



## Isolation and Characterization of Soil Bacteria from Industrial Site

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### ABSTRACT

*The present research was conducted to study the relationship between soil and soil microbial community. Soil microorganisms were varying at different site, bacterial communities highly varied in diversity, richness, and structure according to environmental factors. At the Kota city scale, taxa area relationship was significant, supporting spatial structuration of bacterial communities. Spatial variations in community diversity and structure were mainly driven by soil physicochemical parameters. Soil sample was collected from Industrial DCM Factory, A Kota. The isolated bacterial species in industrial area (DCM) soil were Bacillus spp., Proteus spp., Pseudomonas spp, Klebsiella spp, and Escherichia coli. These isolates were considered tolerant to alkaline pH and elevated level of waste materials from the industrial area. Minimum average of bacterial population was observed in industrial area in other area comparatively (unpublished data). The bacterial isolation and identification have been done in Agriculture Microbiology laboratory of CPU and all identified bacteria have been tested.*

**Keywords:** Bacteria, Population, Soil, Industry.

### INTRODUCTION

The Microbial communities vary substantially in space and time, between soil types and under different land management. The mechanisms that control the spatial distributions of soil microbes are largely unknown as we have not been able to adequately upscale a detailed analysis of the micro biome in a few grams of soil to that of a catchment, of some area or continent to better understand the relationship between soil bacterial communities, physio chemical properties, land use and geographical distance. We investigated industrial site, revealed that

bacterial communities highly varied in diversity, richness, and structure according to environmental factors. This analysis identified specific indicators of land use in industrial area and soil conditions (pH, organic C, and other soil texture). Nitrogen cycling bacteria are sensitive to the total nitrogen, total phosphorus, ammonia nitrogen, and nitrate nitrogen, and pH of the soil and other soil fertile bacteria are sensitive to industrial waste pollution of the environment with toxic waste is spreading throughout the world with industrial progress.

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Manmade organic compounds and various industrial, agricultural and military operations have released huge amounts of toxic heavy metals into the environment with deleterious effects on soils, water and air (Sobolev & Begonia, 2008; Zhang et al., 2014; Ahmad, 2019, & Hanirah et al., 2015). Soil contamination by chemical fertilizer and other transports pollution consequently the most critical environmental problems as it poses significant impacts to the human health as well as the ecosystems (Octavia, 2013; Tamiru et al., 2014 & Owolabi & Hekeu, 2014) because they cannot be naturally degraded like organic pollutants and they accumulate in different parts of the food chain (Šmejkalová et al., 2014). Therefore, several past studies have demonstrated that microbial parameters may be useful as indicators of changing soil condition caused by chemical pollution (Oliveira & Pampulha, 2006). Microorganisms are the first biota that undergoes direct and indirect impacts of industrial waste. In polluted soil, the microbe's response to waste material toxicity depends on the concentration and the availability of waste and on the action of factors such as the type of waste material, the nature of medium and microbial species (Hassen et al., 1998). Adverse effects of chemical waste on soil microbes result in decreased decomposition of organic matter, reduced soil respiration, decreased diversity and declined activity of several soil enzymes (Lenart & Boroń, 2014). However, some microorganisms have developed several mechanisms to tolerate such high concentrations of chemical waste and remediation by different processes including chemical waste uptake (bioaccumulation) and often they are specific to one or a few toxic waste.), showed that bacterial isolates were capable to growing in the presences of chemical waste material at varying concentrations and were identified to belong to the Bacillus, while in another study it was demonstrated that isolates were categorized under Pseudomonas species, Staphylococcus, Bacillus species, Lactobacillus Lactei species, Streptococcus species and E. coli spp. after

morphological and biochemical tests (Nath et al., 2012 & Tamiru et al., 2014). In this research soil sample was selected from area with known pollution problems where industrial chemical waste material. The objective was determining the effect of chemical waste on soil physicochemical properties and bacterial population, as well as to study distribution of soil bacteria population at selected sites.

## MATERIALS AND METHODS

### Study area

To carry out a comprehensive study of microorganisms, soil samples were collected from rhizosphere soils. These samples were collected aseptically and stored in sterile bags, during transportation stored in iceboxes and transferred to laboratory. The sample containing bags were sealed and stored into 4°C refrigerator in Microbiology Laboratory, until the time of the analysis. Samples were preserved at 4°C in refrigerator for further experiments. About 100 gm of each sample was collected aseptically using UV-rays sterilized polythene bags. The interaction of soil microorganism with different soil environment in Kota was done. The studies have been conducted from July 2015 to October 2018, and the data have been collected using general information of different industries from past literature. The bacterial isolation and identification have been done in Agriculture Microbiology laboratory of CPU and all identified bacteria have been tested on soil sample. The samples then were processed in an isolated process for bacteria using the standard plate method followed by serial dilution for all collected samples.

### Isolation of bacteria

Serial dilutions were used for the isolation of bacteria and the sample were plated on nutrient agar and incubated at 37°C for 24 hours. After successful growth, microorganisms were identified on the basis of colony morphology and named as RS1, RS2, RS3, RS4, RS5 respectively. Serial dilution was prepared by adding one gram of each soil sample to 9 ml of sterile distilled water in

sterilized test tubes, shaken well, a serial dilution of  $10^{-1}$  to  $10^{-6}$  were made by the same method (Aneja, 2003).

### Morphological Characterization

Gram stain was performed to observe the identification of colony morphology was based on colour, margin, and confirmation. The colony morphology was viewed and determined by light microscope (Olympus). Gram staining (Gram, 1884) were performed to observe the cellular morphology and gram nature of the isolated and selected bacterial strains being viewed under light microscope (Olympus).

## RESULTS AND DISCUSSION

### Isolation of bacteria from rhizosphere soil

The 5 bacterial strains were isolated from rhizosphere soil, on the basis of morphology and appearance by nutrient agar plates it is followed by serial dilution. The 5 bacterial isolates named as RS1, RS2, RS3, RS4, RS5. It was reported that, the bacterial isolates were plated with separate colonies through culturing and re-culturing using streaking method followed by serial dilution.

### Morphology Characterization

#### Gram's staining

The results of morphology characterization were confirmed by gram's staining, it was observed that 1 isolate identified as gram positive RS3 and 4 isolates were identified as gram negative RS1, RS2, RS4 and RS5. Bacterial isolates resulted in table 1.

**Table 1: Morphological Characterization of isolated rhizosphere bacteria**

List of bacteria	Gram staining	Conformation	Colour	Margin
RS1	-	Rod	Light Pink	Singly or in Paris
RS2	-	Rod	Creamy	Branching
RS3	+	Rod	Pale Yellow	Circular
RS4	-	Rod	Creamy and Yellow	Entire
RS5	-	Rod	Dark pink	Singly

RS= Rhizosphere soil, += positive, -= negative, RS1 (*Escherichia coli*), RS2 (*Proteus* spp), RS3 (*Bacillus* spp), RS4 (*Pseudomonas* spp), RS5 (*Klebsiella* spp).

Totally five bacterial strains were isolated from rhizosphere soil. Individual colonies were identified by serial dilution and nutrient agar plating method. Earlier study it was reported that 10 bacterial strains were isolated from rhizosphere soil from rice field by serial dilution technique (Chulwuma Simon et al., 2014). Extreme temperature meets very difficult life opportunities (Gaete et al., 2020; Jorquera et al., 2019), these bacteria's isolated from industrial area will be validate for their extraordinary characteristics.

### CONCLUSIONS

Soil microorganisms are the main driver force for the biochemical cycles, so it was important to study the abundance and presence of microbial community in soil. Further studies on the effect of DCM industry on rhizosphere microorganisms, diversity and community structure analysis need to be conducted. Bacteria in rhizospheric soil are metabolically

active which leads to the production of various enzymes and bioactive compounds, so it is important to validate selected bacteria for their extra cellular enzyme activity in future.

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