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Short communication

Isolation of bacterial species capable of hydrolyzing pectin

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Abstract: At present, almost all the pectinolytic enzymes used for industrial applications are produced by fungi. There are a few reports of pectinase production by bacterial strains. In the present study, bacterial strains isolated from rotten citrus fruit samples, were screened for polygalacturonase production. Rotten fruit samples were randomly collected which had high amounts of pectin in their peels. Using Kirby Bauer method, two *Bacillus* strains capable of hydrolyzing pectin were screened Both strains showed good polygalacturonase activity at neutral pH; hence, it would be potentially useful to increase the yield of banana, grape or apple juices, or at alkalinity, it can be 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 pectin waste water treatment.

Keywords: Pectin, rotten samples, Kirby Bauer method.

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Pectin is a very complex polysaccharide. It is produced commercially as a white to light brown powder, mainly extracted from citrus fruits, and is used in food as a gelling agent particularly in jams and jellies. It is also used as a stabilizer and dietary fiber [1]. Pectic substances are also located primarily in the middle lamella between cells in higher plant tissues [2]. They include the negatively charged rhamnogalacturonans, neutral arabinogalactans I and II and L-arabinans. These polysaccharides add viscosity to juices, but may also form hazes and precipitates and retard maximum recovery of juices from the fruit. Ramnogalacturonans in the pectic substance are degraded by pectin methyl esterase and polygalacturonase which is normally present in plant tissues [3]. This enzyme following the microbial derived commercial pectic enzymes added during the production of juices may produce haziness in the end product [4]. Furthermore, the presence of arabinofuranosidase, which degrades L-arabinans, in commercial pectic enzyme preparations, can also cause the haze formation in juices such as apple and pear [5].

Many microbes are responsible for the production of pectinase enzyme, which include both aerobic and anaerobic microorganisms. Whereas, the confirmed strains capable of pectinase production are *Bacillus marcerans, Bacillus polymyxa* and *Clostridium pectiovorum* and *Clostridium felsineum* etc. Moreover, certain yeast strains like *Aspergillus niger* is also said to be a very good producer of pectinase enzyme [6]. Recently, there have been a good number of reports on the application of alkaline pectinases 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 [5].

There is a need to isolate more efficient pectinase producing enzymes. Therefore, we used rotten fruits, most susceptible to producing pectin, to isolate pectinase producing microbial strains. The isolation, identification and pectinase production assay were performed in the Department of Microbiology, University of veterinary and animal sciences, under the supervision of Dr. Farah Khan.

The pectin was prepared by using disposed orange or apple peels collected from the university canteen. They were sorted out manually based on their fine texture and rigidity. The peels were placed in the oven dried at 55°C until constant weight. It was grounded into powder (CPP-citrus peel powder) by using an electrical grinder. This powder substrate was fractionated using sieve. The sieved powder substrate was stored at room temperature and used in further studies. Chemicals of analytical grade were used in all experiments.

In order to isolate pectinase producing bacterial strains, badly rotten lemon and apples were collected from the area of Salamat pura near the premises of the University of veterinary and animal sciences, Lahore, Pakistan. The collected samples were processed in a sterile environment and 1 g of it was shifted to 9 ml autoclaved distilled water and diluted tenfold. A loop full of all the tubes was streaked on to minimal media (KH_2PO_4 3g, Na_2HPO_4 6g, NH_4Cl 2g, NaCl 5g, Pectin 2g, $MgSO_4.7H_2O$ 0.1g, Agar 2g, pH 7) at 37°C for 24-48 hours [5]. The $MgSO_4.7H_2O$ was autoclaves separately, and mixed after cooling. The medium was autoclaved at 15 psi for 20 minutes.

All the suspected colonies from minimal media were isolated and purified in the same agar composition i.e. strain S, T and U. Out of which strain U didn't give the growth and was eliminated. This was the primary isolation. The separated colonies were then grown on

the L-agar for the secondary isolation and for the identification of the bacteria [7].

The biochemical tests for the identification were performed according to the Bergey's Manual of Determinative Bacteriology [8]. The isolated strains namely S and T were Gram stained and observed under microscope. Both strains were Gram positive rods. The strains S and T were then grown on mannitol broth, where no turbidity confirmed the presence of *Bacillus cereus* [9]; however, 16S rRNA identification is required for confirmation.

The strains were tested for pectinase activity by standard Kirby-Bauer disc approximation (KBDD) method [8]. The Kirby plates were made using 0.2% commercially prepared pectin as the only carbon source. The testing conditions featured, standard KB wells (Becton Dickinson Microbiology Systems, BBL, Sparks, MD, for CHRMC; Oxoid Limited, Basingstoke, Hampshire, England, for SCMC) at 37°C overnight incubation (~16 hours) in ambient air. For each strain, wells were made in the agar plate 20 to 25 mm apart. The well depth was measured by using a color stain safranin [10]. After the incubation period, iodine reagent (Iodine 1g, potassium iodide 2g, 20% ethanol 125 ml and distill water 400 ml) was poured onto the plates for 5 minutes and then discarded. The clear zones were produced by both *Bacillus* species, around the incubation site which showed the production of pectinase enzyme. The diameter of clearing zones was measured with a caliper scale which was 14mm and 16mm for strain S and T, respectively.

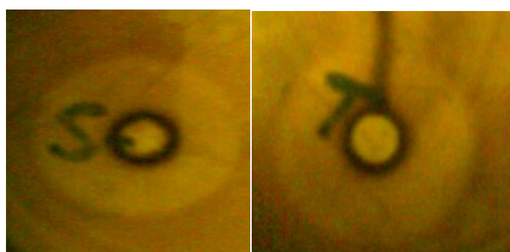

Fig. 1: Zones of pectin degradation by strain S and strains T determined by KBDA method.

The results showed the potential of these *Bacillus* strains to produce pectinase. The *Bacillus* strains were preserved in 30% glycerol stock at -70°C in 1.5 ml appendroff tube.

Pectinase is an enzyme produced by many bacterial and yeast strains as well as is produced commercially for many purposes. Commercially produced pectinase is quite expensive to use in the industry. Producing pectinase from rotten fruits is good theme to utilize the waste products in the manufacturing of useful products [11].

Pectinases are increasing in commercial importance, although a few reports have been published on the isolation of pectinase producing *Bacillus* strains [12]. We have isolated two bacteria from rotten fruits which exhibited pectinase activity on pectin agar plates. It was also observed that pectinase production by *Bacillus* is inducible in nature. It is generally believed that the optimum medium for the enhanced production of extracellular pectinase is that containing pectic material, as an inducer [11]. In this study, *Bacillus* strains produced good amount of polygalacturonase activity at neutral pH; hence, it would be potentially useful to increase the yield of banana juice, grape juice and apple juice, or at alkalinity, it can be 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 treatment.

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