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Research Article

Morphological and Biochemical Characterization of Novel Cellulose and Starch Degrading Bacteria Isolated from the Rhizospheric Soil of *Dendrocalamus minor* and *Musa* sp.

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Abstract

The rhizosphere is the most active region of soil where plants and microorganisms live in close association and exhibit complex interactions. In the present study, rhizospheric soil samples were collected from bamboo and banana growing well in University of North Bengal campus. Cellulolytic and amylolytic bacteria were isolated from these samples through serial dilution technique and were identified on the basis of morphological and biochemical characteristics. The study showed presence of four isolates mostly from genera *Bacillus* sp. from rhizosphere of bamboo capable of degrading cellulose while five isolates (*Rummeliibacillus* sp., *Lysinibacillus*, *Brevibacillus*, and *Bacillus*) from rhizosphere of banana and degrade starch respectively. The study although preliminary but will prove valuable for the extraction of these enzymes from these rhizospheric isolates and will be highly appreciated for their application in biotechnology sector.

Keywords: Amylases, Agricultural biotechnology, Cellulases, Microbial diversity, Soil

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Introduction

The rhizosphere is a dynamic region that acts as an interface between plant roots and surrounding soil. Enormous microbial diversity has been observed to colonise this rhisosphere and these rhizospheric microbes play a huge role in augmenting plant growth and development (Mohanram and Kumar 2019). In addition, there are reports that states commercial importance of these rhizospheric microbes in industries for enzyme production as well as in pharmaceuticals like antibiotics. Although, a number of studies have been carried out to study rhizospheric microbial populations from a variety of plant species (Andreote et al. 2009), a vast diversity of rhizospheric microflora is still unknown that may have huge commercial value.

Bamboo (*Dendrocalamus minor*) and Banana (*Musa* sp.) are important tree species of the Indian subcontinent and have huge commercial applications. Bamboo is known for its role in phytoremediation and sustains growth in heavy metal contaminated sites (Fuke et al. 2021). Banana is a tropical plant and is a high source of carbohydrates in dietary plans for human and animal health (Oyeyinka et al. 2019). Different plant parts of bananas have also been seen to be used for various ceremonial and religious purposes. Due to the high diversity of these plants and subtle growth under varied environmental conditions, it was hypothesized that a great microbial diversity must be associated with the rhizosphere of these plants that sustains their growth (Köberl et al. 2018).

Materials and Methods

Soil samples were collected from the rhizosphere of two different plants *Dendrocalamus minor* (bamboo), and *Musa* sp. (banana) growing inside the University of North Bengal (NBU) campus. Soil samples were placed individually in zipper bags and

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were stored at 4°C in the dark before the isolation of cellulose and starch degrading bacteria.

Isolation of bacteria was done using the serial dilution technique and the resulting diluted suspension was spread into carboxymethyl cellulose (CMC) agar media plates for the isolation of cellulolytic bacteria (Shaikh et al. 2013), while spread in starch agar plates for the isolation of starch degrading bacteria (Choi et al. 2007).

For the screening of cellulase production, CMC plates with bacterial colonies were incubated at 37°C for 48 h, and after incubation, plates were flooded with gram's iodine followed by 1% congo red and 0.1M NaCl solution for 10 min. After 10 min, a hollow zone around the bacterial growth indicates cellulase production by bacterial colonies (Andro et al. 1984). Screening for amylolytic bacteria was carried out by flooding starch agar plates with gram's iodine solution. The formation of a clear zone around the colonies indicated amylase producing bacterial strains (Sharma et al. 2015)

Morphological characterization was done by carrying out the gram staining technique and differences were seen using light microscopy (Zain et al. 2019). Different bacterial isolates were also evaluated for their growth pattern by incubating them under different pH and two media like czapek dox agar (CDA) and yeast malt agar (YMA) media. Biochemical characterization was done by performing various biochemical tests like catalase, citrate, fermentation, indole production, gelatin hydrolysis test, urease production, voges-proskauer (V-P), and growth at different pH. Identification was done by studying both morphological and biochemical characteristics using Bergey's Manual of Determinative Bacteriology.

Results

The present study led to the identification of nine rhizospheric bacterial isolates from two important trees i.e. bamboo and banana. Out of nine isolates, four were isolated from the rhizospheric soil of bamboo (B1, B2, B3, B4), and five from banana (Ba1, Ba2, Ba3, Ba4, Ba5). It was observed that all the rhizospheric isolates (B1, B2, B3, B4) from the tree of bamboo have cellulolytic potential and substantially degraded cellulose under culture conditions (Fig. 1a). In contrast, none of the above isolates (B1, B2, B3, B4) degraded starch under culture.

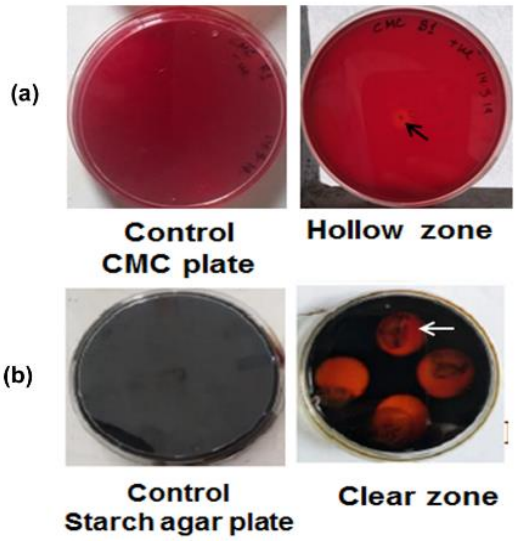

Figure 1. (a) Rhizospheric isolate of bamboo and banana showing (a) cellulose degradation in carboxymethyl cellulose (CMC) agar media plates; (b) starch degradation in starch agar media plate.

However, three of the isolates (Ba2, Ba3, Ba4) of banana exhibit high implications of cellulose hydrolysis. On the other hand, all the isolates (Ba1, Ba2, Ba3, Ba4, Ba5) showed positive results in starch hydrolysis (Fig. 1b). Interestingly, three isolates of banana (Ba2, Ba3, Ba4) have been shown to possess both cellulolytic and amylolytic potential.

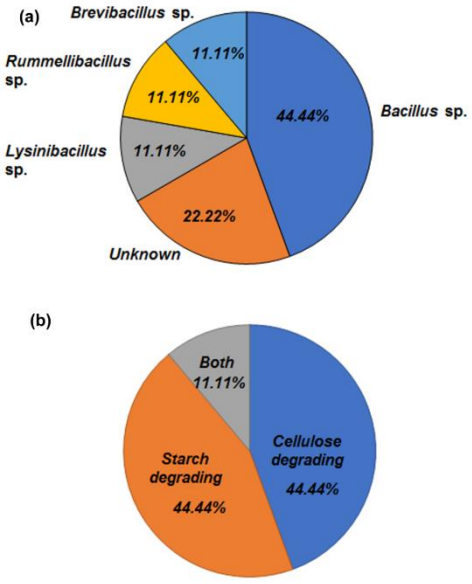

Figure 2. (a) Percent distribution of bacterial genera isolated from the rhizosphere of bamboo and banana. (b) Percent distribution of bacterial diversity based on cellulose and starch degradation ability.

All the rhizospheric isolates were identified both morphologically and biochemically through Bergey's manual of systemic Bacteriology (Bergey,1994). Isolates from the rhizosphere of different plant species show some variations in their morphology on the basis of gram staining (Table 1). Morphological studies revealed that three isolates (B1, B3, B4) of bamboo and four isolates of banana (Ba2, Ba3, Ba4, Ba5) were gram-positive while one isolate of bamboo (B2) and one isolate (Ba1) of banana was gram-negative (Table 1). Interestingly, most of the bacterial isolates were found to be cocci in shape (B1, B2, B4, Ba2, Ba3, Ba4, Ba5) while, two of them were rod shaped (B3, Ba1) (Table 1). Different biochemical tests were carried out in order to identify all the nine isolates.

Table 1. Morphological and biochemical characterization of different rhizospheric isolates from different trees with their identification up to a generic level.

Isolates	Gram stain	Shape of Bacteria	Catalase test	Citrate test	Fermentation of sugars	Indole test	Gelatin hydrolysis	Urease	Voges-proskauer (V-P)	Cellulose degradation	Starch hydrolysis	Identification
B1	Gram positive	Rod	+	+	+	+	+	-	-	+	-	*Bacillus* sp.
B2	Gram negative	Cocci	+	+	+	-	+	+	+	+	-	Unknown taxa
B3	Gram positive	Rod	+	+	+	-	+	-	-	+	-	*Bacillus* sp.
B4	Gram positive	Rod	+	+	+	-	-	+	+	+	-	*Bacillus* sp.
Ba1	Gram negative	Cocci	+	-	-	-	+	-	-	-	+	Unknown taxa
Ba2	Gram positive	Rod	+	-	-	-	+	+	+	+	+	*Rummeliibacillus* sp.
Ba3	Gram positive	Rod	+	-	-	-	+	-	+	-	+	*Lysinibacillus* sp.
Ba4	Gram positive	Rod	+	-	+	-	+	-	+	-	+	*Brevibacillus* sp.
Ba5	Gram positive	Rod	-	-	+	-	+	-	-	-	+	*Bacillus* sp.

B1-B4: isolates from Bamboo;Ba1-Ba5: isolates from Banana

Differential growth pattern of rhizospheric isolates was observed when the isolates were grown in two different media namely czapek dox agar (CDA) and yeast malt agar (YMA). All the isolates of bamboo (B1, B2, B3, B4, B5) showed significant growth under YMA media in comparison to CDA media. On the other hand, isolates of banana (Ba1, Ba2, Ba3, Ba4, Ba5) demonstrated substantial growth in CDA in contrast to YMA media. Isolates growth was optimized under different pH and it was observed that B1, B2, B3, and B4 isolates showed better growth under pH 8 and pH 9. Correspondingly isolates of banana (Ba1, Ba2, Ba3, Ba4, Ba5) had grown significantly under pH 8 and pH 9. From the morphological and biochemical characterization, it was observed that the rhizospheric isolates from bamboo (B1, B3, B4) belong to the genera *Bacillus* sp., and one (B2) needs further tests for identification (Table 1). Among the fiv e isolates from the rhizosphere of banana, Ba5 belonged to the genera *Bacillus,* Ba2, Ba3, and Ba4 were the member of genera *Rummeliibacillus, Lysinibacillus,* and *Brevibacillus* respectively (Table 1). However, Ba1 is still unknown, and further studies are needed for identification. Results showed that out of a total of nine isolates, 44.44% were members of the genera *Bacillus* sp. while 22.22% were not identified through these tests, and the rest 11.11% belonged to genera *Rummeliibacillus, Lysinibacillus,* and *Brevibacillus* respectively (Fig. 2a).

Discussion

This study was carried out in order to isolate cellulose and starch degrading bacteria from the rhizosphere of two important trees. From the nine isolates, 44.44% each were cellulose and starch degrading, while 11.11% were able to degrade both cellulose and starch (Fig. 2b). In rhizosphere, bacteria produce cellulases and amylases to quicken the decomposition of plant residues in soil that results in enhanced soil fertility (Phitsuwan et al. 2013). The result showed that most of the isolated cellulolytic and amylolytic bacteria from the rhizosphere (B1, B3, B4, Ba2, Ba3, Ba4, Ba5) were gram positive and corroborates with other prior reports (Gomashe et al. 2013). Interestingly, isolates from bamboo showed a high capability of degrading cellulose and these can be employed further for the extraction of cellulases for industrial purposes. This enzyme is currently being used in agricultural biotechnology and bioenergy production (Phitsuwan et al. 2013).

In contrast, starch hydrolysis was carried by out bacterial isolates (Ba2, Ba3, Ba4, Ba5) from the rhizosphere of banana and these offer a great source for the extraction of amylases. This thermoenzyme remains stable at high temperatures and dynamic under high pH and salinity which outfit them to industrial solicitations like textile, and food industries (Bruins et al. 2001). These amylolytic

bacteria also the capability to degrade phenol in the liquid wastes of industries such as oil refineries, paper mills, etc., and may be used in bioremediation. Most of the isolates belong to the Bacillaceae family and have been observed to have commercial significance. For example, strains of *Brevibacillus* can improve the shelf-life of fruits (Che et al. 2011) and also show larvicidal activity (Zubasheva et al. 2010). *Lysinibacillus* strains exhibit insecticide degrading properties (Singh et al. 2012), produce toxins to destroy mosquito larvae (Lozano et al. 2011), and can desulfurize dibenzothiophene (Bahuguna et al. 2011). Strains of *Rummeliibacillus* are efficient in converting palm oil mill effluent into biodiesel (Junpadit et al. 2017) and inflating the growth of tilapia (Yih et al. 2019).

Interestingly, one of the isolates of banana i.e. *Rummeliibacillus* sp. may have utmost importance as this isolate showed substantial activity for degrading cellulases and starch and can be utilized at an industrial scale. However, further molecular identification is required to delineate the exact species.

Apart from industrial purposes, these isolated rhizospheric bacteria play a major role in boosting the growth of bamboo and banana by making them tolerant against abiotic stress, producing a huge variety of protecting enzymes, and fixing nutrients so that plants can uptake them effortlessly. Banana has adventitious roots and horizontal roots so it has to be dependent on soil microbes for nutrients. While the rapid growth of bamboo is due to the association of its roots with microflora. The present study laid the foundation for the application of these rhizospheric bacterial isolates in the biotechnology sector as well as the industrial sector. However, this is just a preliminary report additional studies are underway to know the exact mechanism of cellulose and starch degradation and quantitative assessment as well as identification up to species level through molecular identification technique.

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