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Antibacterial activity of endophytes from *Musa Paradisiaca*

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ABSTRACT

This study was aimed to isolate the endophytic bacteria and fungi from the fruit and root of *Musa Paradisiaca*, commonly called as Nendran banana cultivar in the southern Indian state of Kerala. Endophytes are microbes living in plant tissue without harming the plants rather existing as potential beneficial organisms. *Musa paradisiaca* is a less explored variety of banana cultivar which possess anti-disease potential apart from nutritional quality. The total number of twelve root and fruit associated endophytes were isolated. The fruit associated endophytes were seven with four bacterial isolates and three fungal isolates. The number of root-associated endophytes were five with three bacterial isolates and two fungal isolates. The morphological characterisation of both root and fruit endophytes were performed by Gram staining for bacteria and lacto-phenol cotton blue staining for fungi. The bacterial colony examination of the fruit associated culture revealed a specific pink strain with an inhibitory zone proving antibacterial activity which was further investigated through agar well diffusion test. The strain exhibited potential antibacterial activity against five human clinical pathogens. A significant zone of inhibition was observed against *Bacillus subtilis* (22 mm), *Escherichia coli* (21 mm), *Klebsiella pneumonia* and *Pseudomonas aeruginosa* (18 mm) which confirmed the potential antibacterial property of the fruit endophytic strain.

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INTRODUCTION

Microbes that reside in internal tissues of plants without harming them or without abrupt negative effects are regarded as endophytes (Jr. *et al.*, 1997). Endophytes do not cause harm to the plant or produce external structures that arise from these plant tissues (Yates *et al.*, 1997; Holderness *et al.*, 2000). In general, endophytes are fungi and bacteria that play vital roles in the environmental adaptation process of plants (Souza *et al.*, 2014). In other terms, endophytes are a group of microorganisms that reside in the living tissue of different plants (root, fruit, stem, seed, leaf, etc.) instituting mutual relationships. These endophytes have a significant host-pathogen defence mechanism to protect the plant tissue that they dwell from infectious agents and hostile conditions through secretion of bioactive secondary metabolites (Leuchtmann and Clay, 1988; Jr. *et al.*, 1997). The endophytic fungi take part in vital physiological and ecological roles in the life of the plant host (Sandhu *et al.*, 2014). It has been reported previously that this residence of endophytic microbes provides additional defensive properties on the plant immune system and its regulation, as a consequence of the manipulation of direct antimicrobial metabolites such as indirect phytohormones to alkaloids, jasmonic acid, or salicylic acid, etc. (Yan *et al.*, 2019). Studies on occurrence and diversity of endophytic fungi expected to yield potential hub of natural products and bioactive compounds for various uses such as medicinal, agricultural, and industrial uses and also paves a way for new antibiotics as novel potential bio-control agents (White *et al.*, 2000; Yan *et al.*, 2019). Similarly, the endophytic bacteria have also known to enrich plant growth in non-leguminous crops and improve their nutrition through siderophore production, nitrogen fixation, or phosphate solubilisation (Ngamau, 2012; Rungin *et al.*, 2012; Souza *et al.*, 2014). Besides bio fertilization, endophytic bacteria are also reported to stimulate plant growth and efficiency of yield by directly generating phytohormones or enzymes, or biological control of plant pests and diseases or inducing resistance response indirectly (Ngamau, 2012). The alarming increase in the rate of antibiotic resistance globally, demands our attention to tackle the issue and endophytes harbouring naturally in the plant kingdom stand to be a category to be looked up as endophytes after gaining access in plant tissue produce a diverse range of anti-microbial products that can serve as a reliable and promising source of novel antibiotics (Bhore *et al.*, 2013).

There are minimal studies regarding the types of endophytes and its effect on their association with either the wild indigenous bananas or the commercial banana cultivars (Souza *et al.*, 2014). Banana is the common name for herbaceous plants of genus Musa. The fruit length maximum in *Musa Paradisiaca* (generally known as Nendran cultivar) is 23.93 cm (S and P.V, 2017). *Musa paradisiaca* is mainly cultivated in tropical, subtropical region countries and is extensively consumed in the southern Indian state of Kerala for its nutritional values and diverse aroma and taste (Imam and Akter, 2011; S and P.V, 2017). Many studies shows that banana is the richest source of various nutrients that have various health benefits for human (Holderness *et al.*, 2000; Leuchtmann and Clay, 1988). In the recent years, the study on endophytic microorganisms and the determination of their role in plants has taken a considerable interest (de Oliveira Costa *et al.*, 2012; Lopes *et al.*, 2015; Muthuri *et al.*, 2015). Apart from enhancing the plant nutrient absorption, endophytes induce a range of host benefits such as stimulation of growth, the formation of increased numbers of lateral roots and root hairs and thus increase in plant height, shoot weight and shoot diameter (Postma and Rattink, 1992; Larkin, 1996). These microorganisms are considered the potential source of compounds like antibiotics, antioxidants, anticancer drugs, and enzymes (Strobel and Daisy, 2003). An endophytic *Streptomyces* sp. with siderophore producing ability isolated from banana roots was found effective against *Fusarium* sp., the causative agent of wilt disease (Cao *et al.*, 2005). It is hypothesized that wild bananas may host diverse endophytes due to their co-evolution with the soil microbiota. The endophytes are perfect candidates as an environmentally suitable benign agent. Endophytes with growth-promoting properties are necessary not only for the agronomic enhancement of banana but also for their succeeding impact in enhancing tolerance to diseases via growth enhancement (Souza *et al.*, 2014).

The present study was undertaken to isolate endophytic fungi and bacteria from root and fruit tissues of *Musa paradisiaca*. While we carry out the isolation of endophytes from plant tissues, we found a particular fruit strain (FAE 4) unveiled antibacterial activity against all the other strains with a distinct inhibitory zone in the mother culture plate. This encouraged us to assess its antibacterial potential against a set of human clinical pathogens.

MATERIALS AND METHODS

Materials

Nutrient agar, Potato Dextrose Agar, Mueller Hinton agar and broth and antibiotic discs were purchased from Himedia, India. All other chemicals used were of analytical grade and purchased locally.

Bacterial strains

The five human clinical pathogens used in antibacterial susceptibility test include, *Escherichia coli* MTCC 901 (ATCC 13534), *Klebsiella pneumonia* MTCC 109 (ATCC 15380), *Pseudomonas aeruginosa* MTCC 8291, *Bacillus subtilis* MTCC-8114 and the only Gram-positive bacterium included in the study was *Staphylococcus aureus* MTCC 96 (ATCC 9144). All these bacteria were retrieved from Microbial Type Culture Collection (MTTC), Institute of Microbial Technology (IMTECH), and Chandigarh, India and revived and maintained on Nutrient agar (NA).

Plant material

Fully ripened Nendran banana fruits and roots were collected from local banana cultivars in Kerala and authenticated by Botanist. The samples were transported immediately to the laboratory and processed. Banana root was washed in running water to remove soil particles and surface sterilized by sequential immersion in 70% ethanol for 5 min and sodium hypochlorite for 20 min. Samples were then washed in sterile water three times to remove surface sterilization agents (Cao *et al.*, 2005).

Isolation of endophytic bacteria and fungi

The skin of a fully ripen Nendran banana fruit was peeled aseptically and made to suspension using sterile pestle and mortar along with sterile distilled water. The tissue suspension thus obtained was serially diluted, and aliquots were plated on NA and Potato dextrose agar (PDA) by spread plate method for the isolation of the bacteria and fungi respectively. Approximately one cm square blocks were cut from root tips aseptically using sterile cutters and placed on NA and PDA. The plates were then incubated at room temperature for 1-2 days for bacteria and 5-7 days for fungi with the daily examination. Discrete colonies developed were subcultured, and pure cultures were preserved by cold storage for subsequent study.

Morphological characterisation

The colony morphology examination of both root and fruit associated endophytes was done macroscopically for size, shape, colour, texture, etc. for bacteria and colour and appearance for fungi. The microscopic analysis was performed by Gram staining for bacteria to disclose their Gram's reaction and morphology and lacto-phenol cotton blue (LPCB) staining for fungi to reveal their hyphal and spore morphology apart from special structures if present any (Leck, 1999).

Antibacterial susceptibility test

The examination of bacterial endophytes from fruit tissue revealed that a specific pink mucoid colony (FAE 4) forming a zone of inhibition around it preventing the growth of adjacent bacterial colonies which is shown in Figure 1. To confirm its antibacterial efficacy, antibacterial susceptibility test by agar well diffusion method was carried out against five bacterial pathogens. The test and pathogenic bacterial strains were grown on Mueller Hinton agar (MHA), and suspensions were prepared to match 0.5 McFarland standard. 100 μl of each pathogenic bacterial suspension was spread on MHA plates using L-rod. Wells (6 mm diameter) were punched in agar using sterile borer. A test well was loaded with 50 μl of FAE 4 suspension and negative control well was loaded with the same volume of diluent. Gentamycin and ofloxacin were used as a positive control. The plates were incubated at 37ºC for 24 h. The diameter of the inhibition zones was computed in mm.

RESULTS AND DISCUSSION

Isolation of endophytic bacteria and fungi

From the root and fruit tissues of *Musa paradisiaca*, twelve endophytes were isolated. The fruit associated endophytes (FAE) were seven with four bacterial isolates designated as FAE 1, 2, 3 and 4 and three fungal isolates named as FAE 5, 6 and 7. The number of root-associated endophytes were five with three bacterial isolates termed as RAE 1, 2, and 3 and two fungal isolates labelled as RAE 4 and 5 (Table 1).

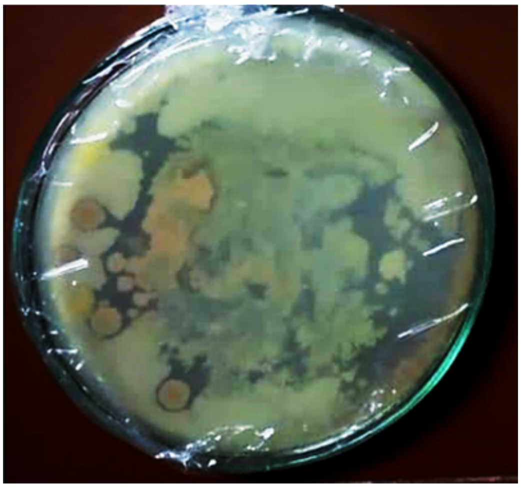

Figure 1: Strain FAE 4 with a zone of inhibition

Morphological characterisation of endophytes

On nutrient agar FAE 1 exhibited large irregular creamy white colonies, FAE 2 appeared large irregular chalky white colonies, FAE 3 displayed circular medium-sized yellow colour colonies, and FAE 4 represented circular medium-sized pale pink mucoid colonies. All the four fruit associated endophytic bacteria were Gram-positive cocci in nature. The three root-associated bacterial endophytes RAE 1, RAE 2 and RAE 3 revealed small circular pale coloured, large irregular creamy, small pale elevated colonies respectively on nutrient agar. Gram staining manifested that RAE 1and 3 were Gram-positive bacilli and RAE 2 was Gram-positive cocci (Table 1). The fruit associated fungal isolate FAE 5 portrayed large circular powdery colonies with bluish-green pigmentation on PDA while FAE 6 and 7 represented round smooth, creamy colonies and large creamy white colonies respectively. LPCB mount of FAE 5 displayed presence of unbranched conidiophore and exogenous chain of conidia suggesting *Aspergillus fumigatus*. FAE 6 and 7 presented circular to oval budding yeast cells. The root-associated fungal isolate RAE 4 produced large circular fuzzy colonies with black pigmentation on PDA, and RAE 5 yielded large circular powdery colonies with bluish-green pigmentation. LPCB staining of RAE 4 revealed unbranched conidiophore bearing black coloured conidia indicative of *Aspergillus niger*. RAE 5 demonstrated branched septate conidiophores with conidia evincing *Penicillium* sp. There are several reports on the isolation of *Aspergillus* sp. and *Penicillium* sp. as endo-

Table 1: Morphological characterisation of fruit and root-associated endophytes

Endophytes	Strain	Strain type	Colony morphology	Gram staining
Fruit associated endophytes	FAE 1	Bacteria	Large irregular creamy white colonies	Gram-positive cocci
	FAE 2	Bacteria	Large irregular chalky white colonies	Gram-positive cocci
	FAE 3	Bacteria	Circular medium-sized yellow colour colonies	Gram-positive cocci
	FAE 4	Bacteria	Circular medium-sized pale pink mucoid colonies	Gram-positive cocci
	FAE 5	Fungi	Large circular powdery colonies with bluish-green pigmentation	Unbranched conidiophore and exogenous chain of conidia
	FAE 6	Fungi	Round, smooth, creamy colonies	Circular to oval budding yeast cells
	FAE 7	Fungi	Round, smooth, creamy colonies	Circular to oval budding yeast cells
Root associated endophytes	RAE 1	Bacteria	Small circular pale coloured colonies	Gram-positive bacilli
	RAE 2	Bacteria	Large irregular creamy colonies	Gram-positive cocci
	RAE 3	Bacteria	Small pale elevated colonies	Gram-positive bacilli
	RAE 4	Fungi	Large circular fuzzy colonies with black pigmentation	Unbranched conidiophore with black coloured conidia
	RAE 5	Fungi	Large circular powdery colonies with bluish-green pigmentation	Branched septate conidiophores with conidia

Table 2: Antibacterial activity of FAE 4

Test Organism	Zone of Inhibition (in mm)	
	FAE 4	Standard
Bacillus subtilis	22 ± 0.5	26 ± 0.3
Pseudomonas aeruginosa	18 ± 0.4	23 ± 0.5
Klebsiella pneumoniae	18 ± 0.5	22 ± 0.6
Staphylococcus aureus	16 ± 0.5	21 ± 0.4
Escherichia coli	21 ± 0.4	25 ± 0.5

phytes from various plants with potential beneficial effects to the hosts (Ngamau, 2012; Yan *et al.*, 2019). Colony morphology and microscopic appearance of all endophytes are provided in Table 1. Microscopic appearance of representative endophytic fungi were shown in Figure 2.

A study on the efficacy of isoflavones disclosed that plants inoculated with *Aspergillus fumigatus* with/without salt stress contained higher levels isoflavones than uninoculated plants (Salas-Marina, 2011; Rai *et al.*, 2014). This suggested that under stress conditions, these endophytic fungi produce phytohormone which influences the secretion of surplus secondary metabolites and also aids plants to withstand stress (Shaaban and Hassan, 2014; Zhou *et al.*, 2013; Khan *et al.*, 2015). Compounds like saponins, phenolic compounds, anthraquinones, steroids, tannins and naphthoquinones were identified in *Aspergillus niger* and *Alternaria alternata*. These two endophytes have exhibited significant antimicrobial activity against an array of pathogenic

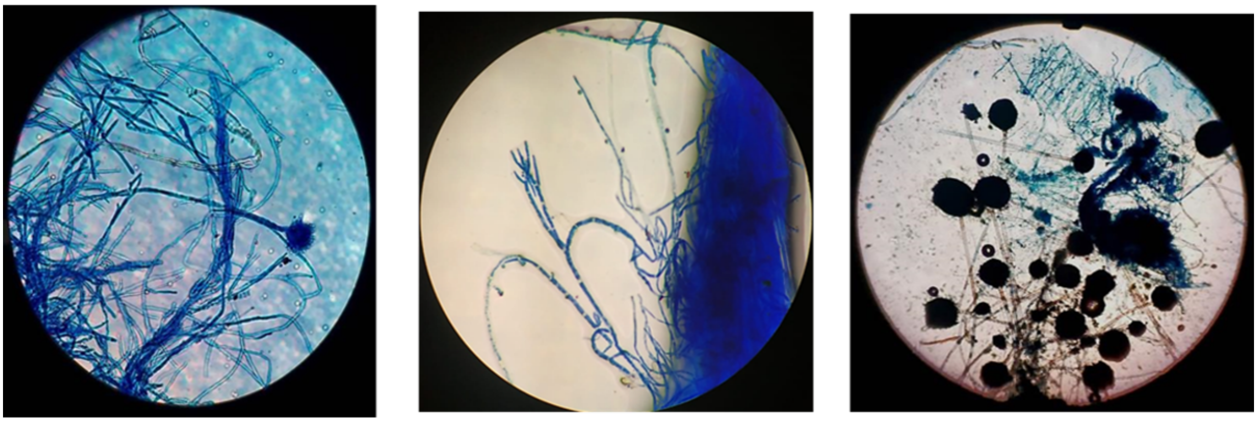

Figure 2: Microscopic appearance of some endophytic fungi

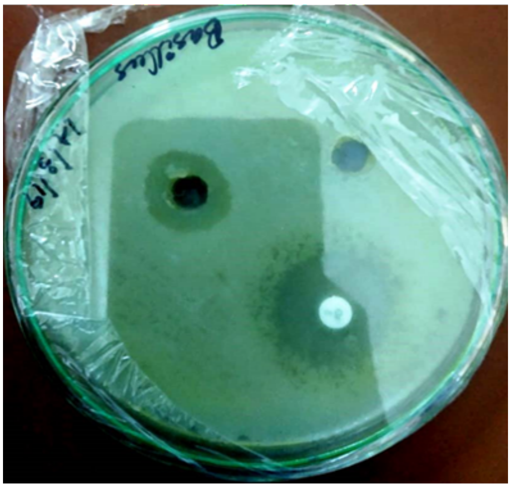

Figure 3: Antibacterial activity of FAE 4 against *Bacillus subtilis*

fungi and bacteria. Probiotic yeast helps to keep the digestive system healthy and balanced (Sadananda *et al.*, 2011). The climatic conditions and soil significantly influence the chemical composition of *Musa paradisicsa* and thus promote the growth of different endophytes (Yates *et al.*, 1997; Ju *et al.*, 2006; Koide *et al.*, 2017).

Antibacterial susceptibility test

The agar well diffusion test was utilised to determine the antibacterial activity of endophytic bacterial strain FAE 4. The results are displayed in Table 2 and Figure 3. The strain exhibited remarkable antibacterial activity against both gram-positive and gram-negative bacteria. A significant zone of inhibition was witnessed against *Bacillus subtilis and Escherichia coli*. The strain displayed the highest inhibition against *Bacillus subtilis* and *Escherichia coli* with 22 and 21 mm diameter zone of inhibition respectively which is close to that of standard antibiotics. The least zone of inhibition of 16 mm was observed against *Staphylococcus aureus.* Moderate zone of inhibition (18 mm) was seen against *Klebsiella pneumonia* and *Pseudomonas aeruginosa* . Endophytes release variety antimicrobial agents and bioactive compounds to competitively inhibit the growth of other microorganisms (Singh *et al.*, 2017). There are several studies around the world on the antimicrobial activities exhibited by several endophytes recovered from the medicinal plants, but studies reported on bananas especially from the fruit and root regions are very minimal (Arunachalam and Gayathri, 2010; Souza *et al.*, 2014; Egamberdieva *et al.*, 2017). The results of the present study were comparable to the previous reports (Souza *et al.*, 2014; Egamberdieva *et al.*, 2017).

CONCLUSIONS

The endophytes are considered as beneficial for growth and development of host plants which produce a huge number of biologically active compounds. As validated in the present study, the isolation of several endophytic bacteria and fungi from fruit and root of banana cultivar *Musa paradisiaca* indicates that these endophytes could play a vital role in promoting plant growth as well as contributing towards defence against pathogens and stress. Endophytes produces a wide range on secondary metabolites which are beneficial to the host. The strain FAE 4 isolated from fruit displayed antibacterial activity against *E.coli* and *Bacillus subtilis* which further emphasize the potential benefits and requirement for the presence endophytes in the plant tissue. Additional study on the nature of the antimicrobial compound needs to be done which enables the design of novel antibiotics since natural compounds have served as a reservoir of various lead molecules and new molecular entities for the development of many potential drugs. Hence we further would like to work on extraction of the active principle from the isolate and its characterization to find out its efficacy as a potential drug candidate.

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Conflict of interest

The authors declare that they have no conflict of interest for this study.

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