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Study the Diversity of Root Associated Microorganisms of Medicinal Plant *Alpinia galanga.*

KR Beula Rani[1], SK Sundar[2], and M Murugan[3]*.

[1]Sathyam College of Engineering & Technology, Sathyam Nagar Aralvaimozhi, Tamil Nadu – 629 301, India.
[2]Research Department of Microbiology, M.R. Govt. Arts College, Mannargudi, Tiruvarur, Tamil Nadu – 641 014, India.
[3]Centre for Biological Science, Noorul Islam Centre for Higher Education, Kumaracoil, Tamil Nadu – 629 180, India.

ABSTRACT

In this present study, diversity of rhizosphere microbial flora present in the medicinal plant's root soil of *Alpinia galanga* was studied. The rhizosphere soil was collected from three different places of Kanyakumari District, Tamil Nadu. Nineteen bacteria, five fungal and two actinomycetes species were isolated and identified by cultural, morphological and biochemical studies. Among these *Bacillus* sp, *Aspergillus* sp and *Streptomyces* sp were found higher percentage of general distribution. These results may helpful for researchers who study the diversity of rhizosphere microflora of plants.

Keywords: Rhizosphere, microflora, medicinal plants, *Alpinia galanga* and root exudates

Corresponding author

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INTRODUCTION

The rhizosphere includes plant roots and the surrounding soil associated microbial flora. The term was firstly coined by Hiltner in 1904 [1]. It is a very important and intensive interaction take place between the plant and microflora [2]. The diversity and functions of microbes in the rhizosphere, a narrow region around the root, are related to the root exudates, biogeochemical reactions and respiration [3].

The rhizosphere contains abundant bacteria, fungi, protozoa and nematodes. Some nematodes are feeding on bacteria and fungi [3]. Root based interactions between plants and organisms in the rhizosphere are highly influenced by EDAPHIC factors [4]. These interactions include signal traffic between roots of competing plants and soil microbes and one way signals that relate the nature of chemical and physical properties to the roots [5].

Plants depend on the ability to communicate with microbes. The converse is also true, many bacteria and fungi dependent on association with plants that are often regulated by root exudates [6]. Root exudates play an active and relatively well documented role in the regulation of symbiotic and protective interactions with microbes [7]. However the role of root secretions in regulating other rhizospheric interactions is less clear.

A wide range of organic compounds secreted by plant roots in the rhizosphere provide a food source for microorganisms increasing microbial density and activity in the rhizosphere than in the bulk soil. They helps to solubilise poorly soluble inorganic phosphorous and mineralize organic phosphorous sources and markedly increase plant growth [8]. The study of rhizosphere bacteria from the medicinal plants is very crucial, as they are well known to have impact on plant growth and also produce industrially important metabolites and improve quality of medicinal product [9]. The objectives of the study is to isolate and characterize the rhizosphere microbial flora of the rhizosphere soil samples of selected medicinal plants

MATERIALS AND METHODS

Sample collection

Rhizosphere soil of medicinal plant *Alpinia galanga* was collected from three different places of Kanyakumari District, Tamil Nadu. The samples were collected by gently uprooting the plants using sterile shovel. The unwanted soil particles of the plants were removed by shaking the plant, the soil adhered to the root was collected to sterile polyethylene bags and transferred aseptically to the laboratory.

Isolation of rhizosphere microflora

Preliminarily, 1gm of sample was made serial dilution using sets of test tubes containing 9 ml of saline water. Dilutions were made up to 10^{-6} dilution and the mixture was agitated with the vortex at maximum speed. An aliquot of 0.1 ml of each dilution from 10^{-3} to 10^{-5} was taken and spread evenly over the surface of Nutrient agar for the isolation of bacteria, Potato Dextrose agar for the isolation of fungus and Starch casein agar for the isolation of actinomycetes. The inoculated plates were incubated at 30- 35°C. Bacteria were counted after 24-48 hours, fungi and actinomycetes were counted after 4 to 7 days of incubation.

Characterization and identification of microflora

The isolated pure colonies of bacterial strains were identified by studying the characterization, such as morphological, physiological and biochemical properties. Gram staining, spore staining, motility test, catalase, oxidase, indole reaction, methyl red test, voges-proscauer test, citrate utilization, phosphate solubilization, nitrate reduction, urease production, triple sugar iron test, starch hydrolysis, gelatin liquefaction, casein hydrolysis, sugar utilization (glucose, sucrose, lactose, dextrose & arabinose). All the above tests were carried out by methods descried in Bacteriological manuals [10] and all the biochemical test reagents were procured from Himedia, Mumbai.

The morphologically different fungal strains were identified by studying cultural, pigmentations, microscopic study by lactophenol cotton blue staining technique. Morphologically different actinomycetes

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strains were characterized by morphological, biochemical and physiological methods. Macroscopically the actinomycetes isolates were differentiated by their colony characters, e.g. size, shape, color, consistency etc. For the microscopy, the isolates were grown by cover slip culture method. Starch hydrolysis and various sugar fermentation tests were performed. The observed morphology of the isolates was compared with the actinomycetes morphology provided in Bergey's Manual for the presumptive identification of the isolates.

RESULT AND DISCUSSION

In this present study, a total of 21 different bacterial colonies (8 colonies from sample 1, six colonies from sample 2 and seven colonies from sample 3), 5 different fungal colonies and 3 different actinomycets colonies were isolated.

The 21 isolated bacterial colonies were most likely identified as *Bacillus* sp I*, Bacillus sp II, Neiserria sp, Bacillus sp III, Bacillus sp IV, Bacillus lichiniformis, Streptococcus sp I, Paenibacillus popilliae, Bacillus sp V, Bacillus sp VI, Neiserria denitrificans, Staphylocoocus sp, Micrococcus sp, Serriatia sp, Pseudomonas sp1, Enterococcus sp, Veillonella sp, Rhizobium sp1,* and *Pseudomonas sp I1.* Among these, bacterial species were found as higher distribution (44%) followed by *Pseudomonas* sp 11%, *Staphylocoocus sp, Enterococcus sp, Rhizobium sp* were 7% and *Neiserria sp, Streptococcus sp, Paenibacillus sp, Micrococcus sp, Serriatia sp, Veillonella sp* were found as 4% (Fig. 1).

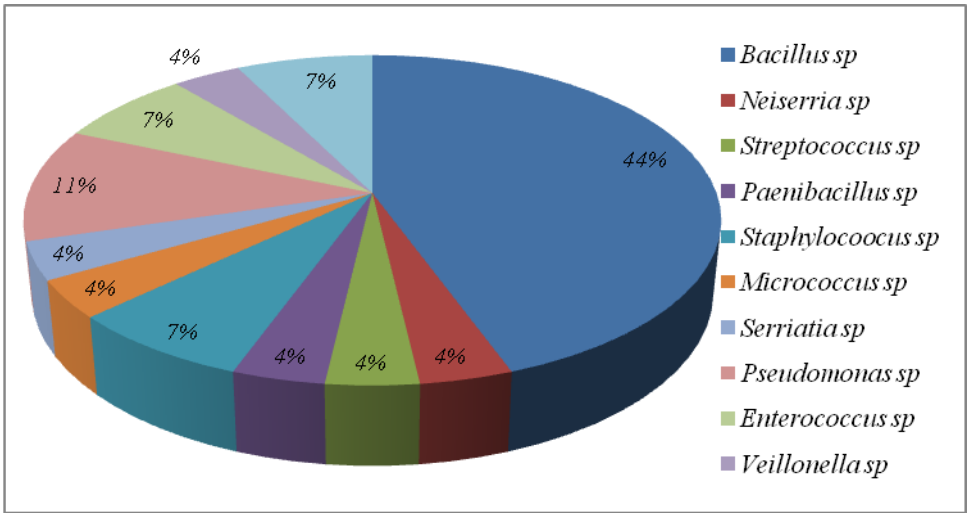

Figure 1: Generic distribution percentage of bacteria

The fungi colonies were probably identified as *Aspergillus sp, Rhizopus sp, Penicillium sp, Trichoderma sp,* and *Fusarium sp.* In this study, *Aspergillus sp* was found as higher distribution (30%), followed by *Rhizopus sp, Penicillium sp, Trichoderma sp* were 20% and *Fusarium sp* was 10% (Fig. 2). Actinomycetes were identified as *Streptomyces sp* and *Frankia sp.* Among these, 75% of population was *Streptomyces sp* (Fig. 3).

In this present study, the bacterial genus *Bacillus* found dominantly among many number of organisms isolated. *Bacillus* are present in anywhere, includes soil, water, air, other sources, and able to produces many important metabolites and enzymes. El-Deeb et al. [11] isolated 28 endophytic bacteria from different organs of the medicinal plant *Plectranthus tenuiflorus*. Among these, 8 isolates were *Bacillus* sp, *Bacillus megaterium, Bacillus pumilus, Bacillus licheniformis, Micrococcus luteus, Paenibacillus* sp, *Pseudomonas* sp. and *Acinetobacter calcoaceticus*. Raichand *et al.* [12] isolated a pigmented novel bacterium *Pontibacter* sp from the rhizosphere of an Indian medicinal plant, *Nerium indicum*. The bacterial population found in the rhizosphere of medicinal plant *Fritillaria thunbergii* was *Proteobacteria*, *Acidobacteria, Actinobacteria* and *Bacteroidetes* [13].

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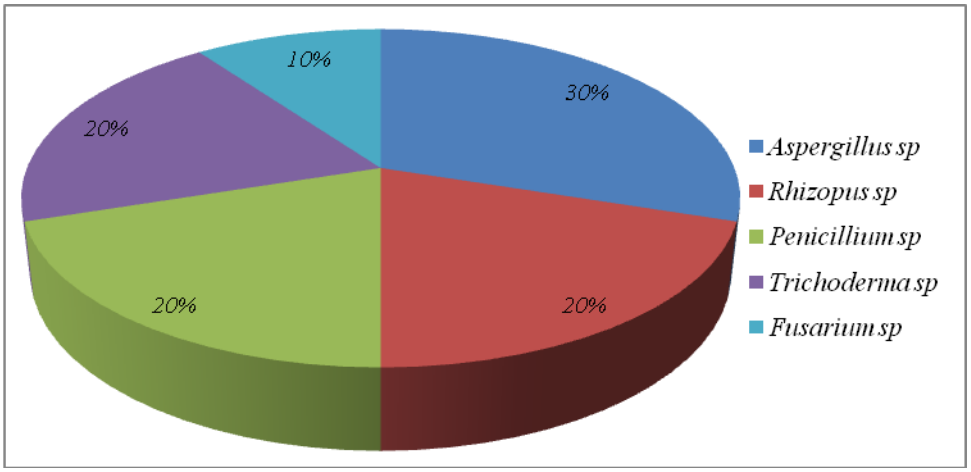

Figure 2: Generic distribution percentage of fungi

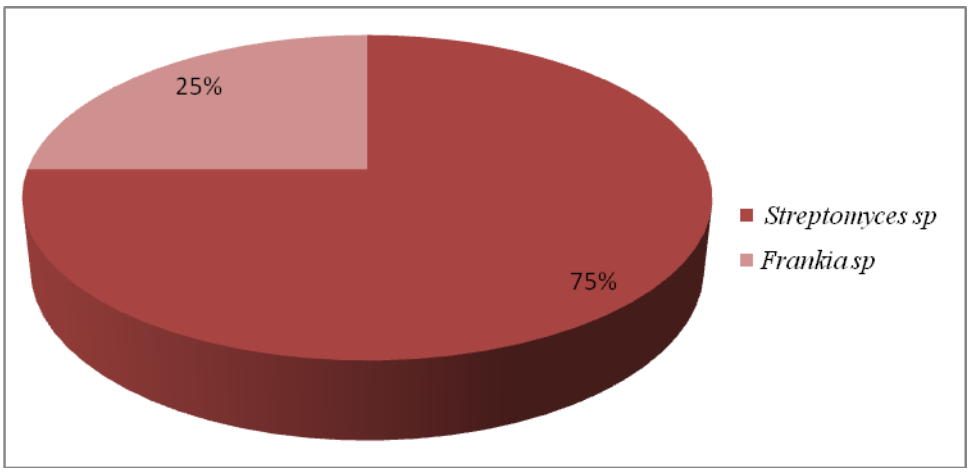

Figure 3: Generic distribution percentage of actinomycetes

Fungal colonisations in the rhizosphere of medicinal plants have been reported widely. However, the distribution in the rhizosphere of medicinal plants may vary depending on host plant species, growing season, soil properties, local climate and environmental factors. The Egyptian henbane (*Hyoscyamus muticus* L.), a medicinal plant of family Solanaceae native to the desert producing pharmaceutically important compounds is colonised by a higher number of fungal species and endophytic fungi [14]. Rhizosphere soil of the medicinal plants (*Centella asiatica* and *Ocimum sanctum*) revealed the presence of 16 species of fungi [15]. Sundar *et al*. [16] identified 21 rhizosphere fungal species in roots of the medicinal plants such as *Eclipta prostrata, Indigofera aspalathoides* and *I. tinctoria*. Tamilarasi et al [17] isolated *Aspergillus, Penicillim, Mucor, Rhizopus* and *Fusarium* sp from the medicinal plants. In actinomycetes isolation, Streptomyces sp was as dominant. This result was supported by Subbarao [18], in the soil 80% of actinomycetes population is *Streptomyces*.

The rhizobacterial strain of *Bacillus subtilis* isolated from the traditional Chinese medicinal herb *Trichosanthes kirilowii* enhances plant growth and inhibits the activity of nematode and has the potential to be a safe and effective microbial pesticide [19]. The bacterial endophytes belongs to the genus *Pseudomonas* sp isolated from medicinal plant *Annona squamosa* L. showed antimicrobial activity [20]. Plant growth promoting rhizobacteria (PGPR) isolated from the medicinal weed, *Cassia occidentalis* are an attractive eco-friendly alternative to chemicals in agriculture [21]. Gupta *et al*. [22] evaluated the potential of phosphate solubilising bacteria, *Burkholderia gladioli, Enterobacter aerogenes* and *Serratia marcescens* for utilizing rock phosphate to improve the medicinal plant growth. The *Aspergillus sp* and *Penicillium pinophilum* fungal present in the rhizosphere of different plants, can effectively solubilise rock phosphate and increase the uptake of phosphorus by the growth of plants [23]. Deleterious effects of some microorganisms on plants, the beneficial effects are usually greater and the overall results are generally demonstrated by growth promotion and faster germination [24].

It was concluded that, the rhizosphere soil of the medicinal plant *Alpinia galanga* contained highest diversity of microorganisms includes bacteria, fungi and actinomycetes. Rhizosphere bacterial populations were recorded higher than fungal and actinomyces. These results may helpful for researchers who study the diversity of rhizosphere microflora of medicinal plants.

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