

Antimicrobial Potential of Paenibacillus Polymyxa AALI Endophyte Isolated from Calotropis Procera

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Abstract – The increased drug resistance spectrum of bacteria pathogens has become a major concern worldwide. The present study addresses the isolation and identification of potent endophytes associated with *Calotropis procera*. Based on 16S rRNA gene analysis, an endophyte strain AALI showing promising antibacterial activity was identified as *Paenibacillus polymyxa*. The cell-free supernatant was extracted with ethyl acetate and the crude extract exhibited feasible antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*. Herein, we highlighted the promising antimicrobial activity of *P. polymyxa* strain AALI endophyte and results suggest the potential use of its crude extract to combat bacterial pathogens, especially *S. aureus*.

Keywords – Antimicrobial, Endophyte, *Paenibacillus*, Screening.

I. INTRODUCTION

Calotropis procera is a shrub plant native to tropical regions and has been used in folk medicine due to presence of various active compounds including alkaloids, triterpenoids, anthocyanins, and tannins [1], [2]. Endophytes are microorganisms living within plant tissues, at least for part of their life cycles, without causing visible symptoms of disease [3]. The emergence of multidrug-resistant pathogens has prompted researchers to find new of biologically active compounds. It has been reported that secondary metabolites produced by endophytic microorganisms mediate promising biological activities [4], [5]. A broad range of biologically active polyketide and peptide compounds with applications in medicine, agriculture, are synthesized by endophytes [6]–[9]. These structurally diverse metabolites include antibiotics, antifungals, antitumor agents, and

immunosuppressive agents [10]–[12]. This study addresses the isolation of *Paenibacillus polymyxa* strain AALI from *Calotropis procera* with promising antimicrobial activity against various bacterial pathogens.

II. MATERIALS AND METHODS

A. Isolation of endophytes

Healthy leaves of *Calotropis procera* were collected and transported to the laboratory. After gently washing in water, leaves were surface sterilized by immersion in 70 % ethanol for 1 min, followed by sodium hypochlorite (2.5 % active chlorine) for 4 min, then in 70 % ethanol for 30 s, and washed three times in sterile distilled water [13]. Several fragments were aseptically cut from the sterilized leaves and homogenized in sterile saline. Afterwards, the homogenate was diluted up to 10⁻⁶ and spread onto tryptic soy agar (TSA)

plates (Himedia, India). The inoculated plates were incubated at 28°C for 72 h. Then several colonies with different shapes were picked and subjected to purification process by streaking on TSA plates three times.

B. Screening for antimicrobial activity

The initial screening of antibacterial activity was conducted using a well-dilution method against *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*. Each bacterial endophyte was inoculated into tryptic soy broth and incubated at 28°C for 72 h then the culture was centrifugated at 15000 rpm for 15 min. The cell-free supernatant of each bacterial endophyte was assessed for antimicrobial activity. Fifty microliters of bacterial suspension (1.5×10^8 CFU/mL) of each pathogen was inoculated on surface of Luria-Bertani (LB) plates and spread uniformly by using a sterile glass spreader. After 20 min, 5 mm-Wells were made in the agar media using sterile cork borer. Afterwards, 50 µL cell-free supernatant of each of the bacterial endophyte was placed into each separate well and plates were incubated at 37°C for 24 h. Plates showing antibacterial activity were confirmed by visualization and measuring of inhibition zones around each well [14].

C. Molecular identification of the most active endophyte

Genomic DNA from the most active endophyte was extracted by GeneJET Genomic DNA Purification Kit (Thermo Scientific, USA). The 16S rRNA gene was amplified using universal primers 27F and 1492R. The PCR product was then was sequenced and the sequence was compared to sequences in National Centre for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov>) database using Basic Local Alignment Search Tool (BLAST) program. The pairwise sequence alignment of 16S rRNA gene sequence was done using ClustalW program and compared with the closest related species. Neighbor joining method was used for construction of phylogenetic tree based on bootstrap values (1000 replications with MEGAX software). The 16S rRNA gene sequence was deposited in GenBank.

D. Ethyl acetate extraction

The cell-free supernatant showing maximum inhibition after preliminary screening was extracted by solvent extraction method using ethyl acetate as the organic solvent. Briefly, an equal volume ethyl acetate was added to the cell-free supernatant in a separating funnel and mixed thoroughly for 10 min and kept for 5 min to obtained two clear immiscible layers. The upper layer was separated,

evaporated, and the resultant residues were dried in a rotary vacuum evaporator to yield the crude extract. The crude extract was then dissolved in 10% (v/v) dimethyl sulfoxide (DMSO) in distilled water and kept at 4°C.

E. Antibacterial activity of the crude extract

The antibacterial activity of the crude extract was evaluated using the standard Kirby-Bauer disc diffusion method. Mueller-Hinton agar plates were swabbed with test bacterial pathogens and discs loaded with 25 µg of crude extract were placed on the surface of the medium. After 30 min, the plates were incubated overnight at 37°C and the zones of inhibition were measured in millimetres.

III. RESULTS

A. Isolation and screening of endophytes

Thirty bacterial endophytes were isolated from surface-sterilized leaves of *C. procera*. Based on the screening results, the most active strain designated AALI showing obvious antimicrobial activity against all investigated bacterial pathogens was selected and subjected to further investigations.

B. Molecular identification and phylogenetic analysis

The strain AALI showing the maximum antimicrobial activity was identified as *Paenibacillus polymyxa*, according to 16S rRNA analysis (Fig. 1). The 16S rRNA gene sequence was submitted to the GenBank under accession number MT122904. It shared 99.48% similarity with *Paenibacillus polymyxa* strain DSM 36 (accession number: NR_114810), 99.04% similarity with *Paenibacillus jamilae* strain CECT 5266 (accession number: NR_042009), 98.67% similarity with *Paenibacillus peoriae* KCTC 3763 strain DSM 8320 (accession number: NR_112161), and 89.15% similarity with *Paenibacillus kribbensis* strain AM49 (accession number: NR_025169).

C. Antibacterial activity of the crude extract

After extraction of the cell-free supernatant of *Paenibacillus polymyxa* strain AALI by ethyl acetate, the antibacterial activity of the crude extract was evaluated using the standard Kirby-Bauer disc diffusion method. The crude extract exhibited promising antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*. The maximum antimicrobial activity was found against *S. aureus* (44 mm), followed by *E. coli* (28 mm), and *K. pneumoniae* (Table 1).

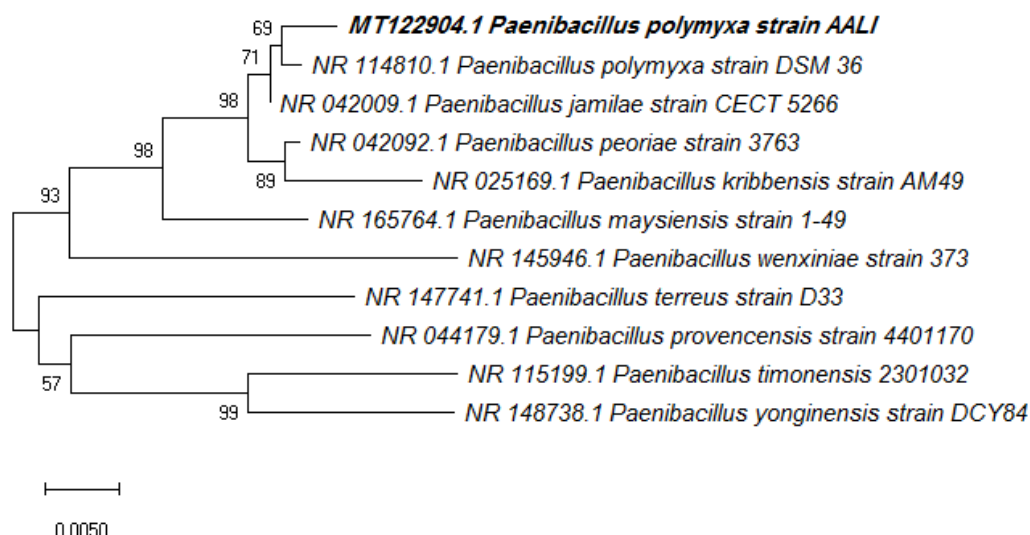


Figure 1: Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between *P. polymyxa* strain AALI and the most closely related species.

Table 1: Antimicrobial activity of *P. polymyxa* AALI crude extract against bacterial pathogens

Bacterial pathogen	Inhibition zone (mm)
<i>Staphylococcus aureus</i>	44
<i>Escherichia coli</i>	28
<i>Klebsiella pneumoniae</i>	25

IV. DISCUSSION

Recently, the increased drug resistance spectrum of bacteria pathogens has become a major concern due to the emergence of multi-drug resistant strains worldwide [15]. The present study aims to isolate endophytes from the selected medicinal plant, *C. procera*, and to explore their antimicrobial activities against a number of human pathogens. Endophytes have been reported to be found ubiquitous in all plant species and contribute to their host plants by production of metabolites that afford protection to the associated plants [16]. In the present study, the endophyte *P. polymyxa* strain AALI has been isolated from leaves of *C. procera* exhibited a potent antimicrobial activity against *S. aureus*, followed by *E. coli*, and *K. pneumoniae*. These findings are in good agreement with previous documents reported the antimicrobial activity of various strains of *P. polymyxa* [15], [17]–[20]. The crude extract of *P. polymyxa* AALI exhibited a notable

antimicrobial activity against both Gram-positive and Gram-negative bacteria, however, it showed a superior activity against *S. aureus* (Gram-positive) compared with *E. coli*, and *K. pneumoniae* (Gram-negative). Generally, Gram-negative bacteria were more resistant to various antimicrobial agents than Gram-positive which may be due to the characteristic difference of the outer membrane between Gram-positive bacteria and Gram-negative bacteria. Recently, a novel lantibiotic, paenibacillin, produced by *P. polymyxa* OSY-DF showing potency against *Listeria monocytogenes*, methicillin-resistant *S. aureus* and other Gram-positive bacteria has been documented [21]. Also, *P. polymyxa* JSa-9 had been found to produce five cyclic LI-F type antibiotics [22].

V. CONCLUSION

The present investigation highlighted the promising antimicrobial activity of *Paenibacillus polymyxa* strain AALI endophyte isolated from leaves of the tropical *Calotropis procera* shrubs. The results suggest the potential use of the crude extract to combat bacterial pathogens, especially *S. aureus*.

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