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Isolation and Screening of Potential Phyllosphere Microorganisms from Mangifera for Its Bioinoculant activity: A Review

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ABSTRACT

Phylloplane of a plant constitutes an important habitat for a variety of microbes many of which play important roles in plant growth. Diversity in epi and endophytic microorganisms from the local Mango variety Imam pasand is investigated in this study. For this purpose, bacterial and fungal strains were isolated from the surfaces and the inner tissues of dominant fruit crop Mango. Leaf wash and homogenized leaf mixture solution were used for the isolations from healthy plants. Our study indicated that complex interactions existed between the host and their epi and endophytic microflora. Each crop has specific bacterial community with the reference of epi and endo phyllosphere. The number and species of bacterial strains varied not only with their host weed plants but also in epi and endo phyllospere. Plants and phylloplane-microbial-interactions result in increased fitness and productivity of agricultural crops. In this study, an attempt was made to isolate and screen phylloplane PGPR in order to better understand the role of phylloplane microbiota in influencing the survival of certain varieties in nutrient limited conditions for agricultural applications.

INTRODUCTION

An abundant and diverse community of microorganisms naturally exists on the surface of above-ground parts of plants, known as the phyllosphere. The phyllosphere can be subdivided into the caulosphere (stems), phylloplane (leaves), anthosphere (flowers), and carposphere (fruits). The phyllosphere is one of the most prevalent microbial habitats on earth and bacteria are by far the most abundant and persistent phyllosphere organisms, with a typical cell density of 10^6–10^7 cells cm^{-2}. Phyllosphere microbial community studies to date have mainly focused on plant species such as *Arabidopsis thaliana* (thale cress), *Lactuca sativa* (lettuce), *Glycine max* (soy bean), *Trifolium repens* (white clover), and *Oryza sativa* (rice) and the greatest microbial diversity has been described using metagenomic tools. Broadly, leaf microbial communities mainly comprise bacteria belonging to the phyla Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes. Further, Proteobacteria species have been reported to comprise about half of the phyllosphere community suggesting that, at higher taxonomic levels, phyllosphere bacterial communities are similar across various host plant species.

Increased knowledge of plant–microbe interactions enable a better understanding of their role during natural plant growth and development, and this knowledge can be translated into improved agricultural biomass production and microbe-assisted phytotechnologies. In this study, the bacterial phylloplane community of ***Mangifera indica*** is explored using culture-dependent and -independent techniques. **This plant is considered to be food crop as it gives a fruit**. Hence it is gives shelter to the bird and animal.

To enhance our understanding about the diversity and function of microbial communities living in the phylloplane, culture-independent approaches are indispensable. Nevertheless, one of the key challenges for microbiologists remains to develop strategies to culture the vast diversity of microorganisms. There has been a recent resurgence in the application of classical culture techniques to interrogate the microbial world, with particular success in environments such as the human gut. In general, a wide diversity of cultured bacteria may be retrieved by increasing the diversity of growth media used to include complex media rich in macro- and micronutrients, and custom media formulations that are more oligotrophic. This includes growth media with low concentrations of mineral salts, the addition of (host) plant extracts, separated preparation of growth medium components, and the use of a range of solidifying agents. Monitoring for colony formation over extended incubation periods is also useful. Once a collection of bacterial isolates is obtained and maintained in the laboratory their functional characteristics can be

evaluated, including plant growth-promoting (PGP) potential through the biosynthesis of PGP hormones and production of specific enzymes.

PHYLLOSPHERE

Phyllosphere is an abode for different kinds of microorganisms. Recent developments in the advancements of molecular and computational tools, high-throughput screening procedures, and amalgamation of omics techniques have significantly enhanced theunderstanding of phyllosphere-associated microbial communities in relation to their structural, functional, and ecological properties. Several research findings indicated that phyllosphere microbiome has played an important role in sustaining crop growth and health management by regulating plant physiological processes under ever- changing environmental conditions.

Cultivation-independent studies have revealed that a few bacterial phyla predominate in the phyllosphere of different plants and that plant factors are involved in shaping these phyllosphere communities, which feature specific adaptations and exhibit multipartite relationships both with host plants and among community members. Insights into the underlying structural principles of indigenous microbial phyllosphere populations will help us to develop a deeper understanding of the phyllosphere microbiota and will have applications in the promotion of plant growth and plant protection.

MATERIALS AND METHODOLOGY

Sample Collection And Microbial Isolation:
The fresh and healthy leaves M***angifera*** were collected from the farm located at Kurumbapalayam, Coimbatore and put separately into sterile bags then taken back to laboratory afresh for isolation of epiphytic and endophytic phyllosphere microorganisms.

To analyze epiphytic microflora, leaf washings were used for the isolation. A leaf sample (was shaken for about 1h in 100 ml of sterile distilled water. An aliquot of 1ml from leaf wash was plated on Nutrient Agar, NB Medium (g/L): Peptone, 5.0; Beef Extract, 1.0; Sodium chloride 5.0; Yeast extract 2.0; Agar, 15.0, was used for bacterial isolation. For endophytic microflora, leaves of each weed were washed through in running water. Sterile leaves were ground in blender with 100ml of sterile distilled water to form a homogenized leaf solution mixture.

Leaf mixture (1 ml) was then plated on NB Medium and PDA agar medium for bacterial and fungal isolation, respectively. The Petri dishes were incubated for 1-2 days at 25-28℃ for the bacterial colony count. Bacterial colonies were counted after 24 hours at 37℃ and purified for further identification To perform morphological taxonomy analysis of bacterial isolates were plated onto petri dishes and incubated for 2 days at 25- 28℃ in darkness to observe the colony morphology and measure their diameters.

Bacterial strains were identified including pigment, colony form, elevation, margin, texture and opacity (Smibert and Krieg, 1981). In addition, bacterial strains were tested with respect to Gram reaction and biochemical characteristics (Holt et al., 1994).

Additionally, for each growth medium, representative bacterial isolates will be purified such that their PGP potential could be evaluated. The information obtained can guide targeted single-colony isolation, focusing on growth conditions that favour certain taxa thereby increasing the likeliness to isolate previously uncultured or underrepresented bacterial species. Identification permits potential candidate Plant growth promoting bacterial species from the weed phylloplane whose reports are meager.

Bioassays For Plant Growth Promoting Trait:
Once the isolated bacteria is screened for Plant Growth Promoting Activities like phosphate solubilization, root colonization and *in vitro* plant growth promotion, IAA production, production of protease, cellulose, amylase, glucanase enzymes production will be screened to confirm as PGPR.

Screening For Phosphate Solubilization:
The phosphate solubilization ability of the bacterial, fungal isolates will be tested by plate assay using National Botanical Research Institute's phosphate (NBRIP) growth medium (Nautiyal, 1999). The medium contained in a litre ; 10 g glucose, 5 g Ca3(PO4)2, 5 g MgCl2, 0.25 g MgSO4, 0.2 g KCl, 0.1 g (NH4)2SO4 and 1.5% agar. The pH of the media was adjusted to 7.0. The plates were incubated at 28°C. Formation of visible halo zones around the microbial colonies/structures in plates containing NBRIP media was an indication of the phosphate solubilization ability of the microorganisms. The halo and colony/structure diameters were measured at 7 and 14 days after inoculation. Halo size will be calculated by subtracting colony/ structure diameter from the total diameter.

Molecular Characterization Of Bacterial Isolates:
Culture DNA will be isolated based on plant growth promoting and biocontrol activities, and be used as template DNA in PCR to amplify the16S rRNA gene for phylogeneticanalysis. The sequencing of 16S rDNA/ 18S rDNA sequence will be done privately as outsource basis.

Indole-3-Acetic Acid Production:
For detection and quantification of indole-3-acetic acid (IAA) production by bacterial isolates, isolated colonies were inoculated into Jensen's broth (Sucrose 20 g, K_2HPO_4 1 g L^{-1}, $MgSO_4$ $7H_2O$ 0.5 g L^{-1}, NaCl 0.5 g L^{-1}, $FeSO_4$ 0.1 g L^{-1}, $NaMoO_4$ 0.005 g L^{-1}, $CaCO_3$ 2 g L^{-1}) (Bric et al., 1991) containing 2 mg mL^{-1} L-tryptophan. The culture was incubated at 28 ± 2°C with continuous shaking at 125 rpm for 48 h (Rahman et al., 2010). Approximately 2 mL of culture solution was centrifuged at 15000 rpm for 1 min, and a 1 mL aliquot of the supernatant was mixed with 2 mL of Salkowski's reagent and incubated 20 min in darkness at room temperature (150 ml concentrated H_2SO_4, 250 ml distilled water, 7.5 ml 0.5 M $FeCl_3.6H_2O$) as described byGordon and Weber (1951). IAA production will be observed as the development of a pink-red color, and the absorbance will be measured at 530 nm using a spectrophotometer.

Root Colonization:
Root colonization by bacterial isolates will be determined according to the protocol of Hossain et al. (2008). Seed bacterization will be done in important crop seeds for *in vitro* PGP activity. Roots were harvested from plants at 7, 14, and 21 days of growth. Root systems were thoroughly washed with running tap water to remove adhering soil particles, then were rinsed three times with sterile water and blotted to dryness. Serial dilutions were prepared and plated on NB agar plates, and the number of colony-forming units (CFU) per gram root was determined after 24 h of incubation at 28 ± 2°C.

Understanding of phyllosphere-associated microbial communities in relation to their structural, functional, and ecological properties. Several research findings indicated that phyllosphere microbiome has played an important role in sustaining crop growth and health management by regulating plant physiological processes under ever- changing environmental conditions.

Cultivation-independent studies have revealed that a few bacterial phyla predominate in the phyllosphere of different plants and that plant factors are involved in shaping these phyllosphere communities, which feature specific adaptations and exhibit multipartite relationships both with host plants and among community members. Insights into the underlying structural principles of indigenous microbial phyllosphere populations will help us to develop a deeper understanding of the phyllosphere microbiota and will have applications in the promotion of plant growth and plant protection.

CONCLUSION AND DISCUSSION

This study gives first insights into the total microbial community of the ***Mango***, including novel bacterial, fungal isolates of Kurumbapalayam, Coimbatore, Tamil Nadu and an evaluation of its culturability using different growth media. We further provide a collection of bacterial isolates underrepresented in current databases, including the characterization of **PGP** (plant growth promoting) profiles. Here we highlight the potential of simple strategies to obtain higher microbial diversity from environmental samples and the useof high-throughput sequencing to guide isolate selection from a variety of growth media. This study paves way for identification and application of bioinoculants from previously unreported plant species.

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