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.

Accepted 23 May, 2020

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 *Fusarium* 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 fungal species to be *Pichiakrudiavzevii*, *Pichiacecembensis*, *Hanseniasporapunia* 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to the non-target organisms and the environment as well as exposes human beings to many health implications (Onianwah, unpublished). 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 paraforce tolerance and utilizing fungi from an arable farmland previously exposed to the herbicide paraforce. 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:** Paraforce 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 Cheesborough (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 paraforce medium**

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

**Molecular characterization of paraforce 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) database 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**

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

Aspergillus sp (grey/green spores)  Aspergillus sp (blank fungi)

Candida sp  Pichia cecembensis MT366876

Pichia kudriavzevi MT366877 sp  Saccharomyces 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

Trichoderma sp

Penicillium sp

Sacharomyces sp

Aspergillus sp

Fasurium sp
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.

**DISCUSSION**

The presumptive identification of fungal isolates based on colonial and cell morphology showed the presence of *Saccharomyces, Aspergillus, Pichia, Candida, Rhizopus, Penicillium, Trichoderma and Fusarium* 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 fungal species to be *Pichia kudriavzevii*, *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 kudriavzevii*, *Pichia cecembensis* and *Hanseniaspora opuntiae*). The molds are filamentous and their mycelia secrete variety of extracellular enzymes (Saparrat et al,
2002 and Das and Chandran, 2011) that degrades paraforce. 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

Paraforce utilizing fungi can be isolated from impacted soil as evident in this work. The ability of these fungi to utilize and grow in paraforce 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 paraforce degradation.

REFERENCES