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Research Article

SCREENING OF THERMOPHILLIC BACTERIAL STRAINS ISOLATED FROM THE DESERT OF NORTHERN INDIA FOR INDUSTRIALLY APPLICABLE PECTINASE

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ABSTRACT
Pectinase is a very important and potential enzyme, especially used in food industry, showing day by day increased demand in global market. The microorganisms growing in unique habitat may possess novel characteristics which have always been the focus of the research. Therefore, thermophilic strains of Bacillus and Enterobacter were isolated from the soil of sand dunes of Northern India. The isolated strains had shown optimum growth on a temperature range of 37-55^{0}C and tolerance to 2 % salt concentration as well. These strains were then screened for the production of pectinase enzyme. The optimum production of pectinase was observed after 24 hr of incubation at 50^{0}C. The thermostable and salt tolerant pectinase enzyme can meet the demand of food industry as well as for economic running of various industrial processes.

Keywords: *Pectinase, Thermophilic Bacteria, Halophytes, Pectin, Pectinolytic Bacteria*

INTRODUCTION
Pectinolytic enzymes are widely distributed in many organisms like insects, nematodes, higher plants and microorganisms (Whitaker, 1990). Pectinases are a heterogeneous group of related enzymes that hydrolyze the pectin substances, present mostly in plants. They are of prime importance for plants as they help in cell wall extension (Jayani *et al.,* 2005) and softening of some plant tissues during maturation and storage (Sakai, 1992; Aguilar and Huirton, 1990). Microbial pectinases are important in the phytopathologic process in plant-microbe symbiosis (Jayani *et al.,* 2005) and several bacterial and fungal strains have been shown to produce different types of pectinolytic enzymes (Gummadi and Panda, 2003). In commercial terms pectinases refer to a mixture of primarily three different enzymatic activities: polygalacturonase, pectinesterace and pectinlyase (Semenova *et al.,* 2006). Pectinases have major applications in fruit processing industries (Pandey *et al.,* 1999), like degradation of plant materials, speeding up the liquefaction, clarification and extraction of fruit juice from fruits, including apples, sapota and citrus fruits (Sakai and Okushnima, 1998; Kaur *et al.,* 2004), wines (Favela- Torres *et al.,* 2005), paper- pulp industry, fabric industry and in improving the quality of black tea (Sharma and Satyanarayan, 2004; Favela- Torres *et al.,* 2005). Prathyusha and Suneetha (2011) had reported that 25% of worldwide sales of enzymes belong to pectinase enzyme due to its potential application in biotechnology and industry.
Pectinases are constitutive or inducible enzymes that can be produced either by submerged (Alkorta *et al.,* 1998) or solid state fermentation (Kashyap *et al.,* 2001). Additions of pectinase lowers the viscosity and causes cloud articles to aggregate to larger units, which sediment and are easily removed by centrifugation (Apsara and Pushpalatha, 2002). Microorganisms can work in many adverse conditions as compared to chemical catalyst. Therefore, microbial enzymes are being used for biotechnological and industrial purposes. Microorganisms are preferred as a source of enzyme because of their short life span, high productivity rate, cost affectivity and also free of harmful chemicals (Chaplin and Bucke, 1990). Enzymatic activity highly depends upon optimum pH, temperature and salt concentration. The demand for commercial pectinase with high stability and novel characteristics to overcome the limitation of existing commercial pectinase is increasing (Khatri *et al.,* 2015). The present study aims to find an optimum microbial source of pectinase enzyme showing thermo stability and salt tolerance to be used as potential raw material in various industrial processes.

MATERIALS AND METHODS

Isolation of Thermophilic Bacteria

Soil samples were randomly collected from different areas of northern India. These samples were serially diluted in nutrient broth up to 10^{-7} dilution followed by plating on nutrient agarand incubated at 50ºC for 24 hrs. Single colonies were picked and purified by streak plate method of which only ten strains were selected randomly and named as BN1 – BN10.

Characterization of Bacterial Strains

Purified strains were plated on nutrient agar and ATCC 697 medium as described in Bergey's Manual (Krieg and Holt, 1984; Sneath *et al.*, 1986) and incubated at 50ºCfor 24 h. Gram's, spore, flagella and capsule staining were performed to observe cell shape, presence or absence of endospores, flagella and capsule formation.

Motility was checked and micrometry was also done to determine cell size. Cultural characterization was recorded by observing pattern of growth in broth and on agar plates and biochemical tests were performed.

Optimization of Growth Conditions

Isolates were grown in nutrient agar and ATCC 697 media plates and incubated for 1-2 days at 10°C, 37°C, 45°C, 55°C and 65°C. The ability of the isolates to grow in high salt concentration was checked by testing on a range of NaCl concentrations (0.9 %, 2%, 5%, and 7%) in nutrient broth. Cultures were incubated for 1-2 days at 50^0C.

The ability of the isolates to tolerate high/low pH was tested in nutrient broth of varied pH (3.5, 7 and 9). Optical densities of cultures in all varied conditions were recorded at 620 nm after 24 h of incubation by spectrophotometer.

Screening for Pectinase Production

The cultures were screened for the production of Pectinase (Phutela *et al.*, 2005). Isolates were inoculated on pectin based medium plates containing 1.7% pectin, yeast extract 1.5 %, peptone 0.9%, agar 4.5% and mineral salt solution gm/100 ml [($NH_4)_2SO_4$ 0.14gm, K_2HPO_4 0.2gm, $MgSO_4$ 0.02gm, $FeSO_4$ 0.05gm, $MnSO_4$ 0.016gm, $ZnSO_4$ 0.14gm, $CaCl_2$ 0.2gm]. Inoculated plates were incubated for 2-3 days at 50^0C. Then 1% cetyl-trimethyl-ammoniumbromide solution was poured onto the surface of the plates. After 10 min incubation at room temperature, colonies with clear zones indicated pectinase activity.

RESULTS AND DISCUSSION

Results

Bacterial Morphology and Culture Characterization

After 24 h incubation at 50^0C in nutrient broth, the cultures showed flocculating growth in aerobic condition. This indicated that these strains can be easily grown at high temperature. The strains showed smooth, moist translucent and milky colonies on nutrient agar and ATCC 697 medium and bacteria were Gram negative and positive, rod shaped, motile as well as non-motile, with cell size of 2.4±1.1µm to6.3±1.4µm (Table 1), having subterminal position of spores (Table 2). Results of biochemical tests are shown in Table 3.

According to Bergey's Manual strains were identified as species of Bacillus (BN 1, 4, 5, 6 and 10) and Enterobacter (BN2, 3, 7, 8, and 9).

Optimum Growth Conditions

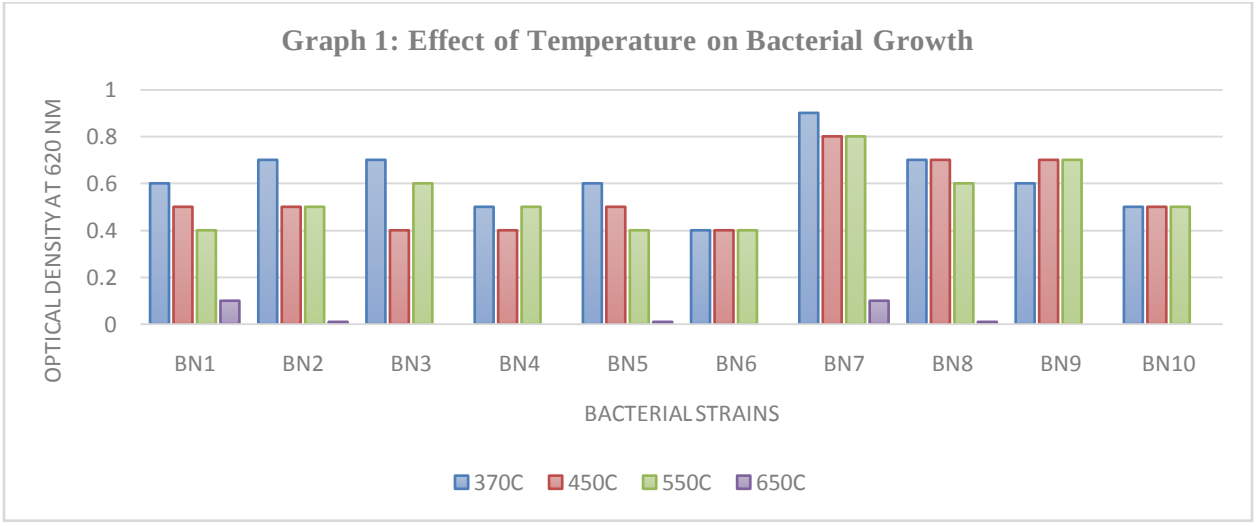
Most of the isolates (90 %) were able to grow at 37^0C as well as 55^0C (Graph 1), they were therefore, called as moderate thermophiles or facultative thermophiles. All the isolates showed optimum growth at 0.9 % salt concentration and were significantly tolerant to 2% NaCl concentration (Graph 2). All the isolates had shown optimum growth at pH 7 (Graph 3).

Screening for Pectinase Production

Out of ten strains, BN2, BN3, BN4, BN5 and BN7 showed clear zone around the streaked area of test organism. These were selected as pectinase producing strains. These strains of bacteria showed significant production of pectinase enzyme at 50^0C for a period of 24 hours (Figure 1).

Table 1: Cell Size of Bacterial Isolates as Measured by Micrometry (Values are Mean of 10 Cells with SD)

S. No	Isolates	Size of Micobes (µm)	Cell Size Range (µm)
1.	BN1	5.2±1.9	3.3- 7.1
2.	BN2	6.3±1.4	4.9- 7.7
3.	BN3	2.4±1.1	1.3-3.5
4.	BN4	5.8±1.5	4.3-7.3
5.	BN5	6.0±1.3	4.3-7.3
6.	BN6	6.1±1.6	4.5-7.7
7.	BN7	5.8±1.5	4.3-7.3
8.	BN8	2.4±1.1	1.3-3.5
9.	BN9	1.9±0.6	1.3-2.5
10.	BN10	7.3±1.1	6.2-8.4



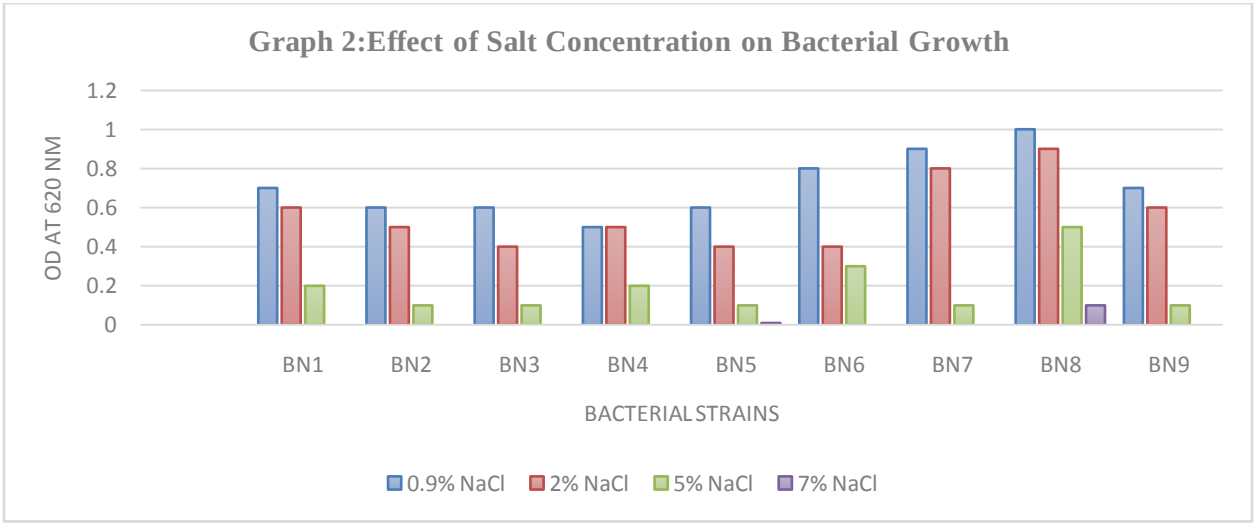
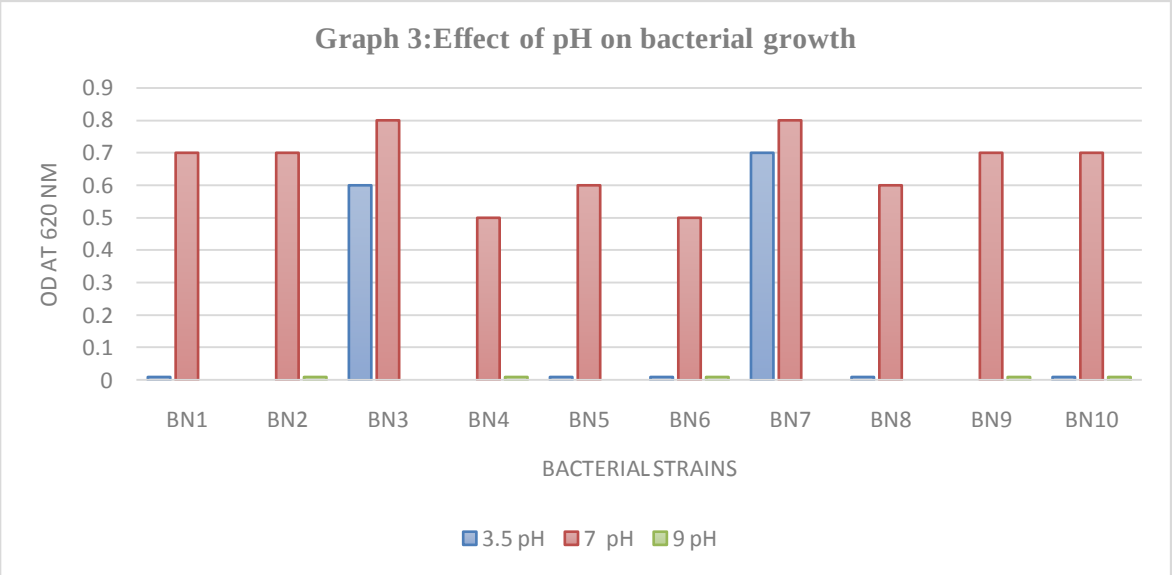


Table 2: Morphological Characters of Bacterial Strains

S. No	Isolates	Gram Staining	Endospore Forming	Position of Spore	Motility	Capsule Formation	Presence of Flagella
1.	BN1	+	Yes	Subterminal	No	No	No
2.	BN2	-	Yes	Subterminal	No	No	No
3.	BN3	-	Yes	Subterminal	Yes	No	Yes
4.	BN4	+	Yes	Subterminal	No	No	No
5.	BN5	-	Yes	Subterminal	No	No	No
6.	BN6	-	Yes	Subterminal	No	No	No
7.	BN7	+	Yes	Subterminal	No	No	No
8.	BN8	-	Yes	Subterminal	No	No	No
9.	BN9	-	Yes	Subterminal	No	No	No
10.	BN10	-	Yes	Subterminal	No	No	No

Table 3: Results of Indole, Methyl Red (MR), Voges-Proskauer (VP) and Citrate Testand Sugar Fermentation Test

S. N.	Isolates	Indole	MR	VP	Citrate	Catalase	Oxidase	Lactose		Sucrose		Dextrose	
								Acid	Gas	Acid	Gas	Acid	Gas
1.	BN1	-	+	-	+	-	+	-	-	-	-	-	-
2.	BN2	-	-	+	-	-	+	+	-	-	-	-	-
3.	BN3	-	+	-	+	-	+	-	-	-	-	-	-
4.	BN4	-	-	+	-	-	+	+	-	-	-	-	-
5.	BN5	-	+	-	-	-	+	+	-	-	-	-	-
6.	BN6	-	-	+	-	-	+	+	-	+	-	-	-
7.	BN7	-	+	-	-	-	+	-	-	-	-	-	-
8.	BN8	-	-	+	-	-	+	+	+	-	-	-	-
9.	BN9	-	-	+	+	+	+	+	-	+	+	-	-
10.	BN10	-	-	+	-	-	+	+	-	-	-	-	-



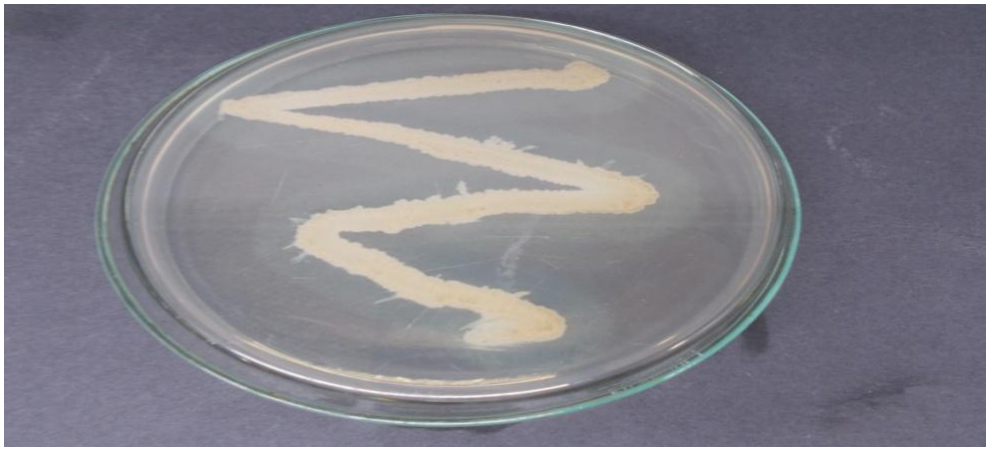


Figure 1: Screening for Pectinase Enzyme

Discussion

There are so many different sources from where pectinolytic enzymes are derived, such as soil, agro wastes, fruit wastes etc (Whitaker, 1990; Banu *et al.*, 2010; Namasivayam *et al.*, 2011). It has been reported by Vibha and Neelam (2010) that microorganisms producing pectinase have advantage over the other sources because they can be manipulated to increase the yield by modifying cultural conditions as microbial production is subjected to environmental and genetic factors. Hirose *et al.*, (1999) was reported enhanced production of pectinase by *Bacillus licheniformis, Bacillus subtilus* and *Enterobactor cloacae* from soil sample at 50°C. They used other substrates (wheat bran, orange peel, sugar cane peel and banana peel) and revealed the preference of banana peels and wheat bran by the isolates for pectinase production. Namasivavam *et al.*, (2011) working on *B. cereus* isolated from market solid waste also reported enhanced pectinase production by wheat bran. Phutela *et al.*, (2005) also reported enhancement of pectinase by wheat bran and industrial pectin from thermophillic *Aspergilus fumigatus* isolated from decomposing orange peels. Bacillus sp. DT7 isolated from soil bacteria has been found to produce significant amounts of extracellular pectinase which was subsequently characterized as pectin lyase (Kashyap *et al.*, 2001). Pectinase can also be isolated from fungi such as *Aspergillus flavus, Penicillium viridicatum*, actinomycetes and moulds (Mellon and Cotty, 2004; Silva *et al.*, 2002; Bruhlman *et al.*, 1994; Fawole and Odunfa, 1992).

Conclusion

Thar Desert is now a day's being explored as rich source of novel genes due to harsh climatic conditions, hence bacterial strains were isolated from the sand dunes of Bikaner region of Northern India. Ten randomly selected strains (BN1 to BN10) were identified as species of Bacillus (BN 1, 4, 5, 6 and 10) and Enterobacter (BN2, 3, 7, 8, and 9) on the basis of cultural, morphological and biochemical characters. All of these strains were tolerant to 55^0C temperature and 2 % salt concentration. All of them were tested for pectinolytic activity using plate assay method of which, BN2, BN3, BN4, BN5 and BN7 were identified as pectinase producing bacteria.

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