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Full Length Research Paper

Microbial analysis of different top soil samples of selected site in Obafemi Awolowo University, Nigeria

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Soil samples were collected from three different locations within the Obafemi Awolowo University (OAU) Ile-Ife Campus complex and labelled according to the site of collections as PAG (park and garden), HOR (hall of residence) and BIS (biological sciences). 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 using established procedures. Bacterial counts were in the order of 10^5-10^7 cfu/g of soil, while fungal counts were in the order of 10^3-10^5 cfu/g of soil. The highest bacterial count was observed in PAG, while the lowest value was seen in HOR. Fungal counts were high in PAG and low in BIS. Similar bacterial and fungal species were encountered in the different sampling locations in course of this study, but their occurrences and levels of predominance were different. *Bacillus* spp dominated the bacterial isolates while *Aspergillus* spp was the most dominant fungus across the different sampling locations. A near neutral pH was observed across the sampling sites. Bacterial and fungal abundance were typical of an environment with high species richness and functional diversity.

Key words: Culturable bacterial, fungi, top soil.

INTRODUCTION

Soil is the region on the earth's crust where geology and biology meet, the land surface that provides a home to plant animal and microbial life (Pelczar et al., 1993). 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 many environmental factors: amount and type of nutrients available, available moisture, degree of aeration, pH, temperature etc (Prescott et al., 1999). Soil bacteria and fungi play pivotal roles in various biochemical cycles and are responsible for the recycling of organic compounds (Wall and Virginia, 1999). Soil microorganisms also influence aboveground ecosystems by contributing to plant nutrition, plant health, soil structure and soil fertility (O'Donnell et al., 2001). Soil is generally a favourable habitat for the proliferation of microorganisms, with micro colonies, developing around soil particles. Numbers of micro organi- in soil habitats normally are much higher than those in fresh water or marine habitats (Atals and Bartha, 1998).

Bacteria make up the most abundant group of microorganisms in the soil ($3.0 \times 10^6 - 5.0 \times 10^8$) per gram of soil, followed by the actinomycetes ($1.0 \times 10^6 - 2.0 \times 10^7$), fungi ($5.0 \times 10^3 - 9.0 \times 10^6$), yeast ($I.0 \times 10^3 - 1.0 \times 10^6$), algae and protozoa ($1.0 \times 10^3 - 5.0 \times 10^5$) 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). Soil fungi may occur as free-living organisms or in mycorrhizal association with plant roots. Fungi are found primarily in 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). They grow and carry out active metabolism when conditions are favourable which implies adequate moisture, adequate aeration and relatively high concentrations of utilizable substrates (Postage, 1994; Miyanoto et al., 2002).

In this research we isolate culturable heterotrophic bac teria and fungi from different top soil samples collected from Obafemi Awolowo University (OAU) community with

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Table 1. Soil pH of sampling locations.

Sample	pH
PAG Soil	7.58
BIS Soil	7.03
HOR Soil	6.80

All values are means determined by using four independent cultures. PAG, Parks and Garden Soil; BIS, Biological Sciences Soil; and HOR, Hall of Residence Soil.

a view to understanding the microbial flora that are found in the different top soils of this community. The findings of this study shall shed light into the microbial resources present in this community in lieu of designing ways and means by which their potentials may be optimally harnessed.

MATERIALS AND METHODS

Sampling

The soil samples used for this work were collected from 3 different locations in Obafemi Awolowo University (OAU) Community. The samples were labeled according to the site of collection as PAG – Parks and Garden samples, BIS – Biological Sciences samples, HOR – Hall of Residence samples. Randomly located 5- X 5 m were used to attain six sub samples of the topsoil (7.5 cm depth) using an auger 8.5 cm diameter. The samples were transported in polyethylene bags in ice pack to the laboratory. When samples could not process immediately, they were stored at 4°C for no longer than 18 to 24 h.

Sterilization techniques

The polyethylene bags were cold-sterilized in uv-radiation box for at least 12 h (usually overnight), while glassware was treated in the hot-air oven at 160°C for 2 h. Growth media and diluents (distilled water) were autoclaved at 121°C for 15 min.

Microbiological analyses

The soil sample was mixed, and a suspension of 1 g (dry weight equivalent) in 10 ml of sterile water was prepared. One ml of the soil suspension was then diluted serially (ten-fold) and used in the estimation of aerobic heterotrophic bacterial and fungal populations by standard spread-plate dilution method described by Seeley and VanDemark (1981), in triplicate. Nutrient agar containing 0.015% (w/v) nystatin (to inhibit fungi growth) was used for bacteria isolation and incubation was at 35°C for five days. 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 seven days. Pure isolates of representative communities were maintained on agar slant at 4°C. Identification of isolates was based on cultural, microscopic, and biochemical characteristics with reference to Bergey's manual of determinative bacteriology (1989) for bacteria, and Talbot (1978) for fungi.

Soil pH was determined according to the procedure described by Akpor et al. (2006). 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. All statistical analysis was performed using SPSS 11.0 software.

RESULTS

Total bacterial counts

The mean total bacterial counts (TBC) of each soil sample ranged from 9.5×10^7 colony forming units (cfu) per gram of soil, 8.0×10^5 cfu/g of soil, 6.5×10^5 cfu/g of soil of PAG, BIS and HOR respectively as shown in Figure 1. Although there were differences in the averages total bacterial counts of the different sampling locations, these differences were not statistically significant. However, highest counts were observed in PAG, and lowest count was observed in HOR.

Total fungal counts

The mean total fungal counts (TFC) of each soil sample ranged from 7.5×10^5 cfu/g of soil, 5.1×10^4 cfu/g of soil, 6.4×10^4 cfu/g of soil of PAG, BIS and HOR respectively. Highest counts were observed in PAG, lowest counts were observed in BIS as shown in Figure 2. Differences in the average total fungal counts of the sampling locations were not statistically significant.

Soil pH

The pH values ranged from 6.80 – 7.58. The soil pH in PAG was higher than BIS and HOR as shown in Table 1. However, differences in the soil pH values of the different sampling locations were not observed to be statistically significant.

Variations in bacteria types in sampling locations

Throughout the sampling locations, a total of eleven (11) distinct strains of bacteria were recorded from PAG with *Bacillus* genera being the most dominant. The least genera were *Chromobacterium* and *Nocardia,* In BIS nine (9) genera were recorded, *Bacillus* and *Staphylococcus* genera were the most and least dominant, respectively as shown in Table 3. The total bacteria cultural types in HOR were eleven (11) genera. The genus *Bacillus* was the most dominant, while *Aeromonas* were the least dominant. The total number of bacteria cultural types recorded were statistical significant ($P<0.01$) i.e. between PAG vs BIS, PAG vs HOR and BIS vs HOR.

Variations in fungi cultural types in sampling locations

Throughout the different sampling locations a total of

Table 2. Occurrence and abundance of some genera of aerobic heterotrophic bacteria of sampling location.

Bacteria	Occurrence			Abundance		
	PAG Soil	BIS Soil	HOR Soil	PAG Soil	BIS Soil	HOR Soil
Lactobacillus	+	-	+	4	-	4
Bacillus	+	+	+	11	8	10
Proteus	+	-	-	2	-	-
Pseudomonas	+	+	+	5	5	3
Corynebacterium	+	+	+	4	3	4
Flavobacterium	+	+	+	5	3	4
Aeromonas	+	+	+	3	3	1
Staphylococcus	+	+	+	4	1	2
Chromobacterium	+	+	+	1	2	2
Nocardia	+	+	+	1	3	2
Micrococcus	+	-	-	2	-	-
Streptococcus	-	+	+	-	3	2
Alcaligenes	-	-	+	-	-	2

PAG, Parks and Garden Soil; BIS, Biological Sciences Soil; and HOR, Hall of Residence Soil. + = Presence; - = absence.

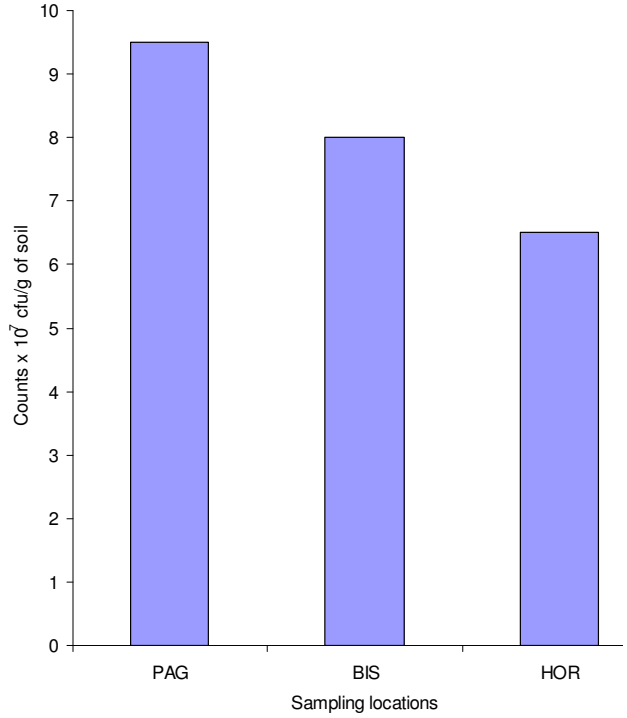

Figure 1. Average total bacterial count (TBC) of the sampling locations. PAG, Parks and Garden Soil; BIS, Biological Sciences Soil; and HOR, Hall of Residence Soil.

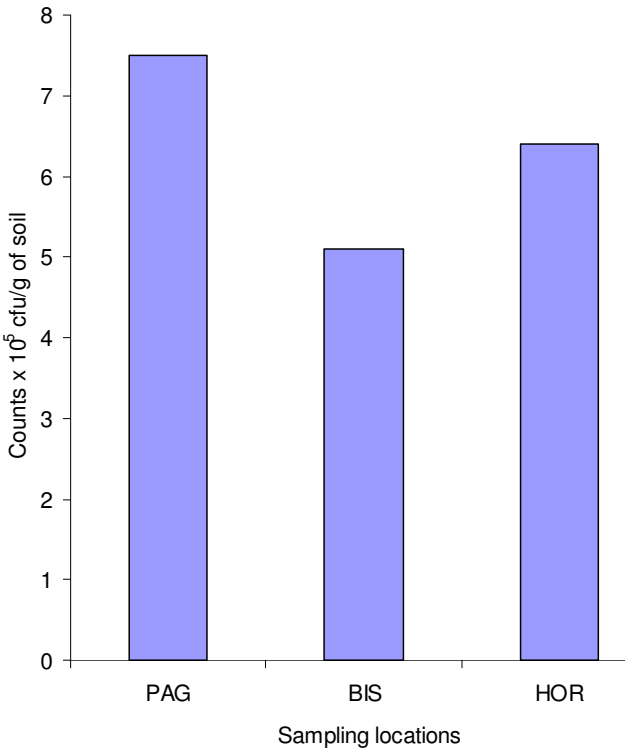

Figure 2. Average total fungi count (TFC) of the sampling locations. PAG, Parks and Garden Soil; BIS, Biological Sciences Soil; and HOR, Hall of Residence Soil.

eleven (11) distinct types of fungi belonging to nine (9) genera were recovered from PAG with *Aspergillus niger* and *Gliocladium* sp being the most and least dominant respectively. A total of ten (10) distinct types and eight (8) genera were recovered from BIS. Nine (9) species of fungi belonging to seven (7) genera from HOR. In all the samples *A. niger* was the most dominant while *Microsporium* for BIS and HOR respectively as shown in Table 4. The different distinct types of fungi isolated from were statistically significant ($P<0.01$) for PAG vs BIS, PAG vs HOR and BIS vs HOR.

DISCUSSION

The results obtained for the total bacterial counts ranged

Table 3. Occurrence and abundance of some aerobic heterotrophic fungi of sampling location.

Fungi	Occurrence			Abundance		
	PAG Soil	BIS Soil	HOR Soil	PAG Soil	BIS Soil	HOR Soil
Aspergilus niger	+	+	+	11	10	11
Aspergillus fumigatus	+	+	+	3	3	4
Aspergillus flavus	+	+	+	6	4	3
Botrytis	+	+	+	2	1	2
Cladosporium	+	+	+	2	3	2
Penicillium	+	+	+	10	6	6
Mucor	+	+	+	5	3	4
Trichophyton	+	+	+	3	4	2
Cephalosporium	+	+	-	2	1	-
Microsporium	+	+	+	2	3	1
Gliocladium	+	-	-	1	-	-

PAG, Parks and Garden Soil; BIS, Biological Sciences Soil; and HOR, Hall of Residence Soil. + = Presence; - = absence.

Table 4. Occurrence and Abundance of some aerobic heterotrophic fungi of sampling location.

Fungi	Occurrence			Abundance		
	PAG Soil	BIS Soil	HOR Soil	PAG Soil	BIS Soil	HOR Soil
Aspergilus niger	+	+	+	11	10	11
Aspergillus fumigatus	+	+	+	3	3	4
Aspergillus flavus	+	+	+	6	4	3
Botrytis	+	+	+	2	1	2
Cladosporium	+	+	+	2	3	2
Penicillium	+	+	+	10	6	6
Mucor	+	+	+	5	3	4
Trichophyton	+	+	+	3	4	2
Cephalosporium	+	+	-	2	1	-
Microsporium	+	+	+	2	3	1
Gliocladium	+	-	-	1	-	-

LEGEND
PAG Parks and Garden Soil
BIS Biological science Soil
HOR Hall of Residence Soil
+ Indicate presence
- Indicate absence

from 10^5-10^7 cfu/g of soil, and fell within the range reported by earlier workers (Okoh et al., 1999). 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 (Ferando et al., 1994; Ingham et al., 1989; Okoh et al., 1999). Differences in bacterial counts between the different samples were not significant. This finding corroborates that of Amir and Pineau (1989) and Okoh et al. (1999). The fungal counts in this study were in the range of 10^3-10^5 cfu/g of soil. These values also fell within the range reported by other worker (Amir and Pineau, 1998). The non-significance of the differences between total fungal counts of the different samples, irrespective of sampling locations supports the finding of Fernando et al. (1994); Amir and Pineau (1998). Also, there was a highly significant correlation between the pH of soil in different locations ($r = 0.601$, $P<0.01$). There was a significant difference between the soil pH in PAG and that of BIS and HOR ($P<0.01$). The soil in PAG had a higher pH than those of BIS and HOR. Similar findings have been reported by Okoh et al. (1999). All the soil pH in this study was near neutral ranges, which favours microbial growth.

The composition and diversity of culturable, heterotrophic bacteria observed in this study were similar for the different sampling locations. This corroborates with the finding of Okoh et al. (1999). However, the variation of species differed from one sample to another. Most of the bacteria isolated in this study have been reported by other workers (Amir and Pineau, 1998 and Okoh et al.,

1999). Amir and Pineau, (1998) reported that, among the topsoil they investigated, species of *Actinomycetes* were the most dominant. The results of this study however revealed that, in all the soil samples.

Summary and Conclusion

The outcome of this study has been able to show that diverse types of bacteria and fungi were isolated from Obafemi Awolowo University Community Ile-Ife, Nigeria. Although samples were collected from different locations, the distinct types of bacteria and fungi isolated were generally similar, though occurring at different locations during the period of the study.

The abundance of bacteria and fungi in this study were typical of environment with high species richness and functional diversity. Despite the fact that it is possible that a number of bacteria and fungi may be missed in this study, the isolates could be readily assigned dominant (e.g. *Bacillus* sp, *Aspergillus* sp) or transient/successional roles in the isolation of organisms form different sampling locations, which form the basis of this study. In additions to the implications of the determination of the number of microorganisms during soil sampling, one should consider the qualitative aspect of the preservation of important species and groups of microorganisms and of the changes in these biochemical characteristics resulting from the variations in these counts.

Although the results of this study would not be considered to be exhaustive, as it was done within the limits of facilities available in the laboratory, an insight into the population dynamics and distribution of culturable aerobic bacteria and fungi diversity has been elucidated. This is without prejudice to the possible influence which a substantial proportion of bacteria and fungi that are not culturable *in vitro* could have on the overall picture of event. It would require more modern technology (nuclei acid probes) to obtain such detailed overview of microbial diversity. This should be a subject of extension of this investigation in future.

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