Direct RESEARCH JOURNALS
Vol. 2 (1), pp. 8-12, December 2016
ISSN: 4372-2608
Article Number: DRJA40913572

Direct Research Journal of Biology and Biotechnology
http://directresearchpublisher.org/journal/drjbb

Research Paper

Isolation and identification of fungi in soil around Sokoto cement company environment

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Received 10 October 2016; Accepted 26 November, 2016

Fungi were isolated from soil in three different sites around Sokoto cement company environment. Soil samples from the three different sites were serially diluted and dilution factors of up to $10^{-4}$and were cultured on the appropriate agar media. The spread plate technique was adopted using potato dextrose agar for the determination of the total fungal counts. Fungal isolates were *Aspergillus niger, Rhizopus species, Candida albicans, Histoplasma capsulatum, Aspergillus nidulans, Epidermophyton floccosum*, and *Fusarium species*. Fungal counts ranged from ($1.0x10^3$ cfu/g) in sample C_2 to ($8.7x10^4$cfu/g) in sample B_2. *Aspergillus niger* was the most common isolated fungi (25%) while *Aspergillus nidulans, Epidermophyton floccosum, and Fusarium species (8.33%)* were the least isolated. In conclusion, *Aspergillus niger* is the most fungi isolated in soil, and it is of medical importance to the soil and also to humans.

Keywords: Isolation, cement company, soil fungus, *Epidermophyton floccosum*

INTRODUCTION

Soil is the mixture of minerals, organic matter, gases, liquids, and the countless organisms that together support life on Earth. Soil is a natural body known as the pedosphere and which performs four important functions: it is a medium for plant growth; it is a means of water storage, supply and purification; it is a modifier of Earth's atmosphere; it is a habitat for organisms; all of which in turn modify the soil. Soil is a major component of the Earth's ecosystem. The world's ecosystems are impacted in far-reaching ways by the processes carried out in the soil, from ozone depletion and global warming, to rain forest destruction and water pollution. Soil is the largest surficial global carbon reservoir on Earth, and it is potentially one of the most reactive to human disturbance and climate change. As the planet warms, soils will add carbon dioxide to the atmosphere due to its increased biological activity at higher temperatures. Thus, soil carbon losses likely have a large positive feedback response to global warming (Powlson and David, 2005).

The physical properties of soils, in order of decreasing importance, are texture, structure, density, porosity, consistency, temperature, colour and resistivity. Soil texture is determined by the relative proportion of the three kinds of soil particles, called soil separates: sand, silt, and clay. At the next larger scale, soil structures called peds are created from the soil separates when iron oxides, carbonates, clay, silica and humus, coat particles and cause them to adhere into larger, relatively stable secondary structures. These properties may vary through the depth of a soil profile. Most of these properties determine the aeration of the soil and the ability of water to infiltrate and to be held within the soil (Nyle *et al*., 2009).

The mineral components of soil are sand, silt and clay, and their relative proportions determine a soil's texture. Properties that are influenced by soil texture include porosity, permeability, infiltration, shrink-swell rate, water-holding capacity, and susceptibility to erosion. In the

illustrated USDA textural classification triangle, the only soil in which neither and, silt nor clay predominates is called "loam". While even pure sand, silt or clay may be considered a soil, from the perspective of food production a loam soil with a small amount of organic material is considered ideal. The mineral constituents of a loam soil might be 40% sand, 40% silt and the balance 20% clay by weight. Soil texture affects soil behavior, in particular its retention capacity for nutrients and water. Sand is the most stable of the mineral components of soil; it consists of rock fragments, primarily quartz particles, ranging in size from 2.0 to 0.05 mm (0.0787 to 0.0020 in) in diameter. Silt ranges in size from 0.05 to 0.002 mm (0.002 to 0.00008 in). Clay cannot be resolved by optical microscopes as its particles are 0.002 mm (7.9×10−5 in) or less in diameter. In medium-textured soils, clay is often washed downward through the soil profile and accumulates in the subsoil. Soil components larger than 2.0 mm (0.079 in) are classed as rock and gravel and are removed before determining the percentages of the remaining components and the texture class of the soil, but are included in the name. For example, a sandy loam soil with 20% gravel would be called gravelly sandy loam (Brown, 2003).

The kingdom Fungi is one of the most diverse groups of organisms on Earth, and they are integral ecosystem agents that govern soil carbon cycling, plant nutrition, and pathology. Fungi are widely distributed in all terrestrial ecosystems, but the distribution of species, phyla, and functional groups has been poorly documented. On the basis of 365 global soil samples from natural ecosystems, we determined the main drivers and biogeographic patterns of fungal diversity and community composition (Leho *et al*., 2014). Fungi are microscopic cells that usually grow as long threads or strands called hyphae. Hyphae interact with soil particles, roots, and rocks forming a filamentous body that promotes foraging for food. These networks release enzymes into the soil and break down complex molecules that the filaments then reabsorb. Fungus act like natural recycling bins, reabsorbing nutrients in the soil (James and Natalie, 2001). Fungi are one of the important microbial components of the soil. Since 1860's, research have been carried out on the fungi of different soil types, such as soils of forest, driftwood, grasslands polar region, desert, marine and mangrove habitats and coastal sand belt 24 from various parts of the world. All these studies revealed that the fungi might reside permanently, temporarily for a period in the soil. Their number and species composition in the soil habitat differs from place to place depending upon the physical, chemical and biological factors of the particular habitat (Arnold *et al*., 2001; Madavasamy and Panneerselvam, 2012). Fungi that may be isolated in soil around Sokoto cement Company environment could be dangerous to both individuals and animals leaving around the area, and also it could affect the nutrient in the soil for agricultural purposes due to the residues dispersed from the cement company.

This study was carried out to determine the presence of fungi in soil around Sokoto cement company environment, to determine whether the fungi is dangerous to the environment (People, animal, plant, water) due to the residues dispersed from the cement company. The aim of the study is to isolate fungi from the soil around Sokoto cement company environment.

MATERIALS AND METHODS

The materials used are petri-dish, wire-loop, autoclave, test tubes, oven, incubator, digital weighing balance, cotton wool, rubber trowel, polythene bag, bunsen burner, conical flask, syringe, glass slides, cover slips, masking tape, distilled water, glass rod, aluminium foil, Potato Dextrose Agar (PDA).

Sample collections

The soil samples were collected around Sokoto cement company environment which is located at KM 10, Kalambaina Road from Sokoto metropolis. The samples were collected from three different locations around Sokoto cement company environment in which the samples sites were collected randomly. The sample site was cleared of living plants, plants litter and surface rocks. A clean rubber trowel was used and the samples were collected from the surface and 30 cm beneath the soil surface and dispense into a polythene bag and were covered with masking tape to avoid contamination. Each sample bag was giving a specific designation such as the surface samples were labelled A1, B1, C1 while beneath the soil surface were labelled A2, B2, C2 respectively, all samples were taken to the laboratory for analysis in accordance with Fery and Murphy,(2002) method.

Sample processing

Preparation of the sample

Each soil sample collected was dried until constant mass was achieved, visible organic debris was removed from the soil sample, and aggregates present in each soil sample was crushed and broken up. The soil from each sample was sieved with a clean 2 mm sieve. One sub-sample of 250 g was taken from the less than 2 mm fraction of each sample, each sub-sample was placed into a sturdy plastic container, sealed and labeled as A1, B1, C1 and A2, B2, C2 respectively (Penman *et al.,* 2003). The isolation of the organisms was based on accordance to the method described by Gray and Williams, (1971). That is the indirect method of the

isolation of soil microorganisms. In the method, the soil dilution is to be inoculated onto the agar medium. Potato Dextrose Agar (Thirty nine grams of the agar powder was weighed and suspended in I litre of distilled water in a conical flask. The solution was stirred, and heated for total dissolution. The conical flask was plugged with cotton wool, wrapped firmly with aluminium foil and then autoclaved at 121°C for 15 min. One percent streptomycin was then added aseptically to inhibit bacterial growth) (Cheesebrough, 2004).

Serial dilution

Each soil sample weighed 1 gm on a filter paper weighing balance. For each sample, four tubes were arranged on a test tube rack and the test tubes were designated as follows: 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} respectively, thus obtaining four test tube rack containing four test tubes. With 10 ml measuring cylinder, 10 ml of distilled water was measured and dispensed into the test tubes that are designated as 10^{-1}, and then 9 ml of distilled water was also measured and dispensed into each of the test tubes that were designated as 10^{-2}, 10^{-3} and 10^{-4}. The 1 gm of soil measured was dispensed into the test tubes marked 10^{-1} the suspension were mixed thoroughly with sterile glass rod. Using sterile 1 ml pipette, 1 ml of the 10^{-1} was withdrawn and transferred to the test tube marked 10^{-2}. Another sterile 1 ml pipette was used to draw 1 ml from the 10^{-2} dilution which is transfer to the 10^{-3} test tube. Another sterile 1 ml pipette was used to draw 1 ml from the 10^{-3} dilution which is transfer to the 10^{-4} test tube to make 10^{-4} dilution. This procedure was used for all six samples as described by Fawole and Oso, (1988).

Inoculation of the samples

With two spirit lamps alight on either site of the petri dish, 30 ml of the prepared agar was poured into the petri dish and allowed to congeal. 6 plates of prepared agar were obtained from the same procedure. The plates were labelled reflecting the dilution of the samples that were used for inoculation. A sterile 1 ml pipette was used to draw1 ml from 10^{-3} test tube and transferred to each corresponding plate. The plates were rotated to ensure evenly distribution of the suspension on the surface. This method of plating out soil dilution series was outlined by (Fawole and Oso, 1988).The plate was incubated for seven days at room temperature.

Sub-culture of mixed culture

After seven days of incubation, variety of colonies was found grown on all the culture plates. The plates that appear with different colonies were sub-cultured on another agar medium (Potato dextrose agar). A very small portion of each suspected colony is removed with a wire loop and a needle and was transferred onto the surface of the congeal medium. The sub culture plate was incubated at room temperature for 1week (Cappuccino and Natalie, 1998).

Identification of isolates

All isolates were identified using the identification techniques of James and Natalie, (2001) was adopted using lacto phenol cotton blue stain. The identification was achieved by placing a drop of the stain on a clean slide with the aid of mounting needle, where a small portion of the mycelium from fungal cultures was removed and placed in a drop of lacto phenol. The mycelium was spread very well on the slide with the aid of a needle. A cover slip was gently applied with little pressure to eliminate air bubbles. The slide was then mounted and observed with x10 and x40 objective lenses. The species encountered were identified in accordance with Cheesbrough, (2000).

RESULTS AND DISCUSSION

Viable plate counts

Morphological characteristics of fungi colonies were first observed. The viable count of fungi from the three selected location around Sokoto cement company environment are presented in (Table 1). Fungal counts ranged from ($1.0x10^3$ CFU/g) in sample C_2 to ($8.7x10^4$ CFU/g) in sample B_2. From (Table 2), the results revealed the percentage occurrence of fungi as follows: *Aspergillus niger* (25%), *Rhizopus species* (16.66%), *Candida albicans* (16.66%), *Histoplasma capsulatum* (16.66%), *Aspergillus nidulans* (8.33%), *Epidermophyton floccosum* (8.33%), *and Fusarium species* (8.33%).

The total viable counts from the fungi isolated from the samples were ranged from ($2.0x10^4$ CFU/g) to ($7.7x10^4$ CFU/g) in sample A_1 and A_2 respectively (Table 1). In sample B, viable counts ranged from ($8.7x10^4$ CFU/g) to ($9.0x10^3$ CFU/g) in sample B_2 and B_1respectively. Viable counts for sample C ranged from $1.0x10^3$ CFU/g in sample C_2 to ($1.6x10^4$ CFU/g) in sample C_1. The result of viable counts shows that fungi were higher at the bottom in both sample A and B (A_2 and B_2). While in sample C, fungi were higher at the surface (C_1).*Aspergillus niger* were the most isolated specie (25%), *Rhizopus species* (16.66%), *Candida albicans* (16.66%), *Histoplasma capsulatum* (16.66%), while the least isolated species were *Aspergillus nidulans*, *Epidermophyton floccosum*, and *Fusarium species* (8.33%). From this research, a total of seven species were isolated, these includes *Aspergillus niger, Rhizopus species, Candida albicans,*

Table 1. Colony counts from the samples.

Samples	Fungal counts CFU/g
A_1	$2.0x10^4$
A_2	$7.7x10^4$
B_1	$9.0x10^3$
B_2	$8.7x10^4$
C_1	$1.6x10^4$
C_2	$1.0x10^3$

KEY: A= Quarry site; B= Gidan Gamba area; C= Gyarafshi area;1= Surface; 2= Beneath surface.

Table 2. Frequency of occurrence of fungal isolates.

Species	Frequency	Percentage frequency (%)
Aspergillus niger	3	25.00
Rhizopus species	2	16.66
Candida albicans	2	16.66
Histoplasma capsulatum	2	16.66
Aspergillus nidulans	1	8.33
Fusarium species	1	8.33
Epidermophyton floccosum	1	8.33
Total	12	100

Histoplasma capsulatum, Aspergillus nidulans, Epidermophyton floccosum, and *Fusarium species.* The study results were in agreement with the work of Rakesh and Kavita, (2014) that also isolated related fungi species, and also *Aspergillus niger* were the most isolated in their research. And also with Arunsasi *et al*. (2010) in which 15 fungal species were isolated and Aspergillus species is the most abundant. Study supported by Obire and Anyanwu, (2009) they isolated fourteen fungal genera from soil in which *Aspergillus, Candida, Rhizopus species,* and *Fusarium species* are included. Absence of isolating certain kind of fungi species in this study compared to other previous study might be due to different environmental condition such as high temperature, dry winds and low percentage of humidity in which is not favorable for fungal growth.

Conclusion

From this research, a number fungi which were of medical importance were isolated these are *Aspergillus niger* which were the most isolated specie (25%), *Rhizopus species* (16.66%), *Candida albicans* (16.66%), *Histoplasma capsulatum* (16.66%), while the least isolated species were *Aspergillus nidulans*, *Epidermophyton floccosum*, and *Fusarium species*(8.33%). Related to different researches on fungi isolated from soil, *Aspergillus niger* is the most isolated specie from soil samples.

Recommendation

Fungi is very important in soil, it has more advantages than disadvantages in the soil, to ensure fungi remain in the earth, the following are recommended

(a) The soil environment must be kept as hospitable as possible. This means there must be enough food (organic matter), suitable host plants (if necessary), water and minimal disturbance of the soil.
(b) Tillage has a disastrous effect on fungi as it physically severs the hyphae and breaks up the mycelium so therefore it should be reduced.
(c) Broad-spectrum fungicides are toxic to a range of fungi. Their use will result in a decline in the numbers of beneficial types.
(d) Herbicides are not generally thought to affect fungi directly, though the removal of some plant types may affect the distribution of different fungi types so therefore fungicide use should be reduced.

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