

Original Research Article

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Isolation and Characterization of Microorganisms from Agriculture Soil of *Mangifera indica* Orchard

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ABSTRACT

A broad range of microorganisms are present in soil of mango orchard which involved in various mango plant diseases. In order to preliminary study for plant pathogenesis the soil samples were collected from GKVK, University of Agricultural Science, Bangalore, Karnataka, India. A number of bacterial and fungal isolates were obtained from soil sample. The bacterial isolates were characterized by Gram staining, Catalase test, MR test, VP test, IND test and Citrate test and fungal isolates were characterized by staining. These analyses revealed the presence of various bacterial pathogen including *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Shigella* species, *Bacillus anthracis*, *Bacillus subtilis*, *Staphylococcus* species, *Streptococcus* species, *Corynebacterium*, *Micrococcus*, *Azomonas* species and *Rhizobium* species. Identification of *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, *Penicillium* and *Rhizopus* species characterized as a fungal pathogen. The present study provided baseline information regarding the phytopathogenic bacteria and fungus which associated with soil of mango orchard.

Keywords

Mango, Bacterial pathogen, Fungal pathogen, Biochemical test.

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Introduction

Mango (*Mangifera indica* L.) is an important fruit crop of the tropical and subtropical countries (Litz, 2009). The mango tree is considered to have evolved in the rainforests of South and South-east Asia (Knight, 1980; Krishna and Singh, 2007). India is the largest producer of mango in the world, contributing to nearly 46% of the total world production. The major constrain of mango production is many devastating diseases (Lim and Khoo, 1985; Iqbal *et al.*, 2006; Rajput and Rao,

A range of microorganisms are involved in these diseases such as fungi, algae and bacteria (Litz, 2009). These microbes cause sets of symptoms including dieback, spots, necrosis, mildew scab, blotch, anthracnose and rots in mango trees (Ploetz, 2003; Freeman *et al.*, 1999; Haggag and Abd El-Wahab, 2009). *Pseudomonas syringae* and *Xanthomonas* sp. (causing apical necrosis and bacterial black spot respectively) are among the few known bacterial pathogens of mango

trees (Cazorla *et al.*, 1998; Pruvost *et al.*, 2005; Ah-You *et al.*, 2007). Currently, mango trees in India are suffering from a disease with symptoms like Dieback, Powdery Mildew, Anthracnose/Blossom Blight, Mango Malformation, *Alternaria* Leaf Spot, Bacterial Canker, Stem End Rot, Gummosis and Root Rot (Kumar *et al.*, 1993; Ploetz, 2001; Khanzada *et al.*, 2004; Youssef *et al.*, 2007).

There exists a lot of diversity of regarding the prevalence of microorganism in mango orchard soil of various parts of the world. In India, however, scant information is available about the prevalence of microorganism strains in various parts of the country. Understanding local pathogen genetic diversity is the first step in a successful integrated disease management programme. One of the purposes of the present investigation on isolation of microorganism from mango orchard soils of Karnataka is to characterize biochemically.

Materials and Methods

Soil Sample collection

Soil samples were collected from the six sites as unmoist soil, moist soil, shaded soil, unshaded soil, Aged soil and new sapling soil of mango orchard of University of Agricultural Sciences, Bangalore, Karnataka, India. These different sites helpful for capture the diversity of the microorganisms. The soil samples (0-15cm depth) were collected from each site into freshly unused polythene bags.

Pure culture

For reducing microbial population, 1 g of soil was dissolved in 10 ml of sterile distilled water to make soil suspension. Serial dilution was carried out for getting isolated single colony. In this research, nutrient agar medium was used for bacterial growth and PDA for fungal growth. 28 g of nutrient agar was dissolved in 1000 ml distilled water and 39 g

of PDA is dissolved in 1000 ml of distilled water and sterilized in autoclave for 15 min at 121⁰C. Streaking plate method was used to get single colonies of pure culture.

Sample inoculums

One ml of 10⁻⁵ dilution of soil suspension was plated out as inocula onto freshly prepared sterile nutrient agar medium in petridishes (Bacterial growth).

The inocula were evenly spread on the surface of the nutrient agar plates by using a sterile bent glass rod. After incubation for 24-48 hrs at 37⁰C, mucous colonies were formed over the plates. Similarly for fungal growth 1ml of 10⁻⁷ dilution of soil suspension were plated out as inocula onto freshly prepared sterile Potato Dextrose Agar (PDA) medium in Petri dishes. The inocula were evenly spread on the surface of the PDA plates by using a sterile bent glass rod. After incubation for 48-72 hrs at 28⁰C, fungus colonies were formed over the plates.

Gram staining

A loop full of the bacterium was spread on a glass slide and fixed by heating on a very low flame. Aqueous crystal violet (Himedia) solution (0.5%) was spread over the smear for 30 seconds and then gently washed with slow running tap water for one minute. It was then flooded with iodine for one minute, rinsed in tap water and decolorized with 95% ethanol until colorless runoff. After washing, the specimen was counter-stained with safranin (Himedia) for approximately 10 seconds, washed with water, dried and observed under microscope at 40X using immersion oil (Schaad, 1980).

Biochemical tests

Biochemical tests such as Indole test, Catalase test, MR test, VP test, IND test and citrate test

were carried out to find the enzymatic activity of isolated organism.

Indole test

One percent (1%) of tryptone broth was inoculated with a bacteria colony. Incubate inoculated tubes at 37⁰C for 48 hours. After 48 hours of incubation, add 1ml of Kovac's reagent and then shake the tubes gently and allow standing for 20 minutes. The formation of the red coloration at the top layer indicated positive and yellow coloration indicates negative.

Catalase test

This was carried out by putting a drop of Hydrogen peroxide on all 6 clean slides. With the edge of another slide, a colony of organism was picked and allowed to be in contact with the hydrogen peroxide. Presence of bubbles indicates positive reaction and absence of bubbles indicates negative reaction.

MR-VP test

Prepare a MR-VP broth of pH 6.9 and then pour the 5ml of broth in each of 6 test tubes and sterilize by autoclaving at 15 lb pressure for 15 min. Inoculate the test tubes with test organism and incubate all the tubes at 37⁰C for 48-72 hrs, after which add 5 drops of methyl red indicator to all the tubes, a red color formation signifies a positive methyl red test and yellow color signifies a negative methyl red test. To the rest of the broth tubes add 5 drops of 4% potassium hydroxide (KHO) were added followed by some 15 drops of 5% alpha naphtol in ethanol. Shake the tubes gently for 1min and allow the reaction to complete for about 30-45 min. The red color formation indicates a VP positive test while no color change indicates VP negative test.

Citrate utilization test

Prepare the Simmon's citrate agar pH 6.9. This was carried out by inoculating the test organism in all test tubes containing simmon citrate medium and after inoculation, these test tubes were incubated at 37⁰C for 48-72 hrs. The development of deep blue color after incubation indicates a positive result.

IND test

Inoculate the tryptophan broth with broth culture or emulsify isolated colony of the test organism in tryptophan broth. Incubate at 37⁰C for 24-48 hrs in incubator. Add 0.5 ml of Kovacs reagent to the broth culture. The positive result will show a red color ring formation after the addition of Kovacs reagent. The negative result will show a brown color ring formation after the addition of Kovacs reagent.

Results and Discussion

This study revealed that soil samples were analysed with respect to different types of bacteria and fungi. The bacteria found in all six soil samples were biochemically characterized as *Staphylococcus sp.*, *Streptococcus sp.*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Shigella sp.*, *Micrococcus sp.*, *Bacillus anthracis*, *Bacillus subtilis* and *Cocci sp.*, *Azomonas sp.*, *Corynebacterium sp.*, *Rhizobium sp.* are the dominating species of the soil samples (Table 1 and Fig. 2). This result also supported by previous researcher (Holding, 1971; Kumar *et al.*, 1993; Ploetz, 2001; Khanzada *et al.*, 2004; Youssef *et al.*, 2007; Musliu Abdulkadir and Salawudeen Waliyu, 2012; Khan *et al.*, 2014; Rupali, 2015). Gram staining result reveals that *Cocci*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Azomonas sp.*, *Rhizobium sp.* are Gram-negative (G-ve) and *Micrococcus sp.*,

Staphylococcus sp., *Streptococcus sp.*, *Corynebacterium sp.*, *Bacillus anthracis*, *Bacillus subtilis* are Gram-positive (G+ve). Similarly, when the soil samples were tested for different types of fungi, *Penicillium*, *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus*, *Fusarium oxysporum*.

In this study the isolated fungi were identified on the basis of cultural, microscopic and morphological characteristics (Fig. 1). Nayak (2015) also isolated similar type of fungus from the rhizosphere of mango plant.

Fig.1 Identified fungi and their microscopic image. A and B; *Aspergillus niger*, C; *Aspergillus flavus* D. *Fusarium sp.*

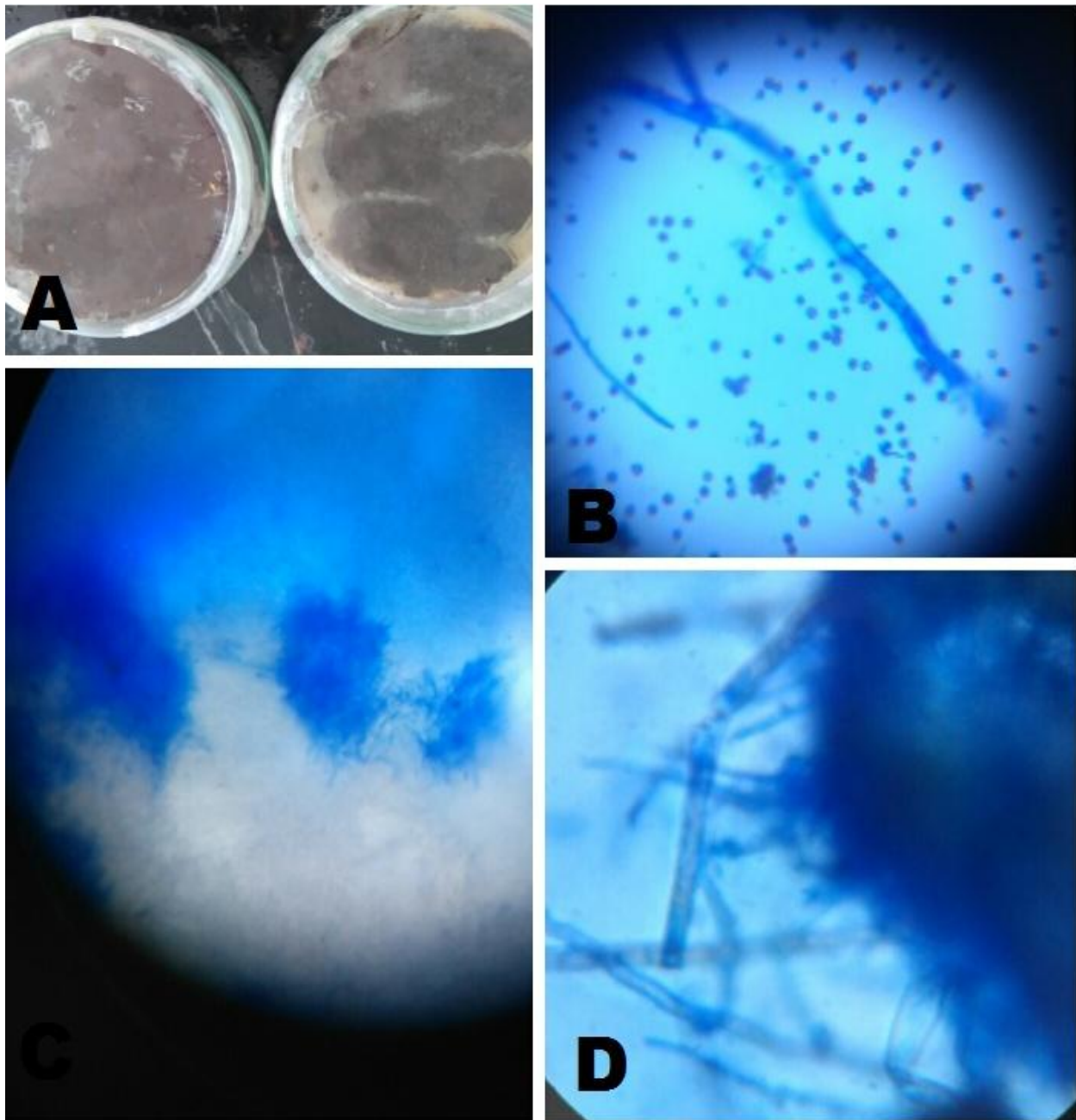


Fig.2 Biochemical characterization of the soil isolate

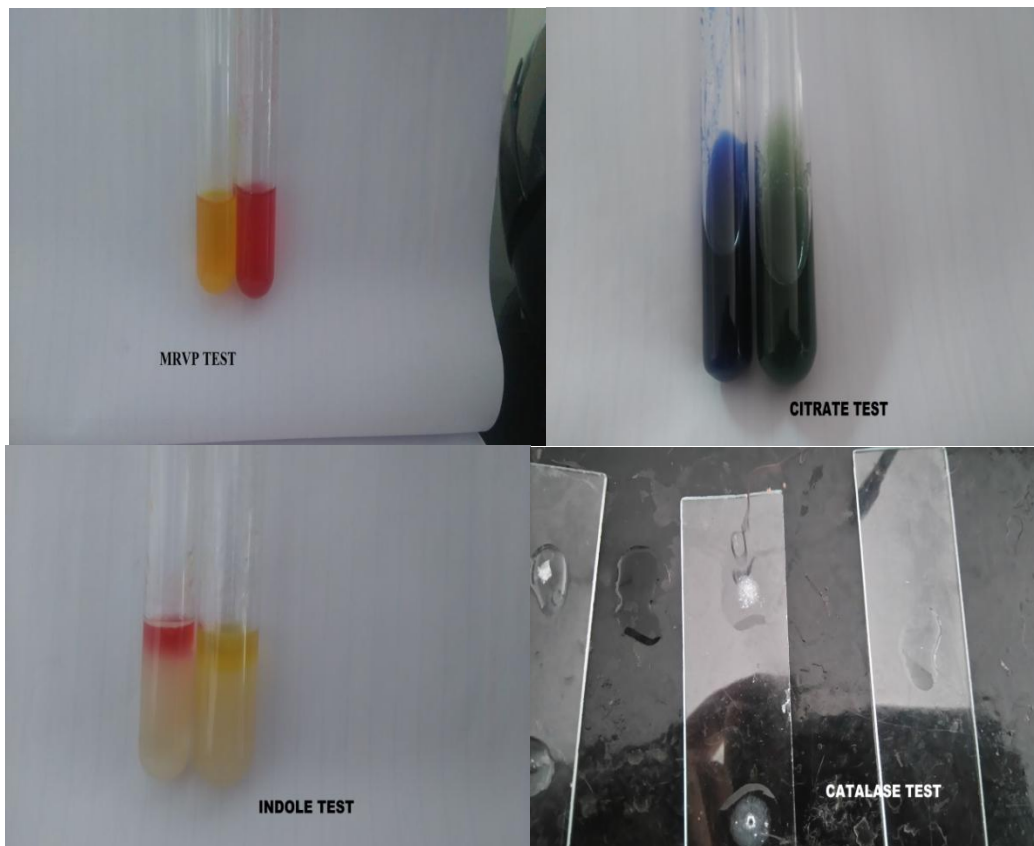


Table.1 Morphology and biochemical characterization of bacterial isolates

Sl.No.	Identified Bacteria	Gram stain	Catalase test	MR test	VP test	IND test	Citrate test
1	<i>Klebsiella</i>	-ve(bacilli)	+ve	-ve	+ve	-ve	+ve
2	<i>Enterobacter aerogenes</i>	-ve(bacilli)	+ve	-ve	+ve	-ve	+ve
3	<i>Micrococcus</i>	+ve(cocci)	+ve	+ve	-ve	-ve	+ve
4	<i>Staphylococcus</i>	+ve(cocci)	+ve	-ve	-ve	-ve	-ve
5	<i>Streptococcus</i>	+ve(cocci)	+ve	-ve	-ve	-ve	-ve
6	<i>Corynebacterium</i>	+ve(bacilli)	+ve	-ve	-ve	-ve	-ve
7	<i>Azomonas</i>	-ve (cocci)	+ve	-ve	-ve	-ve	+ve
8	<i>Rhizobium</i>	-ve (Rod)	+ve	-	-	-	-ve
9	<i>Shigella sp.</i>	-ve (Rod)	+ve	+ve	-ve	-ve	-ve
10	<i>Bacillus anthracis</i>	+ve (bacilli)	+ve	-ve	+ve	-ve	-ve
11	<i>Bacillus subtilis</i>	+ve (bacilli)	+ve	-ve	+ve	-ve	+ve

The isolation of various fungal and bacteria species of soil sample is quite rich in microbial flora. In agriculture process soil microorganisms such as bacteria and fungi may play important roles in soil fertility and pathogenesis in the form of loss and gain in the production of grains, fruits, and vegetables. Moreover, it also helps to maintain or enhance the environment quality and conserve natural resources. Identification and characterization of isolated bacteria were performed by morphological, microscopically, biochemical tests such as shape, arrangement, colonies, growth, indole production test, methyl red and Voges-Proskauer test, citrate utilization test, catalase test, growth at 37 °C. This study provides knowledge on microorganisms present in GKVK mango orchid soil habitat.

In conclusion the goal of this research was to collect and characterize the soil sample from mango orchard of Karnataka. In this study, we collected soil sample from six sites of mango orchard and characterized *Bacillus anthracis*, *Bacillus subtilis*, *Cocci*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Micrococcus species*, *Staphylococcus sp.*, *Streptococcus sp.*, *Corynebacterium sp.*, *Azomonas sp.*, *Rhizobium sp.* as a bacterial pathogen and fungi pathogen as an *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporium* and *Penicillium sp.*

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