
Isolation and Screening of Cellulolytic Fungi from Municipal Solid Waste

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ABSTRACT

This Study was conducted to isolate and screen cellulolytic fungi from municipal solid wastes for cellulolytic activity. Twenty two fungi were isolated and characterized from refuse dump soil of different locations in zaria metropolis using cultural method and microscopic technique. Among them, 86.4% belong to the genus Aspergillus. Fungi isolated were screened for cellulase activity after which Aspergillus niger was found to be the most promising among the cellulase producing isolates with diameter of zones of clearance 64, 65 and 73mm respectively. This shows that majority of the fungi that exist in the soil of municipal solid waste are cellulolytic.

INTRODUCTION

Municipal Solid waste (MSW) is composed of 40–50% cellulose, 9–12% hemicelluloses, and 10–15% lignin on a dry weight basis (Gautam *et al.*, 2010). Every unwanted or non-useful solid substance generated in any human population is referred to as solid waste (Kaseva and Mbuligwe, 2003). In Nigeria municipal waste density generally ranges from 280-370 kg/m³, waste generation rate is 25 million tons annually and daily rate of 0.44-0.66 kg/capital/day (Ogwueleka, 2009).

Over time, consumption practices and activities of economic nature have resulted in generation of Municipal Solid Waste (Cointreau, 2006) which is basically waste that is generated from different sectors of a society such as households, agricultural, educational, health and commercial institutions, public places, etc., and which is taken care of either directly or indirectly by the municipal or local authorities (Williams, 2005). European Environmental Agency (EEA) defines MSW as: “...waste from households and other waste which because of its nature or composition is similar to waste from households. Some of this waste is biodegradable, e.g. paper and agricultural residues, kitchen waste and garden waste. Biodegradable waste means any waste that is capable of undergoing anaerobic or aerobic decomposition, such as kitchen waste, agricultural waste, and paper waste (EAA, 2009).

Bioconversion of these cellulosic materials particularly enzyme hydrolysis, had assumed great potential from the point of view of waste management and isolation of suitable microbial strains that could become useful as sources of hydrolytic enzymes. The enzymatic degradation of waste cellulose by fungal enzymes has been suggested as a feasible alternative for the conversion of lignocelluloses into fermentable sugars and biofuel ethanol (Oksanen *et al.*, 2000).

Cellulolytic enzymes play an important role in natural biodegradation processes in which plant lignocellulosic materials are efficiently degraded by cellulolytic fungi. Cellulolytic enzymes are synthesized by a number of microorganisms. Fungi are well known agents of decomposition of organic matter, in general, and of cellulosic substrate in particular (Lynd *et al.*, 2002). Numerous microorganisms are capable of decomposing cellulose, although only a

few of these microorganisms produce significant quantities of extracellular enzymes to decompose cellulose (Sukumaran *et al.*, 2005).

In industry, these enzymes have found novel applications in the production of fermentable sugars and ethanol, organic acids, detergents and other chemicals. Cellulase provide a key opportunity for achieving tremendous benefits of biomass utilization (Wen *et al.*, 2005).

Over the years, solid waste disposal has become a major problem in Nigeria; Landfill and incineration has been reported to be the cheapest known method of final disposal of solid wastes generated by human activities (Leton and Omotosho, 2004). Although, they are reported to cause negative environmental effects such as production of dangerous gases (Van Wyk, 2001).

Improper disposal of solid wastes facilitates multiplication of pathogens causing diseases like cholera and diarrhea and provides good breeding site for disease vectors like mosquitoes (malaria), flies (diarrhea) and rodents (Kassim and Ali 2006; Abul 2010).

Cellulase is expensive and the demand for more thermostable, highly active and specific cellulases is on the increase; and contributes only 50% to the overall cost of hydrolysis due to the low specific activity. This enzyme has enormous potential in industries and is used in food, beverages, textile, laundry, paper and pulp industries etc. (Lekh *et al.*, 2014).

Justification for the Study

Treatment of MSW with either cellulolytic enzymes or microorganism could lead to more efficient degradation of this waste material, promote cycling of nutrients in the environment and reduce the impact of waste accumulation on terrestrial and aquatic ecosystem as well as generation of end products of economic benefits like compost and biofuels (Klemm *et al.*, 2009).

Several microbial species have been isolated from composts and these microbes have shown effectiveness in the degradation of cellulose as well as the conversion of intermediates into useful products like bio-ethanol, animal feed, and methane gas (Tuomela *et al.*, 2000). Exploration for cost effective and efficient cellulose-degrading microbes with “high product yield” and “novel enzymes” capabilities has therefore become imperative.

Objectives

The Specific objectives of the study are to:

1. Isolate, identify and characterize fungi from refuse dump sites soil using cultural method and microscopic technique.
2. Screen the fungal isolates for cellulolytic activity.

MATERIAL AND METHODS

Isolation, identification and screening of fungal isolates

Fungi were isolated from the soil samples collected from different refuse dump sites in Zaria metropolis. namely; Kaura Zaria city, kwarbai, Tudun wada, Muchiya, and Chikaji. Serial dilution method was used for the isolation of cellulolytic fungi. 1ml of the 10^3 and 10^5 dilutions were inoculated by spread plate method on PDA (Potato Dextrose Agar) plates supplemented with 30 mg/L chloramphenicol and incubated at ambient temperature for 6 days. (Paul and Daniel 2007). Colonies were picked and sub-cultured to obtain pure culture. Identification of the fungal isolates was done microscopically under $\times 10$ and then $\times 40$ objectives and observed for nature of hypae, shape and appearance of spores. This was identified by the use of atlas

(Domsch *et al.*, 1980). The isolates obtained were screened on Agar agar medium supplemented with 1% Carboxy Methyl Cellulose and incubated for 6 days at ambient temperature. The plates were flooded with 0.1% Congo red solution and kept for 10 minutes and then washed with 1 M NaCl for 15 minutes (Albin *et al.*, 2014). Cellulolytic fungi create a clearing zone around the colony and these were measured in millimeters. Isolates were then purify and stored in slants.

RESULTS

A total of twenty two fungal isolates were obtained. Among them, 86.3% belong to the genus *Aspergillus*. Table 1 shows the result of the cultural and microscopic characterization of the isolates by microscopic technique. Table 2 shows cellulolytic activities of the isolated fungi after the screening. *Aspergillus niger* was found to be the most prominent among the cellulase producing isolates with diameter of zone of clearance 64, 65 and 73mm respectively.

Table 1a: Cultural and Microscopic Characteristics of Fungal Isolates

Isolates Codes	Cultural Characteristics			Microscopic Characteristics		Tentative Identity
	Colour Change of Colony	Colony Texture	Colour of Reverse	Conidiophore	Phialides	
MR ₁	White-Green	Valvety	Publish red, brownish dark with age	Short smooth only upper portion of vesicle.	Biseriate covers	<i>Aspergillus nidulan</i>
MR ₁	White-Black	Floc/comp	White to yellow	Long smooth entire vesicle.	Biseriate cover	<i>Aspergillus niger</i>
MR ₂	White-Green	Valvety	White	Long smooth most of vesicle.	Biseriate; cover	<i>Aspergillus versicolor</i>
MR ₂	White-Black	Floc/comp	White to yellow	Long smooth entire vesicle.	Biseriate cover	<i>Aspergillus niger</i>
MR ₂	Cinnamon-Brown	Valvety	White to brown	Short smooth	Biseriate.	<i>Aspergillus terreus</i>
MR ₂	White-Green	Valvety	Publish red, brownish dark with age	Short smooth only upper portion of vesicle.	Biseriate covers	<i>Aspergillus nidulan</i>
MR ₃	White-Green	Valvety	White	Long smooth most of vesicle.	Biseriate; cover	<i>Aspergillus versicolor</i>
TR ₁	White-Green	Valvety	White	Long smooth most of vesicle.	Biseriate; cover	<i>Aspergillus versicolor</i>
TR ₂	White-Dark Green	Valvety/powdery	White-tan	Short smooth on upper two thirds of vesicle.	Uniseriate only	<i>Aspergillus fumigatus</i>
TR ₃	White-Green	Valvety	White	Long smooth most of vesicle.	Biseriate; cover	<i>Aspergillus versicolor</i>
CR ₁	White-Green	Valvety	Publish red, brownish dark with age only upper portion	Short smooth	Biseriate covers of vesicle.	<i>Aspergillus nidulan</i>
CR ₂	White-Black	Floc/comp	White to yellow	Long smooth	Biseriate cover entire vesicle.	<i>Aspergillus niger</i>

Key: MR- Muchiya Refuse dump, TR- Tudun wada Refuse dump, CR- Chikaji Refuse dump soil.

Table 1b: Cultural and Microscopic Characteristics of Fungal Isolates

Isolates Codes	Cultural Characteristics			Microscopic Characteristics		Tentative Identity
	Colour Change of Colony	Colony Texture	Colour of Reverse	Conidiophore	Phialides	
CR ₂	White-Dark Green	Valvety/Powdery	White-tan	Short smooth	Uniseriate only on upper two thirds of vesicle.	<i>Aspergillus fumigatus</i>
CR ₃	White-Green	Valvety	Publish red, brownish dark with age	short smooth	Biseriate covers only upper portion of vesicle.	<i>Aspergillus nidulan</i>
KR ₁	Black-White	Valvety	Cream or tan	Tapering	conidiophores	<i>Sporothrix schenkii</i>
KR ₂	White-Dark Green	Valvety/Powdery	White-tan	Short- smooth	Uniseriate only on upper two thirds of vesicle.	<i>Aspergillus fumigatus</i>
KR ₃	White-Green	Valvety	Publish red, brownish dark with age	Short smooth	Biseriate covers only upper portion of vesicle.	<i>Aspergillus nidulan</i>
KRW ₁	White-Green	Valvety	Publish red, brownish dark with age	Short smooth only upper portion of vesicle.	Biseriate covers	<i>Aspergillus nidulan</i>
KRW ₂	White-Green	Valvety	White	Unbranched conidiophores	Flask shaped bear unbranched chain of smooth or rough, round conidia.	<i>Penicillium chrysogenum</i>
KRW ₂	White-Green	Floc/comp.	White to yellow	Long smooth	Biseriate; cover entire vesicle.	<i>Aspergillus niger</i>
KRW ₃	White-Dark Green	Valvety/powdery	White-tan	Short smooth.	Uniseriate only on upper two thirds of vesicle	<i>Aspergillus fumigatus</i>

Table 2: Cellulolytic Activities of the Fungal isolates

Isolate Codes (mm)	Organisms	Zones of Cellulose hydrolysis
MR ₁	<i>Aspergillus nidulan</i>	25.0
MR ₁	<i>Aspergillus niger</i>	65.0
MR ₂	<i>Aspergillus niger</i>	73.0
MR ₂	<i>Aspergillus versicolor</i>	30.0
MR ₂	<i>Aspergillus terreus</i>	30.0
MR ₂	<i>Aspergillus nidulan</i>	18.0

MR ₃	<i>Aspergillus versicolor</i>	26.0
TR ₁	<i>Aspergillus versicolor</i>	10.0
TR ₂	<i>Aspergillus fumigatus</i>	28.0
TR ₃	<i>Aspergillus versicolor</i>	26.0
CR ₁	<i>Aspergillus nidulan</i>	15.0
CR ₂	<i>Aspergillus niger</i>	64.0
CR ₃	<i>Aspergillus nidulan</i>	23.0
CR ₃	<i>Aspergillus fumigatus</i>	21.0
KR ₁	<i>Sporothrix schenkii</i>	0.0
KR ₂	<i>Aspergillus fumigatus</i>	26.0
KR ₃	<i>Aspergillus nidulan</i>	21.0
KR ₃	<i>Sporothrix schenkii</i>	0.0
KWR ₁	<i>Aspergillus nidulan</i>	20.0
KWR ₂	<i>Penicillium chrysogenum</i>	18.0
KWR ₂	<i>Aspergillus niger</i>	48.0
KWR ₃	<i>Aspergillus fumigatus</i>	24.0

Key: MR- Muchiya Refuse dump, TR- Tudun wada Refuse dump, CR- Chikaji Refuse dump, KR- Kaura Refuse dump and KWR- Kwarbai Refuse dump sites.

DISCUSSION

Fungi are the most common groups of organism that are capable of hydrolyzing cellulose to the monomer units because they are ubiquitous and can easily be isolated from the environment. This prompted the search for potential cellulolytic fungi in municipal solid wastes. Among the twenty two fungal isolates obtained, twenty were found to be cellulolytic. This represents 90.9% of the isolates and is an indication that majority of the fungi that exist in the soil of municipal solid waste are cellulolytic. The result further support the hypothesis that microorganisms for enzyme production be assayed in the environment where the substrates are available as suggested by Fossi *et al* (2005).

Aspergillus represents 86.4% of the cellulolytic fungi isolated in this study. These fungi are abundant in the environment and have no special nutritional requirement and hence can be cultivated on most organic and inorganic nutrients. *Aspergillus* is ubiquitous and utilizes a wide range of nutrients due to its ability to secrete a large number of digestive enzymes. In view of this, species of the genus *Aspergillus* form a very large proportion of all the fungi encountered (Saliu, 2012).

CONCLUSION

Different fungi were isolated from different locations of municipal solid waste soils within Zaria metropolis with *Aspergillus* being the most prevalent (86.4%). *Aspergillus niger*-MR₂ was observed to be the best cellulase-producing isolate after screening with 73mm zone of diameter of hydrolysis.

RECOMMENDATIONS

At the end of this research work it was recommended *Aspergillus niger* should be used for cellulase enzyme production because it is easily isolated from the environment especially refuse dump soil.

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