientists have preffered the same location for isolation of pectinase producing microbes. E. Venkata Naga raju *et al.*,(2013) isolated from dump yards of Bangalore market. He isolated 3 bacterial strains *Bacillus licheniformis, Bacillus cerus,* and *Staphylococcus aureus.He isolated 6 prominent pectinase producing bacteria :in our case we isolated 9 pectinase producing bacteria*. D.R. Kashyap *et al.*,(2000) isolated soil bacteria , *Bacillus sp. DT7* which has been found to produce significant amounts of an extracellular pectinase which was subsequently characterized as pectin lyase. Mukesh kumar D J *et al.*,(2012)produced pectinase from *Bacillus sp.MFW7* but used cassava waste for production. R. C. Patil *et al.*,(2012)isolated pectinolytic bacteria from carrot waste.Many scientists also had tried ti isolate pectinase producing fungi. Mellon & Cotty(2004) studied production of pectinases from *Aspergillus flavus.* Angayarkanni *et al.,* (2002) worked on pectinase production by *Aspergillus sp*. Silva *et al.,(*2002) studies pectinase production by *Penicillium viridicatum RFC3*. Pericin *et al.,* (2007) worked on pectinases from*Penicillium roqueforti.* Bruhlmann *et al.*,(1994) worked on actinomycetes pectinases. Fawole & Odunfa,(1992) studied pectinases production from moulds.

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