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Screening and characterization of bacterial endophytes of the Asteraceae for the production of hydrolytic enzymes

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Abstract
The host plants contain endophytic bacteria that have positive impacts on their hosts. In the study, 18 endophytic bacteria were isolated from *Ageratum conyzoides*, *Parthenium hysterophorus*, and *Tridax procumbens*. Furthermore, colony morphology and biochemical assays were used to characterize these bacterial endophytes and screened for hydrolytic enzyme production. Endophyte colony attributes varied with diverse margins, coloration, translucency, and elevation. Out of 18 endophytic isolates, 38.88% of isolates were Gram positive and 61.11% were Gram negative with different rod and cocci shapes. Further, 33.33% were endospore formers and non-endospore formers were 66.66%. Voges-Proskaeur (44.44%), Methyl red (44.44%), H_2S production (44.44%), catalase (88.88%), carbohydrate fermentation (Glucose, 66.66%; Sucrose, 44.44%), citrate utilization (55.55%) and indole production (22.22%) were all determined positive for isolates in biochemical tests. Endophytic isolates were found to produce hydrolytic enzymes confirmed through hydrolysis of starch (77.77%), urea (66.66%), cellulose (5.55%), pectin (5.55%), casein (22.22%) and gelatin (33.33%). The biochemical profiling of endophytes from the Asteraceae plants indicated the hydrolytic activity of diverse bacterial endophytes found in these plants.

Keywords: Bacterial endophyte, diversity, growth promotion, ageratum conyzoides

1. Introduction
Native microorganisms that live inside of plants have developed sole metabolic paths that support plant development. Various macromolecules that support the health of individuals have been identified over time from bacterial endophytes hosting plant systems with no signs of illness. Therefore, endophytes benefit their hosts and thrive in a rather advantaged niche in host plants (Ullah *et al*., 2019) [21]. The relationship between endophytic bacteria and plants has been an exciting field of study with potential applications in cutting-edge farming methods. The bacterial genera identified in plant tissues include *Bacillus*, *Arthrobacter*, *Pseudomonas*, *Flavobacterium*, *Enterobacter*, *Stenotrophomon*, and others (Hallmann and Berg, 2006) [6].
It is recognized that microorganisms synthesize an extensive range of enzymes that are largely used in industry for use in bioremediation, biotechnology, pharmaceutical research, and other sectors. (Singh *et al*., 2016) [19]. Through the synthesize of phytohormones, chief metabolites, stimulation of the plant immune system counter to biotic stress, accessibility of mineral nutrients, and tolerance to abiotic stress, bacterial endophytes function as organisms that either directly or indirectly encourage plant growth (Kandel *et al*., 2017) [8]. The capability and use of endophytes have not been fully investigated, despite the fact that this could be method extensively used to produce biomolecules and aid in stimulating plant development.
Hydrolytic enzymes, also known as hydrolases' split many groups of biomolecules like glycosides, esters, and peptides. They are intricate in the breakdown of carbohydrates, lipids, proteins, nucleic acids, and others. Hence, are the source for many biogeochemical cycles in soil and are extensively utilized for industrial purposes. Endophytes colonize plant tissues through hydrolytic enzymes like pectinase, cellulase, and others, in association with their entry via wounds (Verma *et al*., 2001) [23]. More evaluation is to be made to understand the endophytic bacterial and plant relationships.
Both under favorable and unfavorable environmental situations, weeds predominate. Bacterial endophytes, known for promoting plant development, may benefit from this. Although underutilized, endophytes from weed species and those resistant to drought stress have the possibility to be utilized in environmentally friendly sustainable agriculture, in the synthesis of hydrolytic enzymes, and in other industrial applications.

Bacteria from a wide taxonomic group are found to associate with weed plant species, including *Bacillus*, *Sinorhizobium*, Pseudomonas, *Herbaspirillum*, *Sphingobium*, *Micrococcus*, *Sphingomonas*, *Rhodococcus*, *Microbacterium* and *Marinorhizobium*. (Fatema *et al*., 2019) [4].

The purpose of the current work was to explore the variety of bacterial endophytes from diverse weed species (*Ageratum conyzoides*, *Parthenium hysterophorus*, and *Tridax procumbens*) of the Asteraceae family due to the diverse uses of endophytes in industry, agriculture, and medicine. These weed species are generally present in all environmental conditions. Further, isolates were profiled considering colony features, microscopic observations, and tests for biochemical synthesis and screened for hydrolytic enzyme production.

2. Materials and Methods

2.1 Collection of weed plant species

Ageratum conyzoides, *Parthenium hysterophorus*, and *Tridax procumbens*, three drought-tolerant healthy weed species (Fig. 1), were gathered in plastic bags from the areas of dryness in the UAS, GKVK, Bengaluru, India-560065 (Table 1), and taken to laboratory.

Fig 1: Weed plant species (a) *Ageratum conyzoides* (b) *Parthenium hysterophorus* and (c) *Tridax procumbens*

Table 1: Details of weed plant species sampling sites

Sl. No.	Weed plant species	Location in GKVK campus, UAS, Bangalore	Geographical position of sampling sites	
			Latitude (N)	Longitude (E)
1	*Ageratum conyzoides*	Dryland plot	13°08’89.96”	77°56’80.35”
2	*Parthenium hysterophorus*	Krishi mela ground	13°08’35.64”	77°57’99.05”
3	*Tridax procumbens*	Dryland plot	13°08’57.34"	77°57'25.80"

*Mean sea level = 924 meters

2.2 Isolation of bacterial endophytes

The bulk of epiphytes and the soil sticking to the stem and roots of each plant were removed with tap water. Then stem and roots were cut into 1 cm lengths, and were surface sterilized as follows: treated with 70% ethanol (1 minute), followed by sodium hypochlorite (2%) for 3 min (for the root) & 1 min 30 s (for the shoot) and extensively cleaned using sterile distilled water. By impregnating the surface-sterilized stem and root fragments in nutrient agar, they were nurtured at 30 °C for 48 h (Anjum and Chandra, 2015) [2].

Following, culture growth was seen along the chopped ends of the stem and root portions (Fig. 2). To obtain the pure culture, the culture growth was collected and streaked onto a nutrient agar, which was nurtured at 30 °C for 48 hours. Then under refrigeration at 4 °C, pure cultures were maintained as broth, slants, and in 70% glycerol stock. Further studies were done by subculturing the preserved cultures.

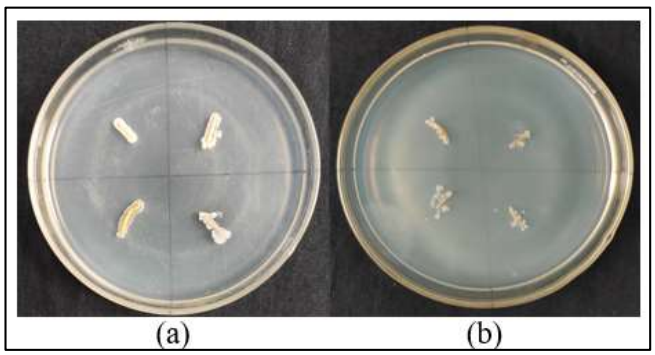

Fig 2: Isolation of bacterial endophytes from (a) stem and (b) root

2.3 Morphological characterization of endophytic cultures

Colony characteristics of bacterial cultures, including color, elevation, translucency, and margin were noted in accordance with Kalaivanan and Mohan's (2017) descriptions.

2.4 Biochemical characterization of endophytic cultures

2.4.1 Gram staining

According to Smith and Hussey's procedure from 2005, the Gram staining for bacterial isolates was done.

2.4.2 Endospore staining

For the 18 endophytic isolates, the Schaeffer-Fulton method, as reported by Gerhardt *et al*. (1981), was employed for endospore staining.

2.4.3 Methyl red

In order to conduct the methyl red test, cultures were inoculated into 5 mL of glucose phosphate broth and nurtured at 28±2 °C for 2 to 5 days (Safary *et al*., 2013) [15]. As growth takes place, five drops of alcoholic methyl red (0.04%) were applied, and the creation of a vivid red color was noticed. A positive result was indicated by this, whereas a negative result was shown by the presence of a yellow color (Van Thuoc *et al*., 2019) [22].

2.4.4 Voges-Proskauer

The endophytic cultures were inoculated in 5 ml of glucose phosphate broth and cultured at 28 ± 2 °C for 48 h (Safary *et al*., 2013) [15]. 1 ml of potassium hydroxide with creatine (0.3%) and 3 mL of the-naphthol solution were added as the growth took place. Within 2 to 5 minutes, a pink color appeared, signifying a favorable reaction (Van Thuoc *et al*., 2019) [22].

2.4.5 H_2S production

Sulfide indole motility (SIM) agar was stab inoculated with bacterial culture and nurtured for 48 hours at 30 °C. H_2S generation is proven as the presence of blackening along the inoculation line (Aneja, 2020) [1].

2.4.6 Carbohydrate fermentation
Cultures were added to the nutrient broth that also contained Durham's tube and phenol red, and was then nurtured at 28±2 °C for 48 hours. Acid and gas generation were shown by the color of the broth turning yellow from red and through the formation of a gas bubble in Durham's tube, respectively described by Li *et al*. (2019) [11].

2.4.7 Catalase activity
On the nutrient agar plates, bacterial endophytes were streaked and cultured for 48 hours at 30 °C. Then colonies received a drop of 3% H_2O_2. The appearance of effervescence confirmed the production of catalase enzyme (Van Thuoc *et al*., 2019) [22].

2.4.8 Indole production
The cultures were cultured in tryptone broth at 28±2 °C for 48-96 hours (Aneja, 2020) [1]. Later shook by adding 0.5 mL Kovac's reagent. Significant reaction was indicated by the alcohol layer developing a pink or red color (Li *et al*., 2019) [11].

2.4.9 Citrate utilization
Cultures were streaked on Simmon's citrate agar to conduct a citrate utilization test described by Safary *et al*. (2013) [15]. Further nurtured at 28±2 °C for 48 hours while being watched for the medium's color to turn from green to blue, indicating a positive outcome (Das *et al*., 2019) [3].

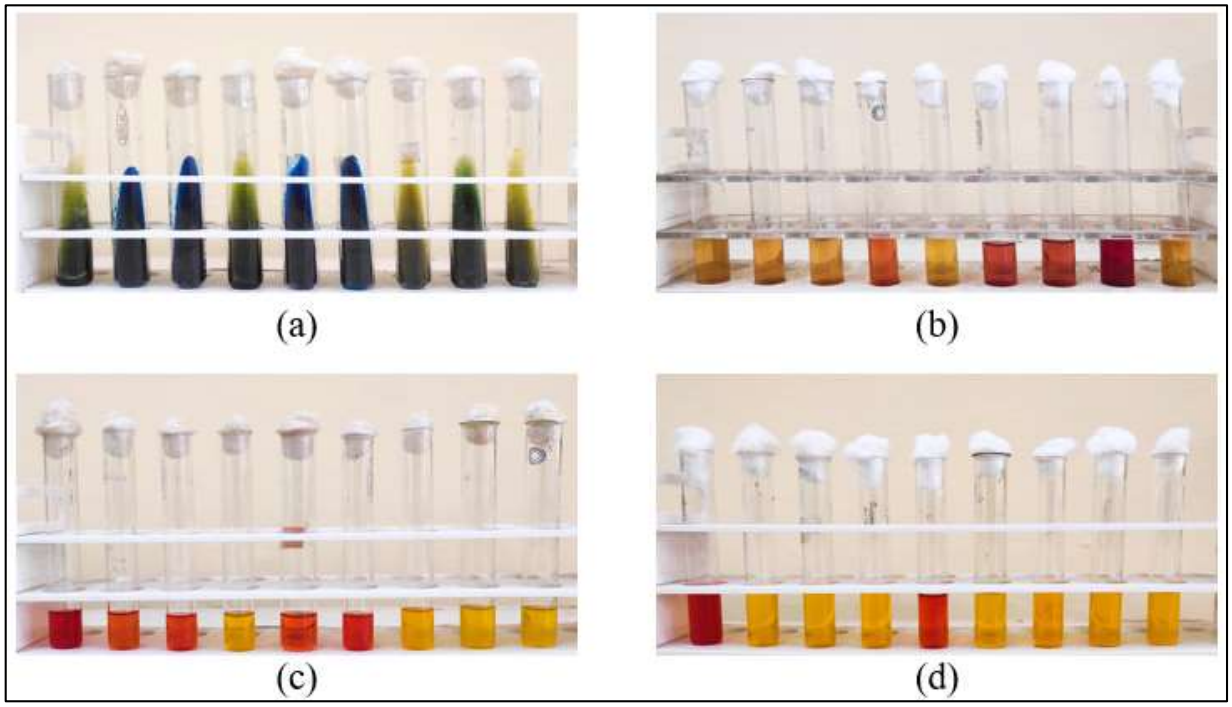

Fig 3: Biochemical tests for (a) Citrate agar test; (b) Methyl red; (c) Sucrose Fermentation; and (d) Glucose fermentation

2.5 Screening for the production of hydrolytic enzymes
2.5.1 Starch hydrolysis
Cultures were streaked on starch agar and nurtured at 28±2 °C for 48 h and flooded with 1% Gram's iodine reagent. A clearing zone around the colonies indicated starch hydrolysis by the activity of amylase (Van Thuoc *et al*., 2019) [22].

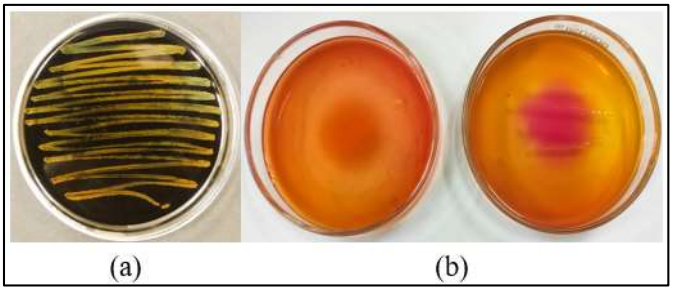

Fig 4: Screening for (a) Starch hydrolysis and (b) Urea hydrolysis

2.5.2 Urea hydrolysis
Cultures were streaked on urea agar, and incubated at 28±2 °C overnight, and the development of purple pink color indicated a positive urease test (Sharma *et al*., 2019) [17].

2.5.3 Cellulose hydrolysis
Bacterial endophytes were streaked on Czapek-mineral salt agar medium containing carboxymethyl cellulose and nurtured at 30 °C for 2-5 d. Later flooded hexadecyltri methyl ammonium bromide (1%). A clearing zone around the colony growth was noted to be significant as a cellulase producer (Aneja, 2020) [1].

2.5.4 Pectin hydrolysis
Bacterial isolates were streaked on Hankin's medium with pH 5 and nurtured at 30 °C for 48-72 h. The microbial growth was flooded with hexadecyltrimethyl ammonium bromide (1 %). A clear zone around the microbial growth was found positive for pectin hydrolysis (Aneja, 2020) [1].

2.5.5 Casein hydrolysis
Skim milk agar with pH 7.2 was inoculated with culture and incubated at 30 °C for 24-48 h. The clearing zone around the streaked line of growth was found to be positive (Aneja, 2020) [1].

2.5.6 Gelatin hydrolysis
Using Simmon's citrate agar, a citrate utilization test was

carried out as described by Safary *et al*. (2013) [15]. Further, incubated at 28 ± 2 °C for 48 h. A positive outcome was determined when the color of the medium changed from green to blue (Das *et al*., 2019) [3].

3. Results and Discussions

3.1 Isolation of endophytic bacteria

In order to isolate bacterial endophytes, stem and root samples of weed species were collected from the areas of dryness in the UAS, GKVK, Bengaluru, India (560065). Based on colony morphology, 18 isolates (Table 2) were chosen to be characterized for the synthesis of different enzymes.

3.2 Morphological characterization of endophytic cultures

Endophytic cultures displayed a varied colony morphology. Cream, white, creamy white, light yellow, yellow, and creamy yellow were among the several colony colors. The colony border has appeared to be entire and erose. The elevation, however, was shown to be flat, elevated, and convex. The cultures could be described as opaque, transparent, and translucent, depending on their degree of opacity. (Table 3).

3.3 Biochemical characterization

Table 5 lists the results of the biochemical tests performed on all 18 endophytic bacterial isolates, including the Voges-Proskauer, methyl red test, H_2S production, catalase activity, carbohydrate fermentation, citrate agar test, and indole production.

Microscopic examinations showed that the isolates of endophytic bacteria are both Gram-positive (38.88%) and Gram-negative (61.11%), with morphologies ranging from rod (55.55%) to cocci (44.44%). Additionally, endophytes were classified as endospore (33.33%) or non-endospore (66.66%) producers via the endospore staining (Table 4).

Table 2: Source of bacterial endophytes from weed species

Isolates	Plant part used	Weed species
ACR1A	Root	*Ageratum conyzoides*
ACR1B		
ACR1C		
ACS1A	Stem	
ACS2A		
ACS2B		
PHR1A	Root	*Parthenium hysterophorus*
PHR2A		
PHR2B		
PHR2C		
PHS2A	Stem	
PHS2B		
TPR1A	Root	*Tridax procumbens*
TPR2A		
TPS1A	Stem	
TPS1B		
TPS2A		
TPS2B		

By adding alcoholic methyl red, approximately 44.44% of endophytic bacterial isolates produced a bright red color during the methyl red test, and 44.44% of the endophytes were discovered positive during the Voges-Proskauer test by producing a pink color. Similar findings were made by Novero and Labrador (2014) [12], who discovered all 8 bacteria of endophytic origin tested positive in the Voges-Proskauer test, catalase, and citrate consumption, but negative for indole and methyl red synthesis. Based on the findings, it was determined that 44.44% of the endophytic bacterial isolates produced H_2S through blackening along the inoculation line. Out of the 7 endophytic bacterial isolates from *Mussaenda roxburghii* that were chosen, Pandey *et al*. (2015) [13] reported that isolates PAK6 and PAK8 produced H_2S.

Table 3: Morphological attributes of endophytic bacterial isolates colony

Isolates	Color	Margin	Elevation	Opacity
ACR1A	Cream	Entire	Convex	Opaque
ACR1B	Cream	Entire	Convex	Opaque
ACR1C	Yellow	Erose	Convex	Opaque
ACS1A	Creamy Yellow	Entire	Convex	Opaque
ACS2A	Creamy Yellow	Erose	Convex	Transparent
ACS2B	Yellow	Entire	Convex	Opaque
PHR1A	Cream	Entire	Raised	Opaque
PHR2A	Cream	Erose	Flat	Transparent
PHR2B	Creamy White	Entire	Flat	Opaque
PHR2C	Cream	Entire	Raised	Opaque
PHS2A	Cream	Entire	Flat	Opaque
PHS2B	Creamy Yellow	Entire	Flat	Opaque
TPR1A	Yellow	Entire	Flat	Translucent
TPR2A	Cream	Entire	Flat	Opaque
TPS1A	White	Entire	Convex	Opaque
TPS1B	Light Yellow	Entire	Raised	Opaque
TPS2A	White	Entire	Flat	Opaque
TPS2B	Creamy Yellow	Entire	Convex	Opaque

Different sources of carbohydrates were added to the fermentation broth in which endophytic bacterial isolates were cultured, and color changes and gas production were monitored. With or without gas production, about 66.66% of the endophytes were discovered to be acid producers, while the remaining 33.33% of endophytes had unsatisfactory results in tests for carbohydrate fermentation when given glucose. While 55.55% of the isolates tested negative for carbohydrate fermentation when given sucrose as a supplement, with or without the formation of gas, 44.44% of the isolates were acid makers. Even Khanam and Chandra (2015) [10] observed that *Serratia marcescens*, a bacterial

endophyte isolated from *Beta vulgaris*, produced sugar that fermented well when glucose and sucrose were added as supplements.

Table 4. Morphological characters of bacterial endophytes

Sl. No.	Isolates	Gram staining		Endospore formation
		Gram reaction	Shape	
1	ACR1A	-	Cocci	-
2	ACR1B	+	Rod	+
3	ACR1C	+	Rod	-
4	ACS1A	-	Rod	-
5	ACS2A	-	Rod	-
6	ACS2B	-	Cocci	-
7	PHR1A	+	Cocci	-
8	PHR2A	+	Rod	+
9	PHR2B	-	Rod	+
10	PHR2C	-	Cocci	-
11	PHS2A	-	Cocci	-
12	PHS2B	+	Rod	-
13	TPR1A	-	Cocci	+
14	TPR2A	-	Cocci	-
15	TPS1A	-	Rod	+
16	TPS1B	+	Cocci	-
17	TPS2A	+	Rod	+
18	TPS2B	-	Rod	-

The exception being 11.11% of endophytes, it was discovered that the majority of the endophytic isolates produced effervescence during a catalase test, indicating a positive result. 66.24 % of the 77 endophytic bacteria discovered in lowland rice roots were able to produce catalase enzyme according to Phuong *et al*. (2021) [14]. At the top of the test tube's alcoholic layer, 22.22% of the 18 endophytic bacterial isolates were brought in a pink to red color, demonstrating their ability to manufacture indole. 55.55% of the isolates were discovered using citrate when incubated slants' medium color changed from green to blue, however, the remaining 44.44% of the isolates were determined to be not utilizing citrate. *Serratia marcescens* had positive citrate test findings (Khanam and Chandra, 2015) [10].

Table 5. Biochemical characters of bacterial endophytes

Isolates	Methyl Red	Voges-Proskauer	H_2S production	Carbohydrate fermentation		Catalase activity	Indole production	Citrate agar test
				Glucose	Sucrose			
ACR1A	-	-	-	-	-	+	-	-
ACR1B	+	+	+	A	AG	+	-	-
ACR1C	+	+	+	A	AG	+	+	-
ACS1A	+	+	-	AG	-	+	-	+
ACS2A	-	-	+	AG	A	+	-	+
ACS2B	-	+	+	AG	A	+	+	+
PHR1A	-	-	-	AG	-	+	+	+
PHR2A	-	+	-	-	-	+	-	+
PHR2B	+	+	+	A	-	+	-	+
PHR2C	+	-	-	-	-	+	-	+
PHS2A	-	-	+	A	AG	+	-	+
PHS2B	+	-	-	AG	-	+	-	+
TPR1A	+	+	-	-	A	-	-	+
TPR2A	-	-	-	AG	A	-	-	-
TPS1A	-	-	+	AG	AG	+	+	-
TPS1B	-	+	+	-	-	+	-	-
TPS2A	-	-	-	AG	-	+	-	-
TPS2B	+	-	-	-	-	+	-	-

3.4 Screening for the production of hydrolytic enzymes

Further, the isolates were characterized for the hydrolysis of starch, urea, cellulose, pectin, casein, and gelatin, and the results obtained are presented in table 6. Hydrolytic enzymes are the main source for the mineralization of organic compounds in soil and hence participate in biogeochemical cycles and bioremediation processes.

Table 6: Production of hydrolytic enzymes by bacterial endophytes

Isolates	Starch hydrolysis	Urea hydrolysis	Cellulose hydrolysis	Pectin hydrolysis	Casein hydrolysis	Gelatin hydrolysis
ACR1A	+	-	-	-	-	-
ACR1B	-	+	-	-	-	+
ACR1C	+	+	-	-	+	-
ACS1A	+	+	-	-	-	-
ACS2A	+	+	-	+	-	-
ACS2B	-	+	-	-	-	-
PHR1A	+	+	-	-	+	-
PHR2A	-	+	-	-	-	+
PHR2B	+	+	-	-	+	-
PHR2C	+	-	-	-	-	+
PHS2A	+	+	-	-	+	+
PHS2B	+	+	-	-	-	+
TPR1A	+	+	-	-	-	-
TPR2A	+	-	-	-	-	-
TPS1A	+	+	+	-	-	-
TPS1B	-	-	-	-	-	+
TPS2A	+	-	-	-	-	-
TPS2B	+	-	-	-	-	-

Endophytic bacterial isolates were found to have amylase activity through starch hydrolysis. Out of 18 isolates 77.77% of the isolates were found to hydrolyze starch and couldn't by 22.22% of the isolates. Khan *et al*. (2017) [9] reported xylanases, phytases, hemicellulases, proteases, asparaginase, pectinases, cellulases, tyrosinase, chitinase, gelatinase and amylases are some synthesized enzymes by bacterial endophytes. Urea is an important source of nitrogen to crop species, made accessible to plants through the urease enzyme. In the present test, 66.66% of the isolates were found to have urease activity through the change in medium color to purple pink. Cellulose in the soil forms the source of organic carbon content through microbial degradation. The presence of cellulolytic activity was detected in around 5.55 percent of the endophytic bacteria. This may be because the isolates were endophytic ones. Sharma *et al*. (2020) [18] isolated a single cellulase-degrading endophytic bacteria *Hafnia* sp. from *Capsicum chinensis*. Whereas, out of 18 isolates 5.55% were found to have pectinolytic activity. *Paenibacillus amylolyticus*, an endophytic bacteria isolated from coffee cherry produced an extracellular pectinase enzyme as reported by Sakiyama *et al*. (2001) [16].

Around 22.22% of endophytic bacterial isolates were positive for casein hydrolysis and 77.77% were negative for the test. Whereas, 33.33% were found positive for gelatin hydrolysis through liquefaction of the inoculated medium even after immersion under an ice bath. Following seven days of incubation at different rates of hydrolysis, Phuong *et al*. (2021) [14] revealed that out of 77 endophytic bacterial isolates from lowland rice roots, 76 (98.71%) exhibited gelatin liquefaction ability and 58 (75.33%) isolates were able to hydrolyze starch. 51 of the 77 isolates tested (66.24%) were capable of producing catalase.

Conclusion

If we know how to approach microorganisms via biotechnological methods, they are what supply us with the most. Bacterial endophytes have the potential to benefit humanity in that situation. The diversity of endophytic bacteria found in the Asteraceae plant species has been demonstrated by this study. These endophytic bacterial isolates' characterization revealed the diverse biochemical activity that they have evolved with and further, the potential of bacterial endophytes was discovered via screening for the synthesis of hydrolytic enzymes via the hydrolysis of starch, urea, pectin, cellulose, casein, gelatine, which also looks at how these microbes are used in bioremediation, agriculture, biotechnology, and in other fields. Therefore, bacterial endophytes are useful scientific instruments, and if they can be used ethically, it might result in a sustainable environment and, consequently, a healthy way of life.

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References

1. Aneja KR. Experiments in microbiology, plant pathology, tissue culture and microbial biotechnology. New Age International Publishers, New Delhi; c2020.
2. Anjum N, Chandra R. Endophytic bacteria: optimization of isolation procedure from various medicinal plants and their preliminary characterization. Asian Journal of Pharmaceutical and Clinical Research. 2015;8(4):233-238.
3. Das, Priyanka, Chatterjee S, Behera BK, Dangar TK, Das BK *et al*. Isolation and characterization of marine bacteria from east coast of India: functional screening for salt stress tolerance. Heliyon. 2019;5(6):e01869.
4. Fatema K, Mahmud NU, Islam T. Beneficial effects of weed endophytic bacteria: diversity and potentials of their usage in sustainable agriculture. Agronomic Crops Publisher: Springer International Publisher. 2019;32:978-981.
5. Gerhardt P, Murray RGE, Costilow RN, Nester EW, Wood WA, Krieg NR *et al*. Manual of methods for general microbiology. ASM Press, Washington DC; c1981.
6. Hallmann J, Berg G. Spectrum and population dynamics of bacterial root endophytes. [(Eds.) B. Schulz B, Boyle C, Sieber, T], Microbial Root Endophytes, Germany; c2006. p. 15-31.
7. Kalaivanan SD, Mohan V. Screening and molecular characterization of salt tolerant bio-control bacterial

isolates from *Casuarina equisetifolia* rhizosphere soil. Asian Journal of Plant Pathology. 2017;11(4):156-166.
8. Kandel SL, Joubert PM, Doty SL. Bacterial endophyte colonization and distribution within plants. Microorganisms. 2017;5(4):77.
9. Khan AL, Shahzad R, Ahmed A, Lee I. Endophytic microbes: A resource for producing extracellular enzymes. Sustainable Development and Biodiversity; c2017. p. 95-110.
10. Khanam B, Chandra R. Isolation and identification of endophytic bacteria producing bright red pigment from the dye yielding plant *Beta vulgaris*. International Journal of Pharmacy and Pharmaceutical Sciences 2015;7(5):220-224.
11. Li, Lan-Yu, Yang Z, Asem MD, Salam N, Xiao, M *et al*. *Georgenia alba* sp. nov., a novel halotolerant actinobacterium isolated from a desert sand sample. Antonie Van Leeuwenhoek. 2019;112(2):203-209.
12. Novero A, Labrador K. Isolation and characterization of bacterial endophytes associated with sago palm (*Metroxylon sagu* Rottb.) in tissue culture. Asian Journal of Microbiology, Biotechnology & Environmental Sciences. 2014;16(4):777-786.
13. Pandey PK., Samanta R, Yadav RNS. Plant beneficial endophytic bacteria from the ethnomedicinal *Mussaenda roxburghii* (Akshap) of Eastern Himalayan Province, India. Advanced Biology. 2015;580510:1-8.
14. Phuong NV, Linh NT, Duy TD, Nam KTL, Thu Giang DD, Thuy LTT *et al*. Identification and evaluation of enzymes gelatinase, amylase, and catalase produced by rice root endophytic bacteria isolated from Hai Duong with antimicrobial properties against *Xanthomonas oryzae* pv. *oryzae*. Academia Journal of Biology. 2021;43(2):107-117.
15. Safary A, Moniri R, Mirhashemi SM, Nikzad H, Khiavi, MA. Phylogenetic and biochemical characterization of a new halo-thermotolerant, biofilm-forming bacillus from saline lake of Iran. Polish Journal of Microbiology 2013;62(4):419-425.
16. Sakiyama CCH, Paula EM, Pereira PC, Borges AC, Silva DO. Characterization of pectin lyase produced by an endophytic strain isolated from coffee cherries. Letters in Applied Microbiology. 2001;33(2):117-121.
17. Sharma A, Kashyap PL, Srivastava AK, Bansal YK, Kaushik R. Isolation and characterization of halotolerant bacilli from chickpea (*Cicer arietinum* L.) rhizosphere for plant growth promotion and biocontrol traits. European Journal of Plant Pathology. 2019;153(3):787-800.
18. Sharma A, Singh P, Sarmah BK, Nandi SP. Isolation of cellulose-degrading endophyte from *Capsicum chinense* and determination of its cellulolytic potential. Biointerface Research in Applied Chemistry. 2020;10(6):6964-6973.
19. Singh R, Kumar M, Mittal A, Mehta PK. Microbial enzymes: industrial progress in 21st century. 3 Biotech. 2016;6(2):174.
20. Smith AC, Hussey MA. Gram staining protocol. ACM Microbelibrary-Laboratory protocols. American Society for Microbiology, Washington, USA; c2005. p. 14.
21. Ullah A, Nisar M, Ali H, Hazrat A, Hayat K, Keerio AA *et al*. Drought tolerance improvement in plants: an endophytic bacterial approach. Applied Microbiology and Biotechnology. 2019;103:7385-7397.
22. Van Thuoc, Doan Hien TT, Sudesh K. Identification and characterization of ectoine-producing bacteria isolated from Can Gio mangrove soil in Vietnam. Annals of Microbiology; c2019. p. 1-10.
23. Verma SC, Ladha JK, Tripathi AK. Evaluation of plant growth promoting and colonization ability of endophytic diazotrophs from deep water rice. Journal of Biotechnology. 2001;91(2-3):127-141.