# Microbial Diversity of Paddy field Soils and Assessment of Enzymatic Activity

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**Abstract**:Soil microorganisms such as bacteria and fungi play an important role in soil fertility and promoting plant health. Soil harbors most of our planet s undiscovered biodiversity. Each ten soil samples of five different agricultural crop fields in and around Rasipuram taluk were investigated for diversity among bacteria and fungi. A total of 12 species fungi belonging to 8 genera and 13 species of bacteria belonging to 7 genera were isolated and identified from agricultural fields at Rasipuram taluk. The highest percentage of occurrence among paddy field isolates was *Bacillus* sp. (93%), followed by *Klebsiella oxytoca*(46%), *Klebsiela pneumoniae*(45%), *Pseudomonas* sp. (45%) and the lowest was *Enterobacter cloacae* (3%).*Aspergillus fumigatus* (54%), *A. niger* (54%), *A. flavus* (32%) *Penicillium sp.* (24%) recorded highest frequency and the lowest was *Paecilomyces variotii* (9%) of occurrence among the paddy field isolates. Among them eight bacterial strains were selected for the assessment of proteolytic, lipolytic and amylolytic activities. Highest enzyme activity for the entire three enzymes was found high in *Protease mirabilis*.

**Key words:** Microbial diversity, bacteria, *Bacillus* sp. and fungi, *Aspergillus fumigatus*

## Introduction

Rice is the world's most important, oldest agronomic plant, with 143 million ha under cultivation globally and 75 percent of the world production managed as irrigated rice. It is the main food for more than a third of the world's population and provides 20% of the human calorie intake. Soil is the major component of earth's ecosystem which contain of organic matter, minerals, gases and large numbers of macro and microorganisms. The soil ecosystem is supported by several interactions among its physical, chemical and biological components (Chandrashekar*et al.*, 2014). The paddy field ecosystem consists of diverse habitats of microorganisms.These fields provide aerobic/anaerobic soil conditions, floodwater, rice roots, paddy straw stubble and composted materials for growth and development of micro-organism. In addition paddy fields, provide gradients from stagnant to percolating water environments with different oxygen levels. These habitats are providing different

microenvironments that could exhibit biologically distinct properties for fields and soil related micro-organism. There is a vast microbial flora inheriting the earth and they are found in all types of soils. Soil contains numerous types of bacteria and fungi. Microbial communities of soil play a significant role in the biosphere particularly in agriculture, improving the soil fertility, regulating the bio-geochemical cycles supplying plant nutrition and vary from soil to soil depends on the environment(Ratna Kumar *et al*., 2015**)**.Various bacteria in soil contribute to decompose soil organic matter and paddy straw. Microorganisms present in paddy field soils are affected by rapid changes between flooding and drainage (Lennartz *et al*., 2009).Agricultural management practices have been reported to influence soil microbial community structures (Sun *et al*., 2004). Analyses of soil microbial diversity and community structures are essential when monitoring environmental influences on soil quality. Bacteria and fungi are the most diverse living beings on earth and only a fraction of them have been identified. This paper aims at the analysis of the diversity of bacteria and fungi found in soil samples from rice-fields ecosystem of Rasipuram Taluk, Namakkal District, Tamilnadu, India and determined the enzyme assay for the selected bacterium. Still no work reported in this direction and these isolates may be useful for other study like plant growth promoting bacteria and other biological control agent in future.

## Materials and methods

### Study area

Soil samples were collected from the paddy fields ecosystem of Rasipuram Taluk, Namakkal District, Tamilnadu, India.

### Sample Collection

The soil sample was collected from ten different paddy fields at five different locations of Rasipuram Taluk, Namakkal District, Tamilnadu, India. The soil caught was emptied into sterilized polyethylene bags. Each sample bag was labelled appropriately by indicating the site of collection, time, date and place of collection. The samples were transferred to the laboratory using sterile container (Aina, *et al*., 2011).

### Bacterial isolation and multiplication

Soil dilutions were made according to Waksman, (1922). 1ml of serially diluted suspension of each concentration were aseptically distributed on nutrient Agar and incubated at 37ºC for 24 hours. Grown colonies were inoculated into

nutrient broth (Moraes *et al.,* 1999) alkaline peptone water (APW), Selenite F broth (SFB) and Brain heart infusion broth (BHI) and incubated at $37^0$C for 24 h. The sample from alkaline peptone water and Selenite F broth were plated onto Thiosulphate Citrate Bile salt Sucrose (TCBS) agar and Xylose Lysine Deoxycholate (XLD) agar respectively. The samples from BHI were inoculated into MacConkey agar, Blood agar and the plates were incubated at $37^0$C for 24 h.

**Bacterial characterization and identification**

The bacterial colonies were identified based on the microscopy (Grams staining and motility test) and the required biochemical tests such as catalase, oxidase, Glucose, Lactose, Indole, Methyl Red, VogesProskauer, Citrate utilization, TSI, Urease test, nitrate reduction test, mannitol, Oxidation and carbohydrate fermentation were carried out according to Ma Faddin (Ma Faddin, 1984).Properties associated with cell structures and secreted enzymes were also studied (Konema*et al*., 1999).

**Fungal isolation and multiplication**

Serially diluted soil samples were inoculated into Sabouraud Dextrose Agar (SDA) and incubated at room temperature for 5 to 10 days. After purification the isolates were sub cultured for further identification. The fungi were identified with the help of standard manuals (Gilman, 2001and Barnett, 1998).

**Fungal characterization and identification**

The grown colonies were identified based on the cultural characteristics (macroscopic - colony texture, topography, exudate production and pigmentation) and morphology of fruiting bodies and spores microscopic characteristics (saptation in mycelium), presence of specific reproductive structures, shapes and structure of conidia (Zafar *et al.,* 2006).

**Screening of Isolates for Enzyme Activity**

The isolates were screened for their ability to produce protease, amylase and lipase enzymes respectively. The selected isolates were tested for amylolytic, lipolytic, and proteolytic activities based on the methods described by Harrigan, (1998). Starch agar, nutrient agar with tween 80, and casein agar were used to test for the presence of amylase, lipase, and protease enzyme respectively. Appearance of clear zone around the colony after addition of 1% iodine solution confirmed amylase production. Clear halo around the colony indicated protease

activity (Williams *et al.,* 1983). Precipitated fatty acids around the colony revealed lipolytic activity (Harrigan and McCance, 1976).

**Results and discussion**

Soil microbial flora is important components of biodiversity which plays an important role in global ecological processes and plant nutrition. In the present study high numbers of 12 fungal sp. belonging to 8 genera (Table -1& Figure -1) and 13 bacterial sp. belonging to 7 genera (Table -2 & figure -2) were identified from different paddy fields soil at five different locations of of Rasipuram Taluk, Namakkal District, Tamilnadu, India. Among the bacterial species the highest percentage of occurrence of *Bacillus sp*. (93%) is found in paddy soil followed by *Klebsiella oxytoca*(46%), *Klebsiella pneumoniae* (45%) and *Pseudomonas* sp. (45%). The lowest number of bacteria is *Enterobacter cloacae* (3%). The *Bacillus* genus presented the highest frequency and the lowest was *Klebsiella, Shigella, Pseudomonas* and *Aeromonas* (RibeiroReche and Mariana Fiuza, 2005). Distribution of microbial flora is depends on the environmental factors such as the soil pH, moisture, temperature, organic carbon and nitrogen. These are the main factors affecting the microbial population and diversity. In case of fungal isolates the dominant was *Aspergillus fumigatus*(54%), *Aspergillus niger*(54%), *Aspergillus flavus*(32%) *Penicillium sp.*(24%) and the least is *Paecilomyces variotii*(9%).*Aspergillus sp*. and *Penicillium sp.* showed the highest prevalence among other fungi (Chandrashekar *et al*., 2014**)**. *Aspergillus sp*. is well-known for its cellulolytic property and has the ability to produce organic acids, enzymes and pigments which play an important role in soil ecology (Kadar *et al.,* 1999). The genus wise relative abundance showed a highest percentage for *Aspergillus sp*. followed by *Penicilliumsp*. Raipuria *et al.,* (2013)and Ratna Kumar *et al.,*(2015). *Penicillium* sp. plays a major role in organic matter decomposition and soil fertility status of the soil.

**Enzymatic characterization of identified isolates**

The bacterial strains such as *Proteus mirabilis*, *Enterobacteraerogenes, Klebsiella oxytoca, Enterobacter dissolvens, Klebsiella pneumoniae*, *Bacillus* sp. *Aeromonas hydrophyla* and *Enterobacter* obacae were selected from the identified strains for the assessment of enzymatic activities. Among them, six

strains showed lipolytic activity except *Enterobacter dissolvens* and *Klebsiella pneumonia. Amylolytic activity showed by Proteus mirabilis*, *Enterobacter aerogenes, Klebsiella oxytoca* and proteolytic activity was found only in *Proteus mirabilis*. Highest enzyme activity for the entire three enzymes was found in *Proteus mirabilis* (Fig-3). It had been reported that enzyme production in most of the bacteria is affected by certain polysaccharides (David Trofa *et al., 2009*).

**Table 1. List of fungal isolates from paddy fields of Rasipuram**

| S.No. | Fungal isolates | Frequency of occurrence (%) |
|---|---|---|
| 1 | *Absidia corymbifera* | 19 |
| 2 | *Aspergillus flavus* | 32 |
| 3 | *Aspergillus fumigatus* | 54 |
| 4 | *Aspergillus niger* | 54 |
| 5 | *Aspergillus* sp. | 9 |
| 6 | *Fusarium* sp. | 14 |
| 7 | *Microsporum audouinii* | 14 |
| 8 | *Mucor* sp. | 15 |
| 9 | *Mycelia sterilia* | 24 |
| 10 | *Paecilomyces variotii* | 9 |
| 11 | *Penicillium* sp. | 24 |
| 12 | *Phialemoniumobovatum* | 19 |

**Fig -1 Percentage occurrence of fungal isolates from Paddy fields of Rasipuram**

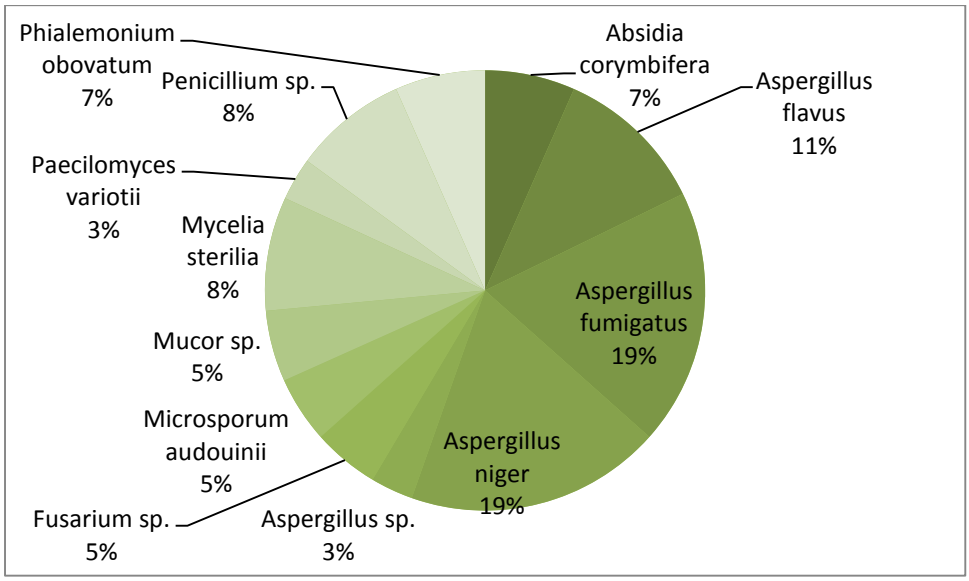



**Table 2. List of bacterial isolates from paddy fields of Rasipuram**

| S.No. | Bacteria isolates | Frequency of occurrence (%) |
|---|---|---|
| 1 | *Aeromonas hydrophyla* | 24 |
| 2 | *Bacillus* sp. | 93 |
| 3 | *Citrobacter freundii* | 19 |
| 4 | *Enterobacter aerogenes* | 27 |
| 5 | *Enterobacter cloacae* | 3 |
| 6 | *Enterobacter dissolvens* | 34 |
| 7 | *Enterobacter* obacae | 41 |
| 8 | *Klebsiella oxytoca* | 46 |
| 9 | *Klebsiella pneumoniae* | 45 |
| 10 | *Proteus mirabilis* | 15 |
| 12 | *Pseudomonas aeruginosa* | 27 |
| 13 | *Pseudomonas* sp. | 45 |

**Fig -1 Percentage occurrence of bacterial isolates from Paddy fields of Rasipuram**

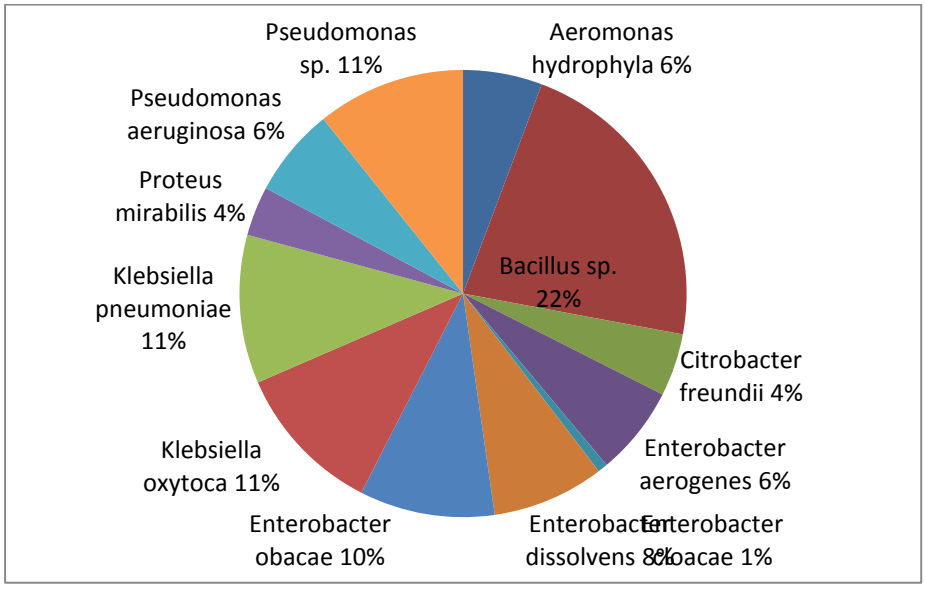
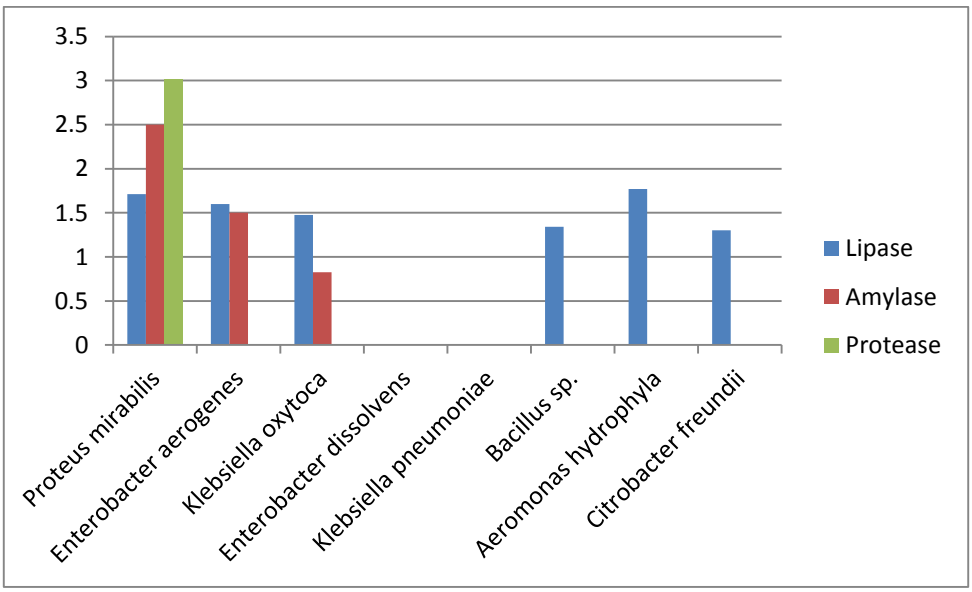



**Fig -3 Enzyme production as depicted by clearance zones (mm) during screening on Solid medium**

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