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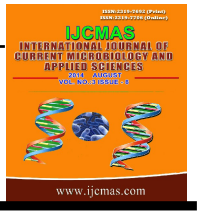

Original Research Article

Study of Pectinolytic Bacteria residing in Mandeepkhol cave, Rajnandgaon district of C.G. India

Arunima Karkun*, Seema Verma and Kalpana Patle

Department of Biotechnology, G. D. Rungta College of Science and Technology, Kohka Kurud Road, India
**Corresponding author*

A B S T R A C T

Some species of bacteria are an important microflora which help in degradation of pectin. For biomass utilization and various other industrial purpose the pectin degrading-enzymes will help to achieve tremendous benefits. In present studies a total of 32 bacterial species were isolated from Mandeepkhol cave which is situated in Chhattisgarh state and is located in deep forest. The studies were conducted to assay for their ability to digest pectin. The assessment was done on the basis of colonial growth and colonial dry weight. Results show that *Brevundimonas diminuta Bacillus thuringiensis* , *Bacillus cereus* and Cream colony I possess greater pectinolytic potency.

Keywords

Pectinolytic Bacteria *Bacillus thuringiensis, Bacillus cereus*

Introduction

Processes of natural bioremediation of pectin includes various organisms specially bacteria and fungi. Laboratory studies on the degradation of pectin, including wood, straw, and cereal grains, have focused mainly on a some bacterial species that grow well in the laboratory and can be readily manipulated in liquid culture to express enzymes of academic interest. Pectinases are group of enzymes that attack pectin and depolymerise it by hydrolysis and transelimination as well as by deesterification reactions, which hydrolyses the ester bond between carboxyl and methyl groups of pectin (Ceci and Loranzo, 1998). These enzymes act on pectin, a class of complex polysaccharides found in the cell wall of higher plants and cementing material for the cellulose network (Thakur *et al*, 1997).

Pectinases accounts for 10% of global industrial enzymes produced and their market is increasing day by day (Stutzenberger, 1992). Pectin is an abundant structure component in plant cell walls, and functions as a matrix holding cellulose and hemicellulose fibers. It is composed of a main chain of (partly) methyl-esterified α-1→4-D-polygalacturonate, and highly branched rhamnogalacturonan (rhamnose-galacturonate stretches), in which the latter can serve as a binding site for 1→4-linked side chains like arabinans, galactans or arabinogalactans.

These pectinases have wide applications in fruit juice industry and wine industry. Pectinases have been used in the paper and pulp industry in addition to cellulases (Reid and Ricard, 2000). A key role of bacteria is based on their capacity to decompose major plant components particularly cellulose, hemicelluloses pectin and lignin(the major components of plant cell walls). Bacteria are potent decomposers and nutrient recyclers of forest litter and debris. Without decomposing bacteria we would soon be buried in debris. Plants are the major locking living entities over the earth which lock nearly 80-90% carbon of the atmosphere. These locked complex organic materials are needed to be degraded up to the level of of simple monomers so that it can be reused by other organisms thereby helping in cycling of organic nutrients.

In present studies the dark caves were focused in search of mesophilic microbes which can degrade the complex materials at a faster speed in mesophilic condition also as mostly degradation occurs in thermophilic condiyion. The selected Mandeepkhol is an adit cave with several branched tunnels. The twilight zone of the cave is hardly 2-3 meters and the remaining part is completely dark. The cave receive a huge amount of plant parts as dried wood, dried leaves etc. along with heavy water streams flowing from surrounding hillocks and entering into the caves. Inside of the cave is highly humid. Bacterial species were isolated from inside the cave.

Materials and Methods

Samples were collected from Mandeepkhol cave from different deposits. The isolation of bacteria were done using nutrient agar media. The samples were serially diluted using serial dilution method in sterilized distilled water. The petriplates were kept for incubation at 26°C. After incubation bacterial colonies were purely cultured in sterile test tube containing nutrient agar media to get axenic culture. The axenic cultured bacteria were then allowed to grow in hankin media where citrus pectin were taken as sole carbon source for testing their growth and were examined for pectinase activity by bacteria. Colonial dry weight method was used to ascertain the bacterial growth.

Culture Media:

Citrus pectin-5gm; $(NH_4)_2$ SO_4-2 gm; KH_2PO_4 -4gm; Na_2HPo_4-6 gm; $FeSO_4$. $7H_2O$-0.2 gm; $CaCl_2$ - 1mg; H_3BO_3 - 10 mg; $ZnSO_4$-10 mg; MoO_3- 10 mg; $ZnSO_4$-70 mg; Yeast extract-1 gm; Agar -15 gm; Distilled water-1000 ml.

20 ml of prepared media was then poured in sterile petriplate and isolated bacteria were inoculated to test their pectinase activity. It was then allowed to grown in 26° C ± 2^0C for 3 days. After incubation fungal plates were recorded at different intervals upto 3 days. To measure colonial dry weight isolates were grown in citrus pectin broth.

Results and Discussion

The organisms were grown on basal agar plates containing citrus pectin as sole carbon source to study colonial growth and then grown in basal media containing citrus pectin as sole carbon source to obtain colonial dry weight.

Table.1 Colonial growth and colonial weight in pectin media.

Isolated Bacteria	Growth rate	Colonial dry weight per 50 ml	Colonial dry weight per 1000 ml
Bacillus cereus	+++	0.11gm	2.2 gm
Bacillus thuringiensis	+++	0.12 gm	2.4 gm
Brevundimonas diminuta	++++	0.14 gm	2.8 gm
Cream colony I	++	0.08 gm	0.16 gm
Cream colony II	+	0.03 gm	0.06 gm
Cream rough colony II	++	0.07 gm	0.14 gm
Cream rough colony II	+	0.04 gm	0.08 gm
Cream rough colony III	++	0.06 gm	0.12 gm
Creamish yellow colony I	++	0.08 gm	0.16 gm
Creamish yellow colony II	++	0.068 gm	0.136 gm
Lemon yellow colony I	+	0.03 gm	0.06gm
Lemon yellow colony II	++	0.07gm	0.14 gm
Orange colony	+	0.05 gm	0.10gm
Transparent colony 4	+	0.029 gm	0.058 gm
Transparent colony II	++	0.06 gm	0.12 gm
White colony II	+	0.04 gm	0.08 gm
Whitish cream colony	+	0.03 gm	0.06 gm
Yellowish rough colony	++	0.07 gm	0.14 gm

+ = poor, ++ = moderate, +++ = good, ++++ = excellent.

Plate 5.1 - *Brevundimonas diminuta*
colonial growth in pectin

Plate 5.2 - *Bacillus thuringiensis*
colonial growth in pectin

Plate 5.3- *Bacillus cereus*
colonial growth in pectin

Incubation period of 3 days were given for all the test organisms. Average of three replicates was considered for each organism. The results are shown in Table-1. Out of 32 species 18 species grown well in media containing pectin as sole carbon source, but *Brevundimonas diminuta* (plate- 1.1 and 2.1), *Bacillus thuringiensis* (plate- 1.2 and 2.2), *Bacillus cereus* (plate- 1.3 and 2.3), Cream colony I, Lemon yellow colony and Yellowish rough colony were proved to be best pectinase producer as their growth rate in pectin media was more as compared to other bacteria and even they showed highest dry weight. Kobayashi *et al.* (2000) purified the first bacterial exo-poly galacturonases from *Bacillus* sp. strain KSM-P443. Mohmoud *et al.* (2008) studied pectinolytic enzymes and their production, characterization and applications and isolated fifty two bacterial isolates, out of which *Paenibacillus polymyxa* showed the highest pectinolytic activities and the exo-polygalactouronase was found to play major role. Bayoumi *et al.* (2008) reported the nutritional and environmental condition requirements for production of

Polygalacturonase by *Bacillus firmus*-I-4071 under solid state fermentation conditions using potato peels. These results are connected with that recorded by Kapoor *et al.* (2000) who found that, *Bacillus* sp. MG-CP-2 produce an alkaline and thermostable Polygalacturonase in degumming of ramie (*Boehmeria nivea*) and Sunn hemp (*Crotalaria juncea*). Kapoor *et al.* (2000) and Bayoumi *et al.* (2008) reported that maximum polygalacturonase production were achieved when media were supplemented with citrus pectin as sole carbon source. However Beg *et al.* (2000a, 2000b) found that, pectinase production from *Streptomyces* sp. QG-11-3 was enhanced by wheat bran. Geetha *et al.* (2012) worked on bacterial species for pectinase production and identified them as *Bacillus sp*. and *Pseudomonas sp*.

According to them *Bacillus sp*. showed best pectinase activity when grown in media with one percent pectin concentration. Kumar *et al.* (2012) studied pectinase production by bacteria and observed that *Bacillus* sp MFW7 showed best pectolytic activity. Janani *et al.* (2011) also studied pectinase producing bacteria from agricultural waste dump soils and isolated ten bacterial strains. Three best pectinase producing strains were identified as *Bacillus* sp whereas Kumar and Sharma (2012) reported *Cocci* sp. as potent producer for pectinase production.

It can be concluded from the present studies that the large caves with high humidity harbour a variety of microbes including molds, bacteria and actinomycetes. These microbes are quite potent in digesting plant materials and animal wastes which are rich in lignin, cellulose and pectic substances. Further, these microbes as they flourish in constant atmosphere through out the year they can be used for the decomposition of complex organic materials to form manure.

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