**Egypt. Acad. J. Biolog. Sci., 5(1): 35-41 (2013) G. Microbiology**
**Email:egyptianacademic@yahoo.com ISSN: 2090-0872**
**Received: 20/6/2013** www.eajbs.eg.net

# Determination of the Soil Microflora of a Soil Near Microbiology Laboratory at the University of Ilorin Main Campus

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## ABSTRACT

The microflora of a soil near microbiology laboratory at the University of Ilorin, Ilorin, Nigeria was analyzed in this study. Total bacterial count and total fungal count of the soil samples were determined using standard spread plate technique. Six soil samples were collected with interval of two weeks between two samples. Identification of the isolates was done using their colonial, morphological, and biochemical characteristics and established procedures were followed. The total bacterial counts ranged from $1.4 \times 10^8$ to $1.7 \times 10^8$ cfu/g and the total fungal counts ranged from $1.4 \times 10^5$ to $1.5 \times 10^5$ cfu/g. The bacteria isolated were *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Acetobacter* species, *Lactobacillus* species, *Erwinia* species, *Klebsiella* species, *Bacillus subtilis*, *Clostridium* species, and *Bacillus cereus*. Isolated fungi were *Rhizopus oryzae*, *Aspergillus niger*, *Aspergillus flavus*, *Mucor hiemalis*, *Penicillium chrysogenum*, *Fusarium oxysporium*, and *Trichophyton rubrum.*

**Keywords:** Microflora, Morphological, Biochemical, Bacteria, Fungi, Soil

## INTRODUCTION

Soil microbial biomass plays a critical role in ecosystem processes, such as carbon cycling, nutrient turnover, or the production of trace gases. Soil microbial activities, populations, and communities are governed by environmental variables and agricultural systems (Alexander, 1998).

Although biomass of all microorganisms living in soil constitute only several percent of organic matter content, they play an important role in the functioning of entire ecosystems because, due to their enormous biochemical and biogeochemical activity, they can exert crucial effects on dynamics of multidirectional microbiological processes (Alexander, 1998).

Microorganisms, being a part of all natural and man-made ecosystems, compose biocenoses which are significant and essential biochemical elements responsible for the entirety of biogenic element transformation in soil environment, which exert critical effects on biochemical activity and ecological stability, and biological productivity of many field, forest, and grassland ecosystems. They are involved in biochemical transformations of mineral fertilizers, particularly NPK fertilizers, synthesis of biologically active substances (amino acids, vitamins, antibiotics, and toxins) and nitrogen fixation from the air (Kennedy A.C and Papppendick J.R., 1995; Lynch J.M. and Poole N.J., 1979.

Soil is the naturally occurring, unconsolidated, or loose covering on the earth's surface. Soil is made up of broken rock particles that have been altered by chemical and environmental conditions, such as weathering and erosion (Chesworth, 2008). Soil is a mixture of mineral and organic constituents that are in solid, gaseous, and aqueous states (Voroney, 2006). Soil organisms include bacteria,

fungi, algae, virus, protozoa, nematodes, and arthropods. These organisms carry out numerous biological functions such as organic decomposition, creation of humus, suppression of pathogens, and they also improve soil properties (Kennedy & Papppendick, 1995).

Soil bacteria decompose organic matter. Nitrogen gas from the air is chemically bound by bacteria into soluble or insoluble organic compounds that degrade in time releasing soluble nitrogen compound like ammonia. Certain bacteria such as rhizosphere bacteria are also useful in legumes for fixing nitrogen into a form that is available to plants. Other bacteria denitrify nitrates while retaining nutrients like sulphur. Bacteria will also convert insoluble mineral phosphorus and iron into soluble products that plants can use. The waste products of bacteria become humus. Some bacteria are very useful in compost piles, while others will thrive in anaerobic composts, which generates odour and are harmful to plants. There are also bacteria that cause diseases in plants. Bacteria often live in colonies of thousands of individuals and produce substances that hold soil particles together. A teaspoon of forest soil can contain twenty million to two billion bacteria cells (Kennedy & Papppendick, 1995).

It has been estimated that the population of soil bacteria may range from hundred million to three billion in a gram of soil. Bacteria are capable of very rapid reproduction by binary fission in favourable conditions. One bacterium is capable of producing sixteen million bacteria cells in just twenty four hours. Most soil bacteria live in close proximity to plant root and are often referred to as rhizobacteria. Bacteria live in soil water, including the film of moisture surrounding soil particles and some are able to swim by means of flagella (Atlas and Bartha, 1998).

Important roles of bacteria include nitrification, nitrogen fixation, and denitrification. Nitrification is a very important part of the nitrogen cycle. In nitrification, certain bacteria such as *Nitrosomonas* and *Nitrosococcus* are able to transform nitrogen in the form of ammonium, which is produced by the decomposition of proteins into nitrates which can be utilized by the growing plants. Nitrification is the biological oxidation of ammonia with oxygen into nitrite and further oxidation into nitrate. The oxidation of ammonium into nitrate is performed by two groups of organisms; ammonia oxidizing bacteria and ammonia oxidizing archaea (Treasch *et al.,* 2005).

Part of the roles of bacteria in the soil is nitrogen fixation. Nitrogen fixation ensures a constant addition of nitrogen into biological circulation. Nitrogen fixation is carried out by free-living nitrogen-fixing bacteria in the soil or water such as *Azotobacter* or by those which live in close symbiosis with leguminous plants, such as rhizobacteria. These bacteria form colonies in nodules they create on the roots of peas, beans, and related plants. These are able to convert nitrogen from the atmosphere into nitrogen containing organic substances (Ingham, 1998).

Denitrification is another process carried out by soil bacteria that works against nitrification. Denitrification returns an approximately equal amount of nitrogen to the atmosphere. Denitrifying bacteria tend to be anaerobes or facultative anaerobes. Denitrifying bacteria include *Achromobacter* and *Pseudomonas*. The putrefaction process caused by oxygen-free condition converts nitrates and nitrites in soil into nitrogen gas or into gaseous compounds such as nitrous oxide or nitric oxide. If denitrification proceed excessively, it can lead to overall losses of available soil nitrogen and subsequent loss of soil fertility (Ingham, 1998; Atlas and Barthas, 2009).

Soil fungi are microscopic and they usually grow as long threads or strand called hyphae, which push their way between soil particles, roots, and rocks. Hyphae are usually only several thousandths of an inch in diameter. A few fungi such as yeasts are single cells (Ingham, 1998). Hyphae sometimes group into masses called

mycelium or thick, cord-like "rhizomorphs" that look like roots. Fungal fruiting structures (Mushrooms) are made of hyphal strands, spores, and some special structures like gills on which spores form (Ingham, 1998).

Fungi perform important services related to water dynamics, nutrient cycling, and disease suppression. Along with bacteria, fungi are important as decomposers in the food web. They convert hard-to-digest organic material into forms that other organisms can use. Fungal hyphae physically bind soil particles together, creating stable aggregates that help increase water infiltration and soil water holding capacity (Atlas and Barthas, 2009). Soil fungi can be grouped into three general functional groups based on how they get their every day nutrients. Decomposers-Saprophytic fungi-convert dead organic material into fungal biomass, carbondioxide, and small molecules, such as organic acids. These fungi generally use complex substances, such as the cellulose and lignin, in wood, and are essential in decomposition of carbon ring structures in some pollutants. A few fungi are called "sugar fungi" because they use the same simple substrates as do many bacteria. Like bacteria, fungi are important for immobilizing or retaining nutrients in the soil. In addition, many of the secondary metabolites of fungi are organic acids so they help increase the accumulation of humid–acid rich organic matter that is resistant to degradation and may stay in the soil for hundreds of years (Ingham, 1998; Moore, 1990).

In a mutualistic relationship, mycorrhizal fungi colonize plant roots. In exchange for carbon from the plant, mycorrhizal fungi help solubilise phosphorus and bring soil nutrients to the plants. One major group of mycorrhizae, the ectomycorrhizae grow on the surface layer of the roots and are commonly associated with trees. The second major group of mycorrhizae are the endomycorrhizae that grow within the root cells and are commonly associated with grasses, row crops, vegetables, and shrubs. Arbuscular, mycorrhizal fungi (AM) are a type of endomycorrhizal fungi (Ingham, 1998).

The third group of fungi is made up of pathogens or parasites that cause reduced production or death when they colonize roots and other parts of plants. Root pathogenic fungi such as *Verticillum*, *Pythium,* and *Rhizoctonia,* cause major economic losses in agriculture. Fungi have been used as biocontrol agents. For instance, nematode-trapping fungi that parasitise disease causing-nematodes, and fungi that feed on insects (Adams, 1990; Ingham, 1998).

In this study, a scientific analysis on the microflora of a soil near microbiology laboratory at the University of Ilorin, main campus was conducted. Emphasis was laid on the isolation, characterization, and identification of bacteria and fungi from the soil.

## MATERIALS AND METHODS

### Collection of Soil Samples

The soil samples were collected on six different occasions using two weeks interval. On each occasion, soil samples were taken from three distinct points with five meters between two points. Soil samples were taken from all the sides of the Microbiology laboratory of the University of Ilorin, main campus.

Debris was removed from the topsoil and the ground was dug to about 20cm depth before samples were taken with sterile trowel. The samples were packed in sterile polythene bags and properly tied. The samples were taken to the laboratory for analysis and were kept in the fridge until needed (Olutiola *et al.,* 2000).

### Isolation of Microorganisms

A series of test tubes were prepared for the isolation of bacteria and fungi. 9ml of sterile distilled water was put into each of the test tubes. To the first test tube, one gram of the soil sample was added to give a dilution of $10^{-1}$. The contents were shaken properly and 1ml of the solution was taken and added to the next test tube containing 9ml of sterile distilled water to make a concentration of $10^{-2}$. The serial dilution was made up to $10^{-5}$

dilution for the soil samples. 0.1ml of the $10^{-5}$ dilution was cultured on the nutrient agar plates using the spread plate technique. A sterile spreader was used to spread the inocula over each plate. The plates were then incubated upside down at $37^0$C for 24 hours. These plates were examined after 24 hours for the isolation of bacteria.

For the isolation of fungi, malt extract agar plates were seeded with 0.1ml aliquot from $10^{-2}$ dilution of the soil samples using the spread plate technique. A sterile spreader was used to spread the inocula on the plates and the plates were incubated at room temperature for 48 hours. The plates were examined for fungal growth after every 24 hours. The plates were prepared in triplicate and the average CFU/ml was recorded after growth.

**Characterization and Identification of Microorganisms Characterization of the Bacterial Isolates**

Colonial characteristics of the bacterial isolates were determined using parameters such as size, elevation, pigment, surface, opacity, edge, and shape. Cellular characteristics of the isolates were determined through the following experiments, Gram's staining, Motility Test, Spore Staining, Capsule Staining, Catalase Test, Oxidase Test, Methyl Red Test, Indole Test, Starch Hydrolysis, Citrate Utilization, Sugar Fermentation, and Oxygen Relationship.

**Characterization of the Fungal Isolates**

The fungal isolates were characterized using their colonial morphology on the plates. Parameters such as colour of the colonies, nature of the hyphae, appearance of the colonies, and the growth rates were considered for proper characterization of the isolates.

Microscopic examination of the isolates was also carried out. Reproductive and vegetative structures were observed. Nature of spores, nature of sporangia, branching of the hyphae, and presence of septa were all considered during microscopy. Microscopic examination of the fungi was done following the procedures of Samson and Van, 1998.

**Identification of Microorganisms**

Characterization of the isolates was used as the basis for their identification. Appropriate texts were consulted for the identification of the bacterial isolates (Prescott *et al.,* 2002; Willey *et al.,* 2009). Appropriate text books were also consulted for the identification of the fungal isolates (Samson and Van, 1998; Prescott *et al.,* 2002; Willey *et al.,* 2009).

## RESULTS

**Bacteria Isolated**

A total of nine bacteria were isolated. The bacteria included *Pseudomonas aeruginosa, Staphylococcus epidermidis, Acetobacter* species, *Lactobacillus* species, *Erwinia* species, *Klebsiella* species, *Bacillus subtilis, Clostridium* species, and *Bacillus cereus* as presented in Table 1.

Table 1: Colonial morphology, cellular morphology, and biochemical characteristics of the bacterial isolates.

| Isolate | Cellular shape | Colonial elevation | Colonial edge | Colonial opacity | Colonial surface | Colonial pigmentation | Cellular arrangement | Gram's staining | Motility test | Spore staining | Capsule staining | Catalase test | Methyl red test | Starch hydrolysis | Citrate utilization | Oxygen reaction | Action on simple carbohydrates | | | | | probable microorganism |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | | Lactose | Glucose | Sucrose | Maltose | Fructose | |
| B1 | Rod | Raised | Entire | Translucent | Smooth | Yellowish Cream | Chain | -ve | +ve | -ve | -ve | -ve | +ve | -ve | -ve | AE | -ve | A | A | A | AG | *Pseudomonas aeruginosa* |
| B2 | Cocci | Raised | Entire | Opaque | Smooth | Creamy White | Clusters | +ve | -ve | -ve | -ve | +ve | -ve | +ve | +ve | FAN | AG | A | A | A | -ve | *Staphylococcus epidermidis* |
| B3 | Rod | Raised | Lobate | Translucent | Rough | Cream | Single | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | AN | A | A | -ve | -ve | -ve | *Acetobacter* species |
| B4 | Rod | Raised | Lobate | Opaque | Smooth | Creamy White | Clusters | +ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | FAN | A | A | A | A | AG | *Lactobacillus* species |
| B5 | Rod | Raised | Entire | Opaque | Smooth | Yellow | Single | -ve | +ve | -ve | -ve | -ve | -ve | -ve | -ve | FAN | -ve | A | A | A | A | *Erwinia* species |
| B6 | Rod | Raised | Entire | Translucent | Smooth | Pink | Chain | -ve | -ve | -ve | +ve | -ve | -ve | +ve | +ve | FAN | +ve | -ve | AG | AG | AG | *Klebsiella* species |
| B7 | Rod | Flat | Lobate | Opaque | Dull | White | Clusters | +ve | +ve | +ve | +ve | +ve | +ve | -ve | -ve | FAN | AG | A | A | A | A | *Bacillus subtilis* |
| B8 | Rod | Raised | Entire | Opaque | Rough | Cream | Chain | +ve | -ve | +ve | -ve | -ve | -ve | +ve | -ve | AN | A | AG | A | AG | A | *Clostridium* species |
| B9 | Rod | Raised | Lobate | translucent | Dull | Cream | chain | +ve | +ve | +ve | +ve | +ve | +ve | +ve | -ve | AE | -ve | AG | A | AG | AG | *Bacillus cereus* |

Key: -ve = Negative AE = Aerobic AN = Anaerobic A = Acid production
+ve = Positive FAN = Facultative anaerobe AG = Acid and Gas production

Among the bacterial isolates three were methyl red positive (i.e. *Pseudomonas aeruginosa, Bacillus subtilis,* and *Bacillus cereus*). During the sugar fermentation test, *Klebsiella* species showed acid and gas production in three out of the five sugars tested, while *Lactobacillus* species showed acid and gas production in only one of the sugars. *Acetobacter* species showed acid production in two of the sugars tested.

*Klebsiella* species, *Bacillus subtilis,* and *Bacillus cereus* occurred four times each during the sampling period. *Pseudomonas aeruginosa* was found to be the most abundant occurring five times during the sampling period as shown in Tables 2 and 3.

Table 2: Occurrence of the bacterial isolates

| Designation | Bacterial Isolate | Sampling Period (Week) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 3 | 5 | 7 | 9 | 11 |
| B1 | *Pseudomonas aeruginosa* | + | + | + | - | + | + |
| B2 | *Staphylococcus epidermidis* | + | - | + | - | - | + |
| B3 | *Acetobacter* species | + | + | - | + | - | - |
| B4 | *Lactobacillus* species | + | - | - | + | - | - |
| B5 | *Erwinia* species | - | - | + | + | - | - |
| B6 | *Klebsiella* species | - | + | + | + | + | - |
| B7 | *Bacillus subtilis* | + | + | + | - | - | + |
| B8 | *Clostridium* species | + | + | - | + | - | - |
| B9 | *Bacillus cereus* | + | + | - | - | + | + |

Key: + = Present - = Absent

Table 3:- Bacterial counts.

| Periods of Sampling (Week) | Bacterial counts (cfu/g of soil) |
|---|---|
| 1 | $1.6 \times 10^8$ |
| 3 | $1.5 \times 10^8$ |
| 5 | $1.4 \times 10^8$ |
| 7 | $1.6 \times 10^8$ |
| 9 | $1.5 \times 10^8$ |
| 11 | $1.7 \times 10^8$ |

## Fungi Isolated

The fungal isolates were *Rhizopus oryzae*, *Aspergillus niger*, *Aspergillus flavus*, *Mucor hiemalis*, *Penicillium chrysogenum*, *Fusarium oxysporium*, and *Trichophyton rubrum*.

*Rhizopus oryzae* was found thrice in the first, third, and seventh week while *Mucor hiemalis* was found only in the third and fifth week. *Fusarium oxysporium* was the most abundant occurring in the first, third, fifth, and eleventh week as shown in Tables 4 and 5.

Table 4: Occurrence of the fungal isolates.

| Designation | Fungal Isolate | Sampling Period (Week) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 3 | 5 | 7 | 9 | 11 |
| F1 | *Rhizopus oryzae* | + | + | - | + | - | - |
| F2 | *Aspergillus niger* | + | - | + | - | + | + |
| F3 | *Aspergillus flavus* | - | - | + | + | + | - |
| F4 | *Mucor hiemalis* | - | + | + | - | - | - |
| F5 | *Penicillium chrysogenum* | + | + | - | - | + | - |
| F6 | *Fusarium oxysporium* | + | + | - | + | - | + |
| F7 | *Trichophyton rubrum* | - | + | + | - | - | - |

Key: + =Present - =Absent

Table 5: Fungal counts.

| Sampling periods (Week) | Fungal counts (cfu/g of soil) |
|---|---|
| 1 | $1.4 \times 10^5$ |
| 3 | $1.5 \times 10^5$ |
| 5 | $1.4 \times 10^5$ |
| 7 | $1.4 \times 10^5$ |
| 9 | $1.5 \times 10^5$ |
| 11 | $1.5 \times 10^5$ |

## DISCUSSION

The total bacterial counts of the soil samples ranged from $1.4 \times 10^8$ to $1.7 \times 10^8$ cfu/g of soil and the total fungal counts ranged from $1.4 \times 10^5$ to $1.5 \times 10^5$ cfu/g of soil. The predominance of bacteria over fungi that were isolated from the soil was similar to the discoveries of other researchers (Ingham *et al.*, 1989). The bacteria that were isolated included *Pseudomonas aeruginosa*, *Staphylococcus epidermis*, *Acetobacter* species, *Lactobacillus* species, *Erwinia* species, *Klebsiella* species, *Bacillus subtilis*, *Clostridium* species, and *Bacillus cereus*. Occurrence of the heterotrophic bacteria as the highest occurring organisms could be attributed to the tolerance of these microbes to wide variations of the soil properties, which prevailed in both seasons. It followed the same trend reported for soil bacterial populations by Brady, 1984. Fungal isolates from the soil included *Rhizopus oryzae*, *Aspergillus niger*, *Aspergillus flavus*, *Mucor hiemalis*, *Penicillium chrysogenum*, *Fusarium oxysporium*, and *Trichophyton rubrum*.

The abundance and distribution of the microorganisms in the soil of the research site indicated that the general condition of the soil supported the survival of the soil's Microflora. This was stated in the work of Oyeyiola and Agbaje, 2013. Soil microbes feed on the bodies of dead plants and animals. Bacteria and fungi in particular, digest the complex organic compound that makeup living matter and reduce them to simpler compounds that plants can use for food. A typical example is the formation of ammonia from the animal and plant proteins.

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