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Thermophillous fungi associated with dimili and gwana warm springs of yankari game reserve, Bauchi state Nigeria

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Abstract
A research was carried out on thermophilous fungi associated with Dimili and Gwana warm springs in Yankari Game Reserve in Bauchi State of Nigeria. The warm springs in question included Dimili and Gwana. Samples of water were collected from the test springs and transported to the Department of Plant Science and Biotechnology Applied Microbiology Postgraduate Laboratory aseptically with the aid of 1 litre sterile glass bottles. Such samples of water were then plated out on nutrient Agar, yeast starch Agar and Exp. Cellulose Agar. A total of 30 culture plates were employed for each culture medium. The resultant culture plates were divided in 3 batches, each containing 10 culture plates. The first batch of plates was incubated at 25ºC for isolation of mesophilic fungi. The second batch of plates was incubated at 37ºC for the isolation of thermotolerant fungi while the last batch of 10 plates was incubated at 45ºC for the isolation of thermophilic fungi. Control plates (10 for each incubation temperature) were also set up. The plates were examined after 4–7 days for the development of fungal colonies. The plates were re-examined after 14 days for the development of more fungal colonies. The fungi that developed were duly purified and examined under the microscope A total of 27 Thermophillous fungi were isolated from both the Dimili and Gwana hot springs coastal soil during the rainy season and dry season. The implication of the various results obtained have been discussed.

Keywords: thermophilous fungi, dimili, gwana, yankari game reserve

Introduction

Thermophilic fungi are small assemblage in mycota that have a minimum temperature of growth at or above 20^0 C. As the only representative of eukaryotic organism that can grow at temperatures above 45^0C, the thermophilic fungi are valuable experimental system for investigations of mechanisms that allow growth at moderately high temperature yet limit their growth beyond 60 to 62^0C (Smith *et al.,* 2011). Although widespread in terrestrial habitats, they have remained underexplored compare to thermophilic species of eubacteria and archaea. However, thermophilic fungi are potential sources of enzymes with specific and commercial interests. Thermophilic fungi can grow in minimal media with metabolic rates and growth yields comparable to those of mesophilic fungi.

Thermophilic fungi have a powerful ability to degrade polysaccharide constituents of biomass. Some extracellular enzymes from thermophilic fungi are being produced commercially, and a few others have commercially prospects. A number of different groups of fungi are found in water, including mastigomycotina (zoosporic fungi), some are zygomycotina, ascomycotina, yeasts and a few basidiomycotina.

Thermophilic fungi have been isolated from many habitats in the past few years. Thermophilic fungi often occur in large numbers in natural geothermal sites; for example, geothermal soils, hotsprings and hot spring effuluent channel (Tansey *et al.,* 2001).

Marine fungi are species of fungi that live in marine or estuarine environments. They are not a taxonomic group, but share a common habitat. Obligate marine fungi grow exclusively in the marine habitat while wholly or sporadically submerged in sea water. Facultative marine fungi normally occupy terrestrial or freshwater habitats, but are capable of living or even sporulating in a marine habitat. About species of marine fungi have been described, including seven genera and ten species of basidiomycetes, and 177 genera and 360 species of ascomycetes.

Statement of problem

While there is detailed scientific report on the occurrence of thermophilic and thermotolerance fungi in Nigeria soil and stored products, there is non- existent information on the occurrence of these fungi in Nigerian Hot springs. The present study has therefore been designed to find out the species of thermophilic and thermotolerant fungi associated with Dimil and Gwana hot springs in Yankari Game Reserve,Bauchi State, Nigeria. It hopes that such information will enrich our present knowledge on the occurrence of these organisms in Nigeria.

Aim

The research is aimed at determining the thermophilous fungi associated with Dimil and Gwana hotsprings in Yankari Game Reserve, Bauchi State, Nigeria.

Objectives

1. To isolate and identify different species of thermophilic fungi associated with Dimil and Gwana hotsprings in Yankari Games Reserve, Bauchi State, Nigeria.
2. To determine the seasonal variation in the frequency of occurrence of the fungal isolates

Study area

Yankari Game Reserve was constituted as a Bauchi Native Authority Forest Reserve in 1957 and open to the public in

1962. Since then, the North Eastern State Government and later the Bauchi State Government both managed the Game Reserve. The park was also managed by the Federal Government of Nigeria, through the National Parks Service from 1991 to 2006.

Yankari Game Reserve lies in the Southern part of the Sudan Savanna. It is composed of Savanna grassland with well – developed patches of woodland. It is also a region of rolling hills, mostly between 200m and 400m above sea level. It falls within the latitudes 9º 50'N and 10º 31' E lying in Bauchi State. The vegetation is composed mainly of conbretaceous trees and shrubs, *Afzelia spp, Anogeissus spp* and *Detarium spp*. Savanna woodland (Geerling, 1976). The park features four hot springs and one cold spring which included;

Dimili hot spring	Latitude N 09.66106º E010.49380º
Gwana hot spring	Latitude N 09.675386º E 10.50008º

Samples Collection

Thermophilous fungi are that thrive very well at high temperatures. In the first experiment, a survey was carried on the seasonal occurrence of species of aquatic phycomycetes in water samples. Water samples were collected from the Hot springs of Yankari Games Reserve. The Hot Springs included.

1. Dimil Hot Spring with an average temperature of 35ºC
2. Gwana Hot Spring with an average temperature of 32ºC

The water samples were collected during the wet season (June to September, 2020). Each water sample was collected with the aid of sterile 250ml conical flask.The said water samples were quickly rushed for the sampling of the presence to the laboratory in ice jars presence of Aquatic Phycomycetes.

1. Isolation of Thermophilous Fungi from the Coastal or Riverine soils of the Hot Springs

In a different experiment, portions of the soil samples collected from the riverine or coastal soils of the Hot Springs were plated out on both Eggins and Pugh Cellulose Agar and Yeast Starch Agar (YSA) using the Warcup plate method. In this method, 0.03g of soil sample was dispensed into a sterile dish and then covered with 15 mls of Agar medium. The plate was then swirled to enable even mixing of the soil and the culture medium. A total of 30 culture plates were employed for each soil sample collected from each Hot Spring Coastal or Riverine Soil. The resultant culture dishes were then divided into 3 batches of 10 plates. The first batch of culture plates was incubated at 25ºC for the isolation of mesophilic fungi. The second batch of 10 plates was incubated at 45ºC for the isolation of thermophilic fungi. A total of 10 plates that contained only culture media were set up at each incubation temperature and these acted as control plates.

2. Identification of the Fungal Isolates

The various fungi that developed were identified according to their morphologies. References were made to existing fungal monographs like Coker, 1923 and Khulbe, 2001.The experiment was repeated for each of the water samples. Scorings were made for each aquatic fungas that was identified in terms of their occurrence in each of the water samples that were collected from the Hot Springs.

2.1 Purification of the Fungal Colonies

The fungal colonies that developed were subjected to sub-culturing processes until pure cultures were obtained.

2.2 Fungal Identification

Slides of fungal isolates were prepared and subjected to microscopic examinations. References were made to existing stock cultures and to Fungal Monographs like Coker (1923) and Khulbe (2001)

The physico-chemical analysis of the coastal soil from five (5) different springs of Yankari Game Reserve was carried out by adopting a standard analytical procedure as described by ArshiIram and TI Khan (2018).

Results

Table 1: Rainy Season Thermophilous Fungi isolated from the Yankari Hot Springs Coastal Soils

Thermophilous Fungal Isolates	**Hot springs costal soils**		
	Dimil	Gwana	Total
Thermophiles:			
Chaetomiumthermophilum La Touche	+	+	2
C. thermophilum var. *coprophilum*	+	+	2
Corynascusthermophilus (Ferqus&Sinden) v. Klopotek	-	+	1
*Humicola*grisea var.*thermoidea* Cooney & Emerson	+	+	2
H. insolens Cooney and Emerson	+	+	2
*Mucor*miechei Cooney and Emerson	-	+	1
*M. pusillus*Lindt	+	+	2
Myceliophthorathermophilum (Apinis) v. Dorschot (=*sporotrichumthermophilum*)	+	+	2
Myrioconiumthermophilum (Ferqus) v.d. Aa *(=Papulaspora thermophile*Ferqus)	+	+	2
Scytalidiumthermophilum (Cooney & Emerson) Austwick (= *TorulaThermophila* (Cooney & Emerson)	+	+	2
*Talaromycesthemophilus*Stolk(=*Talaromy cesdupontii*) [Griffon &Maublanc] Emerson	+	+	2
*Thermomycesibadanensis*Apinis&Eggins	+	+	2
*T.lanuginosus*Tsiklinski	+	+	2
T. stellatus(Bunce) Apinis	-	+	2
Thielaviaterrestris (Apinis) Malloch& Cain	+	+	2
Thermotolerants:			
Absidiacorymbifera (Cohn) Sacc.&Trotter	+	+	2
*Aspergillusfumigatus*Fres.	+	+	2
A. terreus Thom	+	+	2
*Chaetomium*virginicum Ames	+	+	2
*Corynascus*sepedonium (Emmons) v. Arx	-	+	1
*Emricella*nidulans (Eidam) Wint	-	+	1
*Mortierella*wolfiiMehrotra&Baijal	+	+	2
*Paecilomyces*variotii	-	+	1
Rhizopusmicrosporus van Tieghem	+	+	2
R. oligosporus Saito	+	+	2
*Sporotrichumpulverulentum*Novobranova	-	+	1
TOTAL	20	27	47

1 = Dimil Hot Spring 2 = Gwana Hot spring

Table 2: Dry Seasonal Occurrence of Thermophilous Fungi in the Coastal Soils of the Experimental Hot Springs

Thermophilous Fungal Isolates	Dimil	Gwana	Total
Thermophiles:			
*Chactomium*thermophilum la Tauche	+	+	2
C. thermophilum var. coprophilum	+	+	2
Corynascusthermophilus	-	+	2
*Humicola*grisea var. thermoidea	+	+	2
H. insolens	+	+	2
*Mucor*miechei	-	+	1
M. pusillus	+	+	2
*Myceliophthora*thermophilum	+	+	2
*Myrioconium*thermophilum	+	+	2
*Scytalidium*themophilum	+	+	2
*Talaromyces*thermophilus	+	+	2
*Thermomyces*ibadanensis	+	+	2
T. ianuginosus	+	+	2
T. stellatus	-	+	1
*Thielavia*terrestris	+	+	2
Thermotolerants:			
*Absidia*corymbifera	+	+	2
*Aspergillus*fumigatus	+	+	2
A. nidulans	+	+	2
A. terreus	+	+	2
*Chaetomium*virginicum	-	+	2
*Corynascus*sepedonium	-	+	1
*Emericella*nidulans	+	+	2
*Mortierella*wolfii	+	+	2
*Paecilomyces*variotii	-	+	1
*Rhizopus*microsporus	+	+	2
R. oligosporus	+	+	2
*Sporotrichum*pulverulentum	-	+	1
Total	20	27	47

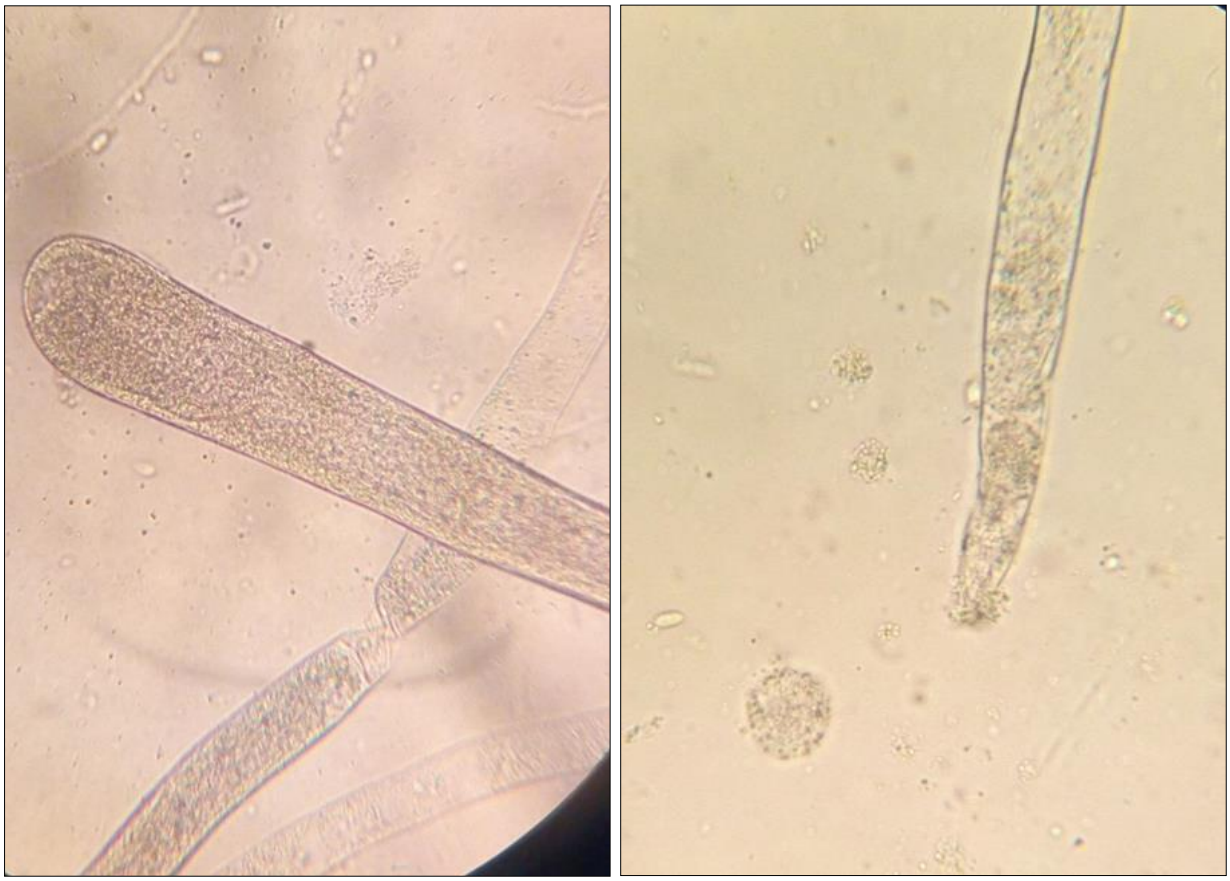

Fig 1: saprolegnia species isolated

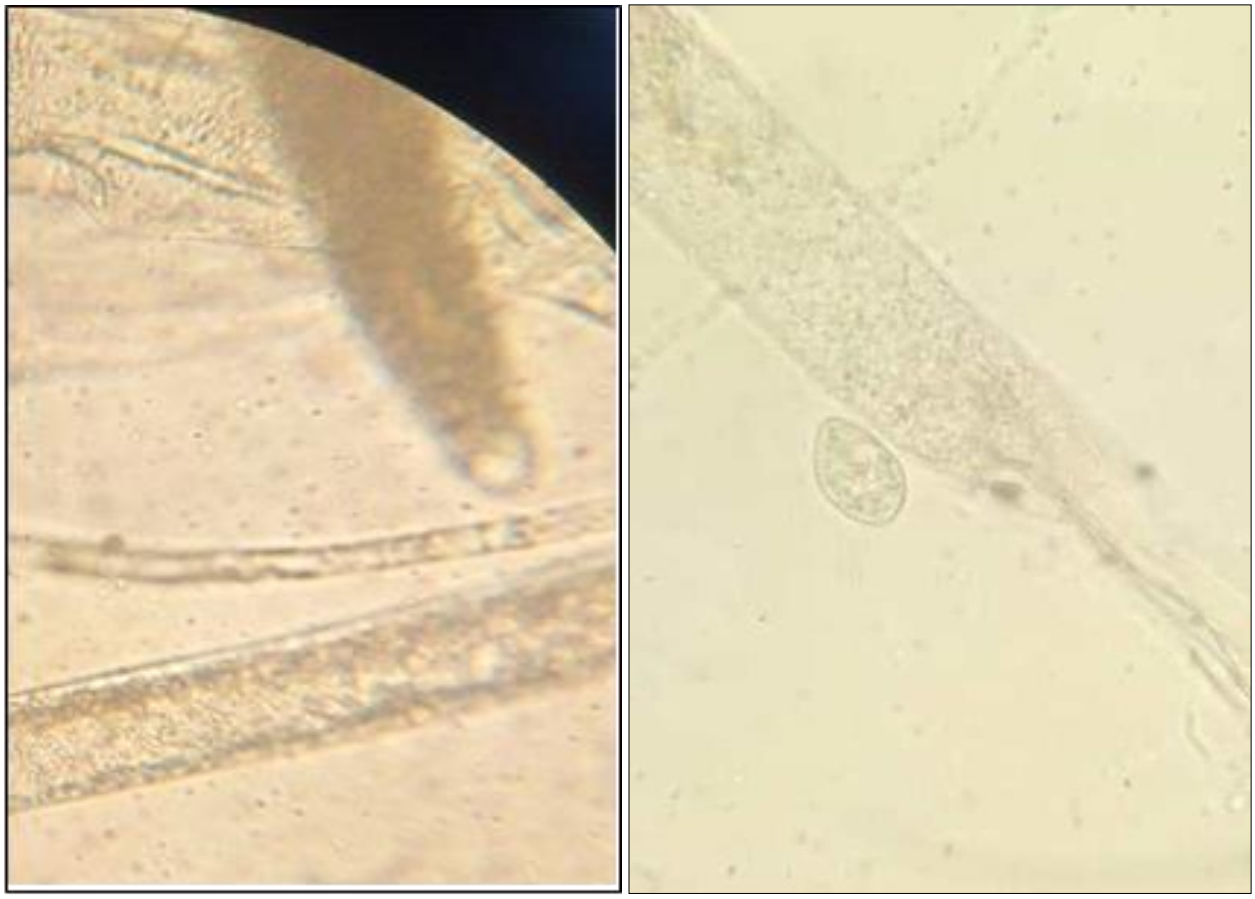

Fig 2: achlya species isolated

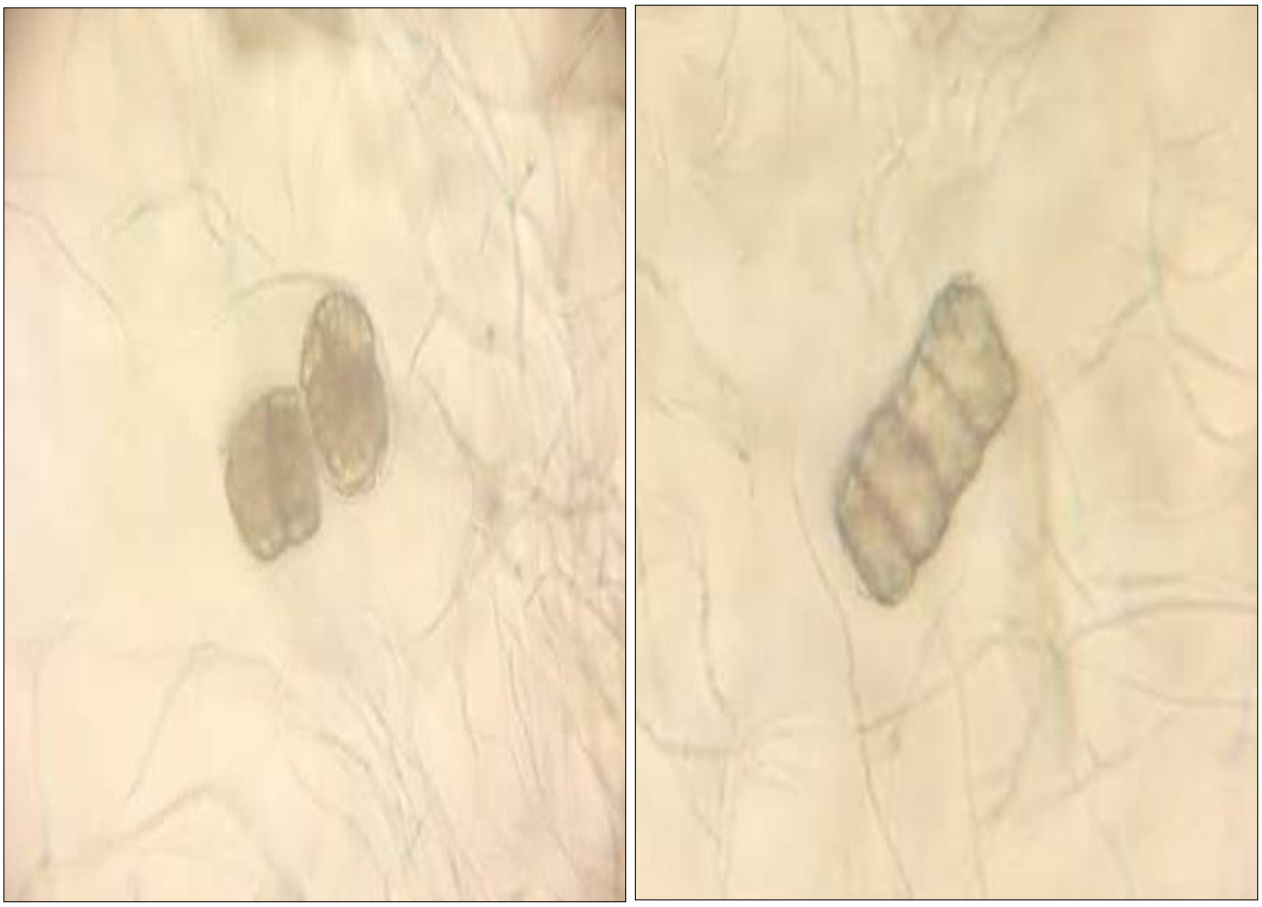

Fig 3: unidentified isolates.

A total of 27 thermophilous fungi were isolated from the hotsprings coastal soils during the rainy season. of these 20 species were isolated from Dimil hotsprings coastal soil and 27 species from Gwana hotsprings coastal soils.

Of the 27 thrmophilous fungal isolates from the hotsprings coastal soils,15 were thermopholic while 12 were thermotolerants. The details are shown inTable 3.

A total of 54 species of phycomycetes were isolated from the hotsprings during the Dry season,of these 52 species were isolated from Dimil hotspring and 50 species from Gwana hotsprings.The details are shown in Table 1.

A total of 27 species of Thermophilous fungi were isolated from the hotsprings during the dry season.Of these, 20 were isolated from Dimil hotspring and 27 were isolated from Gwana hotspring. The highest number of Thermophilous fungi (27) were isolated from the Gwana hotspring.Of the total 27 species of thermophilous fungi isolated from the experimental hotsprings coastal soils in the dry season,15 were thermophillic while 12 were thermotolerants. The details are shown in Table 2.

There seems not be any significant difference in the occurrence of these organisms in the experimental hotsorings in the rainy season. The PH range recorded for the springs (6.60 and 6.80) was within the range that could support the growth of these fungi in pure culture. The temperature range recorded for the experimental hotsprings 35 and 32 was the range that could support the growth of the thermotolerant strains of these fungi in pure culture.

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