DNA BARCODING OF FEW INDOOR PLANTS AND MOLECULAR CHARACTERIZATION OF ITS SYMBIOTIC BACTERIA

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Abstract. The health benefits of indoor plants are widely explored now a days and methods of psychological assessment are explored. In this regard we have collected few indoor plants and authenticated them to further investigate for its symbiotic relationship with bacteria. As traditional taxonomic studies possess intrinsic limitations with plant species identification, combinations of DNA barcodes have been considered a powerful tool to discover undetected genetic variation within species across large geographic areas, providing more precise estimates of biodiversity. Therefore, many genes have been targeted for DNA barcodes as standardizing regions is vital for identifying them efficiently and discriminate the plant species. In this study, we targeted different genes such as ITS2, matK, RbCl2 and PetB for sequencing. The plants were identified as *Draceana senderiana,Pandanus sanderi* and *Hylotelephium ewersii*. Once the species are authenticated they were further used to identify its bacterial relationship. The symbiotic bacteria present in roots were identified as *Acinetobacter calcoaceticus* and *Pseudomonas stutzeri.* This information will further help in exploring the properties of bacteria which are involved in enhancing the overall growth of plants.
Keywords: *DNA barcoding, indoor plants, symbiotic bacteria, isolation, identification, molecular characterization*

Introduction

DNA barcoding is a method used to identify unknown species based on their specific regions of DNA. The main aim of DNA barcoding is to ensure that data is available publically to understand conserved and utilised worlds biodiversity (Vere et al., 2014). Traditionally taxonomy of morphology-based studies provides an ambiguous phylogenetic evidence of large diversified plants genera (Dayrat, 2005). Hence, to overcome this problem sequencing genomic DNA can serve as standard method for species identification as more closely related species hold more homologous DNA sequences with respect to the distantly associated species (Hebert et al., 2003). Most of the times it is useful to identify species from material like roots, seeds and pollen or in mixtures of plants sampled from the air, soil or water. It is regarded as a promising method for proper identification of species using short region of specific DNA sequence efficiently (Meyer and Paulay, 2005). In plants due to its variability in genome several chloroplast loci and combinations of these loci have been proposed as a promising DNA barcode in plants (CBOL Group, 2009). In addition to plastid DNA sequence, nuclear ribosomal internal transcribed spacer (ITS) region is also being used in plants. As, it endures complications in amplification rendering its feasibility as a universal barcode for land plants (Chase et al., 2007; Giudicelli et al., 2015). Despite these complications,

many researchers proved that ITS can perform better amplification in comparison to other coding or non-coding plastids markers. Although the same regions of DNA should be used for DNA barcoding, wide range of protocols are available and approaches that have been used to generate plant DNA barcodes (Fazekas et al., 2012).

Since ancient times, human beings have good relationship with plants and placing them indoor has proved to have various health benefits and increases ones involvement. Indoor plants shows positive psychological effect on health and decreases the levels of stress. It also acts as indoor air purifiers and its an efficient method to reduce pollutants naturally, hence are widely used now a days. They also have potential applications in other fields including solar power, sensing, acoustic, people's health and luxury (Adachi et al., 2000). Indoor plants reduce components of indoor pollution specifically volatile organic compounds like benzene, xylene and toluene. This is done by sucking up the pollutant with their stomata during gas transfers to get rid of these compounds .The role of the plants is to determine and maintain the species specific root-zone microbial communities (Orwell et al., 2006).

Dracaena is one among the foremost wanted houseplants within the world. They absorb the chemicals within the air and remove up to 87% of volatile organic compounds every 24 hours. It helps remove formaldehyde, trichloroethylene, benzene and CO2. These are the chemicals which are linked with health problems like headaches, anemia, respiratory problems, marrow disease, renal disorders etc. The advantages of dracaena are often seen within the psychological, cognitive, physical and social realms. The plants are linked to increased self-esteem, improved mood and sense of well-being, increased sense of stability and control also as increased feelings of relaxation, calm and optimism. Pandanus leaves serve double duty as a spice and medicine. Leaves of this plant is used for alleviating stomach cramps, soaked leaves in warm copra oil is used as poultice to alleviate arthritis pain. Add finely chopped leaves to a shower for a soothing soak. The fresh, crushed leaves or plant extract made up of fresh plants are usually used externally in traditional herbal medicine. These plants have often been regarded to possess diuretic and laxative properties. Additionally, it had been used as an herbal remedy for ringworm, diphtheria, to trigger abortion and also scurvy due to high content of vitamin found within the plant. It is also used to treat itchy rashes, ulcers and for the topical treatment of warts, pimples, acne dermatitis and corns.

There are diverse group of microorganisms called symbiotic bacteria which colonize in roots of plants. They help plants in many ecological processes like nutrient acquisition, nitrogen and carbon cycling formation of soil. Microbial symbionts comprise highly complex diverse communities in the root of the plant and hence are called rhizosphere microbiome. Whereas few plants establish a symbiotic relationship with bacteria present in the soil and thus form nodules in the root which facilitates the conversion of atmospheric nitrogen to ammonia. The cytokines play a major role in the formation of root fixing nodules which is necessary for nitrogen fixation (Frugier et al., 2008). Hence, symbionts play significant roles in plant growth and development providing nutrition for plants to protect themselves from abiotic and biotic stress. In certain plants the leaves and stem contain few bacteria known as phyllosphere bacteria on the surface which absorb air pollutants. These can detoxify pollutants by means of degradation, transformation or sequestration process (Barrett et al., 2012). According to few hypothesis interaction of different groups of symbionts enhances plant productivity and plant diversity. The four groups of symbiotic microbes are Rhizobiaceae, arbuscular mycorrhizal fungi (AMF) in the phylum Glomeromycota, specific strains of fungi in the

Ascomycetous genus *Trichoderma* and fungi belonging to order Sebicales, exemplified by *Piriformaspora indica*. These organisms are phylogentically different and are found colonized in plant roots referred as endophytes. These four groups are true plant symbionts in that they confer advantages to the plants whose roots they colonize, while at the same time, they derive nutrients and other benefits from their plant hosts (Harman et al., 2004). Few indoor plants like Asparagaceae, Pandanus and Crassulaceae families were taken and DNA bar coded using ITS2, Matk, Pet B and Rbcl genes through Sanger method. And this paper is aimed at investigating the microbes that are exclusively present inside the root and in rhizosphere by molecular techniques.

Materials and Methods

Collection of plant materials

Plant samples like Asparagaceae, Pandanus and Crassulaceae families were collected from the indoor garden. *Table 1* depicts the samples taken for DNA bar coding and endophytic bacterial culturing from root.

Table 1. *List of plants taken for studies.*

Family	Plants
Asparagaceae	*Dracaena sanderiana*
Crassulaceae	*Hylotelephium sieboldii*
Pandanus	*Pandanus sanderi*

Isolation of DNA

Leaf samples were weighed about 0.5g and washed with distilled water to remove dust particles and grinded it with 500µl of CTAB buffer using motar and pestle. It was followed by vortexing and incubated at 60 ºC for 30 minutes. Following the incubation period, homogenate was centrifuged and equal volume of chloroform/isoamyl alcohol (24:1) was added. The aqueous upper phase containing DNA was carefully transferred to new tube and allowed to precipitate by adding 0.7 ml of ice cold isopropanol and incubated at -20°C overnight. This solution was allowed to pass through DNA binding columns, centrifuged at 10000rpm for 1 minute, washed and eluted purified form of genomic DNA. It was treated with RNAase to avoid RNA contamination. 2µl of DNA was mixed with bromophenol blue and loaded on 0.8% agarose gel to visualise the bands under UV light to see DNA concentrations, which was calculated based on standard ladder (1kb). This DNA was further taken for amplification process (Doyle and Doyle, 1987).

Amplication of conserved gene

PCR amplification was done for isolated plant DNA targeting ITS2, Matk, Pet B and Rbcl genes. Amplification was carried out in 25µl reaction mixture by adding 10µM forward and reverse primer, 10µM dNTPs, 0.5µl Taq DNA polymerase, 2.5µl of 2x buffer and 40ng of plant genomic DNA. Gene amplification was performed using thermocycler where it was set for 30 cycles, consisting of an initial denaturation step where the unwinding of DNA occurs and final denaturation step followed by annealing, extension or elongation. Initial denaturation 94°C for 5 minutes, Final denaturation

94°C for 1 minute, Annealing 30°C for 1 minute, Elongation 72°C for 2 minutes. The obtained PCR products were run on gel and eluted to get pure form of template for sequencing.

Sanger sequencing

Sanger sequencing was performed to identify the nucleotide sequence of the DNA using automated sequencing machine. It is called chain termination and automated containing ddNTPs, where in each dNTP has a unique fluorescent label ddNTPs lack 3'-OH group required for phosphodiester bond formation and hence extension ceases on binding. The result of chain-termination PCR is millions to billions of oligonucleotide copies of the DNA sequence of interest, terminated at a random lengths by 5'-ddNTPs. All nucleotide fragments are run in capillary gel electrophoresis within the sequencing machine. The output data will be obtained in the form of electropherogram which shows a florescent peak of each nucleotide along the length of the template. Further this is converted to other formats for data analysis.

Data analysis

The obtained FASTA file was analyzed for the presence of nucleotide pattern and performed blast for further analysis. From the blast, the unknown sequence was predicted based on the percentage similarity and e-value. The obtained DNA sequences were aligned with top hits from BLAST in Clustal omega to construct tree. Phylogenetic tree was constructed to find out the evolutionary relationship of plants taken for study.

Identification of symbiotic bacteria

Collection of root samples

The roots of the three indoor plants taken for DNA bar coding were collected from the garden. The roots were thoroughly washed with sterile distilled water to remove any adhering soil and placed in clean and sterile container. The finely sliced root samples were inoculated on LB agar plates and the obtained pure cultures were used to perform morphological, microscopic and molecular characterization.

Morphological and microscopic identification

Cell morphology was observed thoroughly by understanding nature of colonies obtained. Gram staining method was performed to differentiate the bacteria into Gram Positive and Gram Negative, which further helps in selecting the strains for molecular characterization.

Molecular characterization of bacterial isolates

Genomic DNA was extracted from bacterial cultures by using CTAB method. Polymerase chain reaction (PCR) was used to amplify partial gene regions of 16S rRNA gene. PCR reaction and program was set using 27F and 1492R primers as explained above. Once the program is done the reaction mixture was mixed with dye and subjected to gel electrophoresis. The product obtained was thus gel purified and subjected to Sanger sequencing to obtain sequences of gene. The FASTA sequences were further analysed using BLAST and phylogenetic tree construction.

Results and Discussion

The plants of different families were collected from the indoor garden, and further processed for DNA barcoding and isolation of symbiotic bacteria from the roots of plants. *Figure 1* depicts the plants taken for study and leaf material taken for its genomic DNA isolation. The isolated DNA was quantified using gel electrophoresis. Further they were subjected to amplify ITS2, MatK, RbCl2 and PetB genes using PCR technique. *Figure 2* shows the gel image of genomic DNA of plants and amplified genes when compared to standard 1KB ladder. The obtained PCR products were gel purified to get pure template for Sanger sequencing. Sequencing technique provided fasta sequences of plants which were analysed further using NCBI Blast. *Table 2* shows the predicted hits from individual gene sequences along with similarity percentage, query coverage, E-value and its respective accession number. Whereas *Figure 3* highlights representative electropherogram showing individual nucleotides present in the sequences. From the BLAST hits top 10 sequences were taken and further analysed for its evolutionary relationship. *Figure 4* depicts Phylogenetic tree and the evolutionary relationship of plant *Draceana senderiana.* Similarly, *Figure 5* and *Figure 6* shows phylogenetic tree constructed to understand the evolutionary relationship of plants *Pandanus sanderi* and *Hylotelephium ewersii* respectively.

Table 2. *Highlighting the predicted hits from individual gene sequences using NCBI BLAST.*

Sample Name	Description	Query coverage (%)	E value	% Identity	Accession No.	Gene
A	Dracaena cochinchinensis chloroplast psbA gene, psbA-trnH intergenic spacer, partial sequence, isolate: CHULA-032	99	0.0	100	LC461768.1	ITS2
A	Dracaena sanderiana tRNA-Leu (trnL) gene and trnL-trnF intergenic spacer, partial sequence; chloroplast	89	0.0	100	KC439472.1	RBCL2
A	Dracaena sanderiana chloroplast DNA, psbD-trnT intergenic spacer, partial sequence	59	1e-148	99.63	AB613970.1	PETB
A	Dracaena sanderiana glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene, partial cds	100	0.0	100	MT476380.1	MATK
B	Pandanus sanderi isolate T0129 floricaula (LFY) gene, partial cds	55	9e-157	100	KJ682127.1	ITS2
B	Pandanus tectorius chloroplast, complete genome	65	0.0	99.45	NC_042747.1	RBCL2
B	Pandanus tectorius chloroplast, complete genome	66	4e-133	100	NC_042747.1	PETB
B	Pandanus sanderi isolate T0129 floricaula (LFY) gene, partial cds	78	1e-134	89	NC_04265.1	MATK
C	Hylotelephium ewersii chloroplast, complete	50	4e-105	100	MN794014.1	ITS2

C	genome Hylotelephium sieboldii genes for 18S ribosomal RNA, internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2, 28S ribosomal RNA, partial and complete sequence	68	4e-123	100	AB088567.1	RBCL2
C	Hylotelephium ewersii chloroplast, complete genome	100	0	100	MN794014.1	PETB
C	Hylotelephium ewersii chloroplast, complete genome	46	1e-51	100	MN794014.1	MATK

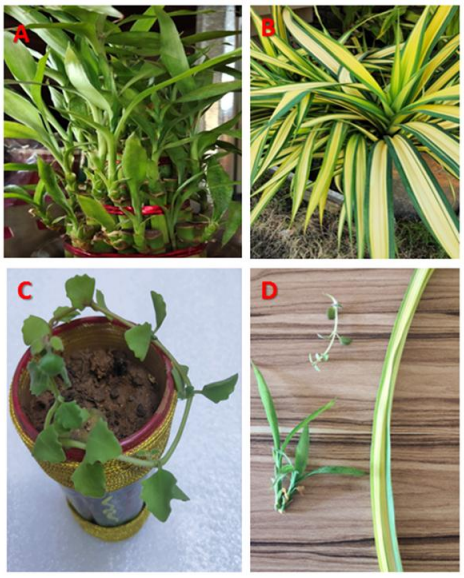

Figure 1. *Indoor plant species taken for DNA barcoding.*

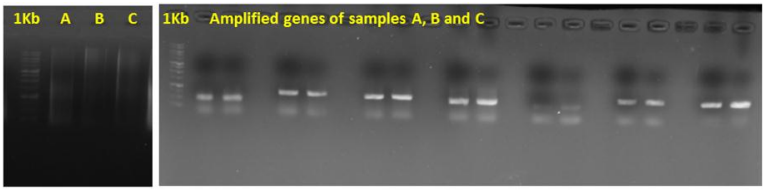

Figure 2. *Gel image showing genomic DNA of plants and amplified genes.*

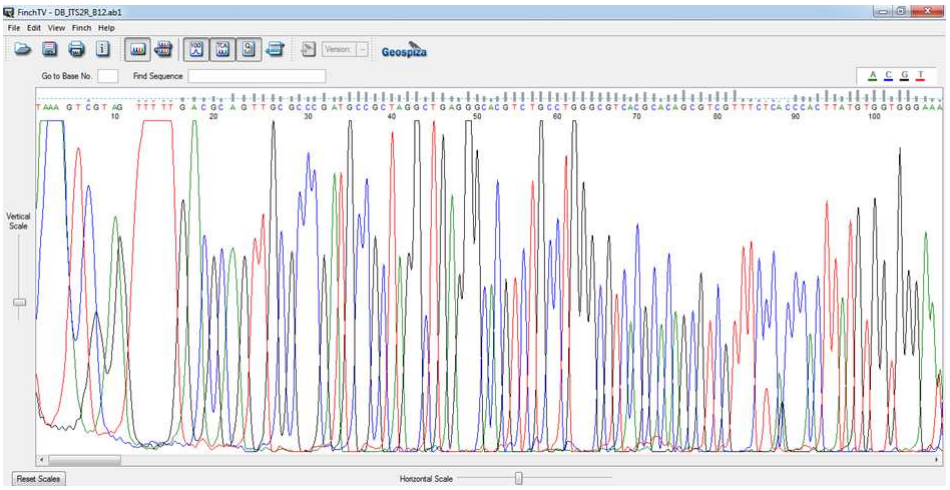

Figure 3. *Electropherogram analysing nucleotides present in the sequences.*

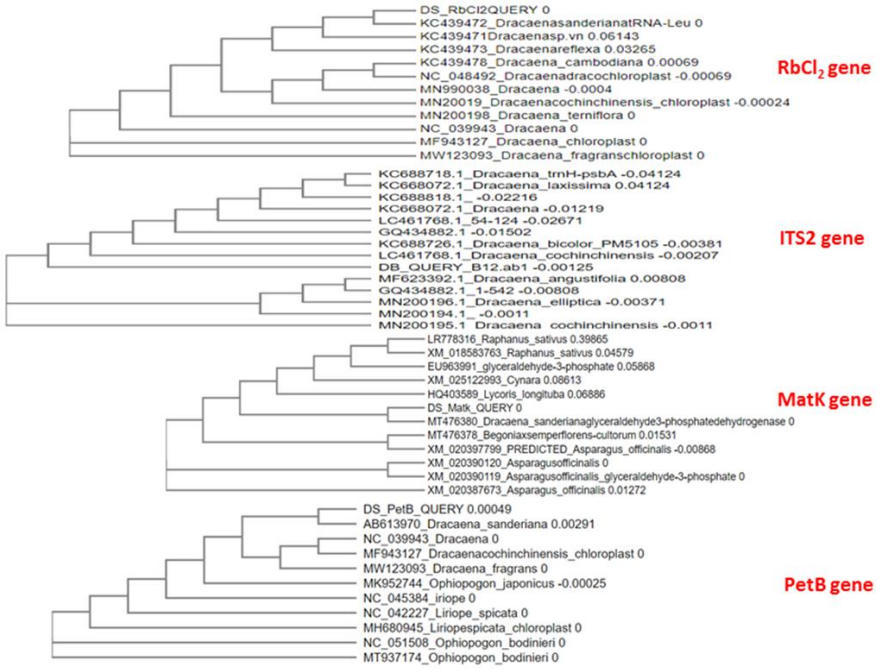

Figure 4. *Phylogenetic tree representing the evolutionary relationship of plant Draceana senderiana.*

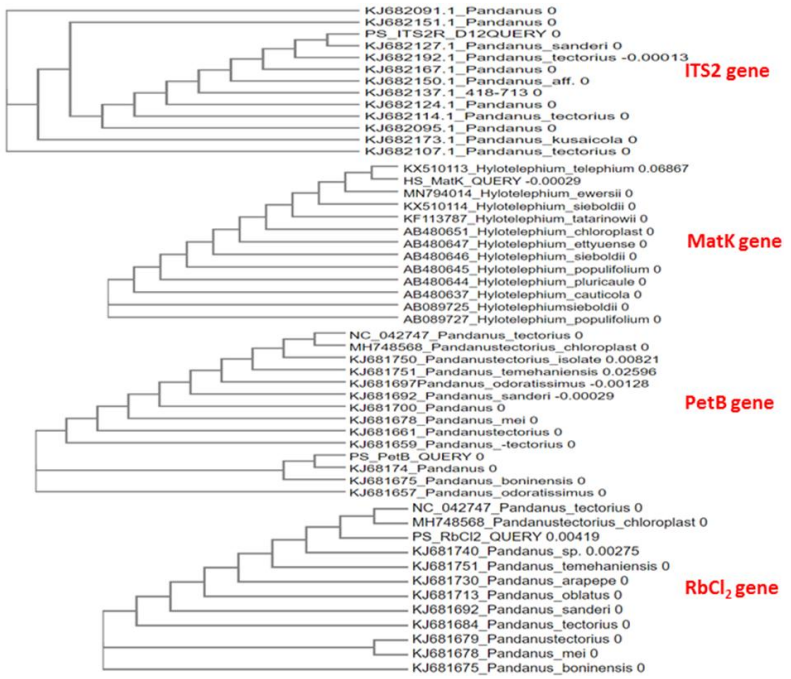

Figure 5. *Phylogenetic tree representing the evolutionary relationship of plant Pandanus sanderi*

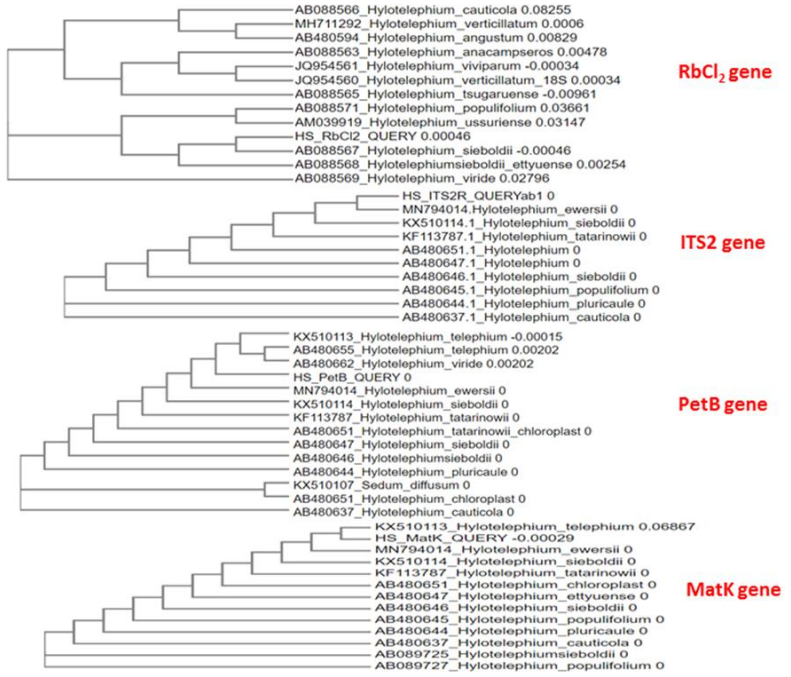

Figure 6. *Phylogenetic tree representing the evolutionary relationship of plant Hylotelephium ewersii.*

Hylotelephium ewersii

After identifying the plants using DNA bar coding technique we further wanted to know the bacteria responsible for growth of these plants. Hence, the root samples were taken, crushed and spread on LB agar plates for growth of bacteria. *Figure 7* shows the growth of symbiotic bacteria from the root samples. Individual colonies were observed morphologically and processed further for genomic DNA isolation. *Figure 8* shows the

isolated genomic DNA from bacteria and amplified 16srRNA gene from bacteria using PCR technique. The sequences got from Sanger method were analysed further using NCBI Blast and closest hits were selected. *Table 3* highlights the predicted bacteria from gene sequences using NCBI BLAST. The bacteria were identified as *Acinetobacter calcoaceticus* and *Pseudomonas stutzeri.* The phylogenetic tree was constructed for obtained bacteria's to understand the evolutionary relationship as shown in *Figure 9*.

Table 3. *The predicted bacteria from gene sequences using NCBI BLAST.*

Sample	Description	Query coverage (%)	E value	% Identity	Accession No.
Bac1	*Acinetobacter calcoaceticus* strain bnj_dkc5 16S ribosomal RNA gene, partial sequence	99	0.0	96.98	DQ074752.1
Bac2	*Pseudomonas stutzeri* strain NB11_4A 16S ribosomal RNA gene, partial sequence	99	0.0	97.84	JX087266.1

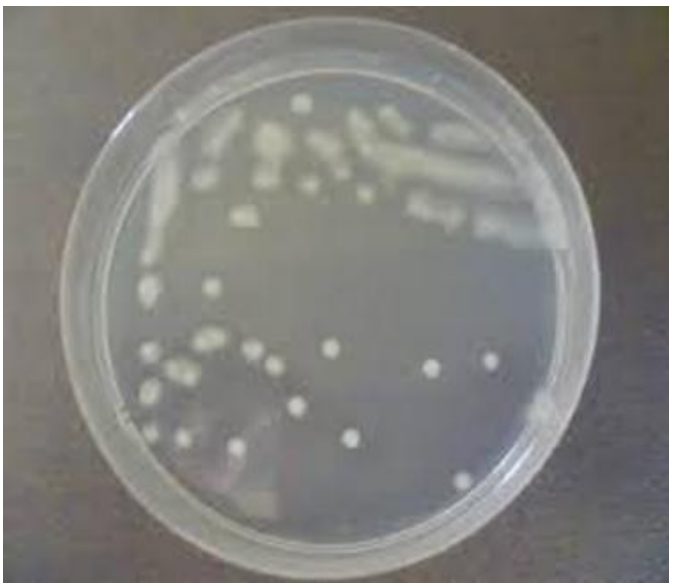

Figure 7. *Growth of Symbiotic bacteria from root samples.*

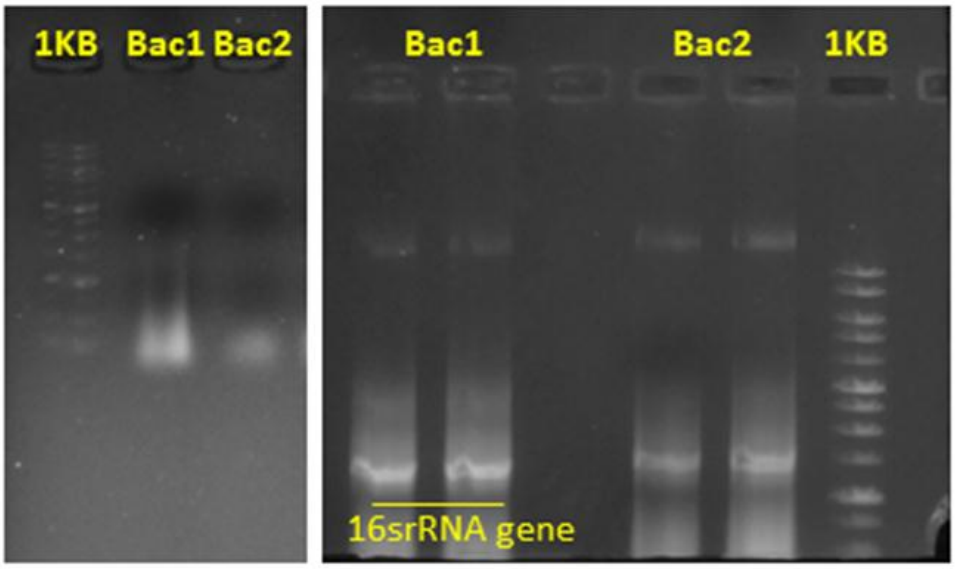

Figure 8. *Genomic DNA and amplified 16srRNA gene from bacteria.*

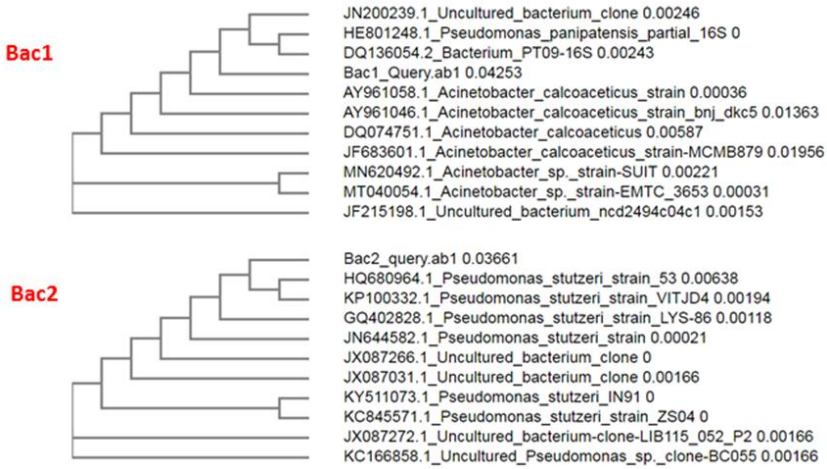

Figure 9. *Phylogenetic tree representing the evolutionary relationship of bacteria.*

Study performed by Lee et al., (2015) presented can explain the mechanism behind the health benefits of active interaction with indoor plants, from the standpoint of the stress response. The data also supported the notion that active interaction with indoor plants can have positive effects on human stress response mediated by cardiovascular activities (Tsunetsugu et al., 2007). The physiological benefits of these plants may result from multiple natural stimuli acting on the senses of vision, hearing, touch, and smell (Park et al., 2008; Lee et al., 2011; Lee et al., 2014). There are so many studies which have reported the positive effects of indoor plants, wherein most of them have been focused on the benefits of passive interaction with indoor plants (Lohr et al., 2005). A number of studies are also underway concerning the physiological and psychological effect of interacting with plants where they relieve physiological stress and negative psychological symptoms (Chang and Chen, 2005). Various investigators have reported endophytic microbes from various plant exists in different ecosystems. Parallely, the opportunity to find new and interesting micro-organism among myriads of plants in different settings and ecosystems is under practise. And the success of DNA barcoding lies in the distinct identification of the clusters in the phylogenetic analysis (Steinke et

al., 2009). Hence, our work has reported the authentication of plant species *Draceana senderiana,Pandanus sanderi* and *Hylotelephium ewersii* using multiple markers of DNA bar coding technique. The symbiotic bacteria were identified as *Acinetobacter calcoaceticus* and *Pseudomonas stutzeri* using microbiological and molecular procedure.

Conclusion

In this study the phylogenetic relationships of 3 indoor plant species were tested by using four barcode regions. The plants were identified as *Draceana senderiana,Pandanus sanderi* and *Hylotelephium ewersii*. Hence, our results suggest that active interaction with these indoor plants can reduce physiological and psychological stress compared with mental work. This might be accomplished through suppression of nervous system activity and diastolic blood pressure and promotion of comfortable, soothed and natural feelings. Further these plants were explored to culture and idenfity symbiotic present in roots. This study evidenced that these bacteria are potential and act asexploited resources for plants. A detailed investigation on these bacteria is needed to prove its further properties and which will leads to the discovery of numerous value metabolites. Future research aiming towards the role of plants on control of pollution is in progress.

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Conflict of interest

Author confirm that there are no conflict of interest inolve with any parties in this research study.

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