Original article

Hydrocarbon biodegradation and transcriptome responses of cellulase, peroxidase, and laccase encoding genes inhabiting rhizospheric fungal isolates

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A B S T R A C T

By using the indigenous micro-organisms of the polluted environment to be treated, bioremediation can be a successful strategy. PCR and RT-PCR molecular techniques were applied to examine the evolution of fungal isolates through putative genes f ligninolytic enzymes like lignin peroxidase (LiP), laccase (LaC), manganese peroxidase (MnP), and cellulase (Cx) as a response to polluting of the environment by hydrocarbons. In this study, isolation of rhizospheric fungal isolates, molecular identification, crude oil tolerance, and enzyme excretions were demonstrated. From the date palm rhizosphere, 3 fungal isolates were isolated and characterized morphologically and molecularly by ITS ribosomal RNA (rRNA) sequencing. The isolates were identified as *Aspergillus flavus* AF15, *Trichoderma harzianum* TH07, and *Fusarium solani* FS12 through using the BLAST tool in NCBI. All fungal isolates showed high tolerance to crude oil and survived with various responses at the highest concentration (20%). *Aspergillus flavus* AF15 and *Trichoderma harzianum* TH07 demonstrated promising oil-degrading tolerance ability based on the dose inhibition response percentage (DIRP) of the fungal isolates. *A. flavus* had a powerful capacity to production Cx, LaC, LiP and MnP with a range from 83.7 to 96.3 mL. Molecularly, nine genes of the ligninolytic enzymes, *cbh* (*cbhI.1*, *cbhI.1*, *cbhII*) *lcc*, *lig* (1, 2, 4 and 6) and *mnp* were tested for presence and expression (by PCR and RT-PCR, respectively). PCR showed that all isolates contained all the nine genes examined, regardless of capacity to enzymes production profiles, so the presence responses of nine genes did not correlate with enzymes-production ability. Gene expression analysis shows a more diverse pattern for tested isolates for example, *Aspergillus flavus* AF15 had over-expression of *lig* and *mnp* genes, *Fusarium solani* FS12 have a weak signal with *lcc* gene while, *Trichoderma harzianum* TH07 showed moderate expression of *mnp* and *lcc* genes. The power of the transcription of the gene leads to increased enzyme secretion by fungal isolates. Fungi are important microorganisms in the clean-up of petroleum pollution. They have bioremediation highly potency that is related to their diverse production of these catalytic enzymes.

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1. Introduction

To satisfy its energy needs, anthropogenic activity is dependent on oil, allowing the petrochemical industry to flourish. The intensive use of petroleum, however, results in environmental disruption (Xue et al., 2015). Spills occur during and/or resulted from petroleum extraction, storage, refining, manufacturing, shipping, oilfield development, leakage from oil pipelines or tankers, and discharges of petroleum hydrocarbons are also of significant concern due to their widespread distribution into the environment like soil or water and harmful effects on humans (Chen et al., 2015; Wang et al., 2018). Oil spillages cause a large hazard leads to damages the biota residing into earthly and marine ecosystems, and beyond a concentration of 3%, it becomes significantly poisonous (Onuoha et al., 2003). The backbone of microbial 'bioremediation' requiring the use of living organisms to remediate toxins in the environment is microbial mineralization and/or degradation processes. This

capacity was well documented in bacteria (Hamamura et al., 2013), fungi (Asemoloye et al., 2018), and algae (Chan et al., 2006), while some investigators have identified plants with soil remediation abilities (Asemoloye et al., 2017). Various methods for the treatment of oil waste, such as bioremediation and phytoremediation, have arisen in the recent years (Jahangeer and Kumar, 2013).

Bioremediation could be defined as a biological method utilizes microorganisms' metabolic ability to remove pollutants (Maiti et al., 2008). For the entire mineralization of toxic hydrocarbons to safer and non-toxic elements e.g. carbon dioxide and water, apparently, bioremediation has proven to be the most efficient, practical, promising, economical, flexible and eco-friendly method (Toledo et al., 2006). In general, two different techniques are applied for bioremediation of contaminated areas via either detection, isolation and utilization of native microorganisms in these areas or application of natural or genetically engineered oil-degrading microorganisms (Ikuesan, 2017). Native microbial species, i.e. fungi and/or bacteria present naturally in ecosystems, interact synergistically producing several hydrolytic and oxidative enzymes with a wide range of primary and secondary bioactive molecules (Sabra et al., 2010). For bioremediation of hydrocarbons resulted from crude oil or petroleum, utilization of fungi is crucial because of their significant ability to decompose hydrocarbons of long chains or multiple rings (Acevedo et al., 2012). Furthermore, via secreting different ligninolytic enzymes, fungi show remarkable ability to degrade various hydrocarbons. Ligninolytic enzymes were originally used to decompose wood; however, some of them were recently reported to play fundamental roles in fungi ability to remediate xenobiotic and recalcitrant contaminants. The existence of different fungal enzymes is significantly affected by fungi genetic material, different physicochemical functions and life cycle (Janusz et al., 2013). Although several fungi can grow in soil, few species have the ability to survive in contaminated soils (Juhasz and Naidu 2000). Hydrocarbons in the environment are mainly biodegraded mostly by fungi, bacteria, and yeast. Recorded biodegradation efficiency ranged from 6% to 82% for soil fungi (Das and Chandran, 2011). Laccases (LaC, EC 1.10. 3.2), manganese peroxidase (MnP, EC 1.11.1.13), lignin peroxidases (LiP, EC:1.11.1) and versatile peroxidases (VPLs, EC 1.11.1.16) are the major fungal extracellular ligninolytic enzymes. Fungal LaC catalyzes simultaneous oxidation of large number of aromatic compounds via the redox ability of copper ions, whereas LiP shows remarkable oxidation potential against nonphenolic and aromatic compounds with high redox-potential (Giardina et al., 2010).

Cellulase (Cx) is a mixture of endo-glucanase, cellobiohydrolase (CBH, EC 3.2.1.91), and β-glucosidase, that act synergistically with hemicellulases to hydrolyse cellulose. A complex regulatory system regulates the expression of genes that encode these plant cell wall-degrading enzymes (Amore et al., 2013). Until now, the transcriptase responses of ligninolytic fungal genes are still vague. Furthermore, fungi producing cellulase and peroxidase laccase and have evolved functional genes to help them utilize hydrocarbon compounds. Therefore, there is a crucial need to identify the genetic basis behind fungal secretion of ligninolytic enzymes and link such genetic changes with the existing mineralization or degradation of hydrocarbon contaminants. Indeed, the deeper understanding of the regulatory mechanisms controlling transcription of genes encoding ligninolytic enzymes is a critical issue (Argumedo-Delira et al., 2012; Asemoloye et al., 2018). Therefore, the current study aimed to isolate and characterize different fungal species present in the date palm rhizosphere. In addition to investigate the tolerance of characterized fungi to crude oil, beside examination of the presence and quantification of genes encoding LaC, Cx, LiP and MnP enzymes using enzyme assay, PCR and RT-PCR techniques.

2. Materials and methods

2.1. Fungal isolates

In a previous study, four fungal isolates were isolated: *Aspergillus terreus* KC462061, *Aspergillus flavus* (AF15), *Trichoderma harzianum* (TH07), and *Fusarium solani* (FS12). These isolates were identified morphologically and molecularly by internal transcribed spacer (ITS) regions according to the methodology of the previous study (Al-Zaban et al., 2020).

2.2. Crude oil tolerance assay

Mycelia radial growth measurement on minimal medium (MM) supplemented with crude oil at different concentrations was used to examine the tolerance and survivability of each fungal isolate in the presence of crude oil via hydrocarbons degradation. Each fungal isolate was cultivated on MM medium supplemented with crude oil according to the method of Anaisell et al. (2014). In this regard, different concentrations (0 "control", 5, 10, 15 and 20%; v/v) of crude oil (Saudi Aramco) were mixed with 5 mL solution of 1×10^4 spores/ml of each isolate. Each treatment was prepared in triplicate and the plates were incubated at 30 °C for 8 days. The following equation proposed by Jonathan et al. (2016) was used to calculate dose inhibition response percentage (DIRP) of the fungal strains to different crude oil concentrations applied.

$$DIRP = \frac{Initial\ radial\ growth\ rate - Final\ radial\ growth\ rate}{Initial\ radial\ growth\ rate} \times 100$$

2.3. Fungal extracellular enzymes

2.3.1. Extraction of fungal enzyme

To examine the enzymatic activity of each isolate, different concentrations (0, 5, 10, 15 and 20%) of crude oil and were mixed with 30 mL of liquid nutrient broth medium supplemented with 20 mL of 0.1 M pH 4.5 succinic/lactic acid buffer. This mixture was used to grow all isolates for 16 days in a 30 °C with control uninoculated with isolate, the method completed as used by (Castillo and Torstensson, 2007). The enzymatic activity was expresses in U/ml.

2.3.2. Cx activity

The reaction mixture was contained 2 mL of crude enzyme; 2 gms of Whatmen No. 1 filter paper and 4 mL phosphate buffer solution (pH5.6). The mixture was incubated at 30 °C for one hr. The activity of cellulase was measured as reducing sugar according to the method of Remero et al. (1999).

2.3.3. LaC activity

The Lac activity were examined using the method described by Novotny et al. (1999) based on 2, 2-Azino-bis-3- benzthiazoline-6-sulfonic acid (ABTS) oxidation. Briefly, 0.5 mL of the culture extracts was mixed with 0.1 mM of ABTS and 100 mM of sodium tartarate at pH 4.5. Reduction of optical absorbance at 30 °C and 490 nm was measured every 5 min.

2.3.4. LiP and MnP activities

Peroxidase activities were calculated following the method described by Paszcymski et al. (1988). In this regard, LiP activity was calculated based on the oxidation of 2 mM veratrole alcohol in 100 mM sodium tartrate (pH 4.5) and 0.4 mM H_2O_2. On the other hand, MnP activity was calculated by mixing 0.5 mL of culture extracts with 1 mM $MnSO_4$, 100 mM sodium tartrate (pH 4.5), 0.1 mM H_2O_2 and 0.01% phenol. Reduction in optical absorbance

at 460 nm was measured every 10 min to calculate LiP and MnP activities.

2.4. Molecular analysis

2.4.1. PCR for detection of cellulase, laccase and peroxidase genes

Presence or absence of genes encoding Cx, LaC, LiP and MnP enzymes in the isolated fungal DNA was examined using PCR with specifically designed primers (Table 1). PCR was carried out as the method described by Brooks et al. (1993), Paul et al. (1995) for cellulase and peroxidase genes Tempelaars et al. (1994) for laccase gene. PCR products were detected with 2% agarose ethidium bromide gels in TAE 1x buffer (40 mM Tris-acetate and 1.0 mM EDTA). A 100-bp DNA ladder (Intron Biotechnology, South Korea) was used as the molecular marker.

2.4.2. RT-PCR for detection of cellulase, laccase and peroxidase genes
2.4.2.1. RNA isolation. Modified CTAB method described by Murray and Thompson (1980) and Raeder and Broda (1985) was used to isolate total RNA from the studied fungal isolates. Nevertheless, 3 M lithium chloride solution was utilized to precipitate RNA instead of isopropyl alcohol (Li et al., 2014). Agarose (1%) gel electrophoresis technique was used to examine isolated RNA quality before further investigations.

2.4.2.2. cDNA synthesis. The mycelial of fungal isolates were grounded in liquid nitrogen to isolate total RNA using the RNeasy plant mini kit (QIAGEN, Germany), according to manufacturer instructions. cDNA synthesis was carried out by reverse transcription using the ProtoScript First Strand cDNA Synthesis Kit (New England Biolabs, UK), according to the manufacturer instructions.

2.4.2.3. Transcriptional expression of the cellulase, laccase and peroxidase genes. The synthesized cDNA of each isolate was amplified via PCR by using the combination of forward and reverse primers outlined in Table 1. The reaction mixture contained 7.4 DEPC water, 8.6 mL of Master mix, 1.5 mL each forward and reverse primer (10 mM), 1 mL Taq DNA polymerase (New England BioLabs, UK), 1 mL cDNA sample, 10 mM Tris HCl (pH 8.3) and 50 mM KCl. The quality of amplified PCR products was examined using 1% agarose gel electrophoresis.

3. Results

3.1. Identification of fungal isolates

The morphological and microscopic fungal isolates characteristics followed by molecular tools used for isolates identification were compared with reference isolates at NCBI Genbank, these four isolates were classified as *Aspergillus terreus* KC462061 (AT), *Aspergillus flavus* (AF15), *Trichoderma harzianum* (TH07) and *Fusarium solani* (FS12) as shown in Table 2.

In the BLAST-based analysis, ITS region sequence appeared of the of each identified fungal isolates showed that isolate AT had 99% similarity with *A. terreus* GU966497 (Table 2). Similarly, AF15 was 98% similar to *A. flavus* (KY488467), while TH07 had 99% similarity with *T. harzianum* (KC569346). The isolate FS12 was 98% similar to *F. solani* MF136402.

3.2. Crude oil tolerance

All the obtained fungal isolates showed moderate tolerance to different crude oil concentrations ranging from 5% to 20% (Table 3). All isolates showed higher growth rates under control treatment (without crude oil) as compared to other treatments supplemented with different concentrations of crude oil. Such alteration in growth indicates the ability of crude oil to disrupt basic fungal physiological processes e.g. mycelia radial growth; however, all the isolates showed remarkable adaptation and were able to survive high concentrations of crude oil up to 20% as evident by the DIRP analysis. The most powerful radial growth tolerance was showed by AF15 and TH07. On the other hand, AT and FS12 showed weaker tolerance.

3.3. Cx, LaC, LiP and MnP activities

The obtained results showed that all the obtained isolates produced the four examined enzymes. All the isolates, however, all the isolates enzymes have more activity with an increasing concentration of crude oil. (Fig. 1).

3.3.1. Cx activity

At 20% crude oil concentration, the highest Cx production was discovered in AF15 and AT isolates (83.7–77.6 U mL^{-1}). Nevertheless, FS12 showed less production of Cx with 12 and 37.1 U mL^{-1} at 5% and 20% crude oil concentration, respectively (Fig. 1a). The

Table 1
Fungal hydrocarbon degrading gene-specific sequences of primers used for PCR and RT-PCR amplification.

Enzyme	Primer name	Gene	Sequence	Annealing temp (C)
Cellulase (cellobiohydrolase)	CBHI.1	*cbhI.1*	ACAATGTTCCGCACTGCTACTT AGGGTGCCCGCGGAGGTGCC	61
	CBHI.2	*cbhI.2*	CACTCCTCGCATTCACTTGTCT CTGCCGGTCTCGGTCCAGTTGC	61
	CBHII	*CbhII*	CCTCAGCCCTTACTACGC CCAATCTACCTCTACAGC	55
Laccase	LaC	*lcc1u*	TGGTA(T/C)CA(T/C)AGTCATTATTC ATGTG(A/G)CA(A/G)TG(A/G)AA(C/G)(A/G)GCCA	60
Lignin peroxidase	LiP	*lig1*	TACATCGAACCACGCGCACGATGATT CATCGCAATTTCGCCCGCCATGGAGGCA	68
		lig2	CATCGCAATTTCGCCCGCCATGGAGGCA ACCTTCTGAACGAATGGCTTCTGGAGC	70
		lig4	GTGCGCCTGGTTCCCCATTCTGCAG AATTGGTCTCGATAGTATCGAAGAC	63
		lig6	GACCTGCTCGAACGGCAAGGTCGTCC CATGATAGAACCATCGGCGCCTCGC	68
Manganese peroxidase	MnP	*mnp1u*	TCCGGTCAACGGCTTGGTATTCCAG GCGATCGTCTTGTTCGGGCGGCCAG	64

Table 2
Identification of soil-borne fungi species by sequencing of ITS1 and ITS 2 and region of 5.8S rRNA gene compared with sequences listed in the GenBank.

Fungal isolate		Fungi of GenBank		
Isolate name	Accession no.	Name	Accession Number	Identity
A. terreus (AT)	KC462061*	*Aspergillus terreus*	GU966497	99%
A. flavus (AF15)	PNUAF015**	*Aspergillus flavus*	KY488467	98%
T. harzianum (TH07)	PNUTH007**	*Trichoderma harzianum*	KC569346	99%
F. solani (FS12)	PNUFS012**	*Fusarium solani*	MF136402	98%

* Accession no. by genbank.
** Accession no. by authors.

Table 3
Radial extension rate (cm day^{-1}) of rhizospheric fungi in response to crude oil concentrations.

Isolate name	Radial extension rate (cm day^{-1})					DIRP
	0% crude oil	5% crude oil	10% crude oil	15% crude oil	%20 crude oil	
AT	2.75 ± 0.43	1.25 ± 0.22	0.95 ± 0.11	0.80 ± 0.024	0.75 ± 0.14	65.90
AF15	3.15 ± 0.91	2.16 ± 0.30	1.95 ± 0.63	1.61 ± 0.67	1.48 ± 0.65	51.85
TH07	3.25 ± 0.27	1.80 ± 0.12	1.51 ± 0.40	1.25 ± 0.38	0.85 ± 0.16	58.37
FS12	1.90 ± 0.16	0.74 ± 0.08	0.61 ± 0.07	0.52 ± 0.04	0.35 ± 0.09	70.78
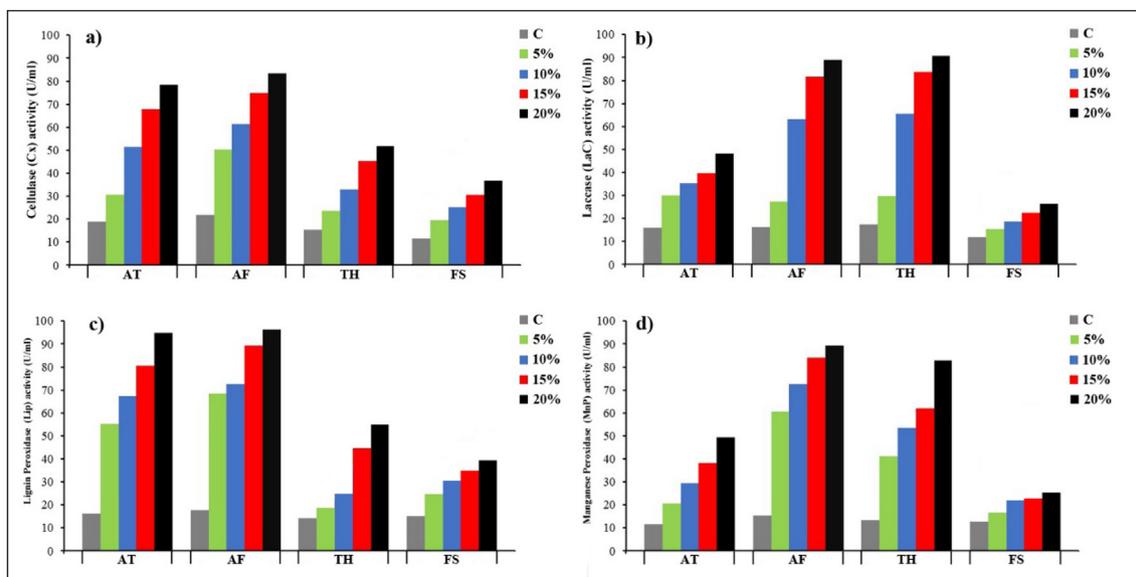
Fig. 1. Crude oil degradation and extracellular enzyme production by crude oil in rhizospheric fungal isolates; AT *A. terreus* (AT); AF = *A. flavus* (AF15); TH = *T. harzianum* (TH07); FS = *F. solani* (FS12). (a) cellulase (b) laccase (c) lignin peroxidase (d) manganese peroxidase.

Highest Cx production was also observed in AF15 and lowest at FS12.

3.4. LaC activity

Furthermore, the highest LaC enzyme production (91.5–88.2 U mL^{-1}) was found in TH07 and AF15. Interestingly, both species showed the highest LaC production under the highest concentration of crude oil (20%; Fig. 1b). However, FS12 have declined at laccase production (28.3 U mL^{-1}/20% crude oil). It seems a stronger correlation between high enzymatic activity and an increase in crude oil concentration at all four isolates expect FS12 have a weak correlation.

3.5. LiP activity

LiP produced highly with increasing contaminant concentration of crude oil, with a range from 38 to 96 U mL^{-1} at 20% crude oil

(Fig. 1c). AF15 (96.3 U mL^{-1}/ 20% crude oil) followed by AT (94.8 U mL^{-1}/ 20% crude oil) and third TH07 (56.4 U mL^{-1}/ 20% crude oil) showed the highest enzyme activity. While FS12 have a weak increase of production LiP enzyme ranging from 38.9 to 38.9 U mL^{-1} at 5% to 20% crude oil, respectively. It appears a high degree of adaptation for four fungal isolates except for FS12 showed at a provided high amount of enzyme at a high concentration of crude oil.

3.6. MnP activity

AF15 and TH07 showed the highest MnP production with 89.4 and 85.2 U mL^{-1} at 20% crude oil concentration, respectively (Fig. 1d). *Fusarium solani* FS12 have lowest MnP production at 27.4 U mL^{-1}/ 20% crude oil. Based on all results of all enzymes, the crude oil contamination increment showed positively affected for enzymes production by AT, AF15 and TH07 isolates as compared to FS12 isolate showed negatively affected. This means that

AT, AF15, and TH07 isolates have a powerful degree of adaptation and FS12 isolate has weakly adaptation.

Molecular analysis

3.7. Detection of Cx, LaC, LiP and MnP genes

3.7.1. PCR

PCR amplification of Cx (*cbhI.1, cbhI.2, cbhII*), LaC (*lcc 1*), LiP (*lig1, lig2, lig4* and *lig6*) and MnP (*mnp*) genes was shown in Fig. 2. The PCR results of the present study showed that all isolates were harbored for all the examined genes. The presence of these genes had a consistent pattern in the isolates, suggesting no differences in the molecular characterization of the isolates.

3.7.2. Analysis of Cx, LaC, LiP and MnP genes transcription

Several genes encoding for in the enzymes biosynthesis pathway. Measuring their transcriptional status should reveal how efficiency of isolates the are capable of producing enzymes. In this study we analysed transcription of nine genes, Cx, LiP, MnP and LaC in four fungal isolates (Fig. 3).

In AF15 and AT have *cbhI.2* and *cbhI.1* genes mRNAs were overexpressed, whereas in TH07 FS12 have very weak expression signal. Highly expressed of the genes leads to the highest enzyme activities of isolates (83.7–77.6 U mL^{-1}).

AF15 and TH07 isolates showed overexpression of LaC gene transcription indicating increasing production of LaC with increment of the crude oil concentrations. AT and FS12 have a weak signal with low enzyme activity. Table 4 showed the level gene expression of four isolates, AF15 was the most powerful isolate indicated to high-level gene expression of eight genes, but FS12 was the weak one with three genes have high-level gene expression. *lig* 1–6 genes transcription can be used as a marker to enzyme production. This transcription gave the best correlation of enzyme production and gene expression. AF15 and AT have the highest enzymatic production related to the over-expression of *lig* genes. TH07 and FS12 have medium amount of enzyme production and moderately expression of *lig* genes. *mnp* gene has the same trend for high enzyme activity meaning overexpression of a gene. The powerful of the transcription of the gene leads to increased enzyme secretion by fungal isolates.
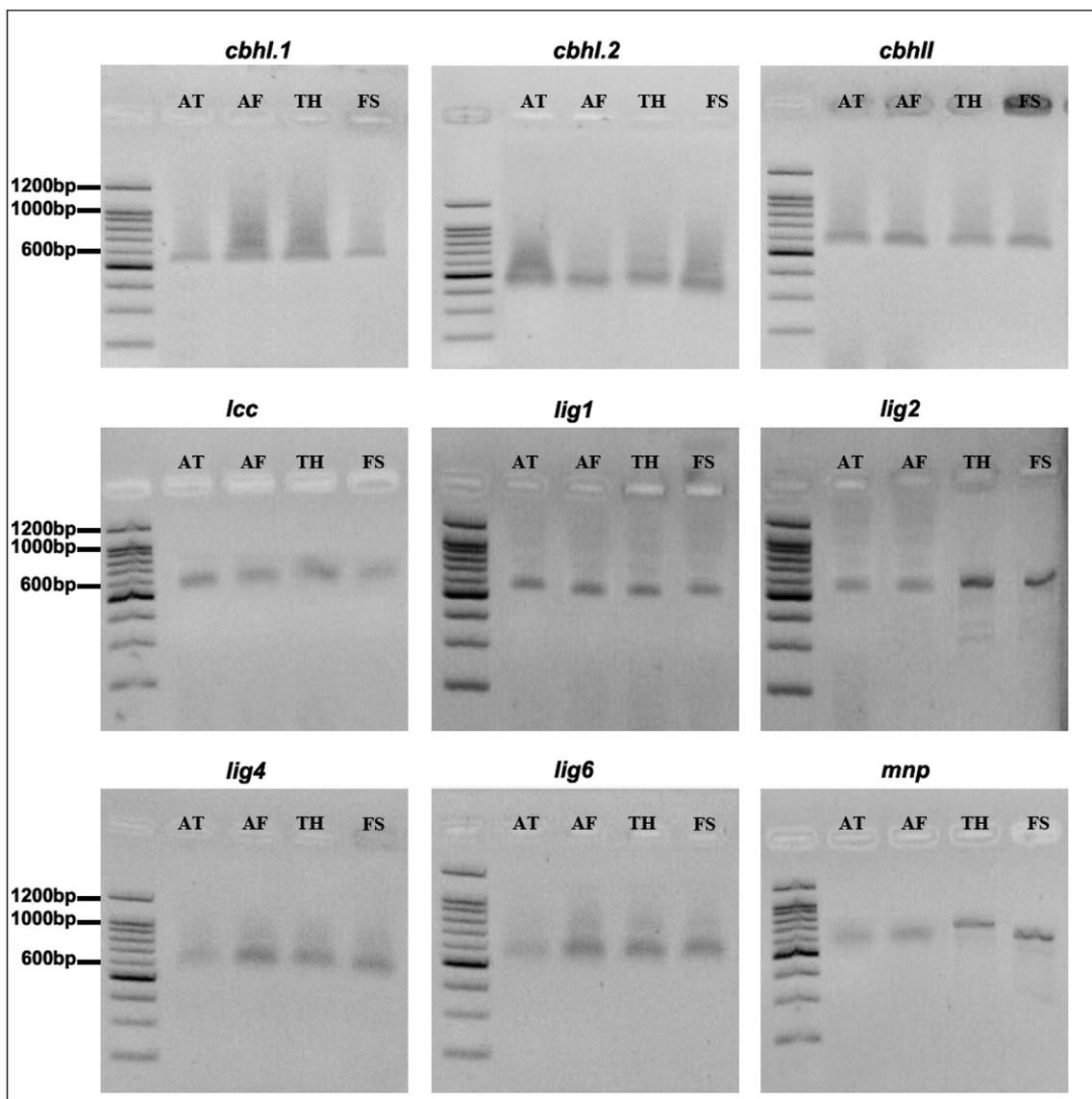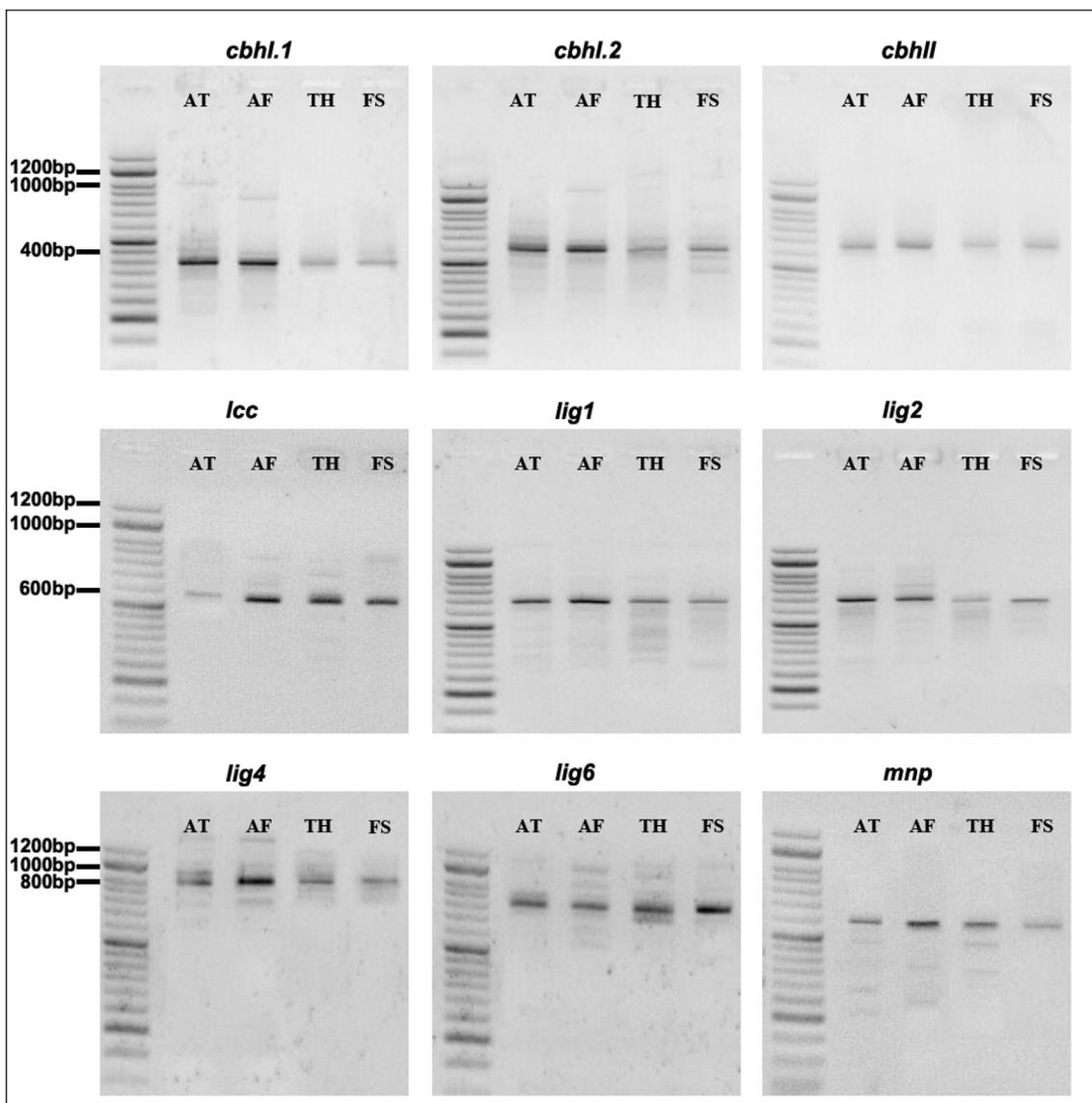

Fig. 2. PCR Detection of cellulase (*cbhI.1, cbhI.2, cbhII*), laccase (lcc) lignin peroxidase (*lig1, lig2, lig4, lig6*), and manganese peroxidase (*mnp*) genes in crude oil in rhizospheric isolates: AT A. terreus (AT); AF = A. flavus (AF15); TH = T. harzianum (TH07); FS = F. solani (FS12).

Fig. 3. RT-PCR of cellulase (*cbhI.1, cbhI.2, cbhII*), laccase (*lcc*) Lignin peroxidase (*lig1, lig2, lig4, lig6*), and manganese peroxidase (*mnp*)) genes in crude oil in rhizospheric isolates: AT A. terreus (AT); AF = A. flavus (AF15); TH = T. harzianum (TH07); FS = F. solani (FS12).

Table 4
Different RT-PCR amplification patterns (level gene expression) in four fungal isolates.[a]

Isolate	Cellulase (cellobiohydrolase)[a]			laccase	Lignin peroxidase				M. peroxidase
	cbhI.1	*cbh1.2*	*cbhII*	*lcc1*	*lig1*	*lig 2*	*lig4*	*lig6*	*mnp*
AT									
AF15									
TH07									
FS12									

[a] The genes have high-level gene expression (black box) or low-level gene expression (grey box) of RT-PCR products of four fungal isolates.

4. Discussion

The recovery of various fungal species isolated from date palm soil of Riyadh rhizosphere in the present study could be referred to soil characteristics, fertilization, pH, root secretions, humidity percentage, microbial and climatic conditions as well as date palm cultivars, tree age, and plant density per unit area. This diversity of fungi was in agreement with the findings of Abdullah et al. (2010), Ndubuisi-Nnaji et al. (2011), The most common fungal isolates were *F. solani*, *F. oxysporum*, *A. flavus*, *A. terreus*, and *A. niger*. Our results were consistent with those reported by Ahmed et al. (2016) and Manzelat (2017).

Molecular identification and discrimination of fungal isolates using the sequences of the ITS1-5.8S-ITS2 regions tends to be a

reliable method Florez et al. (2007). *Aspergillus* spp., *Fusarium* spp. and *Trichoderma* have the broader classification but their examination based on the sequence analysis of the conserved ITS1-ITS2 regions and rDNA region (5.8S rRNA) shows a high percentage of accuracy on molecular identification as ensured by Henry et al. (2000), Gaikwad et al. (2013), Devi et al. (2012).

Oil exploration due to hydrocarbon contamination where is a major problem affect human activities but also on the environment of soil or water and microbial diversity. Therefore, cleanup of these worst pollutants is necessary to have the environment safe and sound (Ijah 2003). Soil fungi are vital in the proper functioning of the ecosystem by helping in the degradation of dead matter, releasing vital nutrients and leading to a safe environment. Fungal isolates from soil can reduce oil pollution by enzyme secretion to remove pollutants from the environment (Clemmensen et al., 2013). A fungal consortium provides a greater spectrum of bio-removal enzyme activity, demonstrated rapid degradation of crude oil by special fungal species belonging to several genera (Silva et al., 2015). In cultures growing in association with crude oil, the level of the four enzymes was noticeably higher compared to the controls; probably because here the enzymes were in increased demand for hydrocarbon breakdown reactions (Mohsenzadeh et al., 2012).

Ligninolytic fungi and enzymes i.e., LaC, MnP, and LiP are the most well-known enzymes, and they occur in ascomycetes, basidiomycetes, and deuteromycetes (Santos et al., 2008). Diverse fungi produced different enzyme combinations, even within the same genera. The different combinations of the development of ligninolytic enzymes that indicate different abilities of the fungi to degrade lignin-containing media, which may be linked to biodegradation strategies (Reid, 1995). Fungal LaC plays a role in detoxification of phenol compounds produced during crude oil degradation and acts synergistically with peroxidases and other enzymes in the breakdown of hydrocarbon pollutants from the environment (Wong, 2009). The fungal isolates displayed good production of LiP, MnP, Cx and LaC enzymes, most of the fungal isolates were previously reported to be involved in the bioremediation of soils contaminated with crude oil because of their significant production of a wide variety of hydrolytic and oxidative extracellular enzymes (Asad et al., 2015).

The scientific evidence concerning the relationship between genetic expression and enzymatic activities of rhizospheric fungi grown in soil contaminated with crude oil is lacking. In the current study, genetic expression of 4 critical enzymes (Cx, LaC, LiP and MnP) involved in the fungal biodegradation of hydrocarbons is investigated and reported in four different fungal species. High concentrations of crude oil in oil-polluted soils may affect the genetic material and the regulatory machinery of the fungal cell altering genetic expression and might lead to overexpression of certain genes that could improve fungal capacity to degrade hydrocarbons (Hadibarata et al., 2009). The theory of environmental genomics is focused on simultaneous genes analysis within environmental microbes (genomics) or an environmental sample (metagenomics) of collective microbial genomes. The availability of whole-genome sequences from many bioremediation-related environmental microorganisms was useful in determining the gene pool of enzymes involved in degradation (Galvao et al., 2005). Recently, functional analyzes of microbial populations are used to predict microbial biodegradation pathways at different rates of transcriptomes, proteomes, and metabolomas. Transcriptomic analyzes have helped researchers to decode the mRNA expression profiles of upregulated or downregulated genes in microorganisms when exposed to hydrocarbon pollutants (Jennings et al., 2009). Transcriptomic or metatranscriptomic techniques are used to obtain functional insight into the behaviors of environmental microbial populations through the analysis of their mRNA transcription profiles (McGrath et al., 2008). It is known that the regulation of fungal laccase's and peroxidase's genes involve a hierarchy of environmental signals such as the concentration of carbon and nitrogen, heavy metal ions, pesticides and hydrocarbons, lengths of daylight and temperature (Ramirez et al., 2010). It has been postulated that some response elements may regulate fungal laccase gene transcripts by an accurate number of mRNA copy. Although this information of about response elements we have a complex picture of laccase expression regulation (Piscitelli et al., 2011). In silico studies of mnp genes highlighted the presence of multiple regulatory elements various distributed along the gene sequence. Each of the known promoter regions consists of one sequence of TATA (Nagai et al., 2007). Several transcription factors involved in cellulase and hemicellulase gene expression have been identified and characterized, including transcriptional repressors CRE1/CreA, Clr2/ClrB and 15 unknown proteins. Of these, Clr2/ClrB, which contains a binuclear zinc cluster, is a key transcriptional activator that is essential for inducing the expression of major cellulases and hemicellulases enzymes. Clr2/ClrB is highly conserved in most filamentous ascomycete fungi such as *Aspergillus* spp., *Trichoderma* spp. and *Penicillium* spp. (Li et al., 2015).

5. Conclusions

In the current study, four fungal isolates were isolated from date palm rhizosphere and molecularly identified and characterized. In each isolate, genes encoding Cx, LaC, LiP and MnP enzymes were examined using various biochemical and molecular techniques. All obtained fungal isolates showed various levels of ability to adapt and survive even under the highest concentration (20%) of crude oil. Differential enzymatic and expressions activities of Cx, LaC, LiP and MnP enzymes and their encoding genes in all isolates indicates their potential role in enhancing fungal survivability in contaminated soil via biodegrading and/or mineralization of hydrocarbons. The results of the current study support the potential large-scale application of the four fungal isolates obtained from date palm rhizosphere in biodegradation of crude oil spill and other bioremediation technology applications.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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