ISOLATION AND CHARACTERISATION OF AQUATIC FUNGI FROM OPEN DRAINS IN PORT HARCOURT MUNICIPALITY, SOUTHERN NIGERIA.

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Abstract
Gari slide culture media was used to isolate fungi from open drains in Port Harcourt Municipality. Fungi grown onto Sabouraud Dextrose Agar (SDA) were purified by repeated sub-culturing unto gari slide culture media. Microcultures of the fungi isolated on SDA were made by transferring spore suspensions of each isolate onto a 1 cm disc of the same medium placed on a microscope slide in a Petri dish. All inoculated slides were incubated at 30±2°C for 5 days, and then examined under the microscope. Fungi isolated on SDA and gari medium were Aspergillus niger, Aspergillus tamarii, Aspergillus versicolor, Cryptococcus neoformans, Mucor spp, Penicillium chrysogenum, Penicillium marneffei, Phoma spp, Rhizopus spp, Scopulariopsis spp and Torulopsis glabrata. Also a micrograph of Torulopsis spp and Cryptococcus spp as yeasts on Gari slide culture medium are presented. However, the patterns and rate of growth of the fungal species were more in sediments than in water samples which could be responsible for the degradation of receiving water bodies.

Keywords: Aquatic fungi, open drains, Gari slide culture media

INTRODUCTION
Microorganisms are present in all natural habitats, as well as in human-made environments such as open drainage channels. Such reservoirs have features such as darkness, long retention times and stagnation zones that favour the proliferation of microorganisms (especially fungi) and biofilm formation, as well as, toxin production (Hageskal et al., 2009). Apart from their significant ecological role in helping nutrient turnover, water can become contaminated or highly impacted by their activities (Hageskal et al., 2006; Besner et al., 2011; Douterelo et al.,
2014, EPA, 2016) causing bad taste and odour. Through the inhalation of spores after aerosolisation of water, especially when water passes through taps and showers water loses its potability (Warris et al., 2001; Hageskal et al., 2009; Anaissie et al., 2011; Richardson and Richardson, 2015).

Studies of the structural characteristics of moulds could be simplified by placing on microscope slides (about 1 cm square) blocks of agar cut from appropriate culture media; the upper edges of the agar blocks are inoculated with the desired moulds; then after adequate incubation of inoculated blocks, the slides along with the agar block are examined under the microscope (Sokari et al., 1996). This is because moulds can be identified to the generic level often without special staining or biochemical techniques since their structural details could be readily visible microscopically. However, the structural differences can be readily observed only if the mould colony is not disturbed during preparation or mounting for microscopic examination (Sokari et al., 1996), and this is achieved by means of monocultures on microscopes slides. The aim of the present study, therefore, was to identify fungi isolated from open drainage channels in Port Harcourt using gari media as an inexpensive, but effective alternative culture medium to conventional dehydrated mycological media for isolating and identifying moulds.

MATERIALS AND METHODS

Collection of sample

Wastewater samples were collected from open drains along the Ntanwogba creek with sterilized plastic bottles. Each sample bottle was rinsed with the appropriate sample before the final collection according to the standard methods (APHA, 2012). To collect the water sample, base of the sterilized sample bottle was held with one hand, plunged about 30cm below the water surface with the mouth of the sample container positioned in an opposite direction to water flow (APHA, 2012). The container was filled with wastewater samples from different locations starting from the upstream (Afan/Kaduna street behind the Winners chapel) to the downstream (at Abacha road, all sites in Port Harcourt Rivers State Nigeria) leaving a gap of about 2cm and then covered.

Sediment samples for analysis were also collected along the same water course. To collect the sediment samples, the bottles were opened and held with the left hand while using the right hand with a plastic scooper to scoop the sediment sample. The sample bottles were filled with sediment sample and covered immediately. Thereafter, each
sample was immediately labelled and transported in a cooler packed with ice blocks for analysis. Sample collection was carried out from February to August 2016.

Mycological Study

Serial dilution

Ten-fold serial dilutions of the samples were made according to Oliveira et al. (2016).

Inoculation and incubation

One milliliter of appropriate ten-fold serial dilution of the sample was inoculated onto appropriate Sabauraud dextrose agar in triplicates using pour plate method of Hageskal et al. (2009) and spread plate method of Oliveira et al. (2016). Inoculated plates were incubated at 28±2°C for 48-72 hours. Visible discrete colonies on incubated plates were counted and expressed as colony forming unit per gram (cfu/g) of sediment samples and colony forming units per milliliter (sfu/ml) of waste water samples.

Fig 1. Map of Port Harcourt showing sampling stations
Maintenance of pure culture

Fungi grown onto SDA were purified by repeated sub-culturing unto gari slide culturing media. Pure cultures were preserved on gari slide culture media at ambient temperature (28±2°C) for further tests.

Garri slide culture method

According to the method of Sokari et al. (1996), individual, fairly large grains of gari were placed in a glass Petri dish and autoclaved at 121°C. Using a flamed forceps, one granule of the gari was transferred from the glass Petri dish unto a glass slide. Thereafter, fungal growth was touched with a wire inoculating needle and then placed on gari granule on glass slide. Inoculated granule was transferred into a sterile Petri dish layered with moistened cotton wool, and incubated at 30°C for 3-5 days. After incubation, structures of fungal growth were enhanced by touching edge of coverslip with cotton blue in lactophenol. Identification of fungal species was done phenotypically based on macroscopic and microscopic morphological features of cultivation in gari slide culture medium.

Characterization and identification of fungal isolates.

Pure cultures of fungal isolates were identified based on cultural parameters, microscopic technique and biochemical tests including carbohydrate utilization as described by Cruickshank et al. (1975). Characterization and identification of fungal isolates was done according to Kelly et al. (2003) and Samson et al. (2004).

RESULTS AND DISCUSSION

Predominant fungal isolates from the open drains were characterized and identified as Aspergillus niger, Penicillium chrysogenum, Aspergillus tamarii, Cryptococcus neoformans, Aspergillus versicolor, Torulopsis glabrata, Rhizopus spp, Mucor spp, Scopulariopsis spp Penicillium marneffei and Phoma spp (Table 1; Figs 1-9 a and b). Species of Torulopsis and Cryptococcus (Table 2; Figs 10-11 a and b) were the only yeast isolates from the open drainage system. Fungi are ubiquitous in soils and wastewater preferring cool and moderate climate, especially with the abundance of organic materials (Nicoletti et al., 2009). However, Cryptococcus neoformans and Rhizopus species were isolated more frequently from both water and sediment samples in the open drains. The patterns and rate of growth of the fungal species were more in sediments than in water samples. Doggett (2000) had earlier reported densities of filamentous fungi and yeasts in similar systems. Their presence in wastewater indicates that there is possible contamination by fungal
The pathogen (Prescott et al., 2005; Oliveira et al., 2016) which may be responsible for the degradation of receiving water bodies. Their presence was most likely through the runoff of fertilizers or sewage which contain excess nutrient that plants, algae and fungi can utilize for growth. Their abundance and activities in drainage systems have profound effect on the physicochemical characteristics of the wastewater especially at the banks of the creek. This is as a result of drastic narrowing of the creek channels induced by the declining riparian vegetation along the system particularly at the degrading wastewater open drains.

Table 1. Morphological Characteristics and identity of fungal Isolates

<table>
<thead>
<tr>
<th>Isolates code</th>
<th>Morphological Characteristics</th>
<th>Microscopic Characteristics</th>
<th>Probable organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>White cottony mycelium</td>
<td>Non-septate hyphae, large globose many spored sporangia on single sporangiophore.</td>
<td><em>Rhizopus</em> spp.</td>
</tr>
<tr>
<td>2</td>
<td>Green dense velvet mycelium</td>
<td>Hyaline conidiophores, phialides borne on versicles. Green chain of conidia with septate hyphae.</td>
<td><em>Aspergillus versicolor</em></td>
</tr>
<tr>
<td>3</td>
<td>Dark green granular dense mycelium</td>
<td>Conidiophores with inflated branches.</td>
<td><em>Pencillium chrysogenum</em></td>
</tr>
<tr>
<td>4</td>
<td>Compact white basal dark colony</td>
<td>Hyaline conidiophore phylides borne on vesicles. Green chain of conidia, with septate hyphae.</td>
<td><em>Aspergillus niger</em></td>
</tr>
<tr>
<td>5</td>
<td>Loose cotton wool-like aerial mycelium</td>
<td>Non septate mycelia, that bear sporangiophores scattered all over the mycelium.</td>
<td><em>Mucor</em> spp.</td>
</tr>
<tr>
<td>6</td>
<td>Oval creamy with slimy surface</td>
<td>Oval yeast cells with single terminal budding.</td>
<td><em>Torulopsis glabrata</em></td>
</tr>
<tr>
<td>7</td>
<td>White with blackish brown pyonidia</td>
<td>Conidia are unicellular, hyaline are ellipsoidal to cylindrical with septate hyphae.</td>
<td><em>Phoma</em> spp.</td>
</tr>
</tbody>
</table>
Plate 1(a) Morphological features of Penicillium chrysogenum as seen on garí medium

(b) Penicillium chrysogenum as seen on slide microcultures

Table 2. Morphological and Biochemical Characteristics of yeast Isolates

<table>
<thead>
<tr>
<th>Isolates Code</th>
<th>Cell morphology</th>
<th>Gram reaction</th>
<th>Oxidation and fermentation</th>
<th>Glucose</th>
<th>Fructose</th>
<th>Maltose</th>
<th>Lactose</th>
<th>Sucrose</th>
<th>Probable organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 1</td>
<td>Oval and creamy</td>
<td>+</td>
<td>F</td>
<td>_</td>
<td>+</td>
<td>+</td>
<td>_</td>
<td>_</td>
<td>Torulopsis spp.</td>
</tr>
<tr>
<td>2. 2</td>
<td>Spherical and piented</td>
<td>+</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>Cryptococcus spp.</td>
</tr>
</tbody>
</table>
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Plate 2 (a) *Penicillium marneffei*

(b) *Penicillium marneffei*

Plate 3 (a) *Aspergillus niger*

(b) *Aspergillus niger* as seen on slide microcultures

Plate 4 (a) *Aspergillus tamarii*

(b) *Aspergillus tamarii*
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Plate 8 (a) *Rhizopus* spp.

Plate 9 (a) *Mucor* spp

Plate 10 (a) *Torulopsis* spp
CONCLUSION
Fungi isolated from all the open drains examined are considered to produce mycotoxins and/or are opportunistic human pathogens. Water storage in drainage systems generates stagnation, stratification, particle accumulation, dead zones, depletion of residual disinfectant, and biofilm formation. These parameters combined with chemical, physical characteristics of the water system (like high turbidity and temperature, pH, total organic carbon and dissolved oxygen), are favorable to microbial growth making reservoirs a potential high risk of water quality degradation by fungi. In this current work, all sampling sites presented *Aspergillus* and other species, and these species are recognized producers of aflatoxins. Paterson *et al.* [1997] and Oliveira *et al.* (2016) demonstrated production of aflatoxins in a cold water storage tank. Water subjected to storage (e. g., reservoirs, cisterns and bottles) for long periods, may contain increased mycotoxin concentrations. In the long term, daily consumption of large amounts of water containing low levels of mycotoxin, may pose a health hazard (Paterson *et al.*, 1997; Skaar and Hageskal, 2015; DEFRA, 2016, Oliveira *et al.*, 2016). Therefore, frequent surveillance for fungi and the setting of limits are required to improve the mycological quality of drinking water. Presence of microorganisms in such environments affects human health, causing various diseases from allergic reaction to more serious systemic infections. The choice of Gari to Sabouraud media was not meant to be selective in separating clearly fungal taxa. This could be explained by the fungi not being under stress conditions due to the low or zero residual chlorine and the presence of considerable amounts of dissolved oxygen and
organic matter in water samples. Furthermore, Sabouraud, as a richer medium, gave better conditions for the water fungi (conidia and propagules) to grow, which is reflected by the number of isolates obtained in this study.

REFERENCES

American Public Health Association (APHA); American Water Works Association (AWWA); Water Environmental Federation (WEF) (2012). Standard Methods for the Examination of Water and Wastewater, 22nd ed.; American Public Health Association; American Water Works Association; Water Environmental Federation: Washington, DC, USA.


