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Molecular Identification and Enzymatic Activities of Endophytic Bacteria of *Ammannia Baccifera* Grown at Hot Spring Area, Laith, Saudi Arabia

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Abstract

The diversity and beneficial characteristics of endophytic bacteria have been extensively studied in different plants. However, information regarding naturally occurring *Ammannia baccifera* plant associated endophytes grown in Hot spring area is limited. Twenty-two endophytic bacteria were isolated from root, stem, and leave of *A. baccifera* collected from Hot Spring area, AL-Lith city (Saudi Arabia). These bacterial endophytes isolates were identified by using morphological characteristics and partial sequencing of their 16S rDNA gene. The isolated endophytic bacteria were identified as *Pseudomonas sp., Pantoeaste wartii, Enterobacter sp, Exiguobacterium sp, Curtobacterium flaccumfaciens, Sphingomonas koreensis, Paenibacillus lautus, Alcaligenes faecalis* and *Bacillus paralicheniformis*. In addition, the enzymatic activities of these bacterial endophytes were screened for degrading of the polysaccharides available in the host plant by using of simpler solid media permits the rapid screening of large populations of bacteria for the presence or absence of specific enzymes, such as cellulose, pectinase, amylase lipase, and protease. The endophytic bacterial isolates showed positive enzymatic production about 18% for cellullase, 81.8% for pectinase, 22.7% for amylase, 40.9% for lipase, and 68% for protease. Furthermore, *Bacillus paralicheniformis* exhibited the strongest activities among other strains in production of extracellular enzymes. The array of enzymes produced differs between endophytic bacteria and often depends on the host and their ecological factors.

Keywords: Ammannia Baccifera; Endophytic Bacteria; Extracellular Enzymes

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Introduction

Plant tissues are a habitat for different microorganisms. Endophytic bacteria are considered the dominant microorganisms present in plants. Endophytes can be defined as bacteria that can be isolated from healthy, disinfected plant tissues that do not cause any damage to the host plant [1]. Endophytic bacteria seem to be distributed in most plant species and have been isolated from roots, leaves, stems, a few from flowers, fruits, and seeds [2]. More than 200 bacterial genera isolated from 16 plants have been reported as endophytes. These include members of the phyla Acidobacteria, Aquificae, Bacteroidetes, Cholorobi, Chloroflexi, Cyanobacteria, Deinococcus-Thermus, Firmicutes, Fusobacteria, Gemmatimonadetes, Nitrospira, Planctomycetes, Proteobacteria, Spirochaetes, and Verrucomicrobiae [3]. The diversity of endophytes inside the plants depends on many factors, including the plant source, the developmental stage of the plant, the genotype of plant, and environmental factors [4]. A single host plant species comprises several genera and species of endophytes; However, the diversity of endophytic communities in the endosphere is regulated by stochastic events, which are influenced by deterministic processes of colonization [5]. It should be added that the microenvironment of soil has an influence on the colonization of plant endophytes by diverse bacteria and their community composition [6]. Generally, plant growing in unique environment having interesting endemic locations possess novel endophytic microorganisms which can equipping new leads. Therefore, Hot spring ecosystem is considered as a type of extreme environment that are unique with respect to physical, chemical and geographical characteristics [7]. Some new species of endophytes have been isolated from different plants growing at extreme habitat and have been found to be able to produce unique active metabolites [8-10].

Many endophytic bacteria may accompaniment certain metabolic properties, such as promoting plant growth, controlling plants pathogens, or helping host plant to defeat stress responses to environmental condition [11,12]. Furthermore, the interactions between plants and bacteria help plants to settle in ecosystem restoration processes [13]. These interactions may increase the ability of plants to utilize nutrients from the soil through increasing root development, nitrate uptake or solubilizing phosphorus, and controlling plants pathogens [14].

Some endophytic bacteria produce extracellular hydrolases as a resistance mechanism against pathogenic invasion and to obtain

nutrition from host, such enzymes include cellulose, pectinase, amylase, lipase, and protase. Moreover, some endophytic bacteria have the abilities of production enzymes for colonization inside plants such as cellulolytic enzyme to hydrolyze exodermal cell walls of the plants [15]. Therefore, plant endophytic bacteria have been widely accepted as an economic resource of vital and novel biomolecules and enzymes having potential application in agriculture, pharmaceutical and food industry. The production of extracellular enzymes for penetration and limited colonization of selected plant cell is a common trait of endophytic bacteria. Few little studies have explored the possibility of endophytic bacteria as biotechnological sources of industrially relevant enzymes [16]. Hence, they occupy a relatively unexplored site and can represent a new source in obtaining different enzymes with potentialities. Hence, the present study was carried out to isolate, identify of endophytic bacteria from *A. bacciferaplant* grown in Hot spring area, and screen new sources of valuable extracellular enzymes from these endophytic bacteria to understand their functional role in the host.

Methods

Collection of Plant Samples

A total of 70 leaves, 7 stems and 7 roots of *A. baccifera* were randomly collected from a stream bank of Hot spring area (N 40˚28'11.4E), which it is located 17 Km North-East of Gomymgah village, and 25 km East of Al-Lith city, Kingdom of Saudi Arabia, during February 2019 at 32°C. The plant samples were brought to the laboratory in sterilized bags for processing.

Isolation and Culturing of Bacterial Endophytes

The leaves, stems, and roots of *A. bacciferawere* rinsed gently in running tap water to remove dust and debris. The plant organs were surface sterilized, according to modified method by immersing the samples in 70% ethanol for 1 minute, then in aqueous solution of sodium hypochlorite (5% available chlorine) for 2 minutes, followed by washing with 70% ethanol for 5-10 second. Finally, the samples were rinsed three times in sterile distilled water and dried on a sterile filter paper [17,18]. After surface sterilization, all plant organs were cut into small segments of pieces 1-3 mm long using a sterilized surgical blade and placed on Nutrient Agar plates (Hi Media, Mumbai, India). To confirm that the disinfection process was successful, the plant organs pieces were pressed onto NA medium plates and aliquots of the sterile distilled water used in the final rinse were also plated onto the same medium. The plates were examined for growth after incubation at 28˚C for one to two weeks to allow growth of endophytes. The Petri dishes were monitored daily to check the growth of bacterial endophytic colonies from the segments. Bacteria growing out of the plant segments were isolated, purified, and identified based on phenotypic characteristics and genotypic identification.

Phenotypic Characterization of the Selected Endophytic Bacterial Isolates

All selected endophytic bacterial isolates were characterized, by studying cell shape, size, colony morphology, pigmentation, and Gram's staining [19].

Genotypic Identification of the Bacterial Isolates by 16S rDNA Sequencing

The selected bacterial isolates were identified by 16S rDNA gene sequences. The genomic DNA of endophytic bacteria was extracted [20] from bacterial colonies by growing them on NA medium for 24 h at 28°C using a commercial bacterial genomic DNA extraction (MQ Bacterial DNA Isolation Kit, MOLEQULE-ON Company, Auckland, New Zealand.

16SrDNA was amplified in polymerase chain reaction (PCR) using the genomic DNA as template and bacterial universal primers, 27 F(5′GAGTTTGATCCTGGCTCA-3′), and reverse primer 1492R (5'-GGTTACCTTGTTACGACTT-3'). Briefly, 30 µl reaction containing 1 µl of template genomic DNA, 0.125 µl Taq DNA polymerase, 3 µl Taq buffer, 0.6 µl dNTP, 2.4 µl $MgCl_2$, and 0.6 µl gene-specific primers 27F and 1492 R with the addition ofsterile deionized H_2O to obtain a final volume of 30 µL. PCR amplification was performed using a thermocycler (Eppendrof® Mastercycler nexus®) for 50 cycles: initial denaturation of 94°C for four min, followed by 94°C for 45 seconds. Then 50°C for 55 seconds, and 72°C for one min with a final extension of 72°C for 10 minutes. The PCR product was visualized by agarose gel electrophoresis (a 1% agarose gel run at 80 volts for 40 min). Finally, the PCR products with the primers were sent in 96 well plates, for sequencing (single pass PCR sequencing) to Macrogen Online Sequencing Company, Korea. Then, the sequencing data were checked by BLAST analysis in the NCBI database for microbial identification.

Detection of Extracellular Enzymes Production by the Selected Bacterial Endophytes

Extracellular enzymes of bacterial endophytes were detected by using qualitative techniques to screen large number of bacteria in a relatively short time. Twenty-two of bacterial isolates were grown on different indicator media:

Cellulase activity: Screening of cellulase bacterial producers were done on carboxy methyl cellulose (CMC) agar according to [21]. 0.5 ml of bacterial suspension isolates were plated on CMC agar (Carboxymethyl cellulose 5 g, peptone 5 g, NaCl 5 g, Beef extract 3 g, Agar 18 g, distilled water 1L). The plates were incubated at 30°C for 4 days. At the end of the incubation, the culture surface was flooded with an aqueous solution of Congo red (1% w/v) for 15 minutes. The Congo red solution was then poured off, and the plates were further treated by flooding with 1M NaCl for 15 minutes. The formation of a clear zone of hydrolysis indicated cellulose degradation.

Pectinase activity: The functional role of pectinase by bacterial endophytes was assessed on pectin agar medium (Pectin 5 g, yeast extract 1 g, agar 15 g, pH 5.0 in 1L distilled water). 10 µl of the bacterial isolates were plated and incubated for 4 days at 30° C. Iodine-potassium/ iodide solution (1.0 g iodine, 5.0 g potassium iodide and 330 ml H_2O) were added to the plate's surface to detect clearance zones [22]. A clear zone formed around the bacterial colony indicated pectinolytic activity.

Amylase activity: Amylase activity was assessed by growing 10 µl the bacterial isolates on Glucose Yeast Extract Peptone Agar (GYP) medium (glucose 1g, yeast extract 0.1 g, peptone 0.5 g, agar 16 g, distilled water1L) with 0.2% soluble starch pH 6.0. After incubation, the plates were flooded with 1% iodine in 2% potassium iodide to detect clearance zones formed around the bacterial colony.

Lipase activity: The lipase activity of the selected endophytic bacterial isolates was determined by growing them on Peptone Agar medium g/l: (peptone 10 g, NaCl 5 g, $CaCl_2$ H_2O 0.1g, agar 16 g, pH 6.0). The medium was supplemented with 1% Tween 20 separately sterilized [23]. At the end of the incubation period, a visible precipitate around the colony due to the formation of calcium salts of the lauric acid by the enzyme indicated positive lipase production.

Protease activity: Endophytic bacterial isolates were screened for their ability of protease production on skim milk agar plate (casein 5g, yeast extract 2.5g, dextrose 10g, skim milk powder 28g, agar 15g, and distilled water 1L), and incubated at 28ºC and after 48 hours [24]. Formation the clearance zone by the enzyme indicated positive protease production.

Results

Isolation and Culturing of Bacterial Endophytes

This is probably the first report to describe the endophytic bacteria that colonize *A.baccifera* plant (Figure 1) at the Hot spring area of Saudi Arabia (Figure 2). Twenty-two of endophytic bacterial isolates were obtained from leave, stem and root segments of *A. baccifera*. *Ammanniabaccifera*, is known as the monarch redstem, is a species in the family Lythraceae. It is annual and herbaceous plant, and can be found in marshes, and water courses at low elevations. The *A.baccifera* plant is branched, smooth, slender, purplish herb 10 to 50 centimeters in height. The flowers are small, about 1.2 millimeters long, purplish color [25]. This plant was found to possess hypothermic, hypertensive, antiurolithiasis, antibacterial and depressant activities [26,27].

Phenotypic Characterization of Endophytic Bacteria

The endophytic bacterial colonies were purified by repeated sub culturing on Nutrient Agar medium. Based on the visible morphological differences, totally 22 bacterial strains were isolated from *A. baccifera* plant samples, and characterized for differences between colonies, in shape, color, elevation, margin, and texture (Figure 3) [28]. In addition, Gram staining were performed to evaluate colony arrangement (Figure 4). It was observed that 36% of the isolates were Gram negative rods, where as 45.5% isolates were Gram negative short rods, 18% isolates were found to be Gram positive bacilli (Table 1). The

Figure 1: Ammannia baccifera (Monarch redstem) plant at Hot spring area.

Figure 2: Hot spring area at Gomygah village, Al-Lith city, Kingdom of Saudi Arabia, a: map, b: location, c: study area.

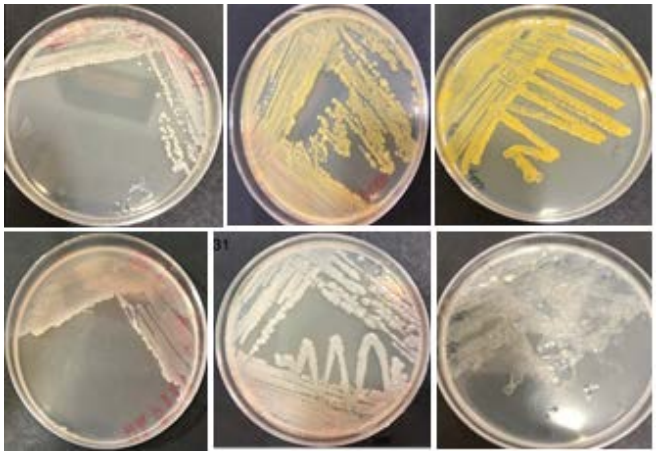

Figure 3: Selected some pure colonies of bacterial isolates on NA agar plates. a: P3_M2. b: P3_M7. c: P3_M11. d: P3_M25. e: P3_M31. f: P3_S2.

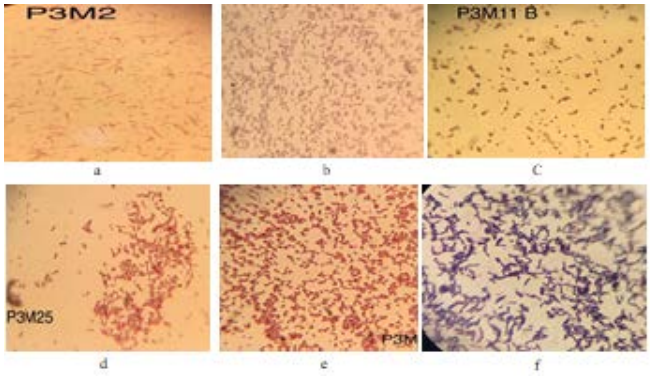

Figure 4: Characterization of Gram staining under the microscope for the selected bacterial isolates. (a) P3_M2. (b) P3_M7. (c) P3_M11. (d) P3_M25. (e) P3_M31. (f) P3_S2.

selected endophytic bacterial isolates to combinatory of morphological characterization, and 16S rDNA gene sequencing provided a specific identification of the bacterial isolates [29].

Genotypic Identification of the Bacterial Isolates by 16S rDNA Sequencing

The identification of 16S rDNA sequence analysis was performed by using the algorithms BLAST (National Center for Biotechnology Information [http://www.ncbi.nlm.nih.gov]. The highest similarities of the bacterial isolates found with different bacterial genera were summarized (Table 2). The 16S rDNA sequences analysis of isolated bacteria showed the maximum identity (93%-100%) to different bacterial species belonging to nine genera of *Enterobacter, Pseudomonas, Bacillus, Exiguobacterium, Pantoea, Curtobacterium, Sphingomonas, Paenibacillus, and Alcaligenes.*

The bacterial isolates (P3_ M11, P3_M23, P3_M24, P3_M28, P3_M31, P3_M41, P3_R10, and P3_R14), were characterized as Gram negative, short rods shaped morphology, belonged to *Enterobacter hormaechei, Enterbacterasburiae,* and *Enterobacter cloacae* with 97% to 100% similarity. While the isolates which they were described as rods shaped morphology (P3_M2, P3_M9, P3_M19, P3_M25, P3_ R29, P3_S1, and P3_M12), were belonged to *Pseudomonas putida, Pseudomonas entomophila, Pseudomonas mosselii, Pseudomonas guezennei,* and *Pseudomonas montelii.* Similarly, bacterial isolate (P3_

Table 1: Phenotypic characterization of bacterial endophytes isolated from *A. baccifera* plant.

Bacterial isolates	Plant organ	Gram morphology	Gram staining	Shape, margin, elevation, size, texture, appearance, pigmentation, optical property
P3_M2	Leave	Rods	Negative	Circular, entire, Convex, medium, smooth, shiny, nonpigmented, opaque
P3_M7	Leave	Short rods	Negative	Circular, entire, Convex, medium, smooth, shiny, nonpigmented (creamy), opaque
P3_M9	Leave	Rods	Negative	Circular, entire, Convex, medium, smooth, shiny, nonpigmented, opaque
P3_M11	Leave	Short rods	Negative	Circular, entire, Convex, medium, smooth, shiny, nonpigmented, opaque
P3_M12	Leave	Rods	Negative	Circular, irregular, Convex, medium, rough, shiny, nonpigmented (creamy), opaque
P3_M19	Leave	Rods	Negative	Circular, entire, Convex, medium, smooth, shiny, nonpigmented, opaque
P3_M22	Leave	Bacilli	Positive	Circular, entire, Convex, medium, smooth, shiny, pigmented (orange), opaque
P3_M23	Leave	Rods	Negative	Circular, entire, Convex, medium, smooth, shiny, nonpigmented, translucent
P3_M24	Leave	Short rods	Negative	Circular, entire, Convex, medium, smooth, shiny, nonpigmented (creamy), translucent
P3_M25	Leave	Rods	Negative	Circular, entire, raised, large, smooth, shiny, nonpigmented, transparent
P3_M26	Leave	Rods	positive	Circular, entire, Convex, punctiform, smooth, shiny, pigmented (yellow), opaque
P3_M27	Leave	Bacilli	positive	Circular, entire, Convex, large, smooth, shiny, pigmented (yellow), opaque
P3_M28	Leave	Rods	Negative	Circular, lobate, raised, small, smooth, shiny, pigmented (yellow), opaque
P3_M31	Leave	Rods	Negative	Circular, irregular, raised, small, smooth, shiny, pigmented (yellow), opaque
P3_M41	Leave	Rods	Negative	Circular, lobate, raised, small, smooth, shiny, pigmented (light orange), opaque
P3_R3	Root	Bacilli	Positive	Circular, entire, Convex, small, smooth, shiny, nonpigmented, transparent
P3_R10	Root	Rods	Negative	Circular, entire, Convex, medium, smooth, shiny, nonpigmented, translucent
P3_R14	Root	Rods	Negative	Circular, entire, Convex, medium, smooth, shiny, nonpigmented, translucent
P3_R29	Root	Rods	Negative	Circular, entire, raised, small, smooth, dull, pigmented (light pink), transparent
P3_R30	Root	Rods	Negative	Circular, entire, Convex, punctiform, smooth, shiny, nonpigmented, opaque
P3_S1	Stem	Rods	Negative	Circular, entire, Convex, large, smooth, shiny, nonpigmented, translucent
P3_S2	Stem	Bacilli	positive	Irregular, entire, Convex, large, smooth, shiny, nonpigmented, transparent

Table 2: Identifying of culturable endophytes bacteria isolates from *A. baccifera* plant.

Bacterial isolates	Accession No.	The most related strain in GenBank	Coverage (%)	Identity (%)
P3_M2	MK934486.1	*Pseudomonas putida*	99%	100.00%
P3_M7	JQ765426.1	*Pantoeastewartii*	99%	93.41%
P3_M9	MK934486.1	*Pseudomonas putida*	99%	100.00%
P3_M11	MK414992.1	*Enterobacter hormaechei*	100%	99.66%
P3_M12	KP267681.1	*Pseudomonas montelii*	100%	100%
P3_M19	KX098359.1	*Pseudomonas putida*	97%	97.63%
P3_M22	MK414873.1	*Exiguobacteriumacetylicum*	99%	99.86%
P3_M23	MH061360.1	*Enterobacter asburiae*	100%	96.80%
P3_M24	MK414992.1	*Enterobacter hormaechei*	99%	100.00%
P3_M25	KJ689793.1	*Pseudomonas entomophila*	99%	94.80%
P3_M26	MH256549.1	*Curtobacteriumflaccumfaciens*	99%	99.74%
P3_M27	JF505986.1	*Sphingomonaskoreensis*	99%	99.75%
P3_M28	MK467572.1	*Enterobacter cloacae*	100%	96.47%
P3_M31	KP305913.1	*Enterobacter cloacae*	99%	97.23%
P3_M41	MK811112.1	*Enterobacter cloacae*	99%	99.73%
P3_R3	MK256307.1	*Paenibacilluslautus*	99%	98.46%
P3_R10	KC853299.1	*Enterobacter asburiae*	99%	96.08%
P3_R14	KC853299.1	*Enterobacter asburiae*	99%	96.16%
P3_R29	MK999975.1	*Pseudomonas mosselii*	99%	100.00%
P3_R30	MK898928.1	*Alcaligenes faecalis*	100%	99.01%
P3_S1	MK825483.1	*Pseudomonas guezennei*	99%	99.87%
P3_S2	MK855179.1	*Bacillus paralicheniformis*	99%	99.86%

R30) had a sequence similarity of 99%, to the type strain of *Alcaligenes faecalis.* The two isolates, (P3_M7) and (P3_M27) were classified to *Pantoeastewarti, and Sphingomonaskoreensis* respectively with 93% to 100% similarity. In addition, Gram positive, rods shaped morphology, (P3_M22, P3_M26, P3_R3, and P3_S2) isolates, denominated with 98% to 99% to be members of *Exiguobacteriumacetylicum, Curtobacteriumflaccumfaciens, Paenibacilluslautus, and Bacillus paralicheniformis* respectively (Table 2).

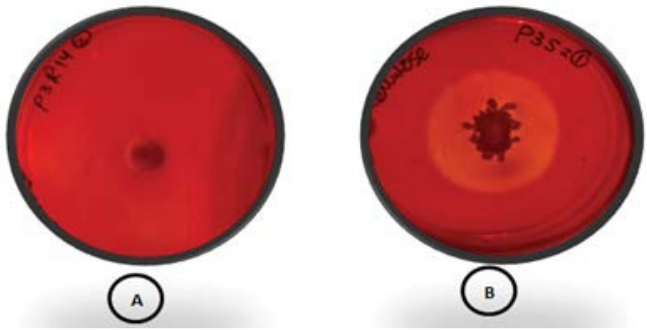

Figure 5: Cellulose activity, A: Negative result, B: Positive result.

Extracellular Enzyme Production by the Selected Isolates of Bacterial Endophytes

The endophytic bacteria isolated from the *A. baccifera* were evaluated for the presence of active hydrolytic enzymes including cellulase, pectinase, amylase, protease, and lipase, (Table 3). Among the isolated bacterial strains, *B. paralicheniformis* showed maximum production of enzymes activities among five tested enzymes activities. The cellulase activity was observed for isolates *Pseudomonas entomophila, Paenibacilluslautus, and Bacillusparalicheniformis* (Figure 5). In addition, most bacterial isolates were showed positive results for pectinase activity (Figure 6) except *Pseudomonas putida* and *Pantoeastewartii.* Moreover, the amylolytic activity was observed for the isolates *Exiguobacteriumacetylicum, Sphingomonaskoreensis, Enterobacter cloacae, Paenibacilluslautus, Alcaligenes faecalis, and B. paralicheniformis* (Figure 7). The Lipolytic activity was only observed for the isolates *B. paralicheniformis*, *Sphingomonas koreensis*, *Exiguobacterium acetylicum* and *Pseudomonas* sp. (Figure 8). These strains have been suggested as producers of extracellular lipase [30]. Protease producer isolates were *Bacillus paralicheniformis*, *Exiguobacterium acetylicum, Enterobacter cloacae, Curtobacterium*

Table 3: Evaluation of extracellular hydrolytic enzyme activity from the endophytic bacteria isolated from A. baccifera plant.

Bacterial codes	Bacterial isolates	Cellulase	Pectinase	Amylase	Lipase	Protease
P3_M2	*Pseudomonas putida*	-	-	-	-	-
P3_M7	*Pantoeastewartii*	-	-	-	-	+++
P3_M9	*Pseudomonas putida*	-	-	-	-	-
P3_M11	*Enterobacter hormaechei*	-	+	-	-	+
P3_M12	*Pseudomonas montelii*	-	+	-	-	-
P3_M19	*Pseudomonas putida*	-	-	-	-	-
P3_M22	*Exiguobacteriumacetylicum*	-	+++	+	-	+++
P3_M23	*Enterobacter asburiae*	-	+	-	-	-
P3_M24	*Enterobacter hormaechei*	-	+	-	-	+
P3_M25	*Pseudomonas entomophila*	+	+	-	++	+++
P3_M26	*Curtobacteriumflaccumfaciens*	-	+	-	-	+++
P3_M27	*Sphingomonaskoreensis*	-	+	+	+	+++
P3_M28	*Enterobacter cloacae*	-	+++	-	+	+++
P3_M31	*Enterobacter cloacae*	-	+++	-	+	+++
P3_M41	*Enterobacter cloacae*	-	+++	-	+	+++
P3_R3	*Paenibacilluslautus*	+++	+	+	-	-
P3_R10	*Enterobacter asburiae*	-	+++	-	+	+++
P3_R14	*Enterobacter asburiae*	-	+	-	-	-
P3_R29	*Pseudomonas mosselii*	-	+	-	+	+++
P3_R30	*Alcaligenes faecalis*	+++	+++	+	-	+++
P3_S1	*Pseudomonas guezennei*	-	+	-	+++	+++
P3_S2	*Bacillus paralicheniformis*	+++	+++	+	++	+++

Note: (-) indicate no clearing zone, (+) indicate clearing zone ≤ 5 mm; (++) indicate clearing zone >5 mm; and (+++) indicate clearing zone ≥ 8 mm.

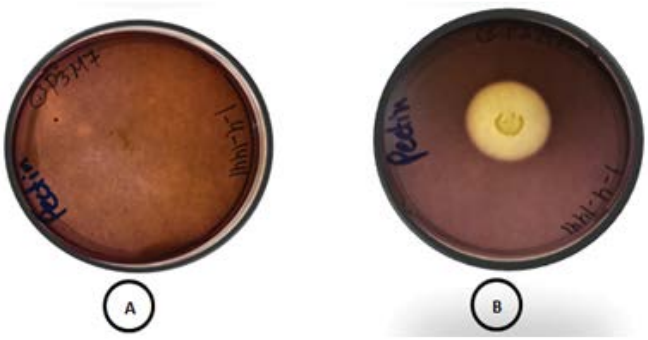

Figure 6: Pectinase activity, A: Negative result, B: Positive result.

Figure 7: Amylase activity. A: Negative result, B: Positive result.

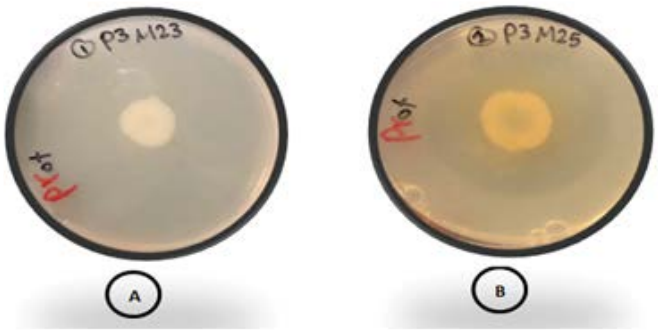

Figure 9: Protease activity A: Negative result, B: Positive result.

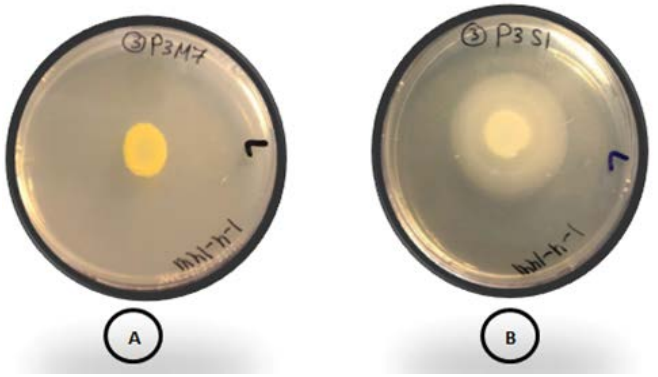

Figure 8: Lipase activity A: Negative result, B: Positive result.

flaccumfaciens, Alcaligenes faecali, and some species of *Pseudomonas* (Figure 9). The presence of proteolytic activity in the *Bacillus paralicheniformis* confirmed that some species of *Bacillus* tended to synthesize proteolytic enzymes during the sporulation process [31]. Researches demonstrated that *Paenbacillus* species are known to produce different hydrolyzing enzymes [32].

Discussion

This study demonstrated that *A. baccifera* plant is relatively unstudied to their endophytic biology and being considered as potential source for natural products to be used in researches or agriculture fields. In particular, endophytes are known to colonize plant tissues

using enzymes involved in the infection process by degrading the plant tissues [33].

In this study, the leaves harbored more endophytic bacteria compared to the stems and roots. This is in line with previous studies that have shown leaves of *Arabidopsis thaliana* harbored more endophytes than roots [34]. This may be attributed to high photosynthetic metabolism in the leaf hence the products could be utilized by the microbes. Moreover, for isolation of endophytes technique, it was demonstrated that the most important treatment prior isolation of endophytes from different parts of plant is surface sterilization. The rinsing of plant parts with sterilized distilled water, and sequential immersion in ethanol and sodium hypochlorite (NaOCl) to ensure the removal of surface microbial flora. These chemical disinfectants have been employed for surface sterilization of excised tissues to remove epiphytes microbes; however, immersion of the tissues in ethanol and NaOCl have shown significant success in different studies [35].

Indeed, the major key in isolating and studying endophytes is to ensure the sterility of the plant surface by dividing the plant parts into small pieces, with sterile surgical blade under aseptic conditions were shifted to the isolation media [17]. In 1998, Schulz and coworkers introduced leaf imprint as a new method for checking the isolation protocols, aiming to eliminate epiphytic organisms [36]. Sánchez and Márquez, used this approach as an excellent sterilization method for isolation of endophytes from (*Dactylis glomerata* L.). Several different species of endophytes can be isolated from a single plant by using sterilization methods [37]. Moreover, the microbial metabolic pathways of colonization may play an important role as determinants of endophyte diversity [38,39]. In addition, many studies illustrate endophyte diversity in different ecologies, there is no reliable estimation of the number of endophytic species, of their host- and tissue-selectivity, since environmental factors have a complex effect on these features [40]. According to [32] the most common taxa of endophytic bacteria isolated from plants include *Bacillus*, *Enterobacter*, *Pseudomonas* and *Flavobacterium*. Some studies reported that the endophytic bacterial diversity exceed over 40 genera, with predominance of *Pseudomonads* and *Bacillus* [41]. *Paenibacillus* has also been found as an endophyte in different woody plants like pine, coffee, and poplar [42]. Moreover, *Exiguobacterium sp*. AM25 was isolated from *Avicenniamarina*as as endophyte and showed high significantly resistance against salinity stress in *Solanum lycopersicum* plant [43]. Additionally, the endophyte bacteria *Sphingomonas* has been isolated from extreme environment, it is considered as a novel strain that has recently been isolated from abandoned heavy metal sites [44], glaciers [45] volcano-associated lakes [46], and the sediment of a eutrophic reservoir [47]. Researchers reported that *Bacillus* was the most frequently genus of bacterial endophytes followed by *Pantoea, Curtobacterium* and *Enterobacter*, which they were isolated from two mangrove species, *Rhizophora mangle* and *Avicennianitida* in Brazil [48].

Additionally, this study showed that the hydrolytic enzymes from bacterial endophytes appear to be important for the colonization of plant roots [17,49]. These enzymes also seem to be important for spreading through the intercellular space of the root cortex and beyond. This hypothesis is supported by the presence of cellulolytic and pectinolytic enzymes produced by numerous endophytic bacteria [50]. Some studies showed that the presence of varying levels of cellulase and pectinase activities in different isolates, possibly affecting their potential for inter/intracellular colonization [50]. In addition, bacteria enter the interior of the root by hydrolyzing wall-bound cellulose, water flow, and wounds, or where the lateral roots branch [51]. Priest [52] showed that there are several possible regulatory mechanisms in the enzymes production including induction. The action of such enzymes gives rise to the possibility that the "genetic recombination" of the endophyte with the host which occur in evolutionary time. This may be the reason why some endophytes can produce some phyto-chemicals originally characteristic of the host. Therefore, the endophytic bacteria isolates subjected to be a new source for commercial enzyme production. Also, researchers reported that hydrolytic enzymes produced by endophytes bacteria are suspected to play an important role in suppression of plant pathogens [53]. In addition, the studies of endophytic bacteria and their relationships with the host plants will shed light on the ecology and evolution of endophytes and their hosts; the evolution of endophyte - plant symbiosis; the ecological factors that influence the strength of endophyte-host plant interaction [54,55].

Conclusion

This study evidenced that *A. baccifera* grown under extreme condition is potential harbor diversity of unique bacterial endophytes. Since the exploited bacteria isolated from *A. baccifera*, showed production diversity of enzymatic activities. The degree of variability of enzyme production by these endophytic bacterial isolates indicates that the enzyme production differs between bacteria and often corresponds to the requirements of its habitat. This may be due to the many factors changing in the host as related to age, environmental factors such as, climatic condition and geographical location may influence the biology of the bacteria. However, knowledge of the types, amounts and characteristics of enzymes produced by endophytic bacteria cited above would be useful for selecting organisms best suited to prove its potential further to the discovery of numerous value metabolites and controlling pathogens (current experiments in our Lab).

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