Effect of Endophytic *Serratia marcescens* Isolated from *Bryophyllum pinnatum* against Clinical Bacterial Isolates

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Abstract. The world's health is being threatened by antimicrobial resistance (AMR). According to the World Health Organization, it is one of humanity's top ten worldwide public health problems. *Serratia marcescens* is an opportunistic pathogen that can be isolated from soil, plants, water, and air. Additionally, *Serratia* species offer a valuable supply of secondary metabolites that are comparatively underutilised and may have anti-MDR pathogenic properties. The present research aimed to determine the antibacterial potential of *Serratia marcescens* isolated from the leaves of *Bryophyllum pinnatum* against clinical bacterial isolates. The leaves of *Bryophyllum pinnatum* were collected, surface sterilised, cultured at 37 °C for 24 hours and identified utilising viteks 2 automated techniques and molecular methods. The crude metabolites extract of *Serratia marcescens* were extracted and utilised for antibacterial susceptibility testing using agar healthy diffusion methods. The data were measured in the diameter zone of inhibition. This study revealed six endophytic bacteria were isolated from *Bryophyllum pinnatum* following standard microbiological culture methods. The endophytic bacteria isolate tag L03 was found to be Gram-Negative Rod. The isolate was tentatively identified as *Serratia ficaria* and molecularly identified as *Serratia marcescens*. The metabolites of *Serratia marcescens* endophytes revealed a significant antibacterial activity on *Klebsiella pneumoniae* with a diameter zone of inhibition of 17.7 mm at 100% concentration, followed by *Staphylococcus sciuri* with a 12.7 mm diameter zone of inhibition. These results suggested that endophytic bacteria *Serratia marcescens* were isolated from the leaves of *Bryophyllum pinnatum* and had shown potent antibacterial activity that could be employed to create new antibacterial agents.

Keywords: Endophytic bacteria; Serratia marcescens; Bryophyllum pinnatum; Antibacterial activity.

INTRODUCTION

Increased antibiotic resistance has recently become a significant obstacle in healthcare, presenting a considerable danger to public health worldwide. Excessive and incorrect usage of antibiotics has expedited the emergence of resistance in disease-causing bacteria, making numerous traditional medicines ineffective [11]. This worrisome pattern highlights the immediate necessity for new tactics to address bacterial infections. Investigating medicinal and endophytic bacteria as possible reservoirs of new antimicrobial substances has gained significant interest.

Bryophyllum pinnatum Lam. a member of the Crassulaceae family, is native to Madagascar and is also found in parts of Asia, America, New Zealand, Australia, and Africa. The leaves of this plant, commonly known as "folha-da-fortuna" in Brazil, are used to treat asthma and bronchitis. Additionally, it is applied externally to treat burns, eye infections, blisters, kidney stones, wounds, ulcers, and insect bites [4].

Endophytes are symbiotic organisms that reside within plant tissues for at least a portion of their life cycles without causing harm or disease [8]. Endophytic bacteria present a hopeful opportunity for identifying novel antibiotics and medicinal substances. Unlike traditional antibiotics from soil bacteria or fungi, endophytes reside in a specific ecological environment within plants. This environment exposes them to various selection pressures and promotes the creation of bioactive chemicals with unique chemical structures and modes of action [15].

Serratia marcescens is an endophyte bacterium that has been shown to affect plants positively. *Serratia marcescens* is beneficial in several scientific journals. This bacterium is classified as a Gram-negative member of a genus with two subspecies and fourteen recognised species. As per [1], *Serratia marcescens* is a rod-shaped, gram-negative *Enterobacteriaceae* bacterium. Its ability to produce prodigiosin, a red pigment, even at room temperature is one of its unique qualities [14].

Serratia are prevalent in the environment and can be found in plants, animals, food, soil, and water. *Serratia* species are a great source of secondary metabolites that have not yet been fully utilised and may be active against Multi-Drug Resistant pathogens.

MATERIALS AND METHODS

Collection of leaves of *Bryophylum pinnatum.* Fresh and healthy leaves of *Bryophyllum pinnatum* were picked and collected from the garden in a sealed zipper bag. They were identified and validated at the Department of Biological Sciences, Edo State University, Uzairue, Nigeria.

Isolation of Endophytic *Serratia marcescens.* The leaves were chopped into little bits of 5.5 mm (length and width) with sterile blades. These parts were cleaned three times using tap and sterile water. The leaflets were washed twice with sterile water, with the parts remaining in each wash for 3 minutes. The leaf pieces were surface sterilised for one minute in 70 % ethanol and then soaked in 2% sodium hypochlorite solution for 5 minutes. The samples were then washed three times in sterile distilled water and placed on Nutrient Agar, and a sterility test was also done by inoculating an aliquot of the last rinse water on NA.

Morphological and Biochemical Characterisation of Endophytic Serratia marcescens. The primary characterisation of *Serratia marcescens* was performed through Gram Staining and biochemical tests using vitek 2 compact systems. The endophytic bacterial isolates were stained using Gram staining techniques [12] and biochemical tests using vitek 2 compact systems as described [16].

Molecular Characterization of Endophytic Serratia marcescens. The isolate underwent another identification round using molecular techniques to verify the primary identification. The DNA of the endophytic bacterial isolate was isolated and purified utilising a zymo bacterial mini prep extraction kit in line with the manufacturer's instructions. Primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-CGGTTACCTTGTTACGA CTT-3') were used to amplify the 16S rRNA gene at the following temperatures: 95^{0}C for two minutes; 30 cycles of 95°C for thirty seconds; 55°C for forty-five seconds; 72°C for ninety seconds; and 72°C for the final elongation of seven minutes. 50 ng of DNA, 30 µl of each primer, and 12.5 µl of Thermo Scientific's 2x Dream Taq PCR Master Mix were all included in the 25 µl PCR mixture. PCR was carried out using an amplifier Master cycler (Thermo Scientific). PCR products were separated in a 1% agarose gel containing 0.01% ethidium bromide. UV light was used to visualise the data. The resultant amplicon, about 1500 bp in size, was extracted from the gel and purified using the BigDye Terminator v3.1 cycling sequencing Kit, manufactured by applied Biosystem and sequenced utilising ABI 3500 sequencer [9].

Extraction of Endophytic Bacteria Secondary Metabolite. The endophytic *Serratia marcescens* were subjected to submerged fermentation in a sterile Nutrient broth medium as described by [2]. An inoculum development was formed by inoculating a few colonies of *Serratia marcescens* from a 24-hour pure culture growth into a 100 ml Erlenmeyer flask containing 15 ml of nutrient broth. It was incubated for 24 hours at 37 °C. Afterwards, the inoculums were transferred onto separate 100 ml Erlenmeyer flasks containing 100 ml of nutrient broth. This was then allowed to stay incubated at 200 rpm for 72 hours, utilising a rotary shaker. Following fermentation, the culture broth was strained, and ethyl acetate was used three times to extract the filtrate; after being run through sodium sulphate, the organic phase evaporated until it was scorched.

Antibacterial Activity of Endophytic Serratia marcescens. Antibacterial evaluation of the *Serratia marcescens* crude metabolites on laboratory strains of *Escherichia coli*, *Staphylococcus aureus, Staphylococcus sciuri,* and *Klebsiella pneumoniae* was carried out using the agar well diffusion method as described by [3]. Meropenem (5 µg/ml) was used as a positive control, and DMSO (100% v/v) was used as a negative control. A volume of 0.1 ml of different concentrations (100, 50, 25%, and 12.5%) of the metabolite's supernatant was transferred into an agar well of plates streaked with *Escherichia coli, Staphylococcus aureus, Staphylococcus sciuri,* and *Klebsiella pneumoniae.* The plates were incubated for 24 hours at 37 °C. The experiment was conducted in triplicate, and the resulting diameter zone of inhibition was measured in millimetres (mm).

Figure 1

RESULTS AND DISCUSSION

Following standard procedures of surface sterilisation and culturing of the fresh leaves of *Bryophyllum pinnatum* for 24 hours at 37 °C, the endophytic bacteria *Serratia marcescens* (L03) was isolated and revealed to be circular, raised, smooth, small, and bright red on nutrient agar plate after 24 hours of incubation. The isolate was determined to be a Gram-Negative rod, as represented in Table 1. A similar result was obtained from the research of [16].

The endophytic bacteria isolate (L03) was identified as *Serratia ficaria* using the substrates card of Vitek 2 bacteriological identification system (BioMeriex) based on the biochemical and substrates characteristics with a probability percentage of 94% identification as displayed in Table 2.

Table 1 – Cultural and Morphological Characteristics of *Serratia marcescens*

Parameters	Endophytic *Serratia marcescens*
Shape	Circular
Elevation	Raised
Colour	Bright red
Size	Small
Transparency	Opaque
Surface	Smooth
Gram stain	Gram-Negative Rod

Table 2 – Vitek 2 Biochemical Identification of *Serratia marcescens*

Tests Substrates	Amount (mg)	LF3
Glutamylarylamidasepna	0.0324	-
Adonitol	0.1875	+
D-Mannose	0.3	+
L-Pyrrolydonyl-Arylamidase	0.018	-
Beta-Galactosidase	0.036	-
L-Arabitol	0.3	+
D-Maltose	0.3	+
D-Cellobiose	0.3	+
L-prolinearylamidase	0.0234	-
Beta-N-Acetyl-Glucosaminidase	0.0408	-
D-Glucose	0.3	+
Fermentation/Glucose	0.45	-
H_20 Production	0.0024	-
Beta-Glucosidase	0.036	+
D-Mannitol	0.1875	+
Urease	0.15	-
Beta-Xylosidase	0.0324	-
Saccharose/Sucrose	0.3	+
Beta-Alanine arylamidasepna	0.0174	-
Lipase	0.0192	-
Palatinose	0.3	+
Tyrosine Arylamidase	0.0276	-
Gamma-Glutamyl-Transferase	0.0228	-
D-Sorbitol	0.1875	+
D-Tagalose	0.3	-
5-Keto-D-Gluconate	0.3	-
D-Trehalose	0.3	+
Ala-phe-pro-Arylamidase	0.0384	-
Malonate	0.15	-
Citrate (Sodium)	0.054	-
L-Lactate Alkalinisation	0.15	-

Tests Substrates	Amount (mg)	LF3
Alpha-Glucosidase	0.036	-
Succinate Alkalinisation	0.15	-
Beta-N-Acetyl Galactosaminidase	0.0306	-
Alpha-Galactosidase	0.036	-
Phosphatase	0.0504	-
L-Histide Assimilation	0.087	-
Ornithine Decarboxylase	0.3	-
L-Lactase Assimilation	0.186	-
Lysine Decarboxylase	0.15	-
Beta-Glucoronidase	0.0378	-
Glucine Arylamidase	0.012	-
Glu-Glu-Arg-Arylamidase	0.0576	-
Coumarate	0.126	+
L-Malate Assimilation	0.042	-
O/129 Resistance (comp. Vibrio)	0.0105	-
Ellman	0.03	-
Characterised as	*Serratia ficaria*	

The isolate (L03) was further subjected to molecular characterisation utilising 16 SrRNA genes, which was confirmed to be identified as *Serratia marcescens* with a similarity percentage of 83.2% with *Serratia marcescens* strain Tc-8 when compared using BLAST at the National Center for Biotechnology Information (NCBI) as shown in Table 3.

As the rule implies, the accession number was not obtained due to the low similarity percentage of 83.2%, below 90%. This finding is similar to the report of several researchers. According to reports, *Serratia marcescens* has previously been identified from a variety of plants, including rice [6], leaves of *Vaccinium uliginosum* [17], and tomatoes [13]. Nonetheless, this is the first time an endophytic *Serratia marcescens* has been identified from the plant *Bryophyllum pinnatum.*

Following fermentation and secondary metabolite extraction from *Serratia marcescens*, the crude metabolites extract was tested for antibacterial activity against four bacterial isolate strains: *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli,* and *Staphylococcus scuiri.* The antibacterial analysis shows that the crude metabolites extract of *Serratia marcescens* displayed a broad spectrum antibacterial activity, with *Klebsiella pneumoniae* having the highest diameters zone of inhibition of 17.7 mm, followed by *Staphylococcus scuiri* 12.7 mm compared to *Staphylococcus aureus* and *Escherichia coli* 2.7 mm and 2.0 mm respectively as showed in Table 4.

Table 3 – Molecular Characterisation of Endophytic Bacteria *Serratia marcescens*

Endophytic bacteria isolate	Molecularly characterised isolate	Pairwise Percentages (%)	Similarity Percentages (%)	Accession Number
Isolates L03	*Serratia marcescens* strain Tc-8	84.9	83.2	NIL

Table 4 – Antibacterial Effect of *Serratia marcescens* Metabolites Crude Extract on Clinical Isolates

Concentrations	Diameter zone of inhibition (mm)			
	Klebsiella pneumoniae	*Staphylococcus aureus*	*Staphylococcus sciuri*	*Escherichia coli*
100	17.7	2.0	12.7	2.7
50	11.3	1.3	6.3	1.7
25	6.7	00	3.7	00
12.5	00	00	00	00
Positive Control (MEROPENEM)	32	11	21	15
Negative Control (DMSO)	00	00	00	00

Authors [10] reported the antibacterial activity of *Serratia marcescens* metabolites on *Staphylococcus aureus* and *Klebsiella pneumoniae*. This could result from the biosynthesis of prodigiosin, an antimicrobial compound Serratia produces.

CONCLUSIONS

In conclusion, *Serratia marcescens* endophytic bacteria was isolated and identified from the leaves of *Bryopphyluum pinnatum*. The crude

metabolites of *Serratia marcescens* displayed moderate antibacterial potential on gram-negative and Gram-positive bacteria.

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Conflict of Interests

There was no conflict of interest in preparing this writing piece.

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