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FIRST REPORT OF *BACILLUS MARISFLAVI* AS A POTENTIAL PECTINASE PRODUCING BACTERIA FROM SAMPLES OF SAVAR, DHAKA

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Abstract

Pectin is a principal component of all plants' primary cell walls which can be hydrolyzed by pectinase enzymes. The present research was aimed at the isolation, screening and identification of pectinase producing bacteria from samples collected from jute degrading soil and water, rotten leaves, sawdust, and kitchen wastes. Out of 14 isolates, nine showed positive pectinolytic activity. Identification of *Bacillus* spp was done on the basis of morphological, cultural and biochemical characterization and *Bacillus marisflavi*(PSD2) by 16S rRNA sequencing. This is the first study to report *Bacillus marisflavi* as a significant pectinase producing bacteria from the world.Among the pectinase producing strains, *Bacillus marisflavi*(PSD2) showed the best pectinase activity (zone size 69 mm) and the pectinolytic activity observed was 0.61 IU/ml.The present study suggests *Bacillus marisflavi* as a potential source for pectinase enzyme and could be used in industrial production upon further investigation.

Introduction

Pectin is a significant component of the plant cell wall and occurs primarily in the non-woody parts of plants. Microorganisms produce pectinase enzyme to breakdown pectin (Mathur *et al*. 2014). Different types of microorganisms have been exploited for the production of pectinase. There are many advantages of using organisms for the production of enzymes through genetic and environmental manipulations to increase the yield (Vibha and Neelam 2010). Pectinases have been used in several conventional industrial processes, such as textile, plant fiber processing, tea, coffee, oil extraction, industrial wastewater treatment etc. Pectinase enzyme also has essential applications in fruit processing industries. It has a share of 25% in the global sales of food enzymes (Oumer and Abate 2017).

Therefore, with the elevated application of pectinases in different fields, there is an increasing demand for discovering new bacterial strains producing pectinases with novel properties. So, this study aimed to search for novel bacterial strain from different habitats of Savar, Dhaka that can degrade pectin substances.

Materials and Methods

Different samples *viz.* sawdust, jute degrading soil, water, kitchen waste and rotten leaves were collected to isolate pectin-degrading bacteria from different areas of Savar, Dhaka. The samples were collected in sterilized polythene bags, and water samples were collected in clean sterile bottles and stored at 4°C. The experiment was carried out in the Laboratory of Microbiology, Department of Botany, Jahangirnagar University.

Pectin degrading bacteria were isolated using proper serial dilution according to the method of Afrin *et al*. (2019). The well diffusion method was used for screening of pectinase-producing bacteria (Lalitha *et al*. 2014).

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Pectinase enzyme activity of the selected isolate was estimated by DNS method. It was quantitatively determined by obtaining the absorbance at 540 nm in UV-VIS spectrophotometer (Oumer and Abate 2018). Absorbance at 540 nm and concentration of D galacturonic acid in mg/ml was then used to prepare a standard curve. Elutes which was obtained by ion exchange chromatography was subjected to DNS assay. The amount of reducing sugar released in the reaction mixture for each of elutes was determined by using standard curve.

Bacterial isolates that hydrolyzed the pectin in well plate assay were characterized morphologically and biochemically. The pectinolytic bacterial strains were morphologically identified by size, shape, color, edges, etc. of the isolates and staining properties. They were biochemically characterized by the Catalase test, Casein hydrolysis test, Carbohydrate fermentation test, Indole test, Starch hydrolysis test and MR-VP test according to the guidelines of Bergey's Manual of Determinative Bacteriology (Rokon-Ud-Doula *et al*. 2021).

The isolate (PSD2) which showed the highest zone in pectin containing media was subjected to PCR for a partial sequence of 16S rRNA of the bacterium. A commercial DNA extraction kit (Promega, USA) was used according to the supplier's instruction for DNA isolation. Partial sequence of the 16S rRNA gene was amplified with universal primer 1492R (5′-TACGGCTACCTTGTTACGACTT-3′) and 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) in the present investigation. Finally, the identification of bacterial isolate was accomplished by comparing the sequences with sequences available in public databases of NCBI Blast (*Basic Local Alignment Search Tool*) using the BLAST search engine (http://blast.ncbi.nlm.nih.gov/Blast.cgi). MEGA version 6 was used for multiple sequence alignment and phylogenetic tree construction (Rokon-Ud-Doula *et al.* 2022). The phylogenetic trees of the isolates were produced using currently obtained sequence data and retrieving the sequence of the NCBI database.

Results and Discussion

Nine bacterial isolates listed in Table 1 were found to have pectinolytic activity based on clear zone around the well. The sizes of zones were ranging from 38 to 69 mm after flooded the plate with iodine solution. The isolate (PSD2) from sawdust was giving the highest pectin depolymerizing zones of 69 mm and pectinase enzyme activity 0.610 IU/ml followed by isolate (PJS1) from the jute degrading soil giving zone of 62 mm and pectinase enzyme activity 0.5042 IU/ml (Table 1). Among the nine bacterial isolates, the highest clear zone producing and

Table 1. Colony size, clear zone and pectinolytic activity of different pectin degrading bacterial isolates.

Serial No.	Type of sample	Pectin degrading isolates	Colony size (mm)	Clear zone (mm)	Pectin lytic activity (IU/ml)
1.	Jute degrading water	PJW1	22	42	0.3120
		PJW2	19	38	0.3566
2.	Jute degrading soil	PJS1	36	62	0.5042
		PJS2	29	57	0.3170
3.	Rotten leaves	PLS1	25	52	0.3215
		PLS2	22	45	0.3140
4.	Kitchen waste	PKW1	20	41	0.4016
5.	Sawdust	PSD1	24	48	0.4890
		PSD2	51	69	0.6100

pectinolytic activity showing isolate (PSD2) was subjected to cellular and biochemical tests (Table 2). Based on the morphological, biochemical, and physiological tests performed, the isolate was initially identified in the laboratory as *Bacillus* sp. 16S rRNA sequencing identified the isolate as *Bacillus marisflavi*(Accession number-MH740896). Generated phylogenetic tree using currently studied 16S rDNA gene sequence, eight different *Bacillus sp.* strains, and *Alcaligenes faecalis* strain as outgroup from Gene Bank have been shown in Fig. 1.

Table 2. Morphological and biochemical characteristics of *Bacillus marisflavi* (PSD2).

Biochemical tests								Morphological tests	
Catalase Test	Casein test	Fermentation test	Indole test	Citrate test	Starch hydrolysis	MR test	VP test	Motility test	Gram staining
+ve	-ve	- ve	- ve	- ve	- ve	- ve	- ve	+ve	+ve

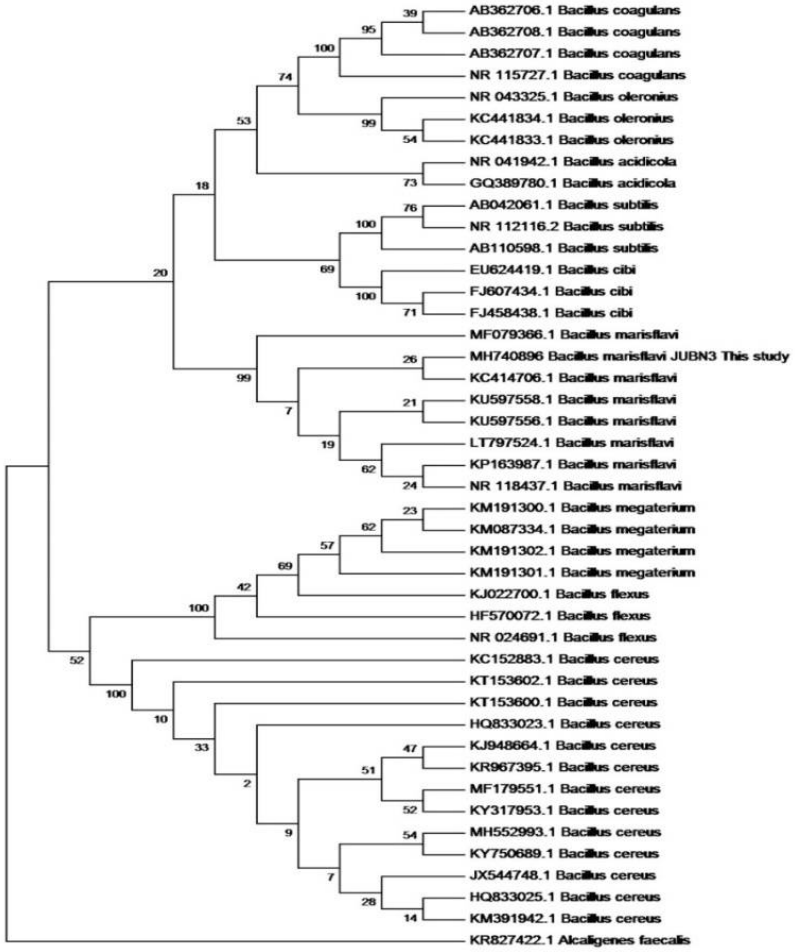

Fig. 1. Phylogenetic position of *Bacillus marisflavi* (JUBN3) based on their 16S rRNA gene sequence. Sequences were aligned with previously published 16S rRNA gene sequences.

In the present study, *Bacillus* sp. has been isolated from soil, water, sawdust, and kitchen waste, which is in agreement of the findings of previous studies where pectinolytic enzyme producing *Bacillus* strains has been isolated from soil (Esmail *et al.* 2013), the water of the jute rotting process (Das *et al*. 2011), and from rotten fruits (Jabeen *et al*. 2015). Besides, Torimiro and Okonji (2013) reported most *Bacillus spp.* as the enhancer of pectinase production. The clear zones indicating pectinase production in this study ranged from 38-69 mm, which is higher than the study reported by Tariq and Latif (2012). The pectinolytic activity of *Bacillus* sp. was 0.61 IU/ml in this study. Kaur *et al*. (2016) found pectinolytic activity of *Bacillus* sp. in the range of 0.32 IU/ml to 0.64 IU/ml which coincides with our study (Kaur *et al*. 2016).

16S rRNA sequencing confirmed the bacterial genus in species level and identified it as *Bacillus marisflavi*(MH740896). To date, no study is available on the production of pectinase enzyme by this species. It was isolated from sawdust. Among the bacterial isolates, *Pseudomonas* sp. and *Bacillus* sp. recorded maximum pectinase activities (Chatterjee *et al*. 2011, Geetha *et al.* 2012), which is also in agreement to the present study in which *Bacillus marisflavi* was identified as the highest pectinase producer.

Pectinase is among the essential enzymes and has great significance in present-day biotechnology. The study revealed that *Bacillus marisflavi* is a highly potential pectinase producing bacteria. This strain could be used for the production and purification of the pectinase enzyme for industrial purposes.

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