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Genetic diversity, microbiological study and composition of soil associated with wild *Pleurotus ostreatus* from different locations in Ondo and Ekiti States, Nigeria

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Abstract

Background: Genetic variation among different strains of wild macrofungi can be expressed and documented using different molecular tools. In this study, the genetic diversity and relatedness of *Pleurotus ostreatus* from different locations in Ondo and Ekiti States were investigated.

Methods: Random amplified polymorphic DNA-polymerase chain reaction marker was adopted to assess the genetic diversity of some wild *P. ostreatus*. Microorganisms associated with the wild *P. ostreatus* and their soil (substrate) were enumerated and identified using standard microbiological methods, while composition of soil around the mushrooms was evaluated using the conventional method.

Results: A total of 114 positive DNA bands were observed among wild *P. ostreatus* based on random amplified polymorphic DNA analysis (RAPD) with 10 primers, showing a total number of polymorphic markers of 91 and average polymorphism of 80.24%. *P. ostreatus* from Ala quarters and Igbatoro Road in Akure related to *P. ostreatus* from Ado-Ekiti, while *P. ostreatus* from Ido-Ekiti and Usi-Ekiti are genetically similar. The bacterial count from wild *P. ostreatus* and associated soil ranged from 1.20×10^4 to 4.70×10^5 and 1.70×10^7 to 6.10×10^8 CFU g^{-1}, respectively. The highest fungal count of 3.40×10^4 and 7.40×10^7 SFU g^{-1} were obtained for wild *P. ostreatus* and associated soil at Usi-Ekiti, which possesses the highest organic content (5.90%). Isolated microorganisms were *Pseudomonas putida, Streptomyces* spp., *Trichoderma* spp., *Penicillium italicum* and others. The highest crude fiber (25.04%) and protein content (24.07%) were obtained in wild *P. ostreatus* from Usi-Ekiti.

Conclusion: RAPD revealed the genetic relatedness and genetic diversity among studied wild *P. ostreatus,* indigenous to two sates in Southwestern Nigeria. This will improve the strain selection for further utilization and documentation.

Keywords: RAPD-PCR, Mushroom growth-promoting bacteria (MGPB), Basidiomycetes, Food nutrient, Soil microorganisms

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Background

Pleurotus species are predominant in both temperate and tropical parts of the world; it naturally grows on decaying woody materials as well as above soil [1]. They are devoid of chlorophyllous tissues and, hence, considered as primary decomposer of organic matter. Mushrooms play important ecological niche by decomposing complex organic matter when their mycelial network extend into the soil to absorb nutrients, thereby contributing to the improvement of soil composition [2, 3].

Generally, species of edible and medicinal mushrooms are gaining much attention for advance biotechnological uses, especially in pharmaceutical and agro allied companies. The biodiversity of *Pleurotus* spp. has created potential utilization with profound biological and economical values [4], but their genetic variability and taxonomic controversies still express some limiting factors [5]. However, exact characterization and identification of *Pleurotus* species is fundamental to reveal their true identity for maximum exploitation in food industries and for research documentation. The improvement in the use of deoxyribonucleic acid (DNA) for molecular technology has been proven to be a successful tool to solve taxonomic chaos [6]. Random amplified polymorphic DNA (RAPD) is one of the cheapest and quickest methods for assessing the variability at DNA level, being explicitly useful in intraspecific analysis and displaying the accurate genotypic identity of fungi [7].

Moreover, microbial ecology and substrate composition are other paramount concerns that affect the relatedness and nutrient contents of wild edible mushrooms. Soil microbial communities are extremely diverse; relatively to their organic contents, water activity and soil nutrient, physical and chemical properties [8]. The microbial succession on wild edible mushroom as well as their substrates is crucial for mushroom farmers to enhance productivity, eliminate post-harvest contamination and maintain quality product. Therefore, microbial community associated with wild mushrooms and their substrate (soil) has not been adequately investigated and revealed. Our study, thus, provides information on the molecular variability of some *P. ostreatus* from different locations and reveals the microorganisms associated with *P. ostreatus* as well as composition of soil around them.

Materials

Collection of wild *Pleurotus* species and soil around them

Wild edible mushrooms, *Pleurotus* spp., were picked from the forest in Ala quarters (116.8 g), Igbatoro Road (97.5 g) in Ondo State, Ido-Ekiti (124.2 g), Usi-Ekiti (145.8 g) and Ado-Ekiti (106.6 g) in Ekiti State, Nigeria (Fig. 1). The soil associated with the growth of wild *P. ostreatus* at different locations was collected using hand soil auger from the surface to a depth of 0–20 cm into sterile cellophane bags. The samples were transferred to the laboratory immediately for analysis.

Methods

Extraction of DNA using cetyl trimethyl ammonium bromide (CTAB)

Each of the mushroom samples (5 g) was air dried and ground in mill machine. The prepared mushroom sample (0.5 g) was transferred into Eppendorf tube; DNA extraction buffer (500 µl) consisting of 2% w/v of CTAB diluted in 100 mM Tris–HCl, 20 mM EDTA, 1.4 M of NaCl and β-mercaptoethanol (2% v/v) was added and incubated at 65 °C for 60 min [9]. Thereafter, the sample was allowed to cool and centrifuged at the speed of 4800*g* at 4 °C for 10 min. 0.5 ml of chloroform and isoamyl alcohol (24:1) were added and vortexed for 10 s, followed by spinning at 5400*g* for 10 min. The supernatant was transferred into a new Eppendorf tube and 0.5 ml of cold isopropanol was added to precipitate the DNA. The sample was kept in the freezer for 1 h and later spun at 5400*g* for 10 min. The supernatant was discarded and the pellet was washed with 70% v/v ethanol. The DNA was dried at room temperature (26 ± 1 °C) and resuspended in 100 µl of sterile distilled water. Thereafter, the DNA concentration was measured using a spectrophotometer (Beckman Instruments Inc., Fullerto CA, USA) at 260 nm.

Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis

Ten (10) RAPD primers were used for the generation of polymorphic fragments of *P. ostreatus* strains (Table 1). Amplifications was performed on 25 µl reaction mixture consisting of the following: 2.5 µl of PCR buffer, 2.5 µl of 10 mM dNTP mix, 2.5 µl of $MgCl_2$, 1.0 µl of DNA, 1.0 µl of 10 pM primer (Operon Technologies, USA), 1.0 µl of Taq polymerase (Boehringer, Germany) and 14.5 µl of distilled water. The reaction volume in Eppendorf tube was amplified in a thermal cycler (Applied Biosystems 9700). The cycling program for amplifying RAPD-PCR assays was: 1st cycle of 94 °C for 3 min for initial denaturation, followed by 45 cycles of 94 °C for 20 s for denaturation, annealing of primer at 37 °C for 20 s and extension at 72 °C for 40 s with a final extension at 72 °C for 7 min.

Gel electrophoresis of PCR amplicons

Agarose gel electrophoresis was used to determine the quality and integrity of DNA by size fractionation on agarose gel. Hence, amplification products were resolved by electrophoresis using 1.0% agarose gel in 100 ml, 0.5× TBE buffer: 20 mM Tris, 89 mM boric acid, 20 mM EDTA, pH 8.0. The gel was allowed to cool down, 10 µl of 5 mg ml^{-1} ethidium bromide was added and mixed

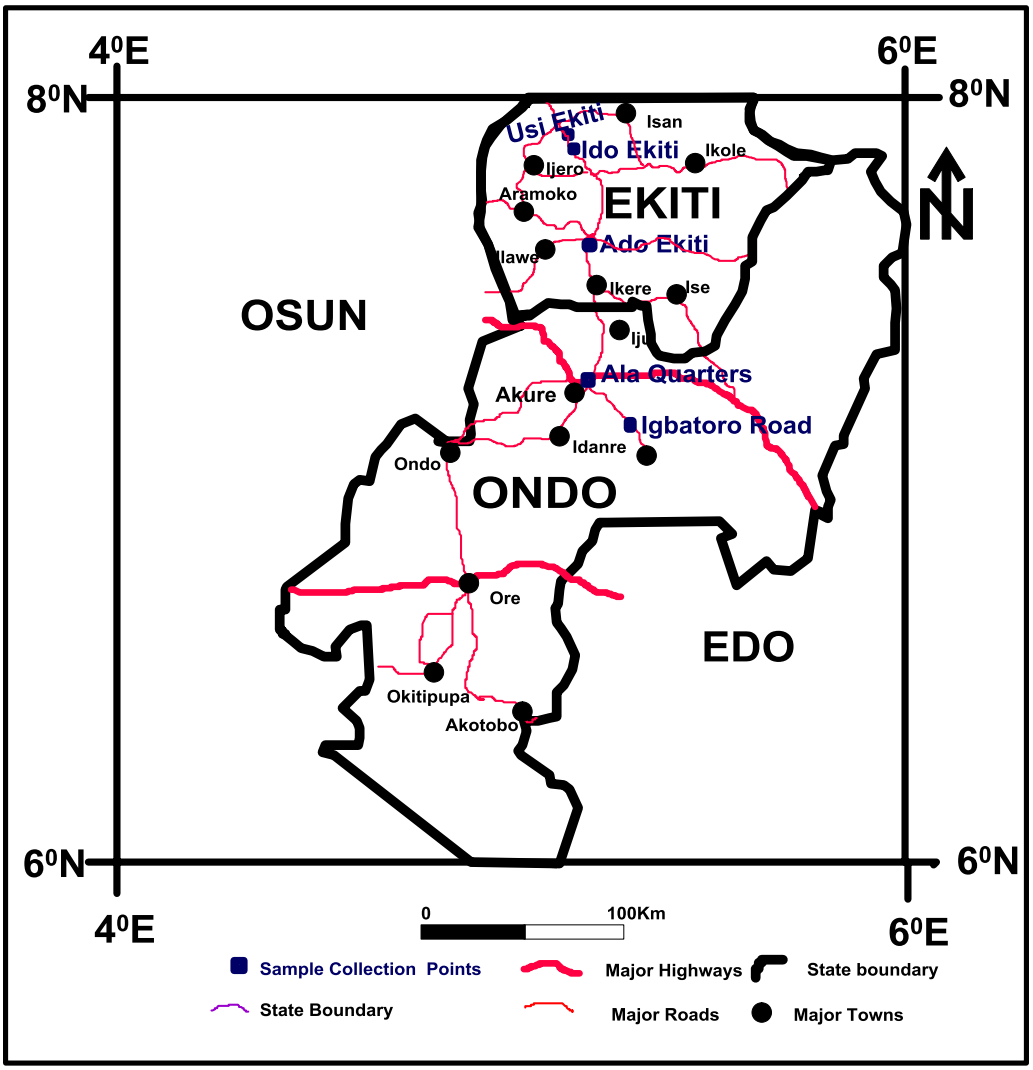

Fig. 1 Map of Ekiti and Ondo States indicating site of mushroom sample collection

together before pouring into an electrophoresis chamber set with the combs inserted. After the gel had solidified, 10 µl of each PCR amplification product was mixed with a 2 µl of 6× loading buffer in the well created on agarose gel. Electrophoresis was done at 100 V for 2 h and the integrity of the DNA was visualized, photographed under UV light and documented with a gel documentation system (UVP, USA).

Cluster analysis

The position of the RAPD bands was scored and transformed into a binary character matrix as "1" stands for the presence, while "0" stands for the absence of an RAPD band at a particular position. Using the NTSYS-pc version 2.02 K package [10], the transformed binary character matrix data are first transferred into the software data collection module, from which pairwise distance matrices are compiled. The genetic diversity parameters were determined using the output data and graphical module of the software, and a phylogenetic tree was created by the unweighted pair-group method arithmetic average (UPGMA) to group individuals into clusters [11].

Isolation of bacteria and fungi from wild *P. ostreatus* and soil

The stock of each sample was prepared by putting 2.0 g of freshly harvested mushroom and 5.0 g of soil into sterilized peptone water of 8 ml and 45 ml, respectively. 1.0 ml of each sample was serially diluted in different test tubes to obtain appropriate dilution. Isolation of bacteria and fungi was done using pour plate method [12]. An aliquot (1.0 ml) of each diluted sample was transferred into a Petri dish with addition of nutrient agar (Lab M Lancashire, UK) for bacteria and potato dextrose agar (Lab

Table 1 RAPD primers used for the analysis of *Pleurotus ostreatus* strains, number of all amplified DNA fragments, number of polymorphic fragments and percentage of polymorphism

Primer	Primer sequence	Number of fragment amplified (bands)	Number of polymorphic markers	% polymorphism
OPI-05	5′-TGTTCCACGG-3′	10	8	80.0
OPI-15	5′ TCATCCGAGG-3′	11	10	91.0
OPL-03	5′-CCAGCAGCTT-3′	12	9	75.0
OPL-13	5′-ACCGCCTGCT-3′	10	8	80.0
OPN-10	5′ACAACTGGGG-3′	12	8	67.0
OPP-11	5′ AACGCGTCGG-3′	13	9	69.2
OPP-18	5′-GGCTTGGCCT-3′	14	12	86.0
OPS-13	5′-GTCGTTCCTG-3′	11	9	82.0
OPS-15	5′ CAGTTCACGG-3′	12	10	83.3
OPQ-06	5′-GAGCGCCTTG-3′	9	8	88.9
	Overall	114	91	
	Average			80.24

M, Lancashire, UK) for fungi. The plates were allowed to solidify, incubated at 37 °C for 24 h and 28 ± 2 °C for 48 h for bacteria and fungi, respectively. Thereafter, the discrete colonies formed on growth media were counted and recorded as colony forming units per gram (CFU g^{-1}) for bacteria and spore-forming unit per gram (SFU g^{-1}) for fungi. The microbial mixture was transferred to the edge of an agar plate with an inoculating loop and streaked out over the surface of fresh agar plate. Pure colonies obtained were maintained on agar slants at 4 °C.

Identification of isolated microorganisms from wild mushrooms and soil samples

The isolated bacteria from wild mushrooms and soil samples were stained and subjected to biochemical tests such as catalase, citrate utilization, methyl red, Voges Proskauer, nitrate reduction, sulfur reduction, indole production, motility (SIM), triple sugar iron (TSI) and other sugar fermentation according to Cappuccino and Sherman [13] and Cheesbrough [12]. The results of biochemical tests were interpreted and identification of bacteria was carried out according to Cowan and Steel [14]. Fungi spores were stained with drops of lactophenol and viewed under a microscope. The microscopic characteristics were interpreted according to Barnett et al. [15].

Determination of soil composition around the wild mushrooms

The total organic matter and total organic carbon were determined by Walkley and Black method through chromic acid digestion [16, 17] with slight modification. Briefly, 0.5 g of each air-dried sample was put into a conical flask. Ten milliliter (10 ml) of 0.167 potassium dichromate ($K_2Cr_2O_7$) and 20 ml of concentrated sulfuric acid (H_2SO_4) were added to the soil. The content was stirred to ensure good mixing of the soil sample with the reagents. After 30 min in a fume cupboard, 200 ml of distilled water, 10 ml of concentrated H_3PO_4 and 1 ml of 0.16% diphenylamine (indicator) were added. The excess dichromate that was not reduced in the reaction was determined by volumetric titration with 1 N $FeSO_4$ solution. The percentage of sand (0.06–2.0 mm), silt (0.002–0.06 mm) and clay (less than 0.002 mm) were read on a textural triangle to determine the soil texture.

The mineral contents of the soil and mushroom were determined using the method of AOAC [18]. Briefly, each sample of mushroom and soil (1.0 g) was digested with HNO_3, H_2SO_4 and $HClO_4$ (3:1:1, v:v:v) with addition of hydrogen peroxide (500 µl) at 200 °C. The resulting solutions were cooled with 10 ml of ultrapure water (Milli-Q system, Millipore, USA) and analyzed for magnesium (Mg), iron (Fe), calcium (Ca), and zinc (Zn) with atomic absorption spectrophotometer (Buck 201 VGP). Flame photometer (Jenway PFP 7, Staffordshire, UK) was used to determine the potassium (K) and sodium (Na) contents of the samples. Each of the mineral standard solutions (1000 µg/ml Merck, Germany) was used for the calibration of the atomic absorption spectrophotometer.

Proximate composition of wild mushrooms *Pleurotus ostreatus*

The proximate composition of dried mushrooms was determined according to the method of AOAC [18]. Briefly, the moisture content in the mushrooms was determined by air oven drying 5 g of each sample at 110 °C for 2 h. The ash content was determined by incinerating the mushrooms (5 g) at 550 °C for 4 h, while the crude fiber in the mushroom samples was determined by

dilute acid and alkali hydrolysis. The fat content in *Pleurotus* mushrooms was determined by extracting with diethyl ether in a Soxhlet apparatus, and the crude protein in *Pleurotus* mushrooms was determined by using Kjeldahl method and multiplying by 4.38. The total carbohydrates in the mushroom samples were calculated by difference using the formula below:

$$\begin{aligned}&\text{Carbohydrates (\%)}\\&\quad = 100 - [\text{moisture (\%)} + \text{crude fiber (\%)}\\&\qquad + \text{fat (\%)} + \text{ash (\%)} + \text{crude protein (\%)}].\end{aligned}$$

Statistical analysis

Data obtained during the experiment were subjected to analysis of variance. Variables with significant effects were characterized using Duncan's new multiple range test at $p \leq 0.05$ with the aid of SPSS version 17.0, Chicago, Illinois, USA.

Results and discussion

A total of 114 positive DNA bands were observed among the wild *P. ostreatus* based on RAPD analysis, showing total number of polymorphic markers of 91 and accounting for percentage of polymorphism ranging from 67 to 91% (Table 1). Typical RAPD gel photographs with marker bands of wild *P. ostreatus* are shown in Fig. 2. The outcome of Jacquard estimates with NTSYS using neighbor-joining cluster (Fig. 3) grouped the studied *P. ostreatus* into two clusters. *Pleurotus ostreatus* obtained from Ala quarters and Igbatoro Road in Akure labeled as 1 and 2, respectively, belonged to the same clade, showing 100% similarity. The harvested *P. ostreatus* from Ido-Ekiti (3) and Usi-Ekiti (4) are also genetically related. The pairing of *P. ostreatus* from Ado-Ekiti (5) in the same clade with *P. ostreatus* in Ala quarters and Igbatoro Road in Ondo State indicated a genetic relatedness. The RAPD cluster of *Pleurotus* species revealed both genetic relatedness

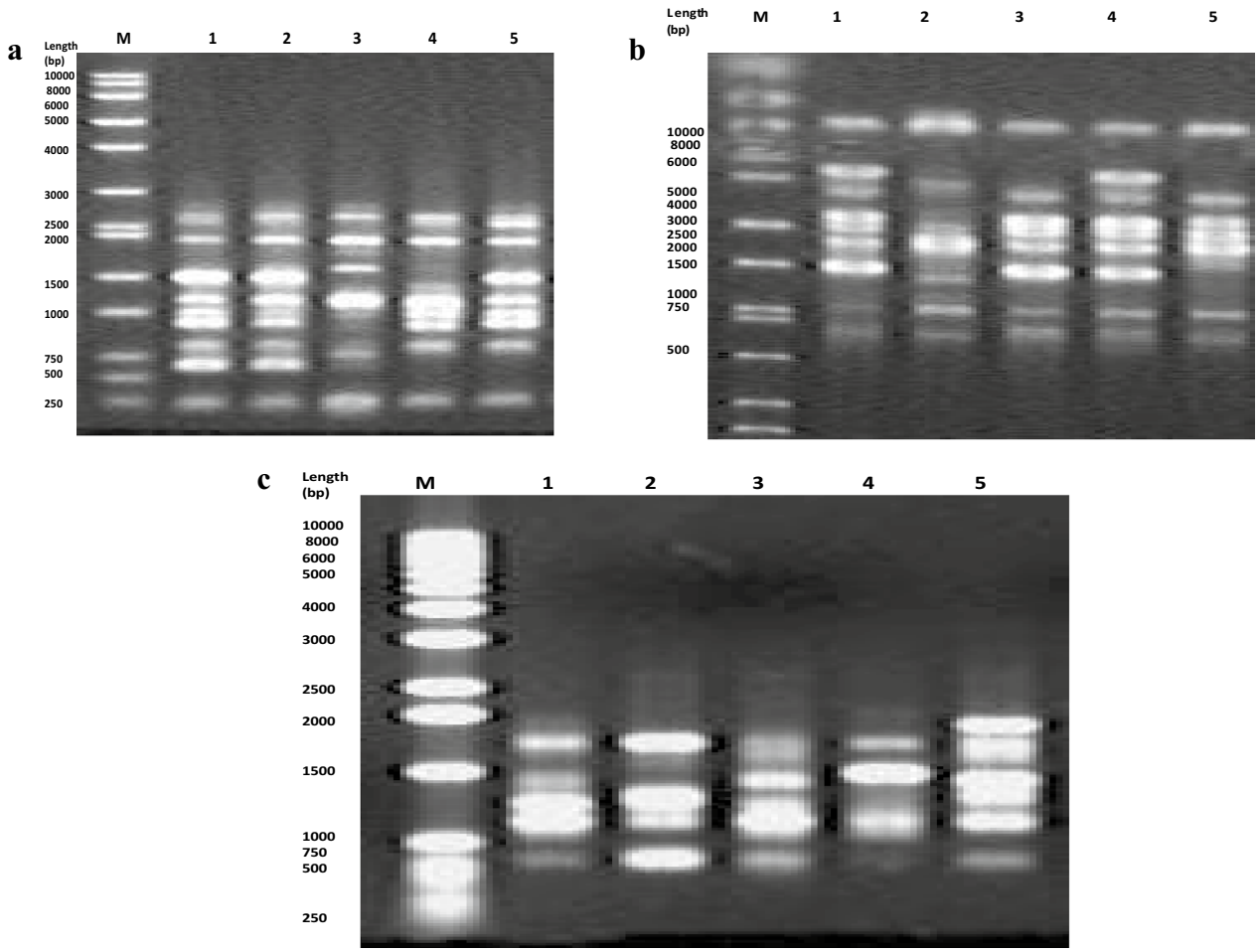

Fig. 2 Typical RAPD gel photographs with marker bands of wild *P. ostreatus*; **a** OPP 18, **b** OPS 15 and **c** OPQ-06, M: marker; 1: *P. ostreatus* from Ala quarters in Akure; 2: *P. ostreatus* from Igbatoro Road in Akure; 3: *P. ostreatus* from Ido-Ekiti; 4: *P. ostreatus* from Usi-Ekiti; and 5: *P. ostreatus* from Ado-Ekiti

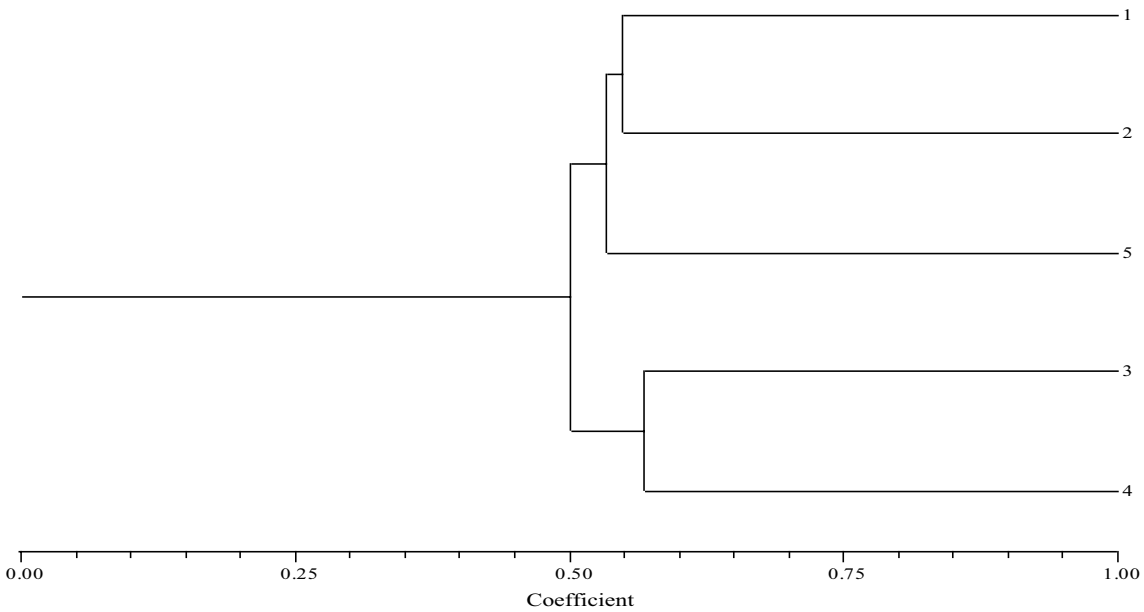

Fig. 3 Dendrogram of wild *P. ostreatus* from different geographic areas and labeled as 1: *P. ostreatus* from Ala quarters in Akure; 2: *P. ostreatus* from Igbatoro Road in Akure; 3: *P. ostreatus* from Ido-Ekiti; 4: *P. ostreatus* from Usi-Ekiti; and 5: *P. ostreatus* from Ado-Ekiti based on polymorphic RAPD markers showing genomic diversity of fungal strains using neighbor-joining cluster analysis method produced from Jacquard estimates with NTSYS Spc Version 2.02

and difference according to their geographical origins. The genetic relatedness could be attributed to the wide dispersal of fungi spore through a short- or long-distance distribution of *Pleurotus* species in the natural population across forest fragmentation, being promoted by human and animal movement [19].

The outcome of phylogenetic relation based on molecular analysis between different or similar populations of *Pleurotus* species provides a better resolution and understanding of their biogeography and speciation [5]. RAPD is a valuable method in distinguishing the different genotypes in mushrooms and evaluating their genetic similarities [7]. Other various biochemical and molecular techniques applied for genetic diversity, relatedness and identification of *Pleurotus* species were highlighted by Maftoun et al. [20] and Correa et al. [4]. These methods have been verified to be efficient in identification, expressed biodiversity in taxonomic system and unique in the selection of fungal strains.

The enumeration of bacteria (1.20×10^4 to 6.10×10^8 CFU g^{-1}) and fungi (1.50×10^3 to 7.40×10^7 SFU g^{-1}) from wild *P. ostreatus* and soil around *P. ostreatus* is presented in Table 2. The microbial count was more correlated to the organic matter than the genetic relationship exhibited by *Pleurotus* mushrooms. It was noted that soil from Usi-Ekiti possesses the highest microbial count: 7.40×10^7 SFU g^{-1} for fungi and 6.10×10^8 CFU g^{-1} for bacteria. The highest organic content (5.90%) in the location may require high microbial communities to improve nutrient cycle. Increase in soil bacteria and fungi creates microbial metabolic function to improve the soil biological characteristics [21]. The occurrence of isolated bacteria and fungi from *P. ostreatus* and their associated soil at different locations is shown in Tables 3 and 4. The presence of *Pseudomonas putida* conformed to the findings of Cho et al. [22] and Young et al. [23]. In their studies, they revealed the bacterium as one of the mushroom growth-promoting bacteria (MGPB) during the growth of *P. ostreatus* and *Agaricus bisporus*. *Pseudomonas putida* initiates primordial development and increases the yield of cultivated mushrooms [24], but Munsch and Alatossava [25] revealed that other members of the genus *Pseudomonas* exhibited saprophytic and pathogenic activities on *A. bisporus* and *Pleurotus* spp.

The species of *Paecilomyces* and *Penicillium* have been associated with white button mushroom in the study of Siyoum et al. [26]. The researchers revealed the microbial succession of compost, casing and mushroom using a plate count technique, denaturing gradient gel electrophoresis (DGGE) and sequencing of 16S and 18S rDNA. *Trichoderma* spp. was isolated with the highest occurrence from the examined *P. ostreatus* (33.30%) and their associated soil (17.10%). The cellulolytic filamentous

Table 2 Total bacterial and fungal count from harvested wild *Pleurotus ostreatus* and soil associated with their growth

Sample	Wild *P. ostreatus*		Soil around wild *P. ostreatus*	
	Bacteria (CFU g^{-1})	Fungi (SFU g^{-1})	Bacteria (CFU g^{-1})	Fungi (SFU g^{-1})
1	$1.20^{a} \times 10^{4}$	$1.50^{a} \times 10^{3}$	$1.70^{a} \times 10^{7}$	$5.00^{b} \times 10^{6}$
2	$1.60^{b} \times 10^{4}$	$2.60^{c} \times 10^{3}$	$2.30^{b} \times 10^{7}$	$5.30^{c} \times 10^{7}$
3	$2.40^{c} \times 10^{4}$	$2.00^{b} \times 10^{3}$	$5.40^{d} \times 10^{7}$	$6.90^{d} \times 10^{6}$
4	$4.70^{d} \times 10^{5}$	$3.40^{d} \times 10^{4}$	$6.10^{e} \times 10^{8}$	$7.40^{e} \times 10^{7}$
5	$4.50^{d} \times 10^{5}$	$2.50^{c} \times 10^{3}$	$4.10^{c} \times 10^{8}$	$4.80^{a} \times 10^{7}$

Values are mean of replicates ($n = 5$)

Mean values with the same superscript letters along the column are not significantly different ($p \leq 0.05$)

1: *P. ostreatus* and associated soil from Ala quarters; 2: *P. ostreatus* and associated soil from Igbatoro Road; 3: *P. ostreatus* and associated soil from Ido-Ekiti; and 4: *P. ostreatus* and associated soil from Usi-Ekiti. 5: *P. ostreatus* and associated soil from Ado-Ekiti

Table 3 Occurrence (%) of bacteria and fungi isolated from wild *Pleurotus ostreatus* at different habitats in Ondo and Ekiti States

	POAL	POIT	POID	POUS	POAE	N	% occurrence
Bacteria							
Aerococcus viridans	−	−	−	−	+	1	5.55
Actinomyces bovis	+	+	−	−	−	3	16.70
Pseudomonas putida	−	−	+	+	+	3	16.70
Fungi							
Fusarium spp.	+	+	−	−	−	2	11.10
Mucor hiemalis	+	−	−	+	−	3	16.70
Trichoderma spp.	+	+	+	+	+	6	33.30

+: present; −: absent

POAL: *P. ostreatus* from Ala quarters in Akure; POIT: *P. ostreatus* from Igbatoro Road in Akure; POID: *P. ostreatus* from Ido-Ekiti; POUS: *P. ostreatus* from Usi-Ekiti; POAE: *P. ostreatus* from Ado-Ekiti; N: number of isolates

Table 4 Occurrence (%) of bacteria and fungi isolated from soil associated with the growth of wild *Pleurotus* species at different locations in Ondo and Ekiti States

	SOAL	SOIT	SOID	SOUS	SOAE	N	% occurrence
Bacteria							
Klebsiella edwardsii	−	−	+	−	−	1	2.80
Bacillus subtilis	−	−	+	+	−	2	5.70
Actinomyces bovis	−	−	−	+	+	2	5.70
Streptomyces spp.	+	+	+	+	−	4	11.40
Pseudomonas putida	+	+	+	+	+	5	14.30
Fungi							
Phialophora verrucosa	−	−	−	−	+	1	2.80
Paecilomyces victoriae	−	−	−	+	−	2	5.70
Fusarium spp.	+	−	−	+	−	2	5.70
Mucor hiemalis	+	+	−	−	−	2	5.70
Microascus brevicaulis	+	+	−	−	+	3	8.60
Penicillium italicum	+	+	+	+	+	5	14.30
Trichoderma spp.	+	+	+	+	+	6	17.10

+: present; −: absent

SOAL: soil associated with the growth of wild *P. ostreatus* in Ala quarters; SOIT: soil associated with the growth of wild *P. ostreatus* in Igbatoro Road; SOID: soil associated with the growth of wild *P. ostreatus* in Ido-Ekiti; SOUS: soil associated with the growth of wild *P. ostreatus* in Usi-Ekiti; SOAE: soil associated with the growth of wild *P. ostreatus* in Ado-Ekiti; N: number of isolates

Table 5 Composition of soil (%) associated with the growth of wild *Pleurotus ostreatus* from different geographical areas in Ondo and Ekiti States

Soil	Sand	Silt	Clay	TOM	TOC	K	Na	Mg	Ca	Fe	Zn
SOAL	$82.00^{d} \pm 1.73$	$5.66^{b} \pm 0.58$	$10.30^{c} \pm 0.58$	$1.41^{a} \pm 0.03$	$0.82^{a} \pm 0.02$	$651.84^{c} \pm 3.90$	$102.89^{a} \pm 5.90$	$218.01^{a} \pm 11.0$	$319.11^{a} \pm 20.17$	$189.1^{d} \pm 8.12$	$161.90^{a} \pm 8.02$
SOIT	$88.33^{e} \pm 1.00$	$3.33^{a} \pm 0.58$	$3.67^{a} \pm 0.50$	$2.94^{b} \pm 0.04$	$1.70^{b} \pm 0.03$	$573.80^{a} \pm 8.09$	$176.11^{b} \pm 0.80$	$309.77^{c} \pm 14.10$	$500.89^{b} \pm 33.8$	$98.11^{b} \pm 0.96$	$204.14^{b} \pm 0.94$
SOID	$66.30^{a} \pm 1.15$	$11.33^{d} \pm 1.15$	$17.70^{d} \pm 0.60$	$2.95^{b} \pm 0.12$	$1.72^{b} \pm 0.05$	$942.71^{e} \pm 14.7$	$376.09^{d} \pm 4.99$	$285.14^{b} \pm 7.90$	$694.66^{d} \pm 27.9$	$108.80^{c} \pm 2.18$	$215.9^{c}7 \pm 1.89$
SOUS	$75.00^{c} \pm 0.74$	$7.80^{c} \pm 1.15$	$7.00^{b} \pm 0.08$	$5.90^{d} \pm 0.70$	$4.32^{d} \pm 0.45$	$614.18^{b} \pm 11.67$	$402.81^{e} \pm 16.0$	$407.07^{d} \pm 19.45$	$548.09^{c} \pm 23.90$	$79.09^{a} \pm 1.98$	$391.09^{d} \pm 3.90$
SOAE	$68.30^{ab} \pm 1.58$	$11.30^{d} \pm 0.03$	$12.33^{c} \pm 1.05$	$5.17^{cd} \pm 0.03$	$3.00^{c} \pm 0.05$	$701.55^{d} \pm 17.7$	$217.15^{c} \pm 8.90$	$394.11^{d} \pm 11.89$	$715.26^{e} \pm 37.8$	$287.90^{e} \pm 2.09$	$217.09^{c} \pm 8.90$

Values are mean $\pm$ SD of replicates ($n = 3$)

Means with the same superscript letters down the column are not significantly different ($p \leq 0.05$)

SOAL: soil associated with the growth of wild *P. ostreatus* in Ala quarters; SOIT: soil associated with the growth of wild *P. ostreatus* in Igbatoro Road; SOID: soil associated with the growth of wild *P. ostreatus* in Ido-Ekiti; SOUS: soil associated with the growth of wild *P. ostreatus* in Usi-Ekiti; SOAE: soil associated with the growth of wild *P. ostreatus* in Ado-Ekiti; TOM: total organic matter and TOC: total organic carbon

Table 6 Proximate composition (on dry basis %) and mineral profile (mg/100 g) of harvested wild *P. ostreatus* from different locations in Ondo and Ekiti States

Fungi	Moisture	Ash	Crude fiber	Fat	Protein	CHO	K	Na	Mg	Ca	Fe	Zn
POAL	$8.10^{b} \pm 0.13$	$6.26^{a} \pm 0.57$	$23.00^{b} \pm 0.25$	$0.95^{a} \pm 0.10$	$17.30^{a} \pm 0.32$	$44.80^{c} \pm 0.54$	$93.80^{b} \pm 8.21$	$0.93^{a} \pm 0.04$	$48.62^{b} \pm 1.30$	$50.30^{c} \pm 1.86$	$22.80^{a} \pm 0.94$	$13.89^{a} \pm 0.58$
POIT	$10.19^{c} \pm 0.16$	$9.17^{c} \pm 0.40$	$24.85^{c} \pm 0.39$	$1.77^{c} \pm 0.67$	$22.65^{c} \pm 0.39$	$33.27^{b} \pm 0.14$	$148.90^{c} \pm 18.70$	$3.01^{b} \pm 0.03$	$79.20^{c} \pm 2.06$	$40.90^{a} \pm 1.00$	$81.03^{e} \pm 1.04$	$43.00^{b} \pm 0.30$
POID	$7.30^{a} \pm 0.05$	$8.45^{b} \pm 0.02$	$19.50^{a} \pm 0.38$	$1.70^{c} \pm 0.05$	$17.90^{a} \pm 0.44$	$44.80^{c} \pm 0.51$	$221.06^{e} \pm 15.11$	$2.91^{b} \pm 0.00$	$111.40^{e} \pm 22.0$	$80.59^{d} \pm 0.81$	$67.77^{d} \pm 0.14$	$25.89^{bc} \pm 0.90$
POUS	$10.05^{c} \pm 0.74$	$7.80^{b} \pm 0.55$	$25.04^{c} \pm 0.09$	$0.85^{a} \pm 0.01$	$24.07^{d} \pm 0.66$	$30.06^{a} \pm 0.33$	$181.50^{d} \pm 23.90$	$1.78^{a} \pm 0.04$	$104.78^{d} \pm 17.0$	$58.80^{b} \pm 4.88$	$26.10^{b} \pm 0.10$	$32.80^{b} \pm 1.45$
POAE	$7.90^{a} \pm 0.03$	$8.31^{b} \pm 0.44$	$19.05^{a} \pm 0.30$	$1.08^{b} \pm 0.25$	$18.36^{ab} \pm 0.23$	$45.10^{c} \pm 0.53$	$71.08^{a} \pm 9.04$	$4.05^{c} \pm 0.01$	$27.00^{a} \pm 2.89$	$101.78^{e} \pm 6.05$	$34.84^{c} \pm 1.09$	$10.71^{a} \pm 1.90$

Values are mean ± standard deviation (SD) of replicates ($n = 3$)

Means with the same superscript letters down the column are not significantly different ($p \leq 0.05$)

POAL: *P. ostreatus* from Ala quarters in Akure; POIT: *P. ostreatus* from Igbatoro Road in Akure; POID: *P. ostreatus* from Ido-Ekiti; POUS: *P. ostreatus* from Usi-Ekiti; and POAE: *P. ostreatus* from Ado-Ekiti; CHO: carbohydrates

fungi are responsible for green mold disease on mushrooms and spoilage of mushroom spawns [27]; the mold causes substantial and economic losses of some edible mushrooms, namely, species of *Pleurotus*, *Agaricus* and *Lentinula* in South Korea, Italy, Hungary, Canada, USA, Iran, New Zealand and Romania [28, 29]. *Streptomyces* spp., *Bacillus subtilis*, *Actinomyces bovis*, *Pseudomonas putida, Penicillium italicum, Fusarium* spp. and others were isolated from the soil around wild *P. ostreatus*. *Streptomyces* spp. produce indole-3-acetic acid (IAA), which acts as biocontrol against soil-borne pathogenic microorganisms. Many microorganisms exert growth-promoting effects by secreting beneficial secondary metabolites and enzymes that support spore germination and fruiting formation of wild mushrooms [30, 31]. The microbial ecology couples with optimal environmental conditions, and available nutrient or substrate composition are also selective parameters for the colonization and growth of mushrooms or other ectomycorrhizal fungi [32, 33].

In addition, soil microorganisms markedly influence soil composition by playing a key role in decomposing organic matter, cycling of carbon, nitrogen, phosphorous and stabilization of soil structure [34]. Therefore, the constituent of soil microorganisms could be a sensitive indicator of the growth of wild mushrooms.

The low occurrence and absence of some microorganisms from the examined mushrooms and soil may be due to shortcomings of traditional methods, that is, routine microbiological method adopted for microbial cultivation. This microbiological technique offers a narrow array for microbial isolation due to the fact that some microorganisms may remain viable but non-cultivable (VBNC). The method is only suitable for fast-growing and non-fastidious microbes, and overgrown fungi capable of producing large amounts of spores suppressing other fungi [35]. Another perception in relation to the low microbial occurrence could be associated with antibacterial, antifungal agents, lytic enzymes and volatile compounds secreted by *Pleurotus* mushrooms and their flora microorganisms, which contribute to the survival strategies of the edible fungi by reducing the occurrence of spoilage microorganisms, since mushrooms are often confronted by different types of bacteria, fungi and viruses [36, 37]. Hence, in associated ecosystem processes, a succession of microbial communities on wild mushrooms and their habitat (soil) involved intra- or interspecific interaction of different associations, which may include antibiosis, competition and mutualism [38].

Soil composition around wild *Pleurotus* mushrooms is presented in Table 5, while the nutrient contents of harvested wild *P. ostreatus* from different geographical locations in Ondo and Ekiti States are presented in Table 6. The sand content in the soil around *P. ostreatus* ranged from 66.30 to 88.33%. The clay contents of 10.30, 12.33 and 17.70% were obtained for soil collected around wild *P. ostreatus* at Ala quarters, Ado-Ekiti and Ido-Ekiti, respectively. They were higher ($p \leq 0.05$) than those of Igbatoro (3.67%) and Usi-Ekiti (7.00%). The highest organic content (5.90%) in the soil around *P. ostreatus* mushroom harvested from Usi-Ekiti could contribute to its highest protein (24.07%) and crude fiber contents (25.04%). Nakalembe et al. [39] reported a high concentration of nutrient in edible mushrooms from the humid zone, which could be as a result of higher organic matter in the soil. Organic substances in the soil support high microbial biomass, metabolic activities and, thus, the assembly of more active nutrients into the food crops [40, 41]. The moisture, ash, dietary fiber, protein and carbohydrate contents in the harvested *P. ostreatus* mushrooms ranged from 7.30 to 10.19%, 6.26 to 8.45%, 19.05 to 25.04%, 0.85 to 1.77%, 17.30 to 24.07% and 30.06 to 45.10%, respectively. These values are within what were reported in the literature for proximate composition of *Pleurotus* spp. by the findings of Maftoun et al. [20]. Essential minerals, potassium (K), magnesium (Mg), calcium (Ca), iron (Fe) and zinc (Zn) in the studied mushrooms ranged from 71.08 to 221.06 mg/100 g, 27.00 to 111.40 mg/100 g, 40.90 to 101.78 mg/100 g, 26.10 to 81.03 mg/100 g and 10.71 to 43.00 mg/100 g respectively. The mineral contents of edible fungi may vary from one species to another based on their varieties, absorption mechanisms from the ecosystem, soil nutrient, mineral concentration in the substrates and environmental conditions [42]. Low fat (0.85–1.77%) and sodium (0.93–4.05 mg/100 g) contents were observed in the *Pleurotus* mushrooms. Several studies have indicated edible mushrooms as a better source of protein, dietary fibers, carbohydrate and essential minerals due to their ability to bioaccumulate nutrients into their fruit body with low or no cholesterol [43].

Conclusion

The study examined *Pleurotus* spp. indigenous to two states in Southwestern Nigeria and revealed their genetic relatedness and diversity. The use of molecular technology needs to be adopted to ascertain proper identification and documentation. The varying degrees in the microbial and nutrient contents of the studied wild *P. ostreatus* could be as a result of environmental factors, substrate constituent and geographical locations.

Authors' contributions

All the authors made substantial contributions to the conception, design and acquisition of the research study. TVF carried out the data collection and OCO did the interpretation. The manuscript was drafted by OCO and revised by all authors to meet up with the intellectual content. All authors read and approved the final manuscript.

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