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Anti-vibrio potential of bacterial and fungal endophytes isolated from *Datura metel*

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Bacterial and fungal endophytes were isolated and characterized from root and shoot of *Datura metel* and studied for their antimicrobial properties. Molecular identification of the endophytes, both bacteria and fungi were done using 16S rDNA and 18S rDNA sequencing, respectively. Out of the total bacterial endophytes, *Bacillus subtilis* was predominant in both the tissues. Of the nine fungal endophytes isolated both from root and shoot, *Aspergillus versicolor* was found to be dominant. These two dominant species of endophytes, *B. subtilis* and *A. versicolor*, were subjected to mass multiplication, and secondary metabolites extraction of the host plant endophytes were performed using solvents of different polarity. The respective extracts were then studied for their antimicrobial activity against different *Vibrio cholerae* strains. Both bacterial and fungal extracts showed impressive activity against the *V. cholerae* strains P5, NE2 and VC7233.

Keywords: Endophytes; bacteria; fungi; *Datura metel*; *Vibrio cholerae*; antimicribial

Introduction

Plants live in close relationship with the microorganisms present in their vicinity, specifically with the ones residing inside the bodies. The progression of the evolutionary impression of ‘plant microbiome,’ which implies the combined genetic structure of microorganisms that thrive in association with their host plants, has shed light upon the advancement of a plant’s internal architechture. Here, natural forces do not merely have an effect on the gene construct of the plant alone, but also on the entire niche, the one that embraces the plant and its associated microbial community[1]. Various bacterial and fungal endophytes has network with higher plants. Symbiotic relationships that exist between plants with their corresponding arbuscular mycorrhizal fungi (AMF) in the roots have been rigorously studied[2-4]. This advocates that, at the least, portions of endophytic populations (both bacteria and fungi) over the course of evolution, coevolved, along with their host plant. Interactions with mutualistic interests that led to malleable profits for the two species sometimes evolved to a higher level of intricate forms that involved more than one species[5]. The recent assumption is that no constricted interactions occurred originally between plants and fungi, but these interactions evolved due to nutritional limitations[6]. The best-known plant-microbe communication is the one that occurs between rhizobia associated with leguminous plants. In primordium cells, bacteria are surrounded by the plant membrane, which forms the symbiosome. Here the atmospheric nitrogen is known to be fixed and transported in exchange for carbohydrates[7]. Symbiosomes are known to have a construction, similar to that of mycorrhizal arbuscules.

Endophytes are microorganisms that include both fungi and bacteria that reside in the underground and aerial tissues of plants in an asymptomatic manner. Davis and Shaw[8] reported that plant-microbe communication with respect to endophytes covers the complete wide range of bacterial/fungal-plant interactions as endophytic species can be mutualists, latent pathogens, temporary residents, commensals or latent saprotrophs. The field of endophyte research has immense application with regard to bio-prospecting. This is because of their ability to yield industrial enzymes of commercial importance[9] and

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novel bioactive compounds[10]. They are also known for their ability to tolerate stress (biotic or abiotic) in plants that harbor them[11-12]. Endophytic fungi and their host plants that show a mutualistic interaction have spawned a diverse interest with regards to its vast potential in research and development[12-15].

Datura metel, a medicinal plant, belongs to the family *Solanaceae*. It is a short perennial shrub to about 4 - 6 ft. The giant flowers can grow to twelve inches. The plant quickly germinates from seed and can bloom in just a few months from planting. All the parts of this plant are very toxic and are advised not to be ingested in any form. The *Solanaceae* is a large, diverse family that consists of trees, shrubs, and herbs, along with 90 genera and not less than 2000 species. Plants belonging to family *Solanaceae* are largely studied for possessing an array of alkaloids. The molecules of such compounds have a signature bicyclic structure that includes scopolamine, atropine, and hyoscyamine. Therapeutically, these compounds are known as anti-cholinergics. This means that they prevent the neurological signals, which are transmitted by the neurotransmitter acetylcholine[16]. The antimicrobial property of this plant has also been confirmed by Rajesh and Sharma[17].

Cholera, caused by the bacteria *Vibrio cholerae* is a diarrheal disease that originates in the small intestine and has a characteristic secretory nature. This leads to loss of water and salt. This disease is associated with mild fever and vomiting. As with other transmittable diseases, with this disease, there exists a scale ranging from healthy carriers, a mild affliction that expands to a massive deluge of watery stools. Such scattering of symptomatic versus asymptomatic disease highlights that the expression of this disease is modulated by constitutional susceptibility and acquired immunity[18]. Behind every explicit case of cholera occurrence in a population, 50 to 400 asymptomatic carriers can be identified[19]. The past 200 years of humanity has seen seven pandemics of the disease since the year 1817[20]. Despite numerous studies about the disease pathology, transmission dynamics and treatment strategies have been extensively done in the Indian subcontinent. Cholera has been successful in dodging such public health programs in the country. A similar conundrum has been observed in other continents like Africa, Asia, and Latin America. Contrary to such reports, health programs in the United States of America and Europe have been struggling in London and Chicago to deal with cholera deaths[21]. The character of endophytes in the accumulation of bioactive compounds with medicinal properties has been an instigating topic of study for the past few decades[22-23]. Ecological appeal of such compounds made by endophytes is still unclear. Yet recent studies have shown that such metabolites are assistance in dissuasion of herbivory and protection against fungal and bacterial pathogens, a characteristic feature of class 3 endophytes[24-27].

In our present study, we have isolated both fungal and bacterial endophytes from both the root and shoot parts of *D. metel* plant and screened them for their antimicrobial potential against the human pathogenic bacterium, *V. cholerae*.

Materials and Methods

Sample Collection

D. metel plants were collected from remote areas of Brahmaputra (Spark No.8, Jorhat), Assam (26.7509° N, 94.2037° E and 26.1173° N, 92.2747° E) in the month of April in their flowering stage. Samples were aseptically transferred into separate sterile polythene zipped bags and transported to the laboratory in room temperature[28].

Isolation of Endophytes

Endophytes were isolated from the tissues of the host plant. Surface sterilization of the plant samples was done using the standard protocol[29]. In brief, the plant roots were cut into small segments approximately 1 - 2 cm in length. Plant segments were rinsed with 70% ethanol followed by 4% sodium hypochlorite solution. This was followed by immersing the segments in 75% ethanol. The standard protocol followed for the isolation of fungi was given by Compant *et al*[30]. Individual plants severed aseptically 3 cm above the soil level for shoot and below the soil level for root were used for the study. A known amount of root and shoot tissues (2 gm) were then crushed by sterile mortar and pestle in sterile PBS buffer and 100 µl of tissue extract was spread plated on the rose bengal agar base media containing streptomycin (30 µg/mL) and nutrient agar media containing cycloheximide (4 µg/mL) for fungi and bacteria, respectively. This whole procedure was carried out in laminar airflow. For fungal growth plate was incubated at 28ºC for a week and for bacteria the plates were incubated at 37ºC overnight. Different morphotypes of fungus and bacterial colony were selected and purified. The tests were done in triplicates.

Mass Multiplication of Endophytes

Pure culture of endophytic fungus (DRF1) was fermented in 1 L Erlenmeyer flasks that contained 600 mL of potato dextrose broth (PDB), with a media composition of 200 g/L potato, 20 g/L dextrose and 100 µg/mL streptomycin at 28°C for the secondary metabolite production.

Molecular Identification of Plant-Associated Endophytes *D. metel*

Genomic DNA isolation from various endophytic isolates was performed by following a standard protocol described by Maniatis *et al*[31]. The 16S rDNA amplification was carried out using 8F (GAGTTTGA TCCTGGCTCAG) and 1541R (AAGGAGGTGA TCCRGCCGCA) as primers. Amplified PCR product was sequenced by the basic local alignment search tool (BLAST).

The amplification of fungal DNA was done using the highly conserved fungal 18S rDNA gene with gene primers. ITS1F (5′-TCCGTAGGTGAACCTGC GG) primer and ITS4R (5′-TCCTCCGCTTA TTGATATGC). The amplified PCR products were sequenced. The obtained data sequences were aligned with the help of BLAST software algorithm at the National Center for Biotechnology Information (NCBI)[32-33] (http://blast.ncbi.nlm.nih.gov).

Sequence Submission to GenBank

The nucleotide sequences were deposited in GenBank of NCBI and the accession numbers were retrieved.

Culture of Pathogenic Bacteria

The bacterial pathogens P5 and VC7233 (01 Ogawa serotype) were obtained from National Institute of Cholera and Enteric Diseases, Kolkata and NE2 was collected from Assam Medical College, Dibrugarh, Assam. *V. cholera* was cultured overnight in Luria Bertani (LB) broth. The density was then adjusted to 0.5 McFarland standard. 100 µl of the suspension was added onto LB agar plates to test antibacterial activity.

Secondary Metabolites Extraction of Endophytes

Secondary metabolite extraction was done from endophytes by using ethyl acetate (EA) solvent. The predominant bacterial endophyte, *B. subtilis* was considered for the study. The bacterial endophyte was cultured in nutrient broth (150 ml) and then incubated for 48 - 72 hrs at 37 ± 2ºC. The broth was centrifuged at 6,000 × g for 5 min. The supernatant obtained was mixed with an equal volumes of EA and incubated in a shaker for 5 - 6 hrs. The solvent containing the metabolites were separated with the help of a separating funnel. The solvent obtained was dried using a rota-evaporator and the extract obtained was used for further study. One fungal endophyte strain, which was predominant (*A. versicolor),* was considered for the study. Fungal biomass (2g/150 ml) was separated from the liquid media by filtration using muslin cloth and was dried at room temperature. After drying, fungal biomass was ground in the presence of EA and placed in a shaker for 24 hrs at 120 rpm, followed by filtration through Whatmann filter paper no. 1. Filtrate was dried at 40ºC and after that it was treated in acetone and this was repeated for solvents with different polarity. Subsequently, extract was evaporated in rota-evaporator at the boiling temperature of the solvent and this was repeated for each solvents. The dried extract obtained was mixed with organic solvents (ethyl acetate, acetone, ethanol and methanol) and stored at 4°C. These extracts were then used for the antimicrobial assay[33-35].

Agar Cup Assay

The antibacterial activities of fractions of ethyl acetate, acetone, ethanol and methanol of the bacterial and fungal extracts were tested by agar cup assay against *V. cholerae* cultures P5, NE2, VC7233. Strain suspension of 2 mL was taken and the bacterial cells were adjusted to 0.5 McFarland standard. Diluted suspension of 2 mL was added to 100 mL of nutrient broth at a temperature of 37°C to make the seed culture. This resulted in a bacterial suspension of 1×10^9 CFU/mL. *V. cholerae* culture of 100 µl was spread on an LB plate using a sterilized spreader and allowed to dry for some time. The agar plugs were prepared using a sterile cork borer (8 mm diameter). Separate plates were made for the bacterial and fungal extracts. Wells were filled with 100 µl of the extracts in various concentrations (0.1, 1, 5 and 10 mg/ml). Positive (ampicillin) and negative controls (extraction solvent) were included in the experiment. The zone of inhibition was measured in millimeters. The experiments were done in triplicates[36-40].

Scanning Electron Microscope (SEM)

The microscopic visualization of the effect of EA fraction of the fungal extract on P5 strain of *V. cholerae* was done using scanning electron microscope (SEM). It was analyzed the instrument ZEISS Evo 18 at Amity University, Noida, India. Both the control and test samples from the agar cup

study was used for the evaluation. Agar pieces were cut and subjected to pre-fixing using 2% glutaraldehyde in 20 mM phosphate buffer pH (6.5) for 1 hour. The samples were then washed with 20 mM phosphate buffer pH (6.5). The postfixing was done using osmium tetraoxide in 50 mM phosphate. For the dehydration process, the samples were subjected to ethanol grades (30% - 80%), with 30 mins in each grade. After that with the help of adhesive carbon tape, the gold coated samples were positioned on metallic platforms. SEM images with a voltage of 20.00 KV and 31 Pa were acquired using a secondary electron detector. Micrographs were taken per sample and the pertinent images were acquired[41].

Thin Layer Chromatographic Analysis of EA Fraction

The ethyl acetate fraction of the fungal strain was subjected to thin layer chromatography (TLC), which would allow for partial separation of the bioactive metabolites. The dried fungal extract was re-dissolved in EA using a stock concentration of 20 mg/ml. From this, 10 µl of the sample was spotted on pre-coated TLC plates (MERCK silica gel F254). The running solvent used was toulene: chloroform: acetone (TCA) in the ratio 45:25:35. The retention factor (R_f) values of all the spots were calculated after exposing the plates to iodine fumigation. The bioactive compounds that belonged to each band were subsequently scrapped off the plate and mixed with 1 ml of EA. These EA portions were centrifuged at 6000 × g for 15 mins and evaporated. The dried metabolites were then dissolved in DMSO a concentration of 100 µg/ml. The antimicrobial properties were determined by the agar cup assay. The zones of inhibition were observed and the diameters were measured[41-42].

Statistical Analysis

All the results of the above experiments are presented in triplicates. In order to test the significance of inhibition of the strains against the *V. cholerae* strains, a one-way analysis of variance (ANOVA) was performed. *P* value (< 0.05) was kept as significant.

Results and Discussion

Identification of the isolated Endophytes

The population of endophytic bacteria in the shoot and root tissues of *D. metel* were found to be 74.2 × 10^2 CFU/g fresh weight and 67 × 10^3 CFU/g fresh weight, respectively. Out of which four different morphotypes from shoot and five different morphotypes from root were selected for further study. Bacterial endophytes were identified by 16S rDNA sequencing. So, a total of nine bacterial isolates were identified, one of which is shown in Figure 1. The strains of bacterial isolates found in *D. metel* plant`s roots and shoots are given in Table 1.

There are total of nine types of endophytic fungi was isolated from the root shoot of the *D. metel* (Table 1). Fungal endophytes were identified by 18S rDNA sequencing using ITS1 and ITS4 primers. One of the fungal isolate is shown in Figure 2. Genomic DNA was extracted by using the standard cetyl trimethyl ammonium bromide (CTAB) method.

The obtained data sequences of both bacteria and fungi were aligned by using online tool BLAST.

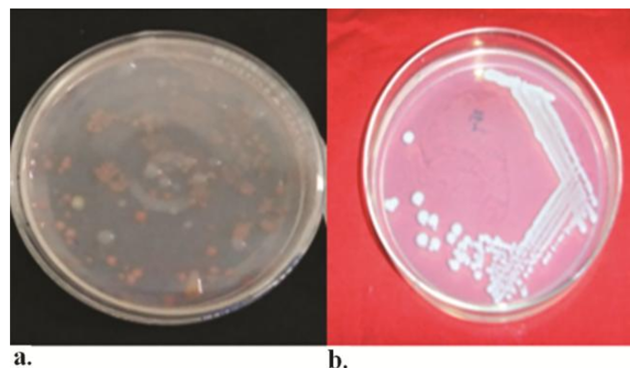

Fig. 1 — Endophytes from *D. metel* a. Different bacterial endophytes; b. Isolated bacterial endophyte.

Table. 1 — List of bacterial and fungal species isolated from root and shoot of *D. metel.*

S. No.	Culture ID	Plant parts	Bacterial species
Bacterial endophytes			
1	NED1	Root	*Bacillus subtilis*
2	NED2	Root	*B. subtilis*
3	NED3	Root	*B. subtilis*
4	NED4	Root	*Staphylococcus warneri*
5	NED5	Root	*S. sciuri*
6	NED6	Shoot	*B. subtilis*
7	NED7	Shoot	*B. safensis*
8	NED8	Shoot	*B. paramycoides*
9	NED9	Shoot	*B. paramycoides*
Fungal endophytes			
1	DRF1	Root	*Aspergillus versicolor*
2	DRF2	Root	*A. sydowii*
3	DRF3	Root	*Emericella striata*
4	DRF4	Root	*A. nidulans*
5	DRF5	Root	*A. sydowii*
6	DRF6	Root	*A. versicolor*
7	DSF1	Shoot	*A. flavus*
8	DSF2	Shoot	*A. versicolor*
9	DSF3	Shoot	*E. qinqixianii*

Molecular Identification and Characterization of Endophytes

The 16S rDNA region was amplified from bacterial genomic DNA and 18S region was amplified from bacterial genomic DNA. The isolates showing distinct bands of ~1500 bp (Fig. 3) and ~600 bp (Fig. 4) was visualized in 0.8% and 12% agarose gel respectively. PCR product was sent for sequencing. The data obtained from sequencing were subjected to BLAST at NCBI to find a comparative identity with endophyte strains depending upon the amplified 16S rDNA and 18S rDNA conserved regions. The 16S rDNA sequences showed 80-100% similarity with *Bacillus* sp., and *Staphylococcus* sp. The largest number of strains comprised of the genus *Bacillus* based on a similarity between the 16S rDNA sequences. Three out of five root isolates showed a similarity to *B. subtilis* sp. one with *S. warneri* and the other with *S. sciuri.* Similarly, the two out of four shoot isolates showed identity to *B. paramycoides,* one with *B. safensis,* and one with *B. subtilis*. The 18S rDNA sequences showed 80-100% similarity with *Aspergillus* sp. and *Emericella* sp (now known as *Aspergillus*) from both root and shoot of *D. metel* plant (Table 1).

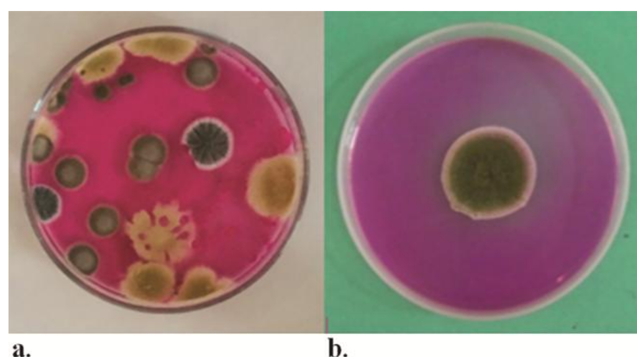

Fig. 2 — Endophytes from *D. metel* a. Different fungal endophytes; b. Isolated fungal endophytes.

The bioactive strains were identified up to the genus level using ITS1 and ITS4 approach[43]. Among the identified fungi, *A. versicolor* (max identity 98.69%), *A. sydowii* (max identity 99.24%), *Emericella striata* (max identity 98.50%), *A. nidulans* (max identity 95.22%), *A. versicolor* (max identity 99.24%), *A. flavus* (query coverage 98%, max identity 99.74%), *A. versicolor* (max identity 99.04%), *E. qinqixianii* (max identity 99.07%) sequences submitted and accession numbers were assigned to them by NCBI. They are MT371217, MT371218, MT371219, MT371220, MT371221, MT371222, MT371223, MT371224, MT371225. The predominant strain was *A. versicolor* in both roots and shoots based on 99-100% similarity with 18S rDNA sequence.

Phylogenetic Analysis

Phylogenetic study of the bacterial strains obtained showed the strains to form two clusters for *Staphylococcus* and *Bacillus*. Again, within the

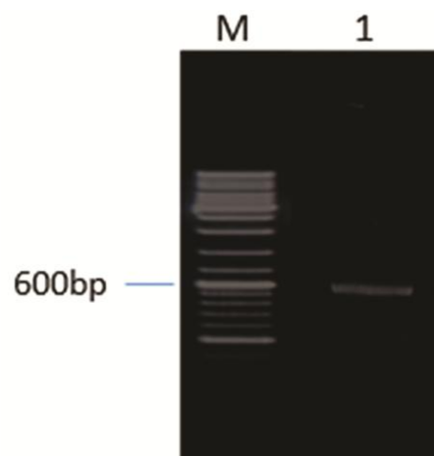

Fig. 4 — Agarose gel of ~600 bp of PCR product (DRF1-lane 1) using 18S rDNA primer (ITS1F & ITS4R). M – 1 kb DNA ladder.

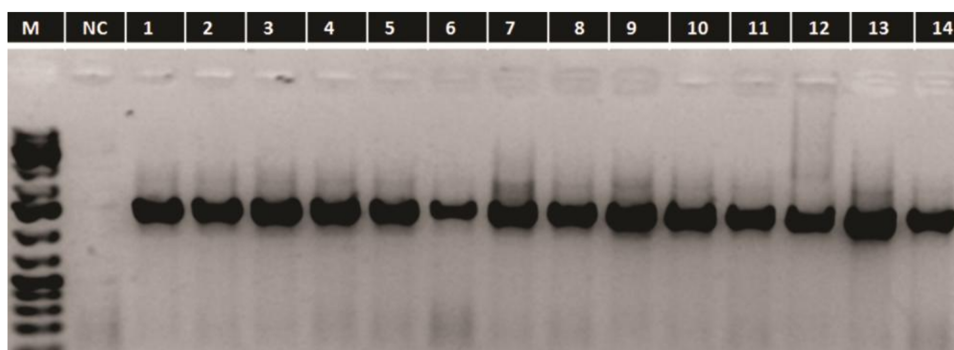

Fig. 3 — Agarose gel of ~1500 bp of PCR product using 16S rDNA primer (8F & 1541R). M – 1 kb DNA ladder; NC – Negative control, 1-9 NED 1-NED 9 Samples.

Bacillus cluster, *B. safensis, B. subtilis,* and *B. paramycoides* form three different branches were obtaibed (Fig. 5). Similarly, the phylogenetic study of fungal strains obtained showed the strains to form two clusters for *Aspergillus* and *Emericella* (Fig. 6). The neighbor-joining (NJ) method was used to check the evolutionary history. The bootstrap tree was inferred from 1000 replicates[44]. Those branches that correspond to partitions that are repeated in less than 50% replicates are collapsed. Maximum composite likelihood method was used to check the evolutionary distances[45]. This analysis involved as many as nine nucleotide sequences. The evolutionary studies were conducted in MEGA X[46].

Figure 7 showed variations and abundance in both bacterial and fungal endophytes from *D. metel* plant.

Antimicrobial Assay

Mass multiplication of the fungal and bacterial isolates *A. versicolor* and *B. subtilis* was done, respectively. The extracts were then evaluated against three different *V. cholerae* strains by agar cup assay. The plates were incubated at 37ºC overnight. In both the cases of the fungal and bacterial endophytes, ethyl acetate extracts consistently showed antimicrobial activity (Table 2 & 3).

SEM Analysis of the Agar Cup Assay against the Pathogenic Bacteria

The SEM study showed visible degradation of the bacterial cells by the fungal EA fractions. Along with cell wall degradation, huge distortion of the cell morphologies of the bacterial cells were observed. Thus, this analysis proved the visible effect of the antimicrobial fractions against the pathogenic bacteria (Fig. 8)

TLC Analysis

The thin layer chromatographic (TLC) study of the EA fractions of both fungal and bacterial extracts revealed various bands of bioactive compounds after iodine fumigation of the TLC plate (Fig. 8). The testing of the bands for their antimicrobial property against *V. cholerae* revealed for the fungal extract, two of the spots had substantial potential to inhibit the

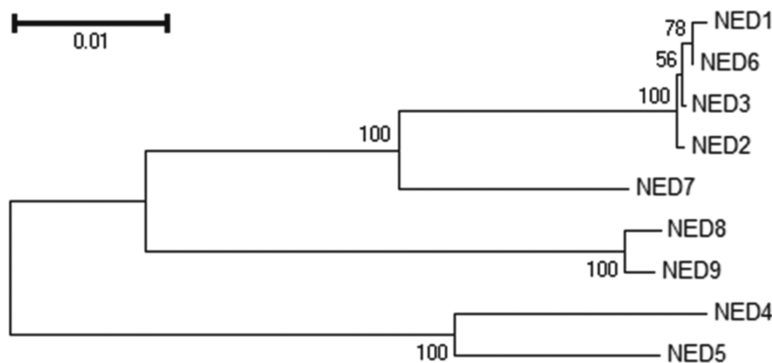

Fig. 5 — Phylogenetic relationships of the nine bacterial strains isolated from *D. metel* plant tissues based on 16S rDNA.

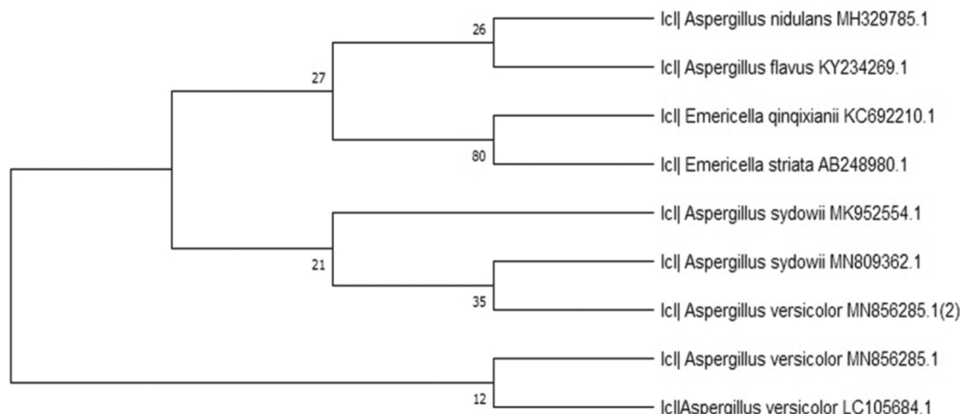

Fig. 6 — Phylogenetic relationships of the nine fungal strains isolated from *D. metel* plant tissues based on 18SrDNA.

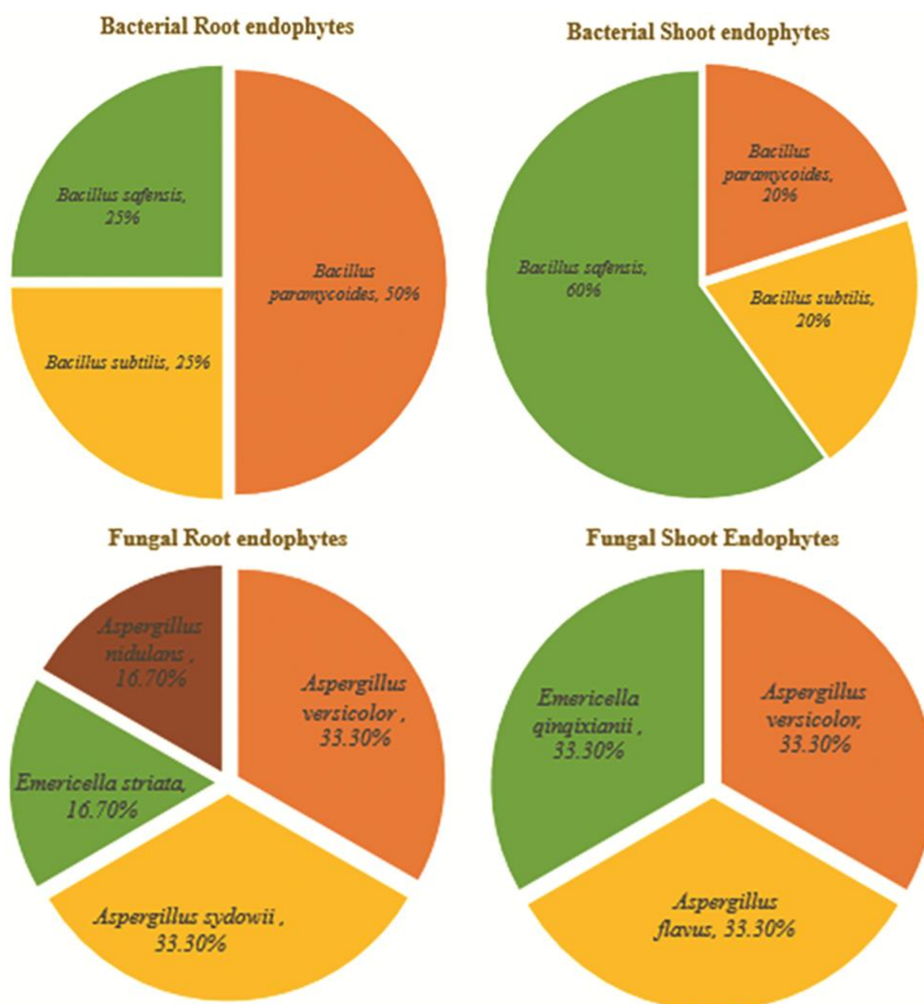

Fig. 7 — Pie-chart showing the abundance of *D. metel*'s bacterial endophytes and fungal endophytes from both root and shoot.

Table 2 — Antibacterial activity of EA extract of the fungal strain.

Concentrations of ethyl acetate fractions (mg/ml)	Diameter of inhibition zones (mm) against *V. cholera* strains		
	P5	NE 2	VC7233
10	15±1.5	14±1.6	14±1.8
5	13±1.0	13±1.2	12±1.8
1	12±1.6	11±1.0	11±1.9
0.1	10±1.7	09±1.6	09±1.5
Ampicillin (1 mg/ml)	18±1.9	20±1.9	20±1.7

Table 3 — Antibacterial activity of ethyl acetate extract of the bacterial strain bioactive spots.

Concentrations of ethyl acetate fractions (mg/ml)	Diameter of inhibition zones (mm) against *V. cholera* strains		
	P5	NE 2	VC7233
10	12 ± 1.5	11 ± 1.6	11 ± 1.8
5	10 ± 1.0	09 ± 1.8	09 ± 1.7
1	09 ± 1.6	08 ± 1.6	08 ± 1.9
0.1	08 ± 1.7	07 ± 1.7	07 ± 1.5
Ampicillin (1 mg/ml)	18 ± 1.5	20 ± 1.3	20 ± 1.6

growth of the pathogenic bacteria. Bands A and B with R_f values 0.27 and 0.35 respectively, showed maximum zones of inhibitions against the pathogenic bacteria (Fig. 9a). The appearance of a huge range of bands was an indication that there are several bioactive metabolites produced by the fungal strain *A. versicolor*. The zones produced by the bioautography assay of the fungal bioactive spots are shown in Figure 10a.

Similarly, the testing of the bands for their antimicrobial property against *V. cholerae* revealed for the bacterial extract, two of the spots had substantial potential to inhibit the growth of the pathogenic bacteria. Bands C and D with R_f values 0.75 and 0.87, respectively showed maximum of inhibitions against the pathogenic bacteria (Fig. 9b). The appearance of a huge range of bands was an indication that there are several bioactive metabolites

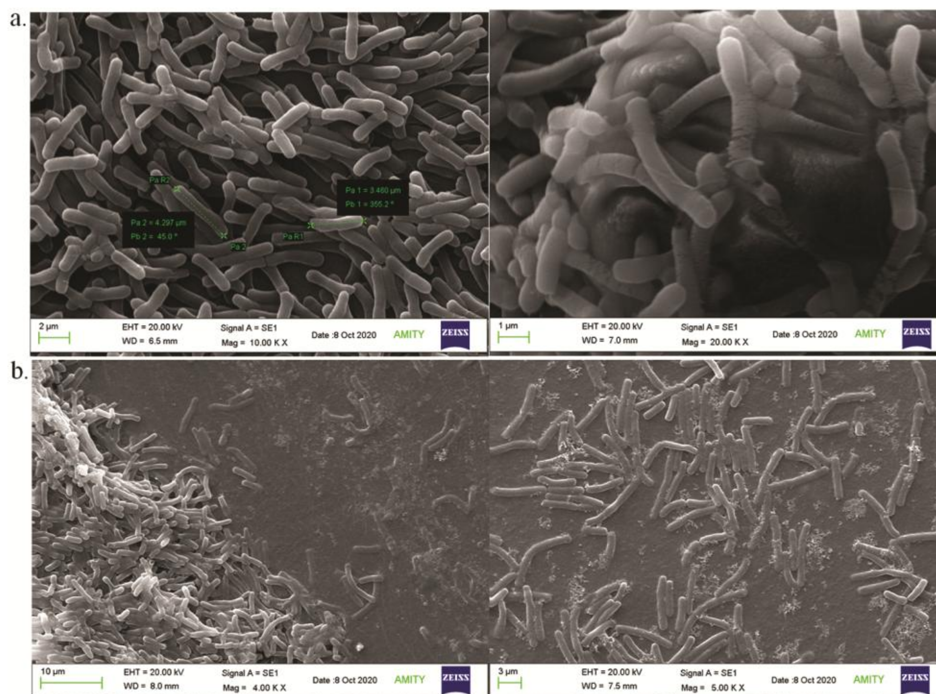

Fig. 8 — Scanning electron microscopy of *V. cholerae* when treated with EA extract of the fungal endophyte by (a). *V. cholerae* (control); (b). Treated *V. cholerae*.

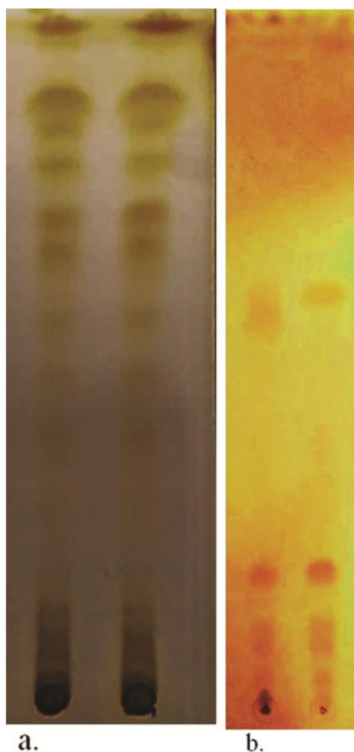

Fig. 9 — a. Thin layer chromatography analysis showed the presence of various bioactive metabolites of the EA fraction of the fungal extract run in TCA running solvent. b. Thin layer chromatography analysis showed the presence of various bioactive metabolites of the EA fraction of the bacterial extract run in TCA running solvent.

produced by the bacterial strain *B. subtilis.* The zones produced by the bioautography assay of the bacterial bioactive spots are shown in Figure 10b.

Statistical Analysis

The one way analysis of variance (ANOVA) result showed that the data was statistically significant at $P < 0.05$ between the groups.

Discussion

The main objective of this study was to explore endophytes (bacteria and fungi) as a source of antimicrobial compounds from the roots and shoots of *D. metel.* The antimicrobial properties of these endophytes were checked against the pathogenic bacteria, *V. cholerae*. Both the fungal and bacterial endophytes showed significant effect against *V. cholerae*. Data in this study about endophytic fungi support previous findings that a relatively small population of endophyte species rule the fungal community, and that most fungal taxa, with a few exceptions, qualify as rare species[47-49]. Endophyte genera *Mycosphaerella, Xylaria, Penicillium* and *Aspergillus*[50-51] are usually isolated from different plant tissues of various species from a wide range of geographical area[52-53]. This clearly indicates their ability to colonize an extensive host range. Ryan *et al*[54] reported that bacterial endophytes actually

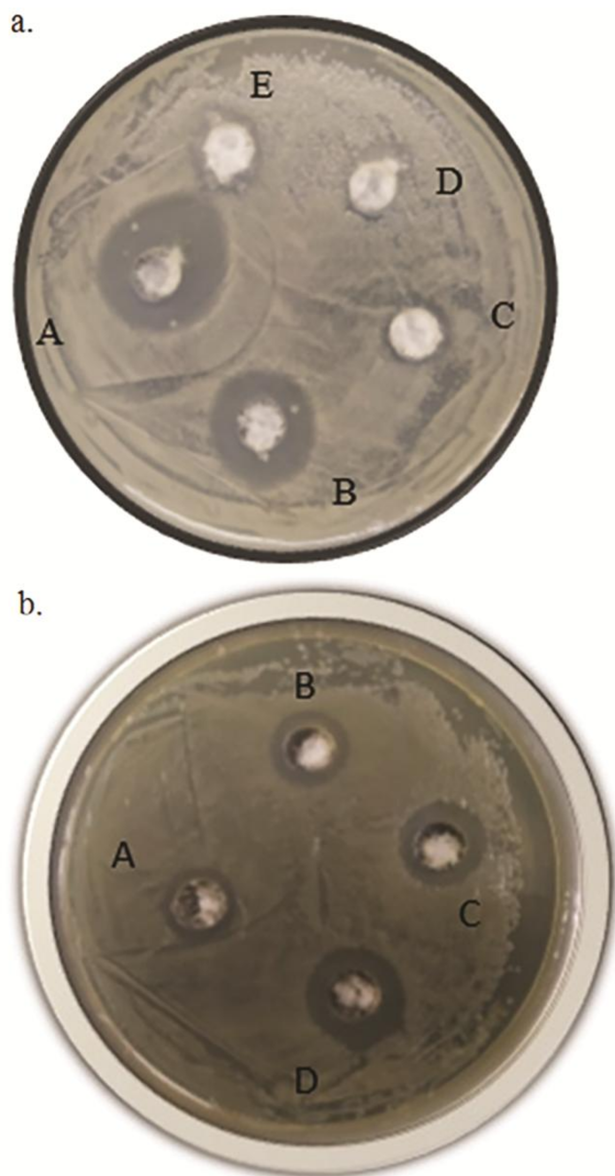

Fig. 10 — a. Agar cup assay of bands (A-E) bioactive spots scrapped from TLC plate of fungal extract (Fig. 9a). b. Agar cup assay of bands (A-D) bioactive spots scrapped from TLC plate of bacterial extract (Fig. 9b).

provide nutrients for host plant's growth advancements, and also to induce either their resistance against biotic and abiotic stress conditions or their tolerance against the same. Devari *et al*[55] showed the presence of a fungal endophyte *Alternaria alternata,* which has the ability to produce capsaicin up to three generations, from the fruits of *Capsicum annuum.* The detection of endophytic fungi and bacteria, which are capable of generating the same bioactive molecules as their respective host plant has raised the expectations that such metabolic compounds could be made in large scale through the process of fermentation, and thus it may appeal to the global market, while alleviating the dependency on their threatened host plants for production of such metabolites. Yet, this expectancy remains hindered mainly because of the low metabolite yields and the diminution of such metabolites after subsequent culturing of the fungal endophytes, both from the mycellium and filtrate[56-57]. Such attenuation could be credited to factors, the ones which cradle from loss of the supposed signal pathways that are delivered by the host or neighboring co-existing endophytes, causing gene silencing in axenic monocultures[58]. *D. metel* is already reported to have antimicrobial activities[59-60] and the antimicrobial activity showed by endophyte *Aspergillus* sps. and *Bacillus* sps. from *D. metel* further confirms synergism.

A report put forward by Janso *et al*.[61] showed that 105 of 123 endophytic actinomycetes from tropical plants belonged to 17 genera. Along with them, *Sphaerisporangium* and *Planotetraspora* as which are rare genera, were isolated, showing nearly 60% bioactive activities[61]. *Streptomyces, Microbispora,* and *Nocardiodes* were reported by Strobel *et al*.[62] as endophytic bacteria with antimicrobial compounds having inhibitory effects against Gram positive bacteria.

Conclusion

In the current study, *D. metel* plants collected from Assam, India were used to investigate their culturable bacterial and fungal endophytes from both its root and shoot regions for their antimicrobial properties. Nine bacterial and fungal isolates as endophytes were obtained from *D. metel*. The reported *Aspergillus* fungal endophyte and the *Bacillus* species of bacterial endophytes could be used as appropriate sources of the antimicrobial substance, specifically against cholera disease. It is already reported that host plant *Datura* produces a vast range of bioactive compounds. Such findings, along with our report could substantiate that the endophytes from this plant might share a mutualistic relationship with the host plant and secrete secondary metabolites with signifcant antimicrobial properties.

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References

1 Hardoim P R, Van Overbeek L S, Berg G, Pirttilä A M, Compant S *et al,* The hidden world within plants: Ecological and evolutionary considerations for defining functioning of microbial endophytes, *Microbiol Mol Biol Rev*, 79 (2015) 293-320.

2 Gherbi H, Markmann K, Svistoonoff S, Estevan J, Autran D *et al*, Sym RK defines a common genetic basis for plant root endosymbioses with arbuscularmycorrhiza fungi, rhizobia, and *Frankia* bacteria, *Proc Natl Acad Sci USA*, 105 (2008) 4928-4932.

3 Kanamori N, Madsen L H, Radutoiu S, Frantescu M, Quistgaard E M *et al*, A nucleoporin is required for induction of Ca2 spiking in legume nodule development and essential for rhizobial and fungal symbiosis, *Proc Natl Acad Sci USA*, 103 (2006) 359-364.

4 Markmann K, Giczey G & Parniske M, Functional adaptation of a plant receptor-kinase paved the way for the evolution of intracellular root symbioses with bacteria, *PLoS Biol*, 6 (2008) 0497-0506.

5 Frey-Klett P, Burlinson P, Deveau A, Barret M, Tarkka M *et al,* Bacterial-fungal interactions: Hyphens between agricultural, clinical, environmental and food microbiologists, *Microbiol Mol Biol Rev*, 75 (2011) 583-609.

6 Brundrett M C, Coevolution of roots and mycorrhizas of land plants, *New Phytol*, 154 (2002) 275-304.

7 Pawlowski K, Prokaryotic symbionts in plants, Springer-Verlag, Dordrecht, Netherlands, (2009).

8 Davis E C & Shaw A J, Biogeographic and phylogenetic patterns in diversity of liverwort-associated endophytes, *Am J Bot*, 95 (2008) 914-924.

9 Suryanarayanan T S, Thirunavukkarasu N, Govinda Rajulu M B & Gopalan V, Fungal endophytes: An untapped source of biocatalysts, *Fungal Divers*, 54 (2012) 19-30.

10 Suryanarayanan T S, Thirunavukkarasu N, Govindarajulu M B, Sasse F & Jansen R, Fungal endophytes and bioprospecting, *Fungal Biol Rev*, *23* (2009) 9-19.

11 Lata R, Chowdhury S, Gond S K & White J F Jr, Induction of abiotic stress tolerance in plants by endophytic microbes, *Lett Appl Microbiol*, 66 (2018) 268-276.

12 Redman R S, Sheehan K B, Stout R G, Rodriguez R J & Henson J M, Thermo tolerance conferred to plant host and fungal endophyte during mutualistic symbiosis, *Sci*, 298 (2002) 1581.

13 Chowdhary K & Kaushik N, Fungal endophyte diversity and bioactivity in the Indian medicinal plant *Ocimum sanctum* Linn, *PLoS One*, 10 (2015) 1-25.

14 Kaaniche F, Hamed A, Abdel-Razek AS, Wibberg D, Abdissa N *et al,* Bioactive secondary metabolites from new endophytic fungus *Curvularia* sp. isolated from *Rauwolfia macrophylla*, *PLOS ONE*, 14 (2019) 1-12.

15 Mapook A, Hyde K D, McKenzie E H C, Jones Gareth E B, Bhat J D *et al,* Taxonomic and phylogenetic contributions to fungi associated with the invasive weed *Chromolaena odorata* (Siam weed), *Fungal Divers*, 101 (2020) 1-175.

16 Soni P, Siddiqui A A, Dwivedi J & Soni V, Pharmacological properties of *Datura stramonium* L. as a potential medicinal tree: An overview, *Asian Pac J Trop Biomed* 2 (2012) 1002-1008.

17 Rajesh & Sharma G L, Studies on antimycotic properties of *Datura metel*, *J Ethnopharmacol,* 80 (2002) 193-197.

18 Panda S, Chatterjee P, Deb A, Kanungo S & Dutta S, Preventing cholerae in India: Synthesizing evidences through a systematic review for policy discussion on the use of oral cholera vaccine, *Vaccine*, 38 (2020) 148-156.

19 Feldman M, Friedman L S & Brandt L J, *Sleisenger and Fordtran's* Gastrointestinal and Liver Disease: Pathophysiology/Diagnosis/Management, 10th ed (Eds.) *Philadelphia: Saunders* (Elsevier) 2016.

20 Pollitzer R, Cholera studies. 1. History of the disease, *Bull World Health Organ*, 10 (1954) 421-461.

21 Rosenberg CE, *The Cholerae years: The United States in* 1832, 1849, and 1866.1st ed. *Chicago and London*: (University of Chicago Press) 1962.

22 Kusari S, Hertweck C & Spiteller M, Chemical ecology of endophytic fungi: Origins of secondary metabolites, *Chem Biol*, 19 (2012) 792-798.

23 Panaccione D G, Beaulieu W T & Cook D, Bioactive alkaloids in vertically transmitted fungal endophytes, *Funct Ecol*, 28 (2014) 299-314.

24 Mousa W K, Shearer C, Limay-Rios V & Ettinger C L, Eisen J A *et al,* Root hair endophyte stacking in finger millet creates a physicochemical barrier to trap the fungal pathogen *Fusarium graminearum*, *Nat Microbiol*, 1 (2016) 161-67.

25 Soliman S S, Greenwood J S, Bombarely A, Mueller L A, Tsao R *et al,* An endophyte constructs fungicide-containing extracellular barriers for its host plant, *Curr Biol*, 25 (2015) 2570-2576.

26 Singh B P, Rateb M E, Rodriguez-Couto S, Polizeli MLTM & Li W J, Editorial: Microbial secondary metabolites: Recent developments and technological challenges, *Front Microbiol*, 26 (2019) 1-2.

27 Payamnoor V, Kavosi M & Nazari J, Polypore fungi of Caucasian alder as a source of antioxidant and antitumor agents, *J For Res*, 31 (2020) 1381-1390.

28 Ratnaweera P B, De Silva E D, Williams D E & Andersen R J, Antimicrobial activities of endophytic fungi obtained from the arid zone invasive plant *Opuntia dillenii* and the isolation of equisetin, from endophytic *Fusarium* sp. *BMC Complem Altern M*, 15 (2015).

29 Schulz B, Guske S, Dammann U & Boyle C, Endophyte-host interactions II. Defining symbiosis of the endophyte-host interactions, *Symbiosis*, 25 (1998) 213-227.

30 Compant S, Mitter B, Colli-Mull J G, Gangl H & Sessitsch A, Endophytes of grapevine flowers, berries, and seeds: Identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization, *Microb Ecol*, 62 (2011) 188-197.

31 Maniatis T, Fritsch E F & Sambrook J, Molecular cloning: A laboratory manual, cold spring harbor, (N Y: cold spring harbor laboratory) 1982.

32 Phookamsak R, Hyde K D, Jeewon R, Bhat D J, Jones Gareth E B *et al*, Fungal diversity notes 929-1035: Taxonomic and phylogenetic contributions on genera and species of fungi. *Fungal Divers*, 95 (2019) 1-273.

33 Rocha P S, Paula V M B, Olinto S C F, Dos Santos E L & De Picoli Souza K, Diversity, chemical constituents and biological activities of endophytic fungi isolated from *Schinus terebinthifolius* raddi, *Microorganisms, 8* (2020) 1-13.

34 Uche-Okereafor N, Sebola T, Tapfuma K, Mekuto L, Green E *et al*, Antibacterial activities of crude secondary metabolite extracts from *pantoea* species obtained from the stem of *Solanum mauritianum* and their effects on two cancer cell lines, *Int J Environ Res*, 16 (2019) 1-12.

35 Eze P M, Nnanna J C, Okezie U, Buzugbe H S, Abba C C *et al,* Screening of metabolites from endophytic fungi of some Nigerian medicinal plants for antimicrobial activities, *The EuroBiotech Journal,* 3 (2019)10-18.

36 Orfali R & Perveen S, Secondary metabolites from the *Aspergillus* sp. in the rhizosphere soil of *Phoenix dactylifera* (Palm tree), *BMC Chemistry*, 13 (2019) 1-6.

37 An C, Ma S, Shi X, Xue W, Liu C *et al,* Isolation, diversity and antimicrobial activity of fungal endophytes from *Rohdea chinensis* (Baker) N.Tanaka (synonym *Tupistra chinensis* Baker) of qinling mountains, China, *Peer J*, 8 (2020) 1-20.

38 Lim K M C, Dagalea F M S & Vicencio M C G, Antibacterial activity of datura metel Linn. (TALONG-PUNAY) fruit extract, *J Pharm Res Int*, 32 (2020) 96-101.

39 Manganyi M C, Regnier T, Tchatchouang C D K, Bezuidenhout C C & Ateba C N*,* Antibacterial activity of endophytic fungi isolated from *Sceletium tortuosum* L. (Kougoed), *Ann Microbiol*, 69 (2019) 659-663.

40 Yuan Z, Tian Y, He F & Zhou H, Endophytes from *Ginkgo biloba* and their secondary metabolites, *Chin Med*, 14 (2019) 1-40.

41 Chatterjee S, Ghosh R & Mandal N C, Production of bioactive compounds with bactericidal and antioxidant potential by endophytic fungus *Alternaria alternata* AE1 isolated from *Azadirachta indica* A. Juss, *PLoS One,* 14 (2019) 1-18.

42 Kaur H, Onsare J G, Sharma V & Arora S D, Isolation, purification and characterization of novel antimicrobial compound 7-methoxy-2,2-dimethyl-4-octa-4′,6′-dienyl-2*H*-napthalene-1-one from *Penicillium* sp. and its cytotoxicity studies, *AMB Expr*, 5 (2015) 1-13.

43 Manter D K & Vivanco J M, Use of the ITS primers, ITS1F and ITS4, to characterize fungal abundance and diversity in mixed-template samples by qPCR and length heterogeneity analysis, *J Microbiol Methods*, 71 (2007) 7-14.

44 Felsenstein J, Confidence limits on phylogenies: An approach using the bootstrap, *Evolution*, 39(1985) 783-791.

45 Tamura K, Nei M, Kumar S, Prospects for inferring very large phylogenies by using the neighbor-joining method, Proc Natl Acad Sci USA, 101 (2004) 11030-11035.

46 Kumar S, Stecher G, Li M, Knyaz C & Tamura K, MEGA X: Molecular evolutionary genetics analysis across computing platforms, *Mol Biol Evol*, 35 (2018) 1547-1549.

47 Arnold A E, Maynard Z & Gilbert G S, Fungal endophytes in dicotyledonous neotropical trees: Patterns of abundance and diversity, *Mycol Res,* 105 (2001) 1502-1507.

48 Mohamad O A A, Li L, Ma J B, Hatab S, Xu L *et al,* Evaluation of the antimicrobial activity of endophytic bacterial populations from chinese traditional medicinal plant licorice and characterization of the bioactive secondary metabolites produced by *Bacillus atrophaeus* against *Verticillium dahliae*, *Front Microbiol*, 9 (2018) 1-14.

49 Gazis R & Chaverri P, Diversity of fungal endophytes in leaves and stems of wild rubber trees (*Hevea brasiliensis*) in Peru, *Fungal Eco,* 3 (2010) 240-254.

50 Arnold A E & Lutzoni F, Diversity and host range of foliar fungal endophytes: Are tropical trees biodiversity hot spots *Ecology*, 88 (2007) 541-549.

51 Tawfike A F, Romli M, Clements C, Abbott G & Young L, Isolation of anticancer and anti-trypanosome secondary metabolites from the endophytic fungus *Aspergillus flocculus* via bioactivity guided isolation and MS based metabolomics, *J Chromatogr B Analyt Technol Biomed Life Sci*, 1 (2019) 71-83.

52 Toghueo R M K, Anti-leishmanial and anti-inflammatory agents from endophytes: A review, *Nat Prod Bioprospect*, 9 (2019) 311-328

53 Douanla-Meli C, Langer E & Mouafo F T, Fungal endophyte diversity and community patterns in healthy and yellowing leaves of *Citrus limon*, *Fungal Ecol*, 6 (2013) 212-222..

54 Ryan R P, Germaine K, Franks A, Ryan D J & Dowling D N, Bacterial endophytes: Recent developments and applications, *FEMS Microbiol Lett*, 278 (2008) 1-9.

55 Devari S, Jaglan S, Kumar M, Deshidi R, Guru S *et al*, Capsaicin production by *Alternaria alternata*, an endophytic fungus from *Capsicum annum*, LC-ESI-MS/MS analysis, *Phytochemistry,* 98 (2014) 183-189.

56 Kusari S, Zuelke S & Spiteller M, Effect of artificial reconstitution of the interaction between the plant *Camptotheca acuminate* and the fungal endophyte *Fusarium solani* on camptothecin biosynthesis, *J Nat Prod*, 74 (2011) 764-775.

57 Kumara P M, Soujanya K N, Ravikanth G, Vasudeva R & Ganeshaiah K N, Rohitukine, a chromone alkaloid and a precursor of flavopiridol, is produced by endophytic fungi isolated from *Dysoxylum binectariferum* Hook.f and *Amoora rohituka* (Roxb), *Phytomedicine*, 21 (2014) 541-546.

58 El-Elimat T, Raja H A, Graf T N, Faeth S H & Cech N B, Flavonolignans from *Aspergillus iizukae*, a fungal endophyte of milk thistle (*Silybum marianum*), *J Nat Prod*, 77 (2014) 193-199.

59 Raheem M A O, Abdul, Sulaiman F A, Abdulrahim H A, Ahmed O, Yusuf K O *et al*., Assessment of Environmental Factors on Secondary Metabolites and Toxicological Effects of Leaves Extracts *Annals of Science and Technology*, 3 (2018) 8-19.

60 Xu S, Liu Y, Xiang L, Xiang L & Zhou F, Metabolites identification of bioactive compounds *Datura taturin A, Datura metel* in I, *N*-trans-feruloyltyramine, and cannabisin f from the seeds of *Datura metel* in rats, *Front Pharmacol*, 9 (2018) 1-9.

61 Janso J E & Carter G T, Biosynthetic potential of phylogenetically unique actinomycetes from tropical plants, *Appl Environ Microbiol*,76 (2010) 4377-4386.

62 Strobel G A and Daisy B, Bioprospecting for microbial endophytes and their natural products, Microbiol. Mol Biol Rev, 67 (2003) 491–502.