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Molecular finger prints of *Pleurotus tuber-regium* from oil contaminated soils

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Abstract

Indiscriminate disposal of engine oil is considered a serious threat to the environment due to its chemical composition. *Pleurotus tuber-regium*, an edible mushroom has been implicated in bioremediation and bioaccumulation of compounds from its environment. The study was to assess the effect of engine oil on molecular finger prints of *Pleurotus tuber-regium* Fr Sing. Loamy soil contaminated with spent and non- spent engine oil at the rate of 2, 4, 6, 8 and 10% concentrations respectively was inoculated with 30g sclerotia. DNA was extracted only from sporophores grown at 2 and 4% oil contamination. Two ITS primers (ITS 1/ITS 4B and ITS 1/ITS 4A) with six RAPD primers (OPM 14, OPL 20, OPK 01, OPA 04, OPD 07 and OPD 11) were used to elucidate variations in the genomic profiles. ITS 1/ITS 4B primer amplified a single band at 600 bp from the fragments while RAPD primers showed PCR product amplifications of variable sizes (500 bp–800 bp). Although similarity existed among fragments from the same contamination level, the dendrogram revealed one major cluster which deviated according to contamination levels irrespective of type of oil; 4% fragments were more closely related than 2%. This indicates that exposure to different doses of oil could affect the molecular finger prints of *P. tuber-regium*. The implication is that engine oil could cause variations in DNA profile.

Key words – DNA profile **–** environment **–** mushroom **–** pollution **–** primers

Introduction

Engine oils are lubricants used for internal combustion engines to reduce wear, clean, inhibit corrosion, improve sealing, and cool the engine. They are composed of hydrocarbons (James & Spearot 2009), additives and esters up to 20% by weight (Klamman 2004). Engine oil is changed periodically in order to enhance performance of the engine and is considered toxic due to its chemical composition (Vazquez–Duhalt 2002). Disposal of spent engine oil has constituted a serious environmental problem in Nigeria as it is indiscriminately disposed on any available land. There seemed to be no existing regulation or enforcement as to appropriate disposal. This is in addition to spillages from trucks in transit during transportation. Oil pollution leads to build up of elements in soil and water supplies with eventual translocation in plant tissues (Vwioko et al. 2006), which may cause metabolic disorders, reduction in soil productivity and growth inhibition (Fernandes & Henriques 1991, Vazquez–Duhalt 2002). Long term effects of these oil on marine

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organisms have been found at concentrations as low as 1 ppm (U.S. EPA). However, mushrooms have been reported to grow and possess the ability to degrade certain recalcitrant toxic compounds (Lau et al. 2003, Gadd 2004, Stamets 2005). Thomas et al. (1999) reported that oyster mushroom, mineralized and metabolized 97% oil after 8 weeks of incubation. *Pleurotus tuber-regium*, an edible, gilled, sclerotial fungus found on various habitats has been implicated in bioremediation of oil polluted sites (Eggen & Sasek 2002, Mansur et al. 2005, Emuh 2009). Morphological aberrations have been reported in plants exposed to crude oil pollution (Akintunde et al. 2011). The study was to assess the effect of different doses of engine oil on molecular finger prints of *P. tuber-regium.*

Materials and Methods

Sample collection

Sclerotium of *P. tuber-regium* was obtained from a local market in Anambra state, Nigeria, while spent and non-spent engine oil were collected from an automobile workshop in Benja Village Ota, Ogun state, Nigeria. Loamy soil was sieved for homogeneity and sterilized at 80^0c for 24 hrs (Onuoha 2007). Thereafter 1 kg was weighed into perforated polyethylene bags and contaminated with spent (S) and non-spent engine (NS) oil at the rate of 2%, 4%, 6%, 8% and 10%. The soil was then moistened with 100 mls of water. Pieces of 30 g *P. tuber-regium* sclerotium, soaked in sterile water for 18 hrs were planted in the contaminated soils. The experiment was set up in triplicate.

DNA extraction and amplification

DNA was extracted using a modified SDS DNA extraction protocol (Lewinsohn et al. 2001). DNA amplification was carried out using two ITS primers (ITS 1/ITS 4A and ITS 1/ITS 4B) and six RAPD primers (Table 1). Polymerase Chain Reaction (PCR) was performed in 25 µl. Each reaction mixture was heated at 95^oC for 10 mins. A total of 30 PCR cycles, each cycle for 0.3 mins at 94^oC for denaturation, annealing (0.45 mins at 50^oC for reactions with ITS 1/ITS 4A and at 55^oC for reactions with ITS 1/ITS 4B), 1.15 mins at 72^oC for extension and 10 mins final extension at 72^oC for 1 min. The PCR products were analysed by electrophoresis on 2% agarose gel in (1x) TBE buffer (50 mM Tris-acetate, 1 mM EDTA, pH 8.0) stained in 0.5 mg/ml ethidium bromide.

RAPD–PCR Amplification

Six RAPD primers (OPM 14, OPL 20, OPK 01, OPA 04, OPD 07 and OPD 11) were selected. Polymerase chain reaction was performed in 25 µl with each of the primers respectively; each reaction mixture was heated at 95 ^oC for 10 mins. A total of 35 PCR cycles, each cycle for 0.3 mins at 94 ^oC for denaturation, 0.45 mins at 36 ^oC for annealing, 1.45 mins at 72 ^oC for extension and a 10 mins final extension at 72 ^oC. The PCR products were analysed by electrophoresis on a 2% agarose gel in (1x) TBE buffer (50 mM Tris-acetate, 1mM EDTA, pH 8.0) stained in 0.5 mg/ml ethidium bromide. The gels were scored for presence or absence of reproducible bands according to Lynch & Milligan (1994). Each band was regarded as a locus with two alternative alleles: present (1) or absent (0).

Results

ITS amplification

The result of ITS primer amplification in (Fig. 1) showed that primer pair, ITS 1/ITS 4B successfully amplified the DNA of four fungal fruit bodies from 2% and 4% oil contamination as a single band with the expected fragment size of 600 bp. ITS 1/ITS 4A primer did not amplify any fragment.

Table 1 ITS and RAPD Primers and their sequences.

No	Primer's Name	Sequence 5' → 3'
1	ITS 1	TCCGTAGGTGAACCTGCGG
2	ITS 4A	GGATCTCTTGGCTCTCGC
3	ITS 4B	TCCTCCGCTTATTGATATG
4	OPM 14	AGGGTCGTTC
5	OPL 20	TGGTGGACCA
6	OPK 01	CATTCGAGCC
7	OPA 04	AATCGGCTG
8	OPD 07	TTGGCACGGG
9	OPD 11	AGCGCCATTG

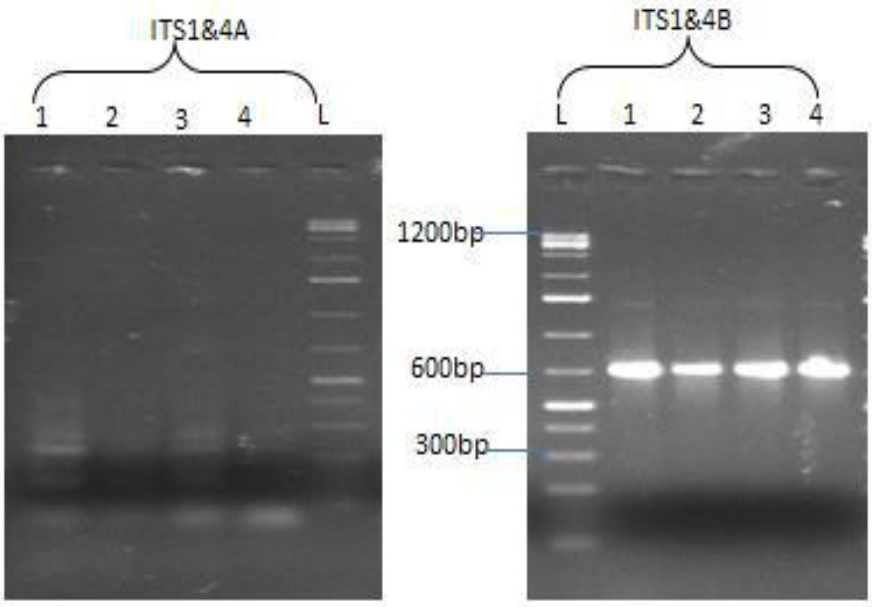

Fig. 1 – Agarose gel electrophoresis of ITS primers on DNA of sporophores harvested from spent & non-spent engine oil contaminated soil. Lane L-Ladder, Lanes 1–4 (2% spent, 2% non-spent, 4% spent, 4% non-spent engine oil concentration.

RAPD–PCR Amplification

The result of RAPD–PCR using six RAPD primers is presented in (Figs 2–4). Among them, OPA 04 and OPK 01 had the highest number of amplified bands (23 & 21 bands respectively) while OPL 20 had the least number of bands (8 bands). Primers OPD 11 and OPK 01 had the highest polymorphism, 75% and 62% respectively while OPM 14 recorded the least (Table 2). Primers OPD 11 and OPA 07 produced similar amplicon sizes of 650 bp and 750 bp respectively for 2NS, 4NS and4S while OPK 01 and OPA 04 produced uniform DNA bands between 600-800 bp respectively. The Dendrogram grouped all the samples under one major cluster with 4% (4N & 4NS) more closely related than 2% (2N & 2NS) (Fig. 5). The genetic distance 0.1 was more prominent between different oil contaminations than same oil level.

Discussion

Extraction of genomic DNA was achieved only with fruit bodies grown in 2% and 4% oil levels. DNA extraction was not successful with fruit bodies harvested at higher oil concentration. This could be associated with bioaccumulation of toxic substances from the oil saturated soil at higher concentration. Akintunde et al (2011) observed that crude oil was toxic to *Pistia stratiotes* plant at

all concentrations in all investigated parameters for as low as 10 ppm. This toxicity was associated with certain metals inherent in the crude oil such as manganese and lead.

Amplification using ITS 1/ITS 4B produced a single band with expected size of 600 bp. ITS 4B had been described as primer specific for basidiomycetes (Gardes & Bruns 1993, Kendall & Paul 2005). ITS 1/ITS 4A did not produce any amplification in all samples. Though the sizes of PCR products were approximately the same, it may not represent similarity in the sequences. Therefore, the genomic DNA obtained was further distinguished using analysing tools such as the Random Amplification Polymorphic DNA (RAPD) and dendrogram.

Generally, the RAPD–PCR amplifications were able to show genetic diversity between oil contamination levels as can be seen in (Figs 2–4). Highest scorable bands was achieved with OPA 04 (23 bands) while OPL 20 had the least number (Table 2). This is contrary to Swarnendu et al (2010) who observed the lowest number with OPA 04. This could be due to differences in the species of *Pleurotus* used for the work. The diversity in the genes is evident from the variations in PCR fragments lengths. This variability could be usefully exploited to achieve genetic gain for persistent growth in oil polluted environment through selection and hybridization of desirable genotypes.

The result corresponds with the phylogenetic analysis which showed genetic similarity at same oil level. The dendrogram revealed that genetic similarity existed between DNA from same oil concentration (Fig. 5). The DNA from different oil contamination level showed higher level of genetic diversity than same contamination level. Hence 4N and 4NS were more closely related and clustered together. Similar trend was observed with 2N and 2NS. These results suggest that there are variations in molecular finger prints of *Pleurotus tuber-regium* from different oil polluted environments.

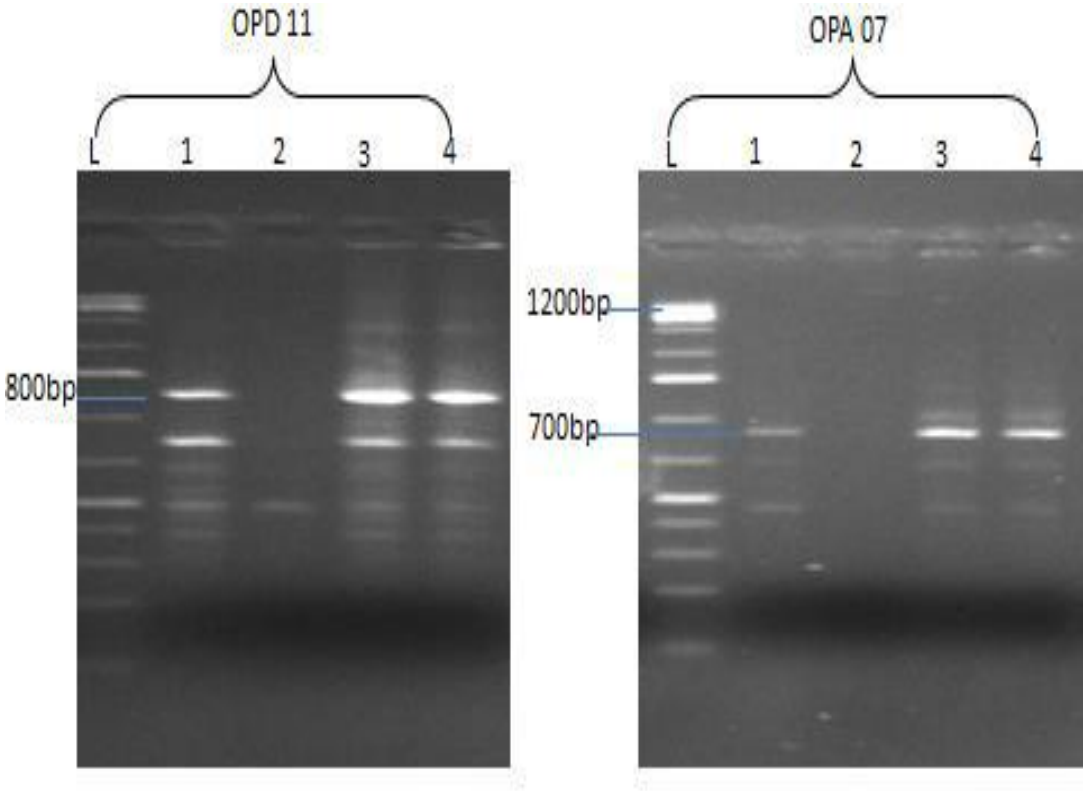

Fig. 2 – Agarose gel electrophoresis of RAPD primers OPD 11 and OPA 07 on DNA of sporophores harvested from spent & non-spent engine oil contaminated soil. Lane L-Ladder, Lanes 1–4 (2% spent, 2% non-spent, 4% spent, 4% non-spent engine oil concentration.

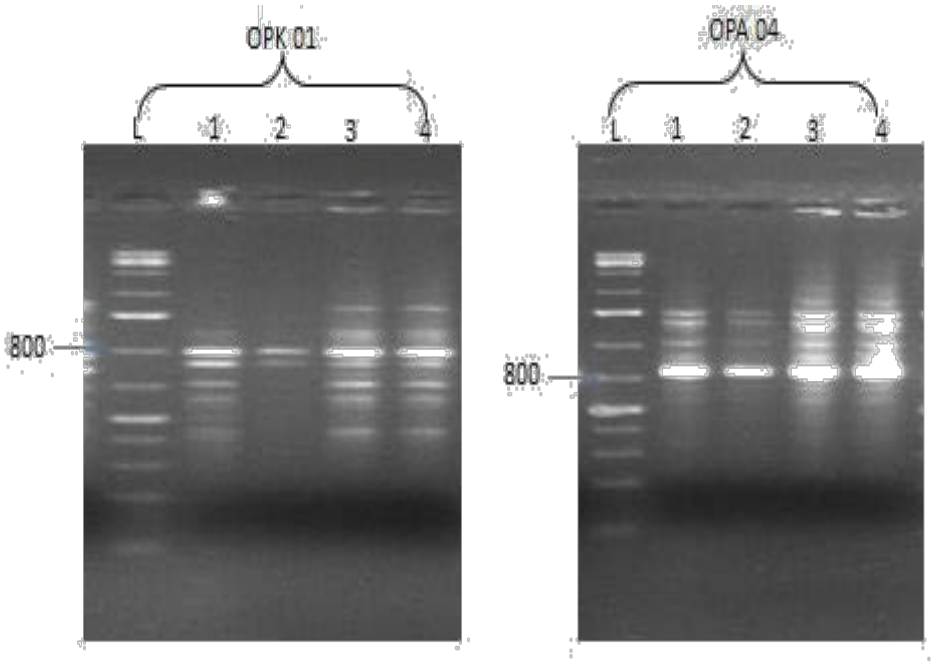

Fig. 3 – Agarose gel electrophoresis of RAPD primers OPK 01 and OPA 04 on DNA of sporophores harvested from spent & non-spent engine oil contaminated soil. Lane L-Ladder, Lanes 1–4 (2% spent, 2% non-spent, 4% spent, 4% non-spent engine oil concentration.

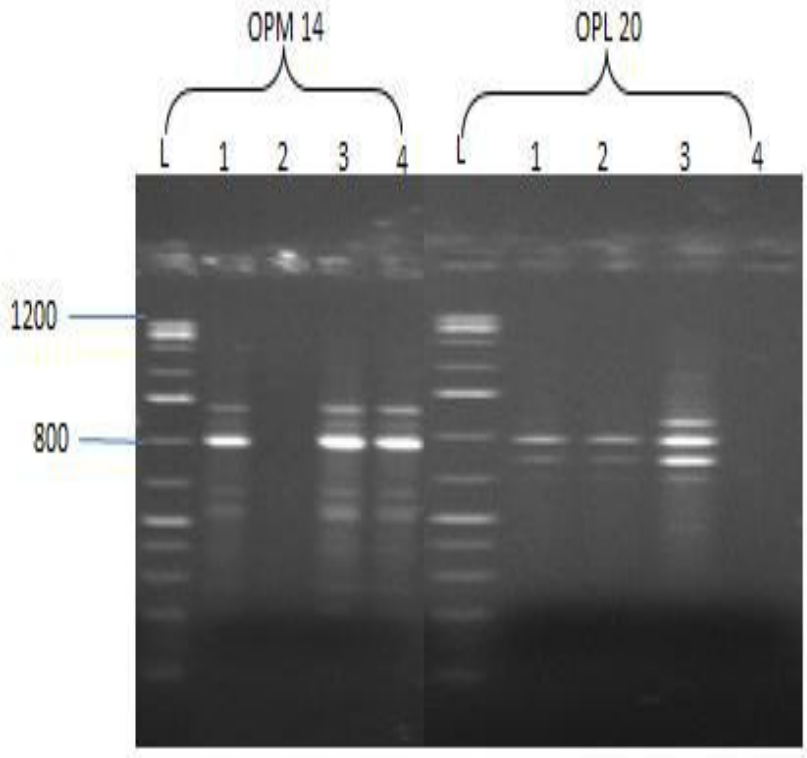

Fig. 4 – Agarose gel electrophoresis of RAPD primers OPM 14 and OPL 20 on DNA of sporophores harvested from spent & non-spent engine oil contaminated soil. Lane L-Ladder, Lanes 1–4 (2% spent, 2% non-spent, 4% spent, 4% non-spent engine oil concentration.

Table 2 RAPD –PCR DNA profiles analysis.

Primer	Total bands	Polymorphic bands	Polymorphic %
OPA 04	23	7	30
OPA 07	11	2	18
OPD 11	16	12	75
OPK 01	21	13	62
OPM 14	14	2	14
OPL 20	8	2	25
TOTAL	93	38	-

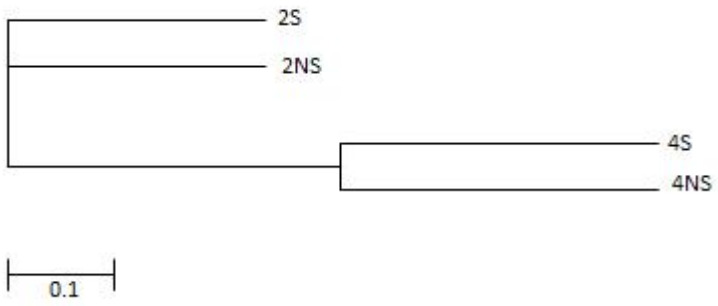

Fig. 5 – Dendrogram showing genetic relationship between DNA profiles of sporophores from 2% (2N & 2NS) and 4% (4N & 4NS) spent and non-spent engine oil contaminated soils

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