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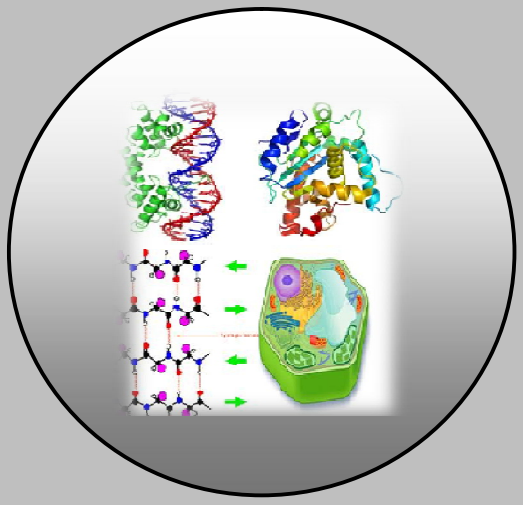

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RESEARCH PAPER

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Isolation, Screening and Biochemical Characterization of Pectinolytic Microorganism from Soil Sample of Raipur City

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ABSTRACT

Pectinase enzymes degrade pectin present in plant material and are cosmopolitan in distribution. The aim of the present study was to isolate, screen and characterize pectinolytic microorganisms from different soil samples of Raipur city, Chhattisgarh, India. Of the sixteen strains tested for pectin hydrolysis thirteen were very good producers of pectinase. Among them the Streptococcus sp (SB6) recorded the maximum zone of clearance of the pectin with high enzyme activity. The probabilistic identification of these microorganisms using Advanced Bacterial Identification software revealed presence of Bacillus gelatini, Staphylococcus aureus anaerobius, Paenibacillus apiaries and Streptococcus sp. The fungi isolate included Mycelia sterilia, Aspergillus sp., Rhizopus sp. and Mucor sp.

Keywords: Pectin, Pectinase, Pectinolytic Micro-Organisms, Soil Samples and Raipur.

INTRODUCTION

Pectin, a heterogeneous polysaccharide, is a major constituent of cereals, vegetables, fruits and fibers, made of α (1-4) D-galacturonic acid residue (Alkorta et al. 1998). Pectinases (EC 3.3.1.15, EC 4.2.2.2) enzyme hydrolyzes pectin and finds applications in various industrial processes. These enzymes break down complex polysaccharides of plant tissues into simpler molecules like galacturonic acid. Acidic pectinases helps in bringing down the cloudiness and bitterness of fruit juices while alkaline pectinases are used in the textile industry for the retting and degumming of fiber crops, production of good quality paper, fermentation of coffee and tea, oil extractions and treatment of pectic waste water (Solbak et al. 2005).

As a result, pectinases are today one of the upcoming enzymes of the commercial sector. Pectinase production occupies about 10% of the overall manufacturing of enzyme preparations (Pedrolli et al. 2009).

Pectinase are obtained commercially from fungi, especially species of *Aspergillus* and *Penicilium* (Silva et al. 2005 and Botella et al. 2007). The pectinase used in food technology are from *Aspergillus niger*, *Corynebacterium, Chromobacterium violaceum* and *Pseudomonas chilinovorans* (Sarvamangala and Dayanand, 2006; Morohoshi et al. 2008). However, efforts are being made to screen new source of pectinase. In view of the potential industrial applications of pectinases, the present investigation was undertaken which covered the isolation and screening of pectin degrading microbial population from the soil sample at Raipur city of Chhattisgarh state; qualitative and quantitative estimation of pectinase produced by the microbial isolates and biochemical characterization with identification of potential pectinolytic microorganisms.

MATERIALS AND METHODS

Sample collection: Samples were collected from fruit and vegetable waste dump soil of Lalpur and Shastri fruit market, garden soil of Pachpedi Naka, Raipur. Decayed banana and tomato and were stored at 4°C till further investigation. All the chemicals and reagents used for the study were of analytical/microbiological grade.

Isolation of pectin degrading micro organisms: Soil samples (10 g) collected from various locations were suspended in sterile distilled water (90 ml). 1 ml was inoculated into standard growth medium and incubated at 37°C for 4 days separately. A loop full of homogenate was then streaked onto standard growth medium supplemented with pectin as sole carbon source and incubated overnight at 37°C. All morphological contrasting colonies (individual colonies) developed were selected and inoculated into standard growth medium and incubated at 37°C for 24 hr. Gram staining for bacteria and lactophenol cotton blue staining for fungi were performed for identification. Morphological identification of fungal species was performed with the help of available literatures and reference slides (Ellis, 1949 and Barnett, 1969).

Screening of pectinase activity by well plate or cup plate assay: Agar plates were prepared by pouring sterilized standard growth medium containing pectin. A well of 5 mm diameter was cut on the agar using a sterile cork borer (Wieringa, 1949). 200 µl of culture suspension was poured into each well. A control was also maintained with 200 µl of sterile water. Plates were incubated at 37°C without inverting for 48 hours. The plates were flooded with iodine solution for 15 minutes, de stained with distilled water for 10 - 15 minutes and the results were observed. The strains were classified as very good producers of pectinase when presented clear halos around the colonies of at least 1.5 cm, good producers when the halos were of at least 1 cm, weak producers when halos were at least 0.5 cm and poor producers when no clear zones were observed.

Quantitative estimation of pectinase was performed by DNS method (Miller, 1959) with pectin as substrate at 540 nm. The concentration of sugar in the sample was calculated with reference to glucose.

One unit of pectinase activity was defined as the amount of enzyme which liberated 1 micromole of glucose per minute. Protein content was also determined (Lowry et al. 1951) using Bovine serum albumin as standard.

Biochemical characterization: Various biochemical tests performed for the identification of the potential bacterial isolates included indole production, methyl red, Voges Proskauer, citrate utilization, catalase test, oxidase test, starch hydrolysis, urea hydrolysis, gelatin hydrolysis, nitrate reduction, H_2S production and carbohydrate fermentation.

RESULTS AND DISCUSSION

Isolation of microorganisms: A total of sixteen organisms were isolated from fruit and vegetable waste dump soil of two market area, decayed banana, tomato and garden soil. Nine bacterial strains, five fungi and two yeasts were able to grow in the medium containing pectin as a sole carbon source.

Screening of pectinase activity by plate assay: All the strains were tested for pectin hydrolysis by plate assay in duplicates, at pH 7.0. The results are presented in Table-1 and Plate-1. Around producer colonies, unstained areas indicated break down of pectin to galacturonic acid by cleaving the α-1,4 glycosidic bonds between two galacturonic acid residues (Soares et al. 1999) while non producing colonies were surrounded by opaque gel containing non degraded pectin.

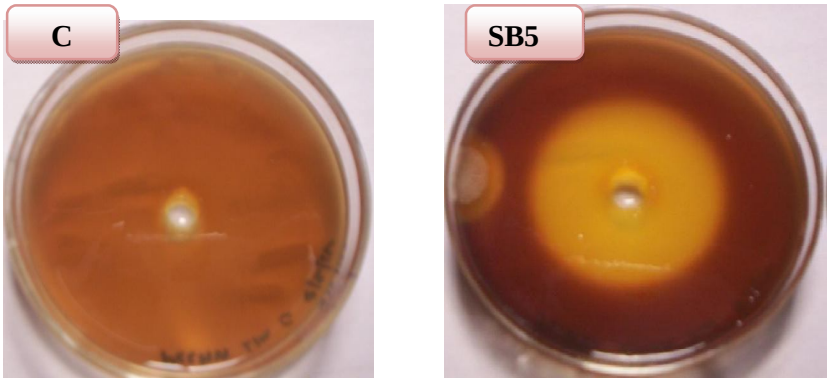


Plate 1. Qualitative Pectinase Assay in *Streptococcus sp* (SB5) and control (C).

The maximum zone of clearance observed in the present study was of the bacterial isolate SB6 *Streptococcus sp* (5.05 ± 0.05 cm), followed by LB3 *Staphylococcus aureus anaerobius* (5.0 ± 0.1 cm) which was isolated from Shastri and Lalpur market respectively. The results so obtained were comparatively much higher than that reported in *Aspergillus niger* (Khairnar et al. 2009) and with *Penicillium chrysogenum* (Banu et al. 2010) Among the decayed fruits, the maximum zone of clearance (4.25 ± 0.05 cm) was exhibited by banana sample. Fungus from garden soil showed the maximum activity (3.35 ± 0.05 cm) when compared among other fungal isolates. The minimal activity among all the isolates was given by yeast isolate PGY1 (1.85 ± 0.15 cm) from garden soil. There were no zone of clearance in SF1, SF2 and LF1 which indicates the poor producers. These results conclude that thirteen isolates were very good producers of pectinase.

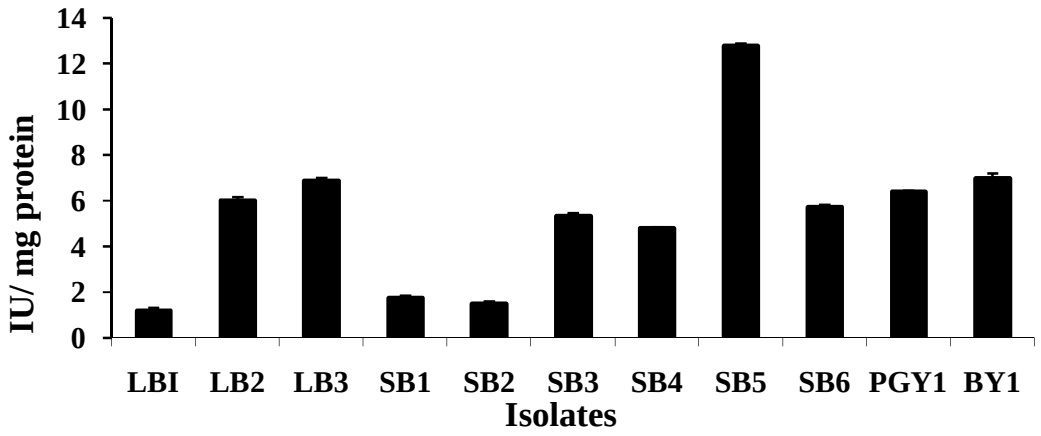
Table-1. Qualitative assay of pectinase from the different isolates by Plate Assay (Mean ± Standard Error).

S. No.	Isolates	Zone Diameter (cm)
1	LB1	2.95 ± 0.05
2	LB2	3.75 ± 0.05
3	LB3	5.00 ± 0.1
4	SB1	3.25 ± 0.05
5	SB2	3.55 ± 0.05
6	SB3	4.05 ± 0.15
7	SB4	3.35 ± 0.15
8	SB5	3.45 ± 0.05
9	SB6	5.05 ± 0.05
10	PGY1	1.85 ± 0.15
11	BY1	4.25 ± 0.05
12	PF1	3.35 ± 0.05
13	TF1	2.05 ± 0.05

LB (1-3) – Lalpur Fruit Market, SB (1-6) – Shastri Fruit Market, PGY 1– Pachpedi Naka Garden Soil, BY1 – Banana, PF1 – Pachpedi Naka Garden Soil, TF1 – Tomato.

Quantitative Estimation of Pectinase: The enzyme activity of all the bacterial and yeast strains are presented in Fig. 1. From the results it was clear that the bacterial strain *Streptococcus sp* SB5 exhibited the maximum activity (12.75 ± 0.12 IU/ mg protein). Therefore SB5 and SB6 both are *Streptococcus sp* and showed high pectinases activity qualitatively and quantitatively followed by yeast strain PGY1 (6.37 ± 0.06 IU/ mg protein) from Shastri market and garden soil respectively. This was comparatively higher than *Aspergillus niger* (Pereira et al. 1993) and Tubercularia *vulgaris* (Fonseca and Said, 1995). However, the activity was lower than Bacillus GK-8 (Dosanjh and Hoondal, 1996) and Bacillus sp. RCPTM1 (Patil et al. 2012). The minimal enzyme activity was observed in bacterial strain SB2 from Shastri market (1.46 ± 0.12 IU/ mg protein).

Biochemical characterization and probabilistic identification of potential pectinolytic micro organisms: All pectinolytic bacterial isolates were biochemically characterized and the details are presented in Table - 2. The probabilistic identification of the bacterial strains was done by using Advanced Bacterial Identification Software (ABIS) and the results are presented in Table- 3. *Bacillus gelatini* and *Staphylococcus aureus anaerobius* was isolated from soil samples of both the sample markets. *Paenibacillus apiaries* and *Streptococcus* sp. was isolated from soil sample of Shastri market.



[LB (1-3) – Lalpur Fruit Market, SB (1-6) – Shastri Fruit Market, PGY1 – Pachpedi Naka Garden Soil, BY1 – Banana.]

Fig. 1. Pectinase activity of bacterial and yeast isolates from soil sample.

Table-2. Biochemical characterization of bacterial strains isolated from the soil sample of Lalpur and Shastri fruit market, Raipur.

Test	LB1	LB2	LB3	SB1	SB2	SB3	SB4	SB5	SB6
Gram staining	-ve rod	+ve cocci	+ve cocci	–ve rod	–ve rod	+ve cocci	+ve cocci	+ve streptococci	+ve streptococci
Indole	-	-	-	-	-	-	-	-	-
Methyl red	+	+	-	-	-	-	-	-	-
Voges proskauer	-	+	+	-	-	+	+	-	+
Citrate	+	-	+	-	+	-	-	+	+
Catalase	-	-	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-	-	-
Urease	-	+	+	-	+	+	+	+	+
Nitrate	-	-	+	-	+	+	-	+	+
H_2S production	-	-	-	-	-	-	-	-	-
Starch	+	+	+	-	+	+	+	+	+
Gelatine	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+
Sucrose	-	+	+	-	+	+	+	+	+
Lactose	-	-	-	-	-	-	-	-	-

LB (1-3) – Lalpur Fruit Market; SB (1-6) – Shastri Fruit Market

Table 3. Probabilistic Identification of the Bacterial strains by using Advanced Bacterial Identification Software (ABIS).

S. No.	Isolate	Bacteria Identified	Percentage of Identity (%)
1	LB1	*Bacillus gelatini*	79
2	LB2	*Staphylococcus aureus anaerobius*	99
3	LB3	*Staphylococcus aureus anaerobius*	85
4	SB1	*Bacillus gelatini*	87
5	SB2	*Paenibacillus apiarius*	84
6	SB3	*Staphylococcus aureus anaerobius*	85
7	SB4	*Staphylococcus aureus anaerobius*	99
8	SB5	*Streptococcus sp.*	-
9	SB6	*Streptococcus sp.*	-

LB (1-3) – Lalpur Fruit Market; SB (1-6) – Shastri Fruit Market.

The fungi identified from Lalpur and Shastri fruit market, Pachpedi Naka garden soil and decayed tomato were respectively *Mycelia sterilia, Aspergillus sp., Rhizopus sp. and Mucor sp.* Although several fungal species were considered as the most efficient organisms for enzyme production (Spinnler et al. 1986; Shevchik et al. 1992; Soares et al. 1999; Morohoshi et al. 2008; Karthik et al. 2011; Namasivayam et al. 2011; Kumar and Sharma, 2012) Several bacterial species belonging to the genera like *Bacillus, Erwinia, Clostridium, Chromobacterium* etc are efficient pectinase producers (Patil et al. 2012). Results are shown in table-3.

CONCLUSION

Thus, in this context, screening of pectinase producing microbial strains from the Raipur soil may help to supplement the ever increasing requirement of these enzymes by the industries. The results obtained in the present study strongly supports the suitability of these species as good candidate for pectinase production and these newly extracted bacterial and yeast strain can be further exploited in future. By harnessing the pectinolytic activity of native microbial population we can produce enough quantity of pectinase at a cheaper rate which will become a boon for our industries which are utilizing these enzymes for a number of processes.

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