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Transcriptomic responses of catalase, peroxidase and laccase encoding genes and enzymatic activities of oil spill inhabiting rhizospheric fungal strains☆

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ABSTRACT

Fungi are well associated with the degradation of hydrocarbons by the production of different enzymes, among which catalases (CBH), laccases (LCC) and peroxidases (LiP and MnP) are of immense importance. In this study, crude oil tolerance and enzyme secretions were demonstrated by rhizospheric fungal strains. Four most abundant strains were isolated from the rhizosphere of grasses growing in aged oil spill sites and identified through morphological characterization and molecular PCR-amplification of 5.8–28S ribosomal rRNA using ITS1 and ITS4 primers. These strains were subjected to crude oil tolerance test at 0–20% concentrations. Presence and transcriptase responses of putative genes *lig* (*1–6*), *mnp, cbh* (*1.1, 1.1 and 11*), and *lcc* encoding lignin peroxidase, manganese peroxidase, catalase, and laccase enzymes respectively were also studied in these strains using RT-PCR. In addition, activities of secreted enzymes by each strain were studied in aliquots. The strains were identified as *Aspergillus niger* asemoA (KY473958), *Talaromyces purpurogenus* asemoF (KY488463), *Trichoderma harzianum* asemoJ (KY488466), and *Aspergillus flavus* asemoM (KY488467) through sequencing and comparing the sequences' data at NCBI BLAST search software. All the isolated strains showed tolerance to crude oil at 20% concentration, but the growth rate reduced with increasing in oil concentrations. All the isolated strains possess the tested genes and *lig 1–6* gene was overexpressed in *A. niger* and *T. harzianum* while *lcc* and *mnp* genes were moderately expressed in all the four strains. Almost 145 $U.mL^{-1}$ of lignin and manganese peroxidase, 87 $U.mL^{-1}$ of catalase, and 180 $U.mL^{-1}$ of laccase enzymes were produced by these strains and it was also observed that these strain mostly produced studied enzymes in response to increasing crude oil concentrations. Considering the robust nature and diverse production of these catalytic enzymes by these strains, they can be exploited for various bioremediation technologies as well as other biotechnological applications.

1. Introduction

Crude oil usually contaminates soils with multiple aliphatic and aromatic hydrocarbons; most of these contaminants form residues that have pose-adverse effect upon human health (Prince, 1993; Wang et al., 1998; US EPA, 2012). The major crude oil fractions are the alkanes (>50%) including linear (n-alkanes), cyclic (such as cycloalkanes) or branched isoalkanes and they exist in the form of solid, liquid and gas (Asemoloye et al., 2017a). In polluted environments, crude oil pollution does not only affect plant and human alone but also the soil microbial population dynamics. However, many soil microorganisms have been reported to develop different survival strategies in oil contaminated environment through the production of several enzymes through modifications in their genetic and metabolic pathways for crude oil degradation and/or mineralization. In these two mechanisms, they convert and utilize hydrocarbons as a sole carbon sources and had been widely studied by many scientists. Microbial degradation and/or mineralization mechanisms form the bedrock for microbial 'Bioremediation'

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which involves the use of living organisms for remediation of environmental pollutants. This capability has been well reported in bacteria (Dong et al., 2005; Arulazhagan and Vasudevan, 2011; Muhammad et al., 2012; Hamamura et al., 2013), fungi (Hadibarata et al., 2009; Basha et al., 2010; Cerniglia and Sutherland, 2010; Sharma and Gupta, 2012; Acevedo et al., 2012; Hanafi et al., 2013), or algae (Munoz et al., 2003; Chan et al., 2006), while some researchers rather reported plants for soil remediation (Abbasi et al., 2016; Ahmad et al., 2016; Asemoloye et al., 2017b).

However, fungi are considered to be very important for crude oil or polyaromatic hydrocarbon remediation based on their ability to degrade recalcitrant longer chained or multiple rings' hydrocarbons (Rodriguez et al., 2008; Messias et al., 2009; Lettera et al., 2010; Venkatesagowda et al., 2012; Acevedo et al., 2012). Moreover, fungi have developed the capability to decompose many hydrocarbons through the secretions of different lignolytic enzymes. These enzymes were formally known to degrade only wood but today, many of them have been reported to take the critical role in fungal degradation of xenobiotic and recalcitrant pollutants. Presences of the diverse number of fungal enzymes and isoforms are associated to fungi life cycle as their important kinetic, physicochemical features and functions (Janusz et al., 2013). Many fungi are capable of growing on plant's roots in soil, and few of them could survive in adverse soil conditions (Juhasz and Naidu, 2000).

Fungi survival in hydrocarbon polluted site is often associated with their ability to degrade the pollutant through their complex enzymatic pathways such as peroxidase and laccase biosynthensis. Fungal manganese peroxidase (MnP) enzyme, for example, is a glycosylated heam protein that is usually associated to certain basidiomycetes' macro-fungi with about 38–63 KDa molecular mass (Hofrichter, 2002). This enzyme was first discovered in 1985 (Glenn and Gold, 1985; Paszczynski et al., 1985), and since then the interest in fungal manganese peroxidases continues increasing due to its immense importance in bioremediation, biobleaching and biopulping. Moreover, peroxidase and laccases regulations in fungi during bioremediation are well documented. Unfortunately, the genetic inference of these fungal enzymes is not yet well exploited. In addition, peroxidase, catalase and laccase producing fungi have developed functional genes that enable them to utilize hydrocarbon compounds, but only few scientists have reported the genetic basis of their abilities to secret enzyme and linked it with concurrent degradation or mineralization of hydrocarbon pollutants (Atagana, 2009; Argumedo-Delira et al., 2012; Husaini et al., 2008). Therefore, this research was designed to isolate and identify the rhizospheric fungal strains from crude oil polluted soil. Moreover, tolerance of isolated strains to crude oil, presence and expression of peroxidase, catalase and laccase encoding genes and enzymes were studied using the molecular techniques such as reverse transcriptase polymerase chain reaction (RT-PCR) and enzyme assay.

2. Materials and methods

2.1. Collection of samples

It was hypothesized that most common (dominant) fungal strains cohabiting the rhizosphere of an aged crude oil polluted soils of grasses over long period of time may have developed special capacity to tolerate and mineralize the hydrocarbon. Therefore, soil samples were collected randomly from different spots in an oil spill site at Ugborodo community, Nigeria (5o34′60N; 5o10′0E). The samples (10 g each) were collected from 20 different spots by uprooting *grasses* (*Panicum maximum, Lolium multiflorum, Lolium perenne, Cynodon dactylon, Panicum virgatum, Paspalum scrobiculatum, Brachiara brizantha*) and collecting the soils that are attached to the roots and placed in sterile cryogenic containers aiming to isolate and identify the rhizosphere fungi. The soil samples were transported into the Mycology Laboratory, Department of Botany University of Ibadan (7260 6.453100N, 3540 51.525400E) for analysis. The soil's physicochemical properties such as the pH, organic carbon, organic matters, micro and macro nutrients and heavy metals were analyzed following the procedures of Asemoloye et al. (2017a) and these are presented in supplementary file (Table S2).

Bonny light crude oil (Composition of bonny light crude oil is in Supplementary Table S3) was collected from Shell Petroleum Development Company (SPDC), Bonny Terminal, Port-Harcourt, Rivers State, Nigeria for isolated fungal strain's tolerance test to crude oil or radial extension rate study.

2.2. Isolation and characterization of rhizospheric hydrocarbon degrading fungal strains

The fungal strains were isolated from the collected grasses rhizosphere soil samples polluted with hydrocarbon by culturing them on potato dextrose agar (PDA). The PDA was prepared according to manufacturer's instruction, sterilized at 121 ± 2 °C and thereafter supplemented with 1% streptomycin sulphate solution to control bacterial growth. Each of the rhizosphere soil sample was subjected to serial dilution in sterile water (10^{-6}) and then inoculated into a prepared sterile Potato-Dextrose-Agar (PDA) plate. This was incubated in 30 °C for 4 days, and then primary isolations of pure cultures were done by picking pure mycelium into another newly prepared PDA plate. The isolated strains were subjected to percentage incidence according the method of Jonathan et al. (2016a) as shown in equation (1):

$$\text{Percentage Incidence } (\%) = \frac{\text{Number of a particular fungal species isolated}}{\text{Total Number of fungal species isolated}} \times 100 \quad (1)$$

The most frequent strains with highest percentage incidence were further studied for their morphological and molecular identification.

2.3. Morphological identification of the fungi strains

Morphological spore color, conidiophore, conidial head, serration of the colony, vesicular shape, plate surface and underside color, and colony diameter were studied using Olympus photomicrograph (BX51) optically fortified with sigma scan (Supplementary Table S1).

2.4. Molecular identification of the fungi strains

2.4.1. DNA isolation

The fungal genomic DNA (gDNA) was extracted using hexadecyl trimethyl ammonium bromide (CTAB) method of Murray