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**Isolation and characterization of bacterial isolates from
agriculture field soil of Roorkee region**

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Naveen Chandra Bahuguna**

Abstract

Microorganisms present in soil plays a major role in enhancing the plant growth. In our present study soil sample was collected from the agriculture field of Quantum Global Campus, Roorkee and bacterial organisms were isolated by serial dilution technique. Well defined isolated colonies were selected and pure cultured. The isolates were named as QAF01, QAF02, QAF03, QAF04 and QAF05. Biochemical characterization of the above mentioned isolates determined that 20% of the isolates were spore formers, 60% were motile in nature, 100% were rod shapers in morphology and 60% were gram positive in reaction. Metabolism of various sugars was done at a maximum rate by the isolates QAF04 and QAF03. Based on their biochemical characterization and carbohydrate fermentation the isolates were identified to be *Bacillus*, *Pseudomonas*, *Streptomyces*, *Azotobacter* and *Alcaligenes*.

Keywords: Agriculture soil, isolates, bacteria, spore formers, motile

Introduction

Ubiquitous microorganisms are in every part of biosphere, including soil, hot springs, inside rocks at least nineteen kilometers deep underground etc., Microorganisms present in soil play an important role in maintaining the biological balance in the life of our planet. All soils contain bacteria, fungi and viruses in varying amounts depending on soil conditions. The permitted degree of acidity and the types of residue added also determine the relative abundance of microbes. The fertility of soil and the accumulation of organic matter within a short time is dependent on the bacterial amount (Kummerer, 2004) [6]. The products and the byproducts of microorganism in soil are beneficial to increase the nutrient contents in soil, plant growth and also play an important role in nutritional chains (Paul and Clerk, 1966; Kumerer, 2004) [10, 6]. Microorganisms in soil also play a major role in changing the nutrients into a form that can be used (Tugel and Lewandowski, 2010) [12]. Microorganisms in soil play a crucial role in biogeochemical cycles and in sustainable development of biosphere (Diaz, 2004) [3]. Microorganisms present in soil produces and consume two or three major naturally occurring green house gases that distinctly influence agriculture (Levine *et al.*, 2011) [7]. In our present study we have collected the soil sample from the agriculture land of quantum global campus, Roorkee. The bactericidal organisms were isolated and characterized.

Materials and Methods

Collection of Soil Sample

The soil sample was collected from uprooted area of the plants without breaking the secondary and tertiary roots and placed in a sterile petriplate and safely transferred to microbiology laboratory. The adhering soils from the root parts were separated carefully and stored at 4°C for further studies.

Determination of physiochemical properties of soil

Fresh soil samples were subjected to determine physiochemical properties. Soil pH was determined according to the procedure described by Martin *et al.* (2013) [8]. The moisture content of the sample was measured in hot air oven at 105°C to constant weight. The

temperature and humidity was determined using thermometer and hydrometer (Pramer and Schmidt, 1964; Iyengar and Bhave, 2005) [11, 5].

Isolation of Bacterial Isolate

The soil microorganisms were isolated by serial dilution technique on nutrient agar medium (NAM). One gram of soil from sample were separately suspended in 10 ml of distilled water and mixed well for 15 minutes and vortexed. Each suspension was serially diluted from 10^{-1} to 10^{-6} . Spread plate technique was carried out to isolate the organism from the diluted sample. 0.1 ml was pipette out onto plates with nutrient agar and spreaded with a glass L shape rod and incubated at 37°C for 24 hours. The most prominent colonies were isolated and maintained at 4°C for further studies.

Identification and Characterization of Bacteria

The shape, size and arrangement of the isolates and their differentiation into gram negative or gram positive bacteria were found. The bacterial isolates were characterized biochemically by various tests like Indole, MR, VP, Citrate etc., including carbohydrate fermentation (Collins and Lyne, 1989; Harold, 2002; Zaved *et al.*, 2008) [2, 4, 13].

Results and Discussion

The soil sample was collected from the rooted region of the plants in the agriculture area field area of Quantum Global Campus, Roorkee. The soil sample was observed for its physiochemical properties. The soil had pH of about 6.5. Soil with such pH conditions enhances the nutrient availability. Queensland department of environment and heritage protection has stated that the optimum pH range for most plants was between 5.5 and 7.5. The moisture content of the soil was 45.8% and the temperature to be 32°C . The temperature notified benefits the plant in availability of nutrients and the moisture content enhances the nutrient availability and enriches the growth of microorganisms which intern aids in plant growth.

Biochemical Analysis of Isolates

Soil sample from the agriculture field of quantum global campus were serially diluted and five well defined colonies (QAF 01, QAF 02, QAF 03, QAF 04 and QAF 05) were selected and were pure cultured. The isolates were subjected to gram reactions and several biochemical characterizations. Among five isolates three isolates were found to be gram positive and two were gram negative. Almost all the isolates were rod shaped formers in which QAF01 was spore former and QAF02, QAF04 and QAF05 were found to be motile (Fig 1).

QAF02 and QAF05 were found to be gram negative and the remaining all other isolates identified namely QAF01, QAF03 and QAF04 were found to be gram positive in reaction. The maximum fermentation of sugars was carried out by QAF04; whereas QAF05 did not undergo fermentation process (Fig. 2). Based on biochemical characterization and fermentation of sugars the isolate QAF1 was identified as *Bacillus* sp, QAF2 was identified as *Pseudomonas* sp, QAF3 was identified as

Streptomyces sp, QAF4 was identified to be *Azotobacter* sp and QAF5 was found to be *Alcaligenes* sp (Table 2).

Chitra Bhattacharya *et al.* (2014) [11] in their research work has stated that from the agriculture soil microorganisms like *E. coli*, *Micrococcus* sp, *Escherichia* sp and *Staphylococcus* sp have been isolated. Several bacteria and fungi were isolated from polyhouse soil using serial dilution method (Nakuteshwar Dut Jasuja, 2013) [9].

Conclusion

Soil contains billions of microorganisms in which some bacteria species are very fragile and may be killed by slight changes in the soil environment. Others are extremely tough, able to withstand severe heat, cold or drying. Some bacteria are dependent on specific plant species. Soil food web supports other soil organisms and the functions of a healthy soil. Diversified nature of soil bacteria can suppress root diseases. The current study establishes that the soil from agriculture field of Quantum Global Campus, Roorkee has bacteria that might enhance the growth of plants and can be used as plant growth promoting bacteria.

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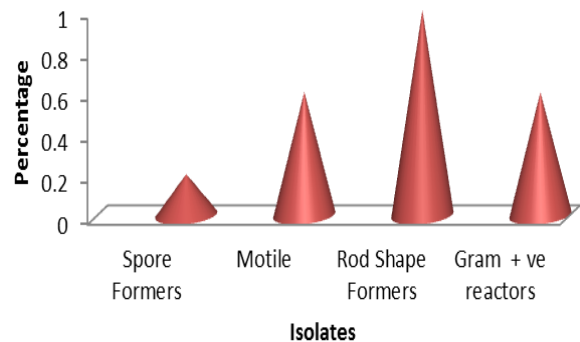


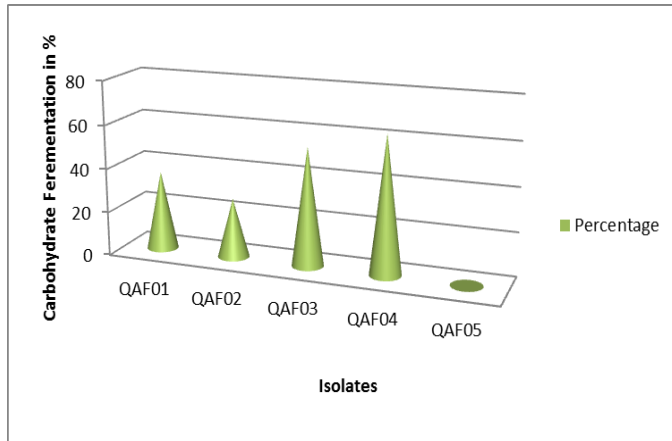
Fig 1: Characterization of isolates from agricultural soil

Table 1: Morphological and Biochemical characterization of bacterial isolates from soil

Isolate	Gram Reaction	Spore former	Motility	Morphology	Indole	Methyl Red	VP	Citrate	TSI	Urea
QAF 01	+	+	-	Rods	+	-	+	-	-	-
QAF 02	-	-	+	Rods	-	-	-	+	-	-
QAF 03	+	-	-	Ariel Mycelium	-	-	-	-	-	-
QAF 04	+	-	+	Rods (Diploid)	+	-	-	+	-	+
QAF 05	-	-	+	Rods	-	-	-	+	-	-

Table 2: Carbohydrate fermentation of bacterial isolates from soil

Isolate	Arabinose	Arabitol	Fructose	Galactose	Glucose	Lactose	Mannitol	Maltose	Starch	Sucrose	Xylose	Identification of Genus
QAF01	-	-	+	+	+	-	-	+	+	+	-	Bacillus sp
QAF02	-	-	-/+	-	+	-	+	+	-	-	-	Psuedomonas sp
QAF03	+	-	+	-	+	-	+	-	-	-	-	Streptomycis sp
QAF04	+	-	+	+	+	+	-	-	-	+	+	Azotobacter sp
QAF05	-	-	-	-	-	-	-	-	-	-	-	Alcaligenes sp

**Fig 2:** Carbohydrate fermentation of agricultural soil isolates

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