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Full Length Research Paper

KERATINOPHILIC FUNGI ASSOCIATED WITH SOILS FROM SELECTED VILLAGES IN UGWUNYEAND OJEBOGENE TOWNS, ENUGU STATE, NIGERIA AND THEIR DISEASE IMPLICATIONS

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ABSTRACT
Keratinophilic fungi are a group of diminutive distinct yet significant fungi that grow and live on different keratinous substances and can be isolated from soil. Fifty-nine (59) fungi were isolated from soils of markets, schools and farmlands of different communities in Ugwunye and Ojebogene towns in Enugu state, by hair baiting technique. The pH and moisture contents of the soils ranged from 4.88 to 6.90 and 0.3% to21.0% respectively. The 59 fungal isolates were fromeight (8) different genera: *Aspergillus, Cladosporium, Coccidioides, Fusarium, Microsporum, Piedraia, Scopulariopsis* and *Trichophyton.* This study exposes the presence and kinds of keratinophilic fungi present in these soils. It also shows the disease potential of these fungi for persons who may be exposed to them.

Keywords: keratinophilic fungi, soil, market, school, farmland

INTRODUCTION
Soil is the main habitat for fungi worldwide and as such, the main reservoir for keratinophilic fungi occurrence and activity. The keratinolytic activity of keratinophilic fungi has attracted many researchers' attention around the world as keratinophilic fungi play an important role in the natural degradation of keratinized residues in the soil (Pakshir *et al.*, 2013). The presence of this keratinous material in soil is the main reason for the incidence and growth of keratinophilic fungi. Thus their distribution worldwide is based on the crucial matter of the presence of keratin from human or animal source(Singh *et al.*, 2009; Zarrin and Haghgoo, 2011).

Keratinophilic fungi are a group of small well-defined and important fungi that colonize various keratinous substrates degrading them to components of low molecular weight (Kumar *et al*., 2013).They are an ecologically important group of fungi often found in soil, that can cycle one of the most abundant and highly stable animal products on earth called keratin which can only be degraded and use as a resource by a few organisms like insects, bacteria, actinomycetes and fungi (Deshmukh and Verekah, 2006; Agu *et al*., 2013; Sharma and Rajak, 2003). These fungi are the largest group of organisms that are able to use keratin as sole carbon and nitrogen source. They also tend to be pathogenic with regard to the keratinized tissues of humans and animals (Sharma and Rajak, 2003). Soils rich in keratin residue are considered a permanent or occasional reservoir for keratinophiles and are a potential source of infection (Yazdanparast *et al*., 2013).

There has been increasing interest worldwide by researchers in keratinophilic organisms due to two factors: the extreme resistance of keratin to biological attack and the pathogenic potential of every keratinophilic, saprophytic species (Yazdanparast *et al*., 2013).In addition to this, the incidence of fungal infections is rising at a disturbing rate, emerging even in locations they were not previously found before and posing great challenges to healthcare personnels (Konkel, 2017; Jain *et al*., 2010). Fungal diseases, which are caused majorly by soil-inhabiting fungi, are now of crucial public health concern as more of the populace seem to be susceptible to it and environmental alterations related to climate change are seemingly favouring soil inhabiting fungi (Centre for Disease Control, (CDC), 2017; Washington State Department of Health (WSDOH), 2014). Most mycoses that are of public health importance are caused by disturbances of fungal mycelia in soiland statistics has shown that invasive fungal infections kill about 1.5 million people worldwide annually (Konkel, 2017). It is therefore, significant to analyze and identify the mycoflora of soils in order to evaluate the presence of keratinophilic fungi in such environments (Shadzi *et al*., 2002).

Ugwunye and Ojebogene towns are located within Udi local government area (LGA) of western Enugu state. Udi LGA falls within Latitudes 6‘45’10N and 6‘4’30N and Longitudes 7’4’0’E and 7’34’30’E and has a population size of about 0.2 million. It has a wet tropical climate with mean annual temperature range of 27-34°C and mean annual rainfall of 1, 609mm (Obeta, 2016; Ngene *et al*., 2015). Prior to this study, there has been no data about keratinophilic fungi in these chosen sites. Thus, this study aimed at isolating and identifying the keratinophilic fungi associated with the soils of selected schools, markets and farmlands in Ugwunye and Ojebogene towns of Enugu state. The study also related these fungi to their possible disease implications to man.

MATERIALS AND METHODS

Soil sample collection: The top surface of the sites was first cleared, and then using sterile soil probe and ziplock bag, 10g of the soils were collected. The bag was then sealed, labelled and conveyed promptly to the lab for analysis. Sites were within Latitudes 6‘45’10N and 6‘4’30N and Longitudes 7’4’0’E and 7’34’30’E in Enugu state.

Determination of soil physical characteristics: The pH of the soils were determined with pH meter and recorded. Percentage moisture content of the soil samples were also determined using the method of Ogbonna and Pugh (1987) and recorded.

Sample inoculation by hair baiting method: The hair baiting technique by Vanbreuseghem (1952) was used. Virgin hair (untreated with chemical) was sterilized in autoclave at 121°C for 15 minutes. A single strand was then aseptically extracted and mixed with 10g of soil sample in sterile Petri dishes (for each soil sample). These were moistened constantly with sterile distilled water and incubated for 2 weeks at room temperature. At the expiration of the 2 weeks, the hair strands were again aseptically removed and inoculated into Sabouraud's dextrose agar (incorporated with chloramphenicol to prevent growth of bacteria) and yeast extract agar prepared in Petri dishes. These were labelled accordingly and incubated at 28°C for 7 days.

Fungi identification: Pure colonies were made from the growth in the dishes by subculture method. The macroscopy and microscopy of each isolate were then carried out and recorded. Macroscopy included standard criteria like general topography, colony colour and texture as well as reverse pigmentation. Fungal hyphaeformations, macroconidia as well as its microconidia were used to identify them microscopically with reference to Beneke and Rogers (1980) and Collins *et al*. (1991).

Occurrence and Percentage Frequency of isolates: The isolates were counted numerically per species growth on media and per genus for each location and their percentage frequencies calculated thus:

Percentage frequency of species =
Number of isolates of a species X 100
Total number of microorganisms

Percentage frequency of genus =
Number of isolates of a genus X100
Total number of microorganisms

RESULTS

Soil Physical Characteristics

The soils of the three schools, three markets and three farmlands (nine (9) selected sites) of Ugwunye town had pH and moisture content ranges of 4.88-6.35 and 0.3-12% respectively. The soils of the three schools, three markets and three farmlands of Ojebogene town also had pH and moisture content ranges of 6.30-6.90 and 2.60-21.00% respectively.

As shown in Tables 1 and 2,the genera of fungi isolated from different locations were *Aspergillus, Cladosporium, Coccidioides, Fusarium, Microsporum, Piedraia, Scopulariopsis* and *Trichophyton.*

The most frequently occurring genus was *Aspergillus fumigatus* followed by *Cladosporium* sp. and *Coccidioides immitis.* The least frequently occurring genera were *Fusarium* and *Scopulariopsis* with 1. 69%. These are shown in Table 3.

Table 1: Characteristics of the different fungal isolates

ISOLATES	MACROSCOPY	MICROSCOPY
Aspergillus flavus	Bright yellow-green, rough and woolly colony turning dull yellow with time. Reverse is colourless.	Conidia are borne on uniserate, crowded phialides which are on entire surface of sphere-like vesicles.
Aspergillus fumigatus	White, velvety colony turning dark brown and having folds with time.	Conidia are borne on uniserate and evenly crowded phialides with columnar head. Flask-shaped vesicles display phialides on about one-third of its head.
Cladosporium sp	Moderate-growing, olive-green, velvety, colonies with folds and a black reverse.	Branching chains of conidia on conidiophores. Conidiophores branch out from septate hyphae with crooked structure.
Coccidioides immitis	Slow-growing, white tufts, of mycelia initially, becoming cottony with time	Short chains of barrel-shaped arthroconidia with hyphae in between them. Segmented branching hyphae also seen.
Fusarium sp	Fast growing, white, fluffy colonies turning pale pink with time	Conidia form in whorls around short conidiophores which are borne on long phialides. Curved microconidia also observed.
Microsporum audouinii	Slow growing, velvety, reddish-brown, flat colony with radiating furrows. Reverse is also reddish-brown	No macro- or microconidia seen.
Microsporum canis	Fast growing, white, velvety growth turning light yellow with time. Reverse is dull yellow	Multiple microconidia borne on septate hyphae having many branches. Septate spindle shaped macroconidia also seen.
Microsporum cookei	Fast growing, brownish, velvety growth with reddish reverse	Rough-walled ellipsoidal macroconidia with few oval-shaped microconidia
Microsporum nanum	Fast growing, white, cottony colony turning pale and granular with time. Reverse is also pale.	Multiple pear-shaped macroconidia with one or two septations. Microconidia are thin and hyphae-like
Piedraia hortae	Slow-growing, flat, greenish-black, velvety colony	Dark and septate hyphae with few irregularly–shaped segments
*Scopulariopsis*sp	Slow growing wrinkled colonies, becoming powdery and brown with time	Septate hyphae with several *Penicillium*-like branches bearing annelid and then conidia at their tips. Few biserate conidia are present
Trichophyton gallinea	Moderate-growing, white, velvety colony with folds turning light pink with time.	No macroconidia are seen. Slender long microconidia are borne singly on short branches of hyphae are seen.
Trichophyton megninii	Slow growing, flat, white, velvety colony turning pink, then deep rose with time. Reverse is rose coloured as well	No macroconidia are seen. Small oval-shaped microconidia seen along hyphae.
Trichophyton rubrum	Slow-growing, white, cottony colony, turning pale pink in patches and powdery with time. Reverse is wine red	Club-shaped rather elongated macroconidia with many septations seen. Oval-shaped microconidia borne on septate, stout hyphae with few branches are also seen.
Trichophyton tonsurans	Slow growing, white, velvety colony with irregular folds and a heaped centre. Colony turns brown with time. Reverse is reddish-brown.	Large oval macroconidia with no septations are seen on hyphae tips. Several oval-shaped microconidia are also seen along hyphae.

Table 2: Fungi isolated from the different locations

	Location/ Locality	Market	School	Farmland
UGWUNYE LGA	**Egede**	*Aspergillus flavus* *Cladosporium* sp	*Aspergillus flavus* *Cladosporium* sp *Coccidioides immitis* *Microsporum cookei* *Trichophyton megninii*	*Coccidioides immitis* *Microsporum audouinii* *Microsporum canis* *Trichophyton rubrum* *Trichophyton tonsurans*
	Affa	*Aspergillus flavus* *Microsporum audouinii*	*Aspergillus fumigatus*	*Aspergillus flavus* *Cladosporium* sp *Microsporum audouinii* *Trichophyton tonsurans*
	Amozalla	*Aspergillus fumigatus* *Trichophyton tonsurans*	*Aspergillus fumigatus* *Microsporum cookie* *Trichophyton tonsurans*	*Aspergillus flavus* *Aspergillus fumigatus* *Microsporum canis* *Trichophyton tonsurans*
OJEBOGENE LGA	**Location /Locality**	**Market**	**School**	**Farmland**
	Abor	*Aspergillus fumigatus* *Coccidioides immitis* *Microsporum cookei* *Piedraia hortae*	*Aspergillus fumigatus* *Coccidioides immitis* *Microsporum cookei*	*Aspergillus flavus* *Cladosporium* sp *Microsporum cookei*
	Awhum	*Cladosporium* sp *Fusarium* sp	*Aspergillus fumigatus* *Cladosporium* sp *Trichophyton gallinea* *Trichophyton tonsurans*	*Cladosporium* sp *Coccidioides immitis*
	Ukana	*Aspergillus fumigatus* *Coccidioides immitis* *Piedraia hortae* *Scopulariopsis* sp *Trichophyton megninii*	*Aspergillus fumigatus* *Coccidioides immitis* *Microsporum cookei* *Microsporum nanum*	*Aspergillus fumigatus* *Cladosporium* sp *Coccidioides immitis* *Microsporum cookei* *Trichophyton megninii*

Table 3: Frequency of Isolates

Fungal isolates	Species Frequency	Percentage Frequency of species (%)	Genus Frequency	Percentage Frequency of Genus (%)
Aspergillus flavus	6	10.17		
Aspergillus fumigatus	9	15.25	15	25.42
Cladosporium sp	8	13.56	8	13.56
Coccidioides immitis	8	13.56	8	13.56
Fusarium sp	1	1.69	1	1.69
Microsporum audouinii	3	5.10		
Microsporum canis	2	3.39		
Microsporum cookie	7	11.86	13	22.05
Microsporum nanum	1	1.69		
Piedraia hortae	2	3.39	2	3.39
*Scopulariopsis*sp	1	1.69	1	1.69
Trichophyton gallinea	1	1.69		
Trichophyton megninii	3	5.10		
Trichophyton rubrum	1	1.69	11	18.64
Trichophyton tonsurans	6	10.17		
Total	**59**	100	59	100

DISCUSSION

The low moisture content of these soils provides favourable conditions for fungal growth because fungi thrive better where there is low moisture content (Stotzky, 2000; Anon, 2016).

The pH of the soils (4.88-6.90) indicated that these soils were slightly acidic. The presence of these fungi in soils with such pH is supported by Böhme and Ziegler (1965) who reported the effect of soil pH on the presence of keratinophilic fungi and found their frequency to be more in weakly acidic than strongly acidic soils. This is contradicts the study of Garg *et al*. (1985), who reported that acidic soils with pH of 5.9 were free of keratinophilic fungi. The works of Asahi *et al.*(1985),who demonstrated that keratinolytic enzymes were produced in soils with pH of between 6 and 9and Pakshir *et al*. (2013), who isolated all the keratinophilic fungi in their study from the soils with pH of between 6 and 9, also support this work.

Keratinophilic fungi were present in every soil sample in this study. Soil is regarded as a major domain for fungi and considered a reservoir for human infection as well as the best media for growth of keratinophilic and saprophytic fungi especially when their humus and organic matter content is high (Ali-Shtayeh and Jamous, 2000; Pakshir *et al*., 2013). It also shows that the fungi genera isolated are *Aspergillus*, *Cladosporium*, *Coccidioides, Fusarium*, *Microsporum, Piedraia, Scopulariopsis* and *Trichophyton.* Many of these fungi have been isolated from various soils all over the world, few of which include the works of Ogbonna and Pugh (1987), Moallaei *et al*. (2006), Ganaie *et al*. (2010), Pakshir *et al*. (2013) and Bisen and Tiwari (2015), which have at least one of these genera as their isolates.

From the table of frequency of occurrence of the isolates, the most abundant keratinophile genera isolated was *Aspergillus*with a frequency of 25.42%.This is similar to works by Hong *et al.*(2010), Jasuja *et al*.(2013) and Soni and Sharma(2014), who separately reported *Aspergillus* sp as the most abundant isolates from Korean, Rajasthani (India) and Bhopal (India) soils respectively.

Aspergillus sp. was found in soils from all but three (3) localities –Egede farmland, Awhum market and Awhum farmland. This fungus is known to cause opportunistic aspergillosis when inhaled especially by immunosuppressed persons (Latge, 1999). It is also known to spoil foods and produce the harmful mycotoxin, aflatoxin, as a secondary metabolite when it colonizes foods stuff such as grains and nuts (Hicks *et al*., 1997; Bhosale *et al*., 1999). At very low levels, aflatoxin instigates strong reactions from its casualties. Its syndrome, aflatoxicosis, is characterized by vomiting, abdominal pain, pulmonary edema, convulsions, coma, and death (Prescott *et al*., 2005, Kimanya, 2013) *Aspergillus* therefore, poses a risk to persons living or working near and around soils it inhabits either by causing disease to them, causing food spoilage or causing food intoxication from its secretion of aflatoxin on stored food products (Tournas, 2005).

Microsporum and *Trichophyton* spp. were the next most abundant species (by genus frequency) and were isolated from all

localities. This is similar to the work of Ogbonna and Pugh (1987) and Agu *et al* (2013), who isolated different species of *Microsporum* and *Trichophyton* from Nigerian soils. Mancianti and Papini (1996), Moallaei *et al.* (2006), Singh *et al.*(2009), Ganaie *et al.* (2010), Gupta, (2012) and Bisen and Tiwari(2015) also isolated *Microsporum* and/or *Trichophyton* species among other fungi in their respective studies on keratinophilic soil fungi.

The presence and abundance of isolates of *Microsporum* and *Trichophyton* species from soil is significant because these fungi cause dermatomycoses, also known as ringworms or tinea (Prescott *et al.,* 2005). These cuteneous fungal diseases are the most common fungal diseases of humans worldwide and include tinea barbae- ringworm of the beard hair, tinea capitis- ringworm of the scalp, tinea cruris- ringworm of the groin, tinea pedis- athlete foot and tinea unguium- ringworm of the nail bed (Woodfolk, 2005).

Cladosporium sp. had a frequency of 13.56%. It is a common environmental mould found on plants, organic matter and many surfaces. It is known cause eye, skin, sinus and brain infections, allergies, asthma and fungal meningitis. It is also a contaminant of steroid injections and can be a risk factor for human inhabitants of such soils (CDC, 2015, Kantarcioglu *et al*., 2002)

Coccidioides immitis also had a frequency of 13.56% and was similarly isolated from soils in San Joaquin valley by Greene *et al*.(2000), in Brazilian soils by Macedo *et al.*(2011) and in Californian soils by Lauer *et al.*(2012). *C. immitis* is a saprobe of semiarid soils and has shown selective advantage over other fungi in saline and alkaline soils and in areas with hot, dry summers and mild winters(Greene *et al*., 2000). It is a pathogenic fungus that causes the systemic mycoses, coccidioidomycosis, when inhaled (Macedo *et al*., 2011). Its arthroconidia can very easily be acquired by just moving through an area where it exists. Ordinary wind turbulence as well as presence of outdoor structures predisposes humans to this fungus in endemic areas (Ruddy *et al*., 2011).

*Piedraia hortae*was isolated only twice in this work (with a frequency of 3.39%). *P. hortae*is the causative pathogen for the superficial mycoses, black piedra and forms black to brown nodules only on the hair shafts (Khatu *et al.,* 2013). The condition constitutes cosmetic problems but does not elicit any immune response from the host (Baron, 1996).

Fusarium sp. was one of the least isolates in this study (with a frequency of 1. 69%). This fungus is known to spoil foods, cause plant diseases and secrete the potent mycotoxin, fumonisin, which causes leukoencephalomalacia and pulmonary oedema in farm animals and oesophageal cancer in humans (Prescott *et al.,* 2005)
Scopulariopsis is a common cosmopolite, saprophytic, filamentous fungi widely isolated from soils, plants and many other organic substrates. It was one of the least isolates in this study with a frequency of 1. 69%. Its invasive infections are rare and occur mostly in immunocompromised persons where it causes infections of the brain, lungs, heart and mostly the sinus.

Invasive sinusitis caused by this fungi is usually severe and fatal (Sattler *et al.*, 2014)
This study shows the abundance of keratinophilic fungi in these soils and the disease potentials of each genus of fungi. A large group of people are exposed to these likely pathogens of man, animals, food and plants. School children, teachers, school premises cleaners, farmers, farm workers, market traders, land tillers and surveyors, even passersby (amongst others), are susceptible to infections by these fungi when they play, inhale, work and walk with bare hands and feet or somehow come in contact with these soils.

CONCLUSION

Keratinophilic fungi play an important role in the natural degradation of keratinized residues in the soil. This suggests its pathogenic potential in colonizing keratin and perhaps gaining entry into the human body. This study exposes the presence and kinds of keratinophilic fungi present in these soils which can be of serious health implication.

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