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Isolation and Characterization of Heterotrophic Microorganisms from Achievers University Top Soils

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ABSTRACT

In this study three (3) samples of soil obtained from three (3) different locations; BH (Boys hostel), GH (Girls hostel) and CF (cafeteria) and at three different depths; (0, 5 and 15) centimetres were analyzed. The total bacterial and fungal counts of the soil samples were estimated using standard spread plate technique. A pH meter was used to monitor soil pH while isolates were identified by their cultural, morphological and biochemical characteristics following established protocols. The pH readings range between 6.92 –7.79. The maximum moisture content of 26.0% was observed in soil samples collected from the girl's hostel and the minimum moisture content 13.0% was found in the soil samples from the cafeteria. Also, the organic matter ranges from 28.1 to 35.5%. A total of six (6) bacterial isolates were recovered from the soil samples namely; *Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Streptococcus pyogenes, and Klebsiellapneumoniae.* Also, a total of four (4) fungal isolates were identified as *Aspergillusniger, Penicilliumnotatum, Mucorpusillus* and *Fusariumsporotrichioides.* The total viable counts range between 2.8 x 10^6 Cfu/g to 9.1 x10^7 Cfu/g. *Bacillus* spp dominated the bacterial isolates while *Aspergillus*spp was the most dominant fungus across the different sampling locations. Bacterial and fungal abundance are typical of an environment with high species richness and functional diversity which can be harnessed to an advantage, hence, overall national development.

KEYWORDS: Achievers University; Bacteria; Fungi; Soil; Microorganisms.

1. Introduction

Soil teems with microscopic life (bacteria, fungi, algae, protozoa and viruses) as well as macroscopic life such as earthworms, nematodes, mites, and insects, and also the root systems of plants. The numbers and kinds of microorganisms present in soil depend on amount and type of nutrients available, available moisture, degree of aeration, pH, temperature and many other environmental factors including extent of human activities (Tate, 2020). Soil bacteria and fungi play vital roles in various biochemical cycles and are responsible for the recycling of organic compounds (Basu*et al.*,2020).Soil microorganisms also influence top soil ecosystems by contributing to plant nutrition, plant health, soil structure and soil fertility (O'Donnell *et al.*, 2001).

Bacteria make up the most abundant group of microorganisms in the soil (3.0 x 10^6 – 5.0 x 10^8) per gram of soil, followed by the actinomycetes (1.0 x 10^6 – 2.0 x 10^7), fungi (5.0 x 10^3 – 9.0 x 10^6), yeast (1.0 x 10^3 – 1.0 x 10^6), algae and protozoa (1.0 x 10^3- 5.0 x 10^5) and nematodes (50 – 200) counts per gram of soil are wide differences in the relative proportions of individual bacteria genera found in particular soils (Atals and Bartha, 1998). Fungi are found primarily at the top 10 cm of the soil and

are rarely found below 30 cm. They are most abundant in well-aerated and acidic soils (Domsch*et al.,* 1980). Most fungi in soil are opportunistic (Zymogenous), thus, they grow and carry out active metabolism when conditions are favorable.Favourable conditions imply adequate moisture, aeration and relatively high concentrations of utilizable substrates (Miyanoto*et al.,* 2002).

Soil characteristics are important factors controlling natural processes, for example, bacterial interaction to soil can be influenced by clay content (Felde*et al.,* 2020). Parameters such as pH and specific ion content and organic matter are critical for shaping the kind of microbial communities that can be present in a particular soil. Microbes in top soils are critical to any natural course. Soil bacteria and fungi play essential roles in a variety of biogeochemical cycles (BGC) (Liu *et al.,* 2020). Soil microorganisms control above-ground ecosystems by providing nutrients to plant, plant physical condition, soil organization and soil productiveness (Kumar *et al.,* 2020).

The two most abundant groups of microorganisms are bacteria and fungi. Fungi have enzymes capable of breaking down virtually all classes of organic compounds, and have the competitive advantage over bacteria in decomposing substrates of low nutrient concentration because of their ability to import nitrogen and phosphorus via their hyphal network(Etesami, and Adl, 2020). Bacteria are single-celled prokaryotes that sometimes perform specialized functions of great ecological significance, such as the chemoautotrophic nitrifiers that are of importance for nutrient cycling soil nutrients (Flint, 2020).

There are two approaches which are widely used for the identification of unknown bacteria. One approach is the biochemical approach which involves different tests which provide information about biochemical characteristics of bacteria as described in Bergey's manual in systematic manner. The other approach is the molecular approach in which bacteria identification can be done according to the information of 16SrRNA sequence (Chalupowicz*et al.,* 2019).

Bacterial and fungal abundance are typical of an environment with high species richness and functional diversity which can be harnessed, hence, the objectives of this study are to isolate, characterize and determine the physico-chemical properties of microorganisms recovered from the top soil obtain from different locations within the Achievers University, Owo community. This is with a view to understanding the microbial flora and it is hoped that it will shed light into the microbial resources in lieu of designing means by which their potentials may be optimally harnessed to an advantage, hence, overall national development.

2. Materials and Methods

2.1 Sample Collection

Top- soil samples were obtained from Boys Hostel, Girls Hostel and Cafeteria and it was labeled respectively BH, GH, CF in Achievers University, Owo, Ondo State, Nigeria. The samples were obtained at depth of (0, 5, and 15) cm in sterilized cellophane nylon as described by Arotupinet al.(2008). A soil auger was used to obtain volume samples with a minimum of 0.5 kg of soil per sampling area and stored at 4 ℃ to maintain field moist and to preserve biological properties.

2.2 Sterilization of Glassware and other Materials.

All glassware were thoroughly, rinsed and allowed to dry before sterilization. The glasswareswerestored wrapped with aluminum foil after sterilization in hot air oven at 170°C for 60 minutes before use. The distilled water used for serial dilutions was autoclaved at 121°C for 15 minutes. The work bench was swabbed with 70% alcohol before and after every experiment.

2.3 Physiochemical Analysis

Physiochemical parameters of soil samples were determined following standard protocols. The pH scale was calibrated before use with buffer solutions of known pH values. Thirty grams (30g) of soil was taken in a 100ml beaker to which 75 ml of distilled water was added. The suspension was stirred at regular intervals for 30 minutes and the pH values were recorded in triplicates of each sample and the mean value estimated.

Water content was determined by weighing to dryness in oven at 110°C for 24 hours. The percent

of water content (w) was calculated using the following formula,

$$wherew = watercontentpercent = \frac{(W_2 - W_3)}{(W_3 - W_1)} \times \frac{100}{1} \quad (1)$$

W1= mass of container with lid with wet soil
W2= mass of container with lid with dry soil
W3 = mass of container with lid

2.4 Microbial Analysis

Serial Dilution

The method of Tan and Eaton (1995) was adopted for this analysis. A portion of 1g of the soil sample was dispensed into test tube containing 9ml of sterile water. Serial dilution to the factor of 10^7cfu/ml was done to reduce the microbial load in the soil before cultivation.

2.4.1 Enumeration and Isolation of Heterotrophic Soil Micro Flora

Aliquots of each dilution was cultured on plates of Nutrient Agar for mean heterotrophic bacterial count and Potato Dextrose Agar (PDA) for mean heterotrophic fungal count respectively by standard pour-plate dilution method described by Ogunfowokanet al. (2008) in triplicates. Nutrient agar containing 0.015% (w/v) nystatin (to inhibit fungi growth) was used for bacteria isolation and incubation was at 35°C for 24 hours. Potato dextrose agar to which 0.05% (w/v) chloramphenicol has been added (to inhibit bacteria growth) was used for fungal isolation, and incubation was at ambient temperature for five days.

2.4.2 Characterization of the Soil Microorganisms

Unique representative bacterial and fungal colonies were sub-cultured on freshly prepared nutrient agar and potato dextrose agar plates. These plates were incubated at 35°C for 24 hours at room temperature (28 ± 2^0C) for 3 days for bacterial and fungal cultures respectively. The colonial characteristics of the sub-cultured isolates were further identified by the identification schemes described by (Kumbar*et al.*, 2013). Isolated bacteria were also cultured on nutrient agar slants and stored at 25°C.

The sub cultured fungal isolates were identified on the basis of their morphological and microscopic features. Their microscopic attributes were examined using the wet mount technique. Both lacto-phenol cotton blue and distilled water were used respectively as mountings. The microscopic structures observed were recorded and compared to illustrations stated by Barnett and Hunter (1972). The fungal isolates were transferred to potato dextrose agar slants and stored in aerated sterile cabinets which served as stock cultures.

2.5. Identification of the Bacteria Isolates

The cellular morphology and biochemical characteristics of the microbial isolates were used for the identification of the isolates according to Garrity and Holt, 2001. Cultural characteristics like shape, color, elevation, surface, edge as well as microscopic features were used for identification. The Bergey's Manual of determinative bacteriology by Buchanan and Gibbons, 1974 was used to compare the characteristics with the results obtained.

2.5.1 Identification of Fungus by Lacto-Phenol Cotton Blue Staining

Lacto-phenol blue solution is a mounting medium and staining agent used in the preparation of slides for microscopic examination of fungi. Fungal elements are stained intensely blue. A drop of lacto-phenol blue solution was placed on a slide. Using an inoculating needle, it was used carefully to spread the fungal culture into a thin preparation. A cover-slip edge was used to place the drop and slowly lowered. It is then observed at low and high power of the microscope.

2.5.2 Gram's Staining

A wet mount of each isolate was prepared, stained with crystal violet for 60 seconds, iodine was then added for 60 seconds, the wet mount was subsequently flooded with 95% ethanol for 30 seconds, washed and Safranin was added to counter stain for 1 min. It was rinsed with water, air dried and examined under the light microscope using x100 oil immersion objective lens. Gram positive organisms appeared purple, while Gram negative organisms appeared red or pink.

2.6 Biochemical Characterization

2.6.1 Catalase Test

The catalase test determines the presence of the enzyme catalase in bacteria. It is essential for differentiating Gram +vecoccus bacteria (e.g. *Staphylococcus* and *Streptococcus*).The catalase enzyme serves to neutralize the bactericidal effects of Hydrogen Peroxide. To test the catalase activity few drops of 3% H_2O_2 was applied over the bacterial

colony of 18-24 hrs. Bubble formation indicates catalase positive.

2.6.2 Oxidase Test

The oxidase test assays for the presence of enzyme cytochrome oxidase. A small piece of filter paper was soaked in 1% Kovács oxidase reagent and dried. The composition of Kovács oxidase reagent is 1% tetra-methyl-phenylenediaminedihydrochloride in distilled water. A well isolated colony was picked from a fresh bacterial plate (18 to 24 hours) and rubbed into the filter paper soaked with Kovács oxidase reagent. Color change indicates the result of this test. Microorganisms are oxidase positive when the color changes to dark purple within 5 to 10 seconds. Microorganisms are delayed oxidase positive when the color changes to purple within 60 to 90 seconds. Microorganisms are oxidase negative if the color does not change or it takes longer than 2 minutes.

2.6.3 Indole Test

The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole by the activity of tryptophanase enzyme. Tryptophan + water = indole + pyruvic acid + ammonia. Indole test was performed as described by Abdulkadir and Waliyu (2012). One percent tryptophan broth was taken in a test tube and inoculated with bacteria colony. After 48 hours of incubation period at 37°C, one militre (1ml) of chloroform was added to the broth. The test tube was shaken gently. 5 drops of Kovács reagent was added directly to the tube. This was also shaken gently and allowed to stand twenty (20) minutes. The formation of red coloration at the top layer indicated positive and yellow coloration indicates negative.

2.6.4 Sugar fermentation

This test was performed in a sugar broth medium to test an organism's ability to ferment sugars as well as ability to produce gas. The medium contains pH indicator (phenol red), the red color in the acidic pH indicates that the organism is able to ferment sugar. The sugars used were glucose, fructose, galactose and sucrose.

Then each sugar broth (contains an inverted Durham's tube) and was inoculated separately with the prepared suspension using a sterile wire loop, the tubes were incubated at 35-37 °C for five days. A change in the color to red indicates fermentation of certain sugars found in the tube following standard protocol.

2.7 Statistical Analysis

Comparisons of means were analyzed statistically, using one-way Analysis of Variance (ANOVA) and Pearson chi–square statistics at probability of $P<0.05$ and $P<0.01$. Relationships were tested for using the Pearson correlation index at the same probability. The Duncan's Multiple Range test will also be used to separate the means at the 5% level of probability. Statistical analysis was performed using SPSS 17.0 software.

3. Results and Discussion

The present study was designed to determine the distribution of microorganisms in soil samples collected from three (3) sites in Achievers University Owo namely Boys hostel (BH), Girls hostel (GH) and Cafeteria (CF) respectively. The soil samples collected from the three sites were analyzed for various physical and chemical properties. The physical properties of the soil include, color, soil type and moisture content while the chemical properties examined includes pH and organic matter content. The soil color showed that the samples from the Boy's hostel were brownish, Girl's hostel (light brown) and Cafeteria (yellowish brown). The soil texture were sandy, sandy –loam and clayey-loam for boys hostel, girls hostel and cafeteria respectively as shown in Table 1.

The pH of the top soil samples from three selected sites were determined and the results showed that for Boys hostel, the pH readings were 6.92, 7.19 and 7.25 for soil depths of 0cm, 5cm and 10cm respectively. The pH readings for the Girl's hostel were 7.21, 7.39 and 7.33 for soil depths of 0cm, 5cm and 10cm respectively. Also, the pH readings for the soil samples from the Cafeteria were 7.18, 7.79 and 7.67 for soil depths of 0cm, 5cm and 10cm respectively. The moisture content ranged from 13.0% to 26.0% as shown in Table 2.

The maximum moisture content 26.0% was observed in soil samples collected from the girl's hostel and the minimum moisture content 13.0% was found in the soil samples from the cafeteria. Moisture content requirement for the growth of microorganism varies from one microorganism to

Table 1: Soil color and texture of the collection sites

Collection sitesn	Soil Texture	Soil color
Boy's hostel (BH)	Sandy	Brown
Girl's hostel (GH)	Sandy-loam	Light brown
Cafeteria (CF)	Clayey-loam	Yellowish brown

Table 2: pH of the different soil samples in relation to depth of collection

Collection depths	BH	GH	CF
0cm	6.92	7.21	7.18
5cm	7.19	7.39	7.79
10cm	7.25	7.33	7.67
Average readings	7.12	7.31	7.56

BH= Boys hostel, GH= Girls hostel, CF= cafeteria

another. These results indicated that all soil samples contain water (moisture). The Organic matter ranges from 28.1 to 35.5%.The highest percentage of organic matter is found in the cafeteria (35.5 %) and the lowest percentage was found in the soil sample collected from the boy's hostel (31.1%). The organic matter of soil samples from girl's hostel is 28.1% as shown in Table 3.

A total of six (6) bacteria isolates and four (4) fungal isolates were isolated from the soil samples examined. The bacterial isolates were *Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Streptococcus pyogenes, and Klebsiellapneumoniae*as shown in Table 4.The fungal isolates identified were *Aspergillusniger, Penicilliumnotatum, Mucorpusillus* and *Fusariumsporotrichioides* as shown in Table 5.

The viable counts of microorganisms were also determined and the result showed that the highest bacterial count was observed in the soil sample from the Cafeteria (CF). The total viable counts of the Cafeteria soil were 9.1 x 10^7 Cfu/g and 6.1 x 10^7Cfu/g and 1.43 x10^7 Cfu/g for 0cm, 5cm and 15cm soil depths respectively. The total viable counts of the Boy's hostel were 3.4 x 10^6Cfu/g, 2.8 x 10^6cfu/g and 8.1 x 10^5Cfu/g for 0cm, 5cm and 15 cm soil depth respectively. The total viable count of the soil samples of the Girl's hostel soil samples were 7.0 x 10^6Cfu/g, 5.3 x 10^6Cfu/g and 3.1 x10^6Cfu/g for soil depth of 0cm, 5cm and 15cm respectively. The total fungal count showed that for the boy's hostel (2.76 x 10^4cfu/g), girl's hostel (3.12 x 10^4cfu/g) and the cafeteria (4.22 x 10^4Cfu/g) respectively for different depths as shown in table 6.

The result on the occurrence and abundance of bacterial isolates showed that a total of five (5) distinct strains of bacteria were isolated from the Boys Hostel (BH) with *Escherichia coli* being the most abundant and *Streptococcus spp* being the least abundant. From the samples collected from the Girl's Hostel (GH), a total of six (6) bacteria strains were isolated with *Bacillus cereus* occurring most and *Klebsiellapneumoniae* being the least abundant. Also, the soil samples collected from the Cafeteria (CF) showed that four (4) distinct bacteria strains were isolated from the samples, *Bacillus subtilis* being the most abundant and *Streptococcus spp* being the least abundant. The total number of culturable bacteria isolated were statistical significant ($P<0.05$) i.e. between BH vs. GH, BH vs. CF and CF vs. GH as shown in table 7.

The result on the occurrence and abundance of fungal isolates showed that a total of four (4) distinct fungal strains were observed from the Boys Hostel (BH) with *Aspergillusniger* being the most abundant and *Penicilliumnotatum* being the least abundant. From the samples collected from the Girl's Hostel (GH), a total of three (3) fungal strains were observed with *Penicilliumnotatum* being the most abundant and *Aspergillusniger* being the least abundant. Also, the soil samples collected from the Cafeteria (CF) showed that three (3) distinct fungal strains were recovered from the samples, with *Aspergillusniger* being the most abundant and *Mucorspp* being the least. The total number of fungal cultural types recorded were statistical significant ($P<0.05$) i.e. between BH vs GH, BH vs

Table 3: Percentage moisture content and organic matter content of the soil samples

Location/site	% Moisture content	% Organic content
Boys Hostel (BH)	20.0	31.7
Girls Hostel (GH)	26.0	28.1
Cafeteria (CF)	13.0	35.5

Table 4: Morphological and biochemical characteristics of bacterial isolates

Isolates	Color	shape	edge	Colony Surface	Gram rxn	catalase	Oxidase	Indole	Glucose	Lactose	Sucrose	fructose	Suspected organism
BH													
1	MILKY	ROD	SMOOTH	DULL	+VE	+VE	-VE	-VE	+VE	-VE	-VE	+VE	*Bacillus Cereus*
2	WHITE	ROD	ROUGH	DULL	+VE	+VE	-VE	+VE	+VE	-VE	+VE	+VE	*Bacillus Subtilis*
GH													
1	BLUE GREEN	ROD	SMOOTH	DULL	-VE	+VE	+VE	-VE	-VE	-VE	-VE	-VE	*Pseudomonas Aeru.*
2	LARGE GREEN	ROD	SMOOTH	SHINY	-VE	+VE	-VE	-VE	+VE	+VE	+VE	+VE	*Klebsiella Spp*
CF													
1	WHITE	ROD	SMOOTH	DULL	-VE	+VE	-VE	+VE	+VE	+VE	+VE	-VE	*Escherichia Coli*
2	MILKY	COCCI	SMOOTH	SHINY	+VE	+VE	-VE	-VE	+VE	+VE	+VE	+VE	*Streptocooccus Spp*

Table 5: Morphological Characteristics of Fungal isolates from soil samples

Isolates	Morphological Characteristics	Probable Fungi Species
A.	Black and powdery-likeConidiophores smooth walled andnon-septate	*Aspergillusniger*
B.	Whitish and cottony-like Round, black conidia, non-septate	*Mucorpusillus*
C.	Brown and cotton- like Long, erect conidiophores, roundshaped conidia	*Penicilliumnotatum*
D.	Yellow-pink creamy, Colonies cylindrical to ovoid conidia,curvedseptate conidiophores	*Fusarium Sporotrichioides*

A: Boys Hostel soil sample; B: Boys Hostel soil sample; C: Girls Hostel soil sample; D Cafeteria soil sample

Table 6: Total viable counts of microorganisms isolated from soil samples

Sampling site	TBC (Cfu/g)	TFC (Cfu/g)
Boy's hostel	4.71×10^5	2.76×10^4
Girl's hostel	5.01×10^5	3.12×10^4
Cafeteria	6.8×10^5	4.22×10^4

CF and CF vs GH respectively as shown in the table 8.

The present study was aimed at determining the distribution of heterotrophic microorganisms present in Achievers University's top soil of different locations. The results obtained for the total bacterial counts ranged from 4.71×10^5 to 6.8×10^5 Cfu/g of soil (Table 6), and fell within the range of earlier reports. Expectedly, the total bacterial counts were generally higher than those of fungi, irrespective of sampling locations. The predominance of bacteria over fungi observed throughout the sampling time has been reported by other workers (Acosta-Martinez *et al.*, 2014). Differences in bacterial counts between the different samples were not significant which corroborates the findings of Amir and Pineau (1998).

The fungal counts in this study were in the range of 2.76×10^4 to 4.22×10^4Cfu/g of soil (Table6). The non-significance differences between total fungal counts of the different samples, irrespective of sampling locations supports the finding of Rafiq et al. (2021).Also, there was a significant correlation between the pH of soil in different locations ($P<0.05$). The significant difference ($P<0.01$) between the soil pH in Boys hostel (BH) and that of Girls hostel (GH) and Cafeteria (CF) may be due to differing activities occurring in the locations. The

soil pH in Cafeteria is higher than that of Girls hostel and Boys hostel (Table 2). Although, the soil pH in this study was near neutral ranges, which favors microbial growth. The composition and diversity of culturable heterotrophic bacteria observed in this study were similar for the different sampling locations (Table 7) this is similar to the findings of Desta, (2021).

Most of the organisms isolated are indigenous to soil environments and their abundance and diversity may be attributable to high human activities and the subsequent discharge of refuse into the surrounding soil, thereby enriching the available nutrients in such soils. It may also be attributable to the destabilization of the soil ecological balance arising from contamination. These findings agreed well with similar findings reported by Orji *et al.*(2021) in which they found that microorganisms of the genera *Bacillus* were predominant in contaminated soilsand can be utilized for bioremediation purposes.

Table 7: Occurrence and abundance of aerobic heterotrophic bacteria of sampling location

Isolates	**Occurrence**			**Abundance**		
	BH	**GH**	**CF**	**BH**	**GH**	**CF**
Pseudomonas spp	+	+	+	5	4	7
Escherichia coli	+	+	-	10	6	-
Streptococcus spp	+	+	+	4	5	5
Klebsiella spp	-	+	-	-	2	-
Bacillus cereus	+	+	+	6	8	8
Bacillus subtilis	+	+	+	7	5	9

BH=Boys hostel, GH= Girls hostel, CF= Cafeteria; + = presence of the organism; - = absence of the organism

Table 8: Occurrence and relative abundance of aerobic heterotrophic fungi of sampling location

Isolates	Occurrence			**Abundance**		
	BH	**GH**	**CF**	**BH**	**GH**	**CF**
Aspergillus niger	+	+	+	9	8	13
Mucorspp	+	+	+	5	7	10
Penicilliumn otatum	+	+	-	6	9	-
Fusarium spp	+	-	+	8	-	12

BH= Boys hostel, GH= Girls hostel, CF= Cafeteria; + = presence of the microorganism; - = absence of the microorganism

4.Conclusion and Recommendations

The outcome of this study revealed diversity of bacteria and fungi present in Achievers University Community Owo, Nigeria. The distinct types of bacteria and fungi isolated were generally similar in all the sample locations. The abundance of bacteria and fungi in this study were typical of environment with high species richness and functional diversity. Achievers University Community top soil is abundant in heterotrophic microorganisms, which implies adequate moisture, aeration and relatively high concentrations of utilizable substrates for microorganisms' favourable growth.

Although the results of this study would not be considered to be exhaustive, nonetheless, an insight into the population dynamics and distribution of cultivable aerobic bacteria and fungi diversity has been elucidated. This microbial resource can be exploited into economical advantage of the country.

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