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Full Length Research Paper

Isolation and Characterization of Paraeforce Tolerance and Utilizing Fungi in Soil Samples Collected From an Exposed Arable Farmland

Onianwah, F. I. Eze, V. C., Ifeanyi, V. O

Department of Microbiology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

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The aim of this study is to isolate and characterize paraeforce tolerance and utilizing fungi from an arable farmland previously exposed to the herbicide paraeforce. Soil samples were collected in sterile containers and aseptically cultured on potato dextrose agar using a streak plate culture technique. The fungal isolates were screened for tolerance and utilization of paraeforce using 50mg/l concentration. The screened fungal species were further characterized using molecular method. The presumptive identification of fungal isolates based on colonial and cell morphology showed the presence of *Saccharomyces, Aspergillus, Pichia, Candida, Rhizopus, Penicillium, Trichoderma and Fasurium* species. The screening test done showed that of these isolates, only *Saccharomyces, Aspergillus* and Candida were able to grow on paraeforce medium. The molecular characterization proved these screened *fumgal* species to be *Pichiakrudiavzevii, Pichiacecembensis, Hanseniasporaopuntiae and Aspergillusspinosus.* However, a more vigorous growth was observed in the mold (*Aspergillusspinosus*) than the yeast counterparts. This is due to their filamentous nature and their ability to produce extracellular enzymes.

Keywords: Isolation, Characterization, Paraeforce, Plate Culture and Molecular Technique.

INTRODUCTION

Paraeforce is used to eliminate weeds in the farm which compete with food crop for limited growth nutrient in the soil. The wrong application of this herbicide results in the contamination of soil which is an important natural resource (Baran *et al.,* 2007). These contaminations pose danger to the non-target organisms and the environment as well as exposes human beings to many health implications (Onianwah, unpublished).

*Corresponding Author's Email: onianwah2208@gmail.com

Some physicochemical methods of paraeforce degradation are quite cumbersome and expensive, and sometimes leave behind toxic metabolic intermediate products that further contaminate the soil. According to Belal *et al* (2008), most microorganisms can detoxify this compound or use it for microbial growth. Biodegradation is achieved through microbial complex enzyme systems and their ability to withstand adverse environmental conditions (Castillo *et al*., 2011). Fungi feature among the nature's most vigorous agent of wastes' decomposition and are essential component of the soil food web (Rhodes, 2012). Baldwin *et al* (1977) found that the most effective

organisms for decomposing herbicides are fungi, isolated mainly from several soils.

The aim of this study is to isolate and characterize paraeforce tolerance and utilizing fungi from an arable farmland previously exposed to the herbicide paraeforce. It is expected to contribute immensely towards enriching the available new technology employed in the remediation of pesticides impacted soils by using fungal living cells.

MATERIALS AND METHODS

Sample site and collection

The site sampled is situated on Gbei road in Nkpolu Rumuigbo, Obio/Akpor local government area of River State. It is a sandy loamy soil used for agriculture with previous known history of exposure to herbicide treatment. Soil samples from the site were collected from different locations at a depth of 20cm in a sterile container and pooled together for use. The physicochemical analysis was done immediately after sample collection. The microbiological analyses were done in Rexall diagnostic and research laboratory, Port Harcourt.

Materials

Culture media: Potato dextrose agar (PDA), nutrient agar and broth from Oxoid Company, UK and were used and prepared according to manufacturer's instruction (Oxoid Manual, 2007).

Herbicide: Paraeforce used is nitrogen based herbicide and served as nitrogen and carbon source. It prepared according to manufacturers' instruction

Isolation of fungal species

Soil sample collected from the farmland was inoculated on PDA culture plates. The plate culture method described by Cheesbourgh (2002) was used. The pure colonies on culture plates were characterized based on cell and colonial morphology (Holt *et al*., 1994 and Aneja, 2005). Cell morphology was based on lactophenol cotton blue wet mount and gram stain reaction (Holt *et al*., (1994). Observed characteristics were compared with the established identification key by Barnett and Hunter (1972).

Screening of fungal isolates for growth on paraeforce medium

The different fungi isolated were inoculated into PDA containing 50mg/L of 1/80 concentration of the test paraeforce (herbicides). The inoculated plates were incubated at 28^0C for 48 to 72hours and growth on plates observed (Okpokwasili and Nwosu, 1990).

Molecular characterization of paraeforce utilizing fungi

This involved DNA extraction using a ZR fungal/bacterial DNA mini prep extraction kit, sequencing using the Big Dye Terminator kit on a 3510 ABI sequencer from Inqaba Biotechnological, Pretoria, South Africa. Sequencing obtained will be edited using the bioinformatics algorithm. Trace edit similar sequences will be downloaded from the National Centre for Biotechnology Information (NCBI) data base using BLASTIN. The evolutionary history will be inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou and Nei, 1987).The bootstrap consensus tree will be inferred 500 replicates (Felsenstein, 1985) taken to represent the evolutionary history of the taxa analyzed.

RESULTS

Ten fungal isolate were presumptively identified from the soil sample as listed in Table 1 above and their colonial cell characteristics illustrated in Plates 1 and 2 below.

Table 1: Colonial and cell Morphological Characteristics of the fungal isolates

Code	Colonial Morphology	Cell Morphology	Presumptive Identity
F1	Whitish, convex, smooth and entire edged colonies	Spherical, sometimes oval and budding yeast cells in pairs or single	*Saccharomyces sp*
F2	whitish flat with rough edge moderate colonies	yeast like cell, budding single, paired and spherical in shape	*Pichia sp*
F3	Dry black, flat colonies with rough edge on PDA	Septate and branched mycelia. Conidia were in chains.	*Aspergillus sp*
F4	moderate colonies, whitish and smooth with entire edge	single, paired and short chain yeast cell, gram positive	*Candida sp*
F5	Abundant cottony mycelia grey to black colonies on PDA, with rough edge and flat surface	Rhizoid with sporangiospores mycelia was non septate and the conidia were separated by septum	*Rhizopus sp*
F6	milky large colonies with entire edge	spherical budding yeast cells single and occasionally paired and elongated	*Candida sp*
F7	A blue mold, velvet in shape with white edge	conidiophores was brush like in appearance which was single or paired and produces conidia. It had septate hyphae	*Penicillium sp*
F8	whitish and cottony mycelium	multi segmented spores branched and segmented conidiospores were sometimes found.	*Fusarium sp*
F9	Grey, rough edged and dry colonies	Spherical, budding cells, single and occasional paired and elongated	*Trichoderma sp*
F10	grey, flat colonies with Rough edge on PDA	Septate and branched mycelia, conidia in chain	*Aspergillus sp*

Colonial morphology of fungal isolates

Aspsergillus sp (grey/green spores)

Aspergillus sp (blank fungi)

Candida sp

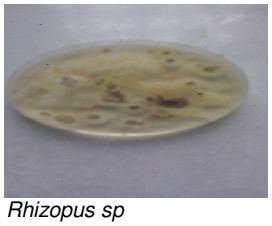
Pichia cecembensis MT366876

Pichia kudriavzevii MT366877 sp

Sacchaeomyces sp

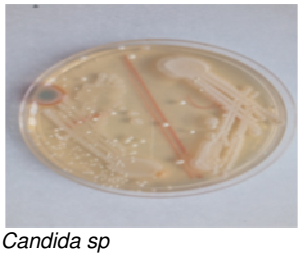
Penicillium sp

Hanseniaspora opuntiae MT366875



Rhizopus sp

Fusarium sp

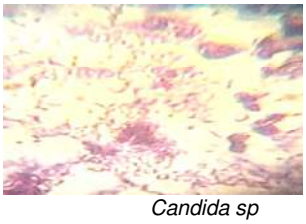


Candida sp

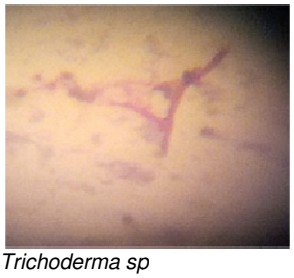
Plate 1: Pure Culture of fungal isolates

Cell morphology of fungal isolates

Candida sp



Candida sp

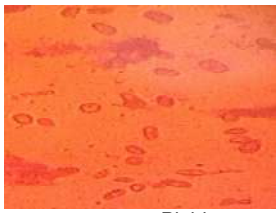


Trichoderma sp

Penicillium sp

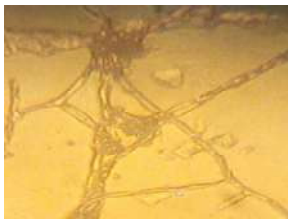
Penicillium sp

Sacharomyces sp



Pichia sp

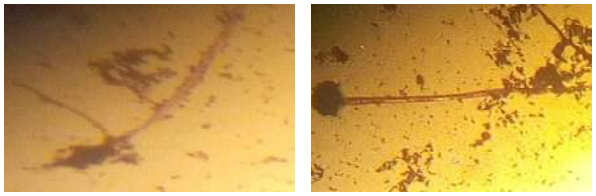
Aspergillus sp



Aspergillus sp

Fasurium sp

Fasurium sp



Rhizopus sp *Rhizopus sp*

Plate 2: Cell morphology of fungal isolates

Table 2: Screening of fungal isolates for growth on paraeforce medium

Isolates	Identity of Isolates
F1	*Saccharomyces sp*
F2	*Candida sp*
F4	*Candida sp*
F10	*Aspergillus sp*

Table 3: Molecular characterization of screened isolates

Isolates	Identity of Isolates
F1	*Hanseniaspora opuntiae MT366875 (Sacharomycetes)*
F2	*Pichia cecembensis MT566876*
F4	*Pichia kudriavzevii MT366877 (formerly Candida cruzei)*
F10	*Aspergillus* spinosus MT366879

Screening of fungal isolates for growth on paraeforce medium

Of the ten fungal species isolated from the soil sample, only four showed tolerance and growth on paraeforce medium. These species are listed in Table 2. These fungal species were subjected to molecular characterization and are confirmed to be yeast cells and *Aspergillus sp* and are listed in Table 3 and illustrated in Figure 1 below.

DISCUSION

The presumptive identification of fungal isolates based on colonial and cell morphology showed the presence of *Saccharomyces, Aspergillus, Pichia, Candida, Rhizopus, Penicillium, Trichoderma and Fasurium* species from the soil sample.. The screening test done showed that of the ten isolates, only four were able to utilize paraeforce, thus; *Saccharomyces, Aspergillus* and two species of *Candida*. The molecular characterization proved these screened fumgal species to be *Pichia krudiavzevii, Pichia cecembensis, Hanseniaspora opuntiae* and *Aspergillus spinosus.* The measurement of growth of these species conformed to the work of Kodama *et al.* (2001) and showed the tolerance of the fungal species at varying degrees over an incubation period of eight days. However, a more vigorous growth was observed in the molds (*Aspergillus spinosus*) than the yeast counterparts (*Pichia krudiavzevii, Pichia cecembensis* and *Hanseniaspora opuntiae*). The molds are filamentous and their mycelia secrete variety of extracellular enzymes (Saparrat *et al*,

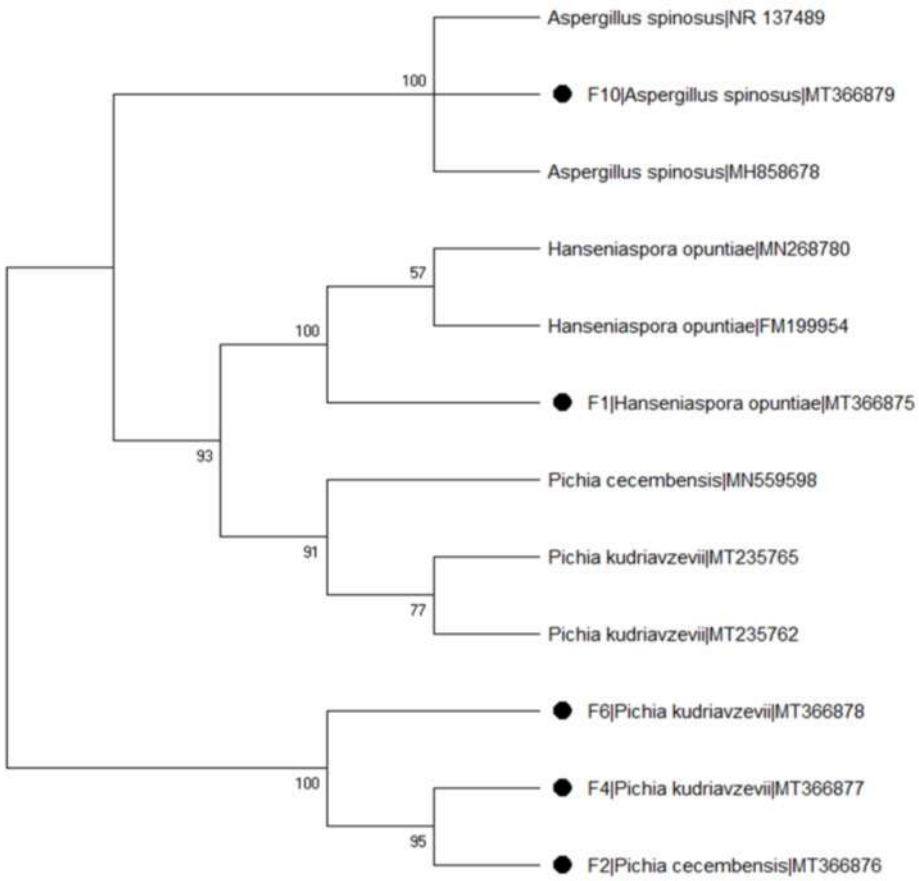


Figure 1: Neighbour joining tree showing evolutionary relationship between fungal isolates and close relatives

2002 and Das and Chandran, 2011) that degrades paraeforce. These extracellular enzymes give the molds advantage over their yeast counterpart (Amakiri, 1982). This quality may probably be due to its filamentous form which enables the mycelia to penetrate the medium and access the pollutant more easily than the non filamentous forms (Onianwah, unpublished). Also, their great biomass production and extensive hyphal growth may account for this strong activity over the non filamentous forms (Gadd, 2001 and Onianwah, unpublished). Fungi are known to produce high level of extracellular enzymes. The ability of these microorganisms to produce these enzymes account for their optimum performance in utilization and metabolism of the herbicide.

CONCLUSION

Paraeforce utilizing fungi can be isolated from impacted soil as evident in this work. The ability of these fungi to utilize and grow in paraeforce medium could be evident of their potential to degrade the herbicide. However, these degraders could be responsible for the restoration of an impacted soil over a period of time, therefore further research is required to explore their potential for paraeforce degradation.

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