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Isolation and Identification of Pectinolytic Bacteria from Soil Samples of Akola Region, India

G.A. Aaisha and D.L. Barate*

Department of Microbiology, Shri Shivaji College Arts, Science and Commerce, Akola, M.S, India
**Corresponding author*

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A B S T R A C T

Pectin is a major component of primary cell wall of all plants and encompasses a range of galacturonic rich polysaccharides. Pectinase hydrolyses pectic substance, they have a share of 25% in the global sales of food enzymes. The present work has been undertaken for the screening and isolation of pectinase producing bacteria from soil samples collected from farms of various regions of Akola. Total 70 bacterial strains were isolated from 12 soil samples. Preliminary screening of pectinase producing bacterial strains was done by spot inoculation on Vincent's agar medium containing pectin. Out of 70 isolates, 36 were found to be positive for pectinolytic activity giving clear zones ranging from 7-35 mm. The pectinase producing isolates were subjected for further identification by Standard Conventional methods. The isolates were found to be *Bacillus spp*, *Pseudomonas spp* and *Staphylococcus aureus*. In further study, 4 most prominent pectinase producing isolates were identified as *Bacillus firmus* (P1), *Bacillus coagulans* (P13), *Bacillus endophyticus* (P57) and *Bacillus vietnamensis* (P58) In the present study, an attempt was made to isolate efficient pectinase producing bacteria from the diverse region of Akola.

Introduction

Enzymes are delicate protein molecules necessary for life. Pectin is a polymeric material having carbohydrate group esterifies with methanol. It is an important component of plant cell wall. It is present in highest concentration in the middle lamella, where it acts as a cementing substance between adjacent cells. Plant pathogens attack target cells by producing number of cell degrading enzyme which facilitate the entry and expansion of pathogen in the host tissue. Pectinases are a group of enzymes that breakdown pectins. The history of pectinases began with an understanding the structure of pectine substances and the mechanism by which pectolytic enzymes degrade pectic substances. Later the microbial production of pectinase became prominent for many decades. Many microorganisms viz. Bacteria, Yeast, Fungi

could produce pectinases. 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 (Marcia *et al.,* 1999).

Among the various pectinase, bacterial extracellular pectinase are the most significant, compared with animals, plants, viruses and fungal extracellular pectinase. Extracellular pectinase produced by *Bacillus* and *Cocci* species are of main interest from a biotechnology perspective, and are not only in scientific fields of protein chemistry and proteins engineering but also in applied fields such as foods, pharmaceutical and paper industries. These pectinases account for 10% of the total worldwide production of enzyme. The genus *Bacillus* and *Cocci* contains a number of industrially important species and approximately half of the present commercial production of bulk enzymes derives from the single class of enzymes which play an important part in the metabolism of almost all organisms. In the present study, an attempt was made to isolate efficient pectinase producing bacteria from the diverse region of Akola.

Materials and Methods

Soil samples were collected from 12 different regions of Akola district. Pectinolytic bacteria were isolated from collected soil samples by serial dilution method and spread on Vincent's 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 upto 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 hours. After incubation mixed cultures were obtained which were purified by streaking on agar plates.

Primary Screening

Screening was done by the spot inoculation method. Isolated cultures were inoculated on the Vincent's agar plate for the screening of pectinase producing bacteria by spots inoculation method and plates were kept for incubation at 37°C for 24 hours, to screen the pectinase activity of the obtained cultures. After incubation period plates were observed for the formation of clear zone around the colonies by flooding Iodine solution on the plates. (Venkata *et al.,* 2013)

Secondary Screening

The secondary screening for pectinase producing bacteria was done by the Well diffusion method (Lalitha *et al.,* 2013). Nutrient broth was prepared and inoculated with each isolate in separate tubes. 1 ml of cell free supernatant was poured on the Vincent's agar well of diameter 5 mm prepared in plates by sterile cork borer. After pouring the broth cultures, plates were incubated at 37° C for 24 hours. After incubation plates were flooded with the Iodine solution, and the zone of clearance was observed. The cultures which showed highest zones were selected for further study.

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.

Growth Curve

In order to detect the growth curve, 100 ml nutrient 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.

Effect of Temperature

Temperature plays an important role for the pectinase production. The effect of temperature on pectinase production was studied by inoculating culture in 100 ml nutrient broth and incubated at various temperatures, 10, 20, 30, 37, 40, 50, 60, 70 and 80° C. After incubation optical density was taken at 600 nm to check optimum growth.

Effect of pH

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

Results and Discussion

Total 70 isolates were obtained from soil samples by serial dilution method and the isolates were screened for the pectinolytic activity. In primary screening, 36 bacterial colonies showed zone of clearance which further were selected for secondary screening. These isolates were classified as good, fair and excellent producers, selected on the basis of zone diameter for their pectinase activity. In secondary screening 4 isolates were found to be excellent producers which were probably identified as, *Bacillus firmus* (P1), *Bacillus coagulans* (P13), *Bacillus endophyticus* (P57) and *Bacillus vietnamensis* (P58). 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.

In the present study, isolation and screening of pectinase producing bacteria was carried out from the agricultural soil. For this soil samples were collected from different places of Akola region. The isolation was carried out first by serial dilution of soil samples on Vincent's agar medium as performed by Kumari *et al.,* The serially diluted soil samples were screened for pectinase producing bacteria on Vincent's agar plate. Out of 70 bacteria, 36 bacteria showed zone of clearance after pouring iodine solution in primary screening. In secondary screening 4 isolates showed high zones of clearance out of 36 isolates.

All 36 bacterial isolates were characterised based on Gram staining and several biochemical reactions. The probable isolates were found to be as, *Bacillus licheniformis* (7), *Bacillus badius* (4), *Bacillus asahin* (2), *Bacillus psychrosaccharolyticus* (4), *Pseudomonas aeruginosa* (7), *Pseudomonas fluroscens* (4), *Staphylococcus sureus* (3), *Bacillus firmus* (1), *Bacillus coagulans* (1), *Bacillus endophyticus* (1), *Bacillus vietnamensis* (1) (Table 1 & 2). This is in agreement with other studies who also reported isolates from these genus are good sources for production of pectinase. (Venkata *et al.,* 2013; Torimiro *et al.,* 2013; Anam *et al.,* 2012; Kashyap *et al.,* 2000; Kumar *et al.,*2012; Sunil *et al.,* 2013; Divakar *et al.,* 2013).

Table.1 Cultural and Morphological Characteristics of Isolates

Characteristics	Isolates			
	P1	**P13**	**P57**	**P58**
Morphological, Cultural Characteristics of isolates				
Size	1-12 mm	1-3 mm	1-3 mm	2-3 mm
Shape	Oval	Circular	Circular	Circular
Margin	Entire	Entire	Entire	Entire
Elevation	Raised	Raised	Raised	Flat
Colour	Cream	White	White	White
Surface	Rough	Smooth	Rough	Smooth
Opacity	Opaque	Convex	Opaque	Opaque
Motility	Motile	Motile	Non-motile	Motile
Endospore formation	Spore forming	Spore forming	Spore forming	Spore forming
Gram character	Gram +ve Long rods	Gram +ve Long rods	Gram +ve Long rods	Gram +ve Short rods
Biochemical Characteristics of Isolates				
Carbohydrate Fermentation Test				
Glu A/G	+/+	+/-	+/-	-/-
Suc A/G	+/-	-/-	+/-	-/-
Man A/G	+/-	-/-	+/-	-/-
Mal A/G	+/-	+/-	+/-	-/-
Lac A/G	-/-	-/-	-/-	-/-
Enzymes Test				
Amylase	+	+	-	+
Urease	-	-	+	+
Gelatinase	+	-	+	+
Catalase	+	+	+	+
Oxidase	+	-	+	+
IMViC Test				
Indol	-	-	-	-
MR	+	+	-	-
VP	-	-	-	-
Citrate	-	-	+	+

Probable isolates	*Bacillus firmus*	*Bacillus coagulans*	*Bacillus endophyticus*	*Bacillus vietnamensis*

+ = Positive - = Negative A = Acid production G = Gas Production

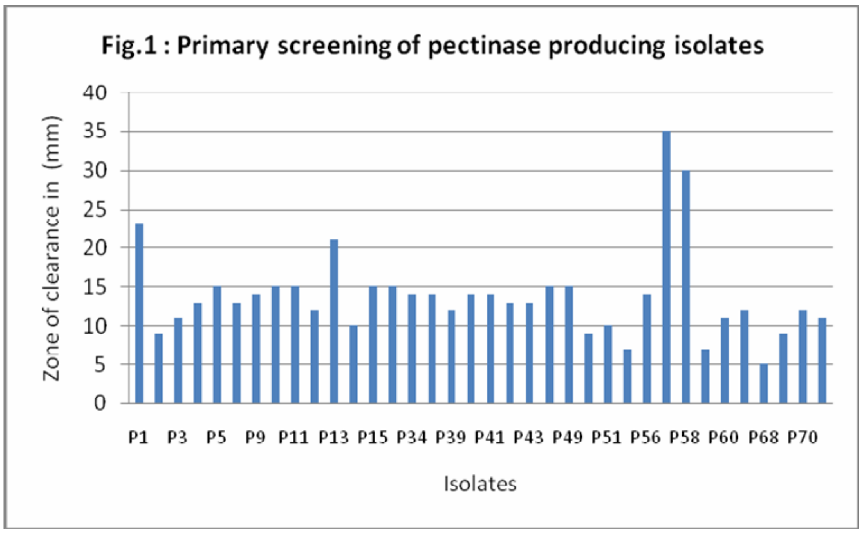


Fig.1 : Primary screening of pectinase producing isolates
Zone of clearance in (mm)
40
35
30
25
20
15
10
5
0
P1 P3 P5 P9 P11 P13 P15 P34 P39 P41 P43 P49 P51 P56 P58 P60 P68 P70
Isolates

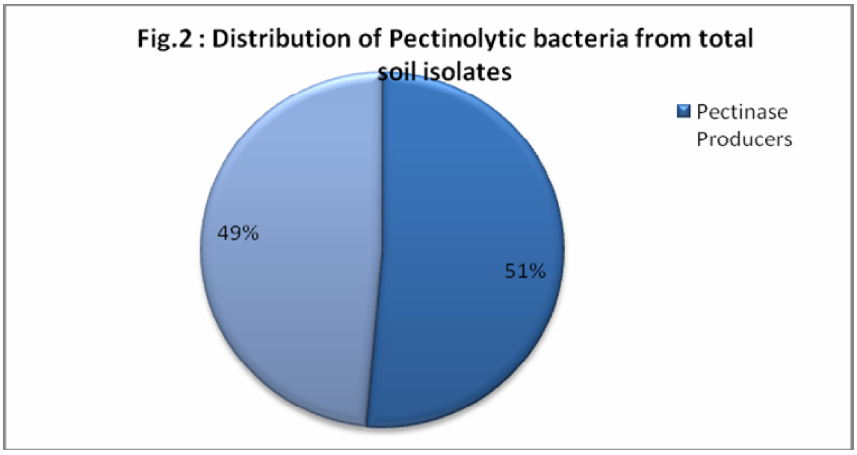


Fig.2 : Distribution of Pectinolytic bacteria from total soil isolates
Pectinase Producers
49%
51%

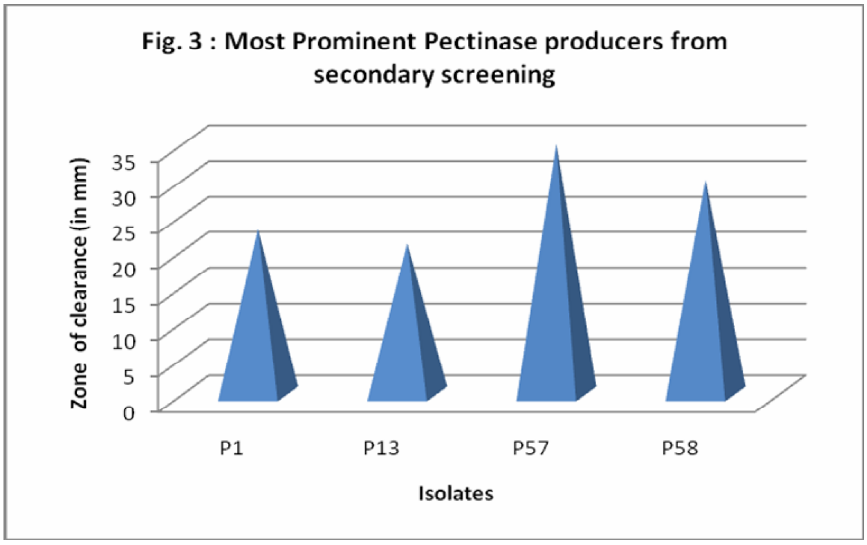


Fig. 3 : Most Prominent Pectinase producers from secondary screening
Zone of clearance (in mm)
35
30
25
20
15
10
5
0
P1
P13
P57
P58
Isolates

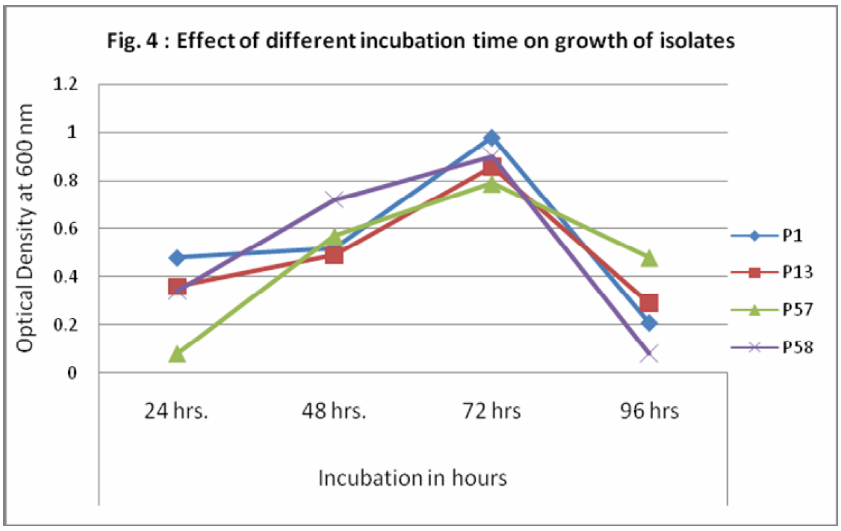


Fig. 4 : Effect of different incubation time on growth of isolates
Optical Density at 600 nm
1.2
1
0.8
0.6
0.4
0.2
0
24 hrs.
48 hrs.
72 hrs
96 hrs
Incubation in hours
P1
P13
P57
P58

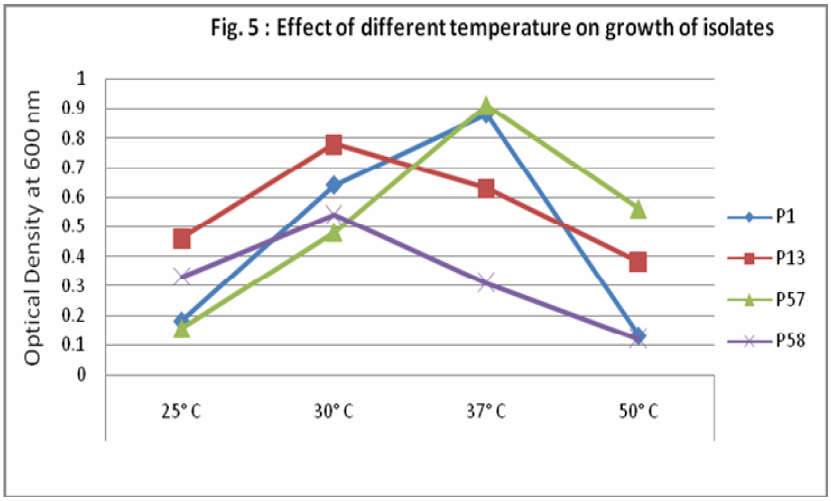


Fig. 5 : Effect of different temperature on growth of isolates
Optical Density at 600 nm
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
25° C
30° C
37° C
50° C
P1
P13
P57
P58

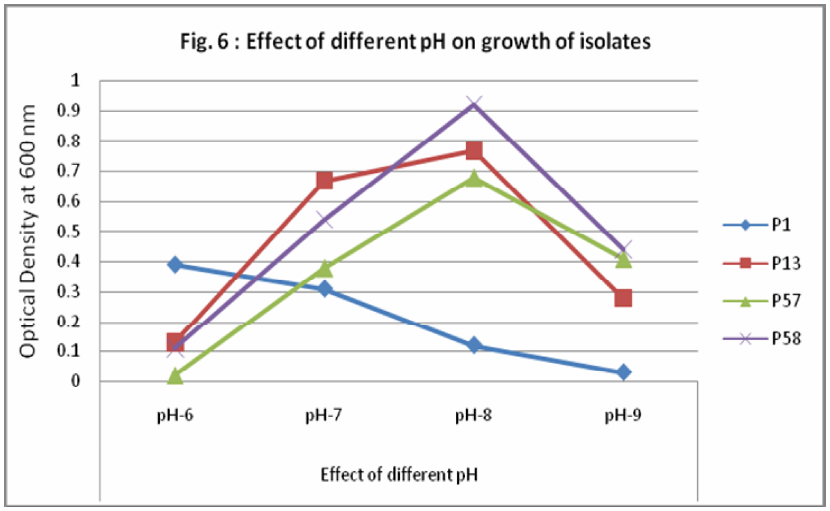
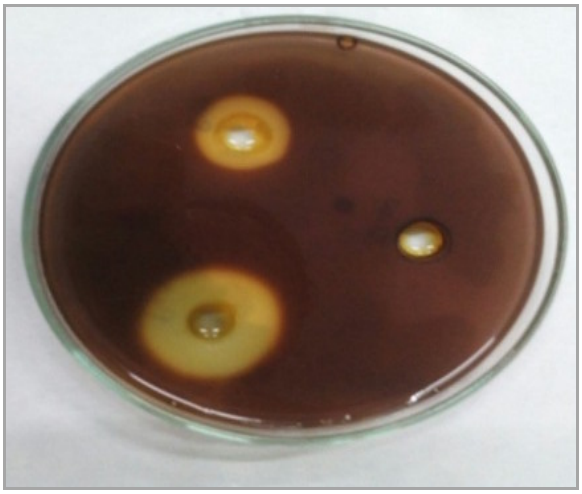


Fig. 6 : Effect of different pH on growth of isolates
Optical Density at 600 nm
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
pH-6
pH-7
pH-8
pH-9
Effect of different pH
P1
P13
P57
P58

Photo.1 Secondary Screening of Pactinase Producers



Optimum cultural conditions for pectinase production by P1 was found to be 37ºC at pH of 6, optimum temperature for P13 was found to be at 30ºC at alkaline pH 8. For P57 optimum temperature was at 37ºC and at alkaline pH 8, optimum temperature for P58 was found to be at 30ºC at alkaline pH 8. Similar sort of result were observed by Marcia *et al*, 1999. He observed that optimum growth was found at alkaline pH for *Bascillus species*. Out of isolates 4 isolates namely *Bacillus firmus* (P1), *Bacillus coagulans* (P13), *Bacillus endophyticus* (P57) and *Bacillus vietnamensis* (P58) was found to be excellent pectinase producers in secondary screening as compared to others isolates. This is in agreement with study of Anna Roosdiana *et al*., in 2013, who also reported that *Bacillus firmus* is able to produce high percentage of pectinase. In other study by Janani *et al.*, (2011), who also reported *Bacillus species* from agricultural soil as high pectinase producers.

In conclusion, the farm soil was found to be rich source for the isolation of pectinase producers. Screening of pectinase producing microbial strain from farm soil samples of Akola region may help to supplement the increasing requirement of pectinase enzyme by the industries as the primary screening showed about 51% isolates were positive for pectinase activity. The identification reveals that the more number of isolates were belong to *Bacillus spp*. Further studies on molecular characterization and optimization of four maximum pectinase producing bacterial isolates from the study may be helpful for the production of industrially important enzyme in near future.

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