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Article

Diagnosis of two local mushroom species (*Pleurotus* spp.) and their production management

Abeer Kadhim Jasim Al-Falahi[1], Ahmed Kareem Alsadaawy[2] and Rukaibaa Ali Chechan[3]*
[1]Ministry of Health and Environment, Baghdad health directorate-Al-kara, Baghdad, Iraq.
[2] Organic Agriculture Center, Plant Protection Directorate, Ministry of Agriculture, Baghdad, Iraq.
[3]Department of Food Science, College of Agricultural Engineering Sciences, Baghdad University, Baghdad, Iraq.
* Correspondence: : roqaibaa.ali@coagri.uobaghdad.edu.iq
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Abstract
The present study confirmed the identity of two Iraqi mushrooms by using the internal transcribed spacer (ITS) sequence; the genomic DNA of two isolates was amplified using ITS1 and ITS4 primers, and the data analyzed through Basic Local Alignment Tool(BLAST) search was achieved using the National Center for Biotechnology Information (NCBI) database. The result shows that the nucleotide sequence of two mushrooms blasted against the sequence from the Gene Bank database that *Pleurotus ostreatus* and *Pleurotus eryngii* matched 99% and 100%, respectively. The second stage of the current study was the comparison of different agro-waste, including wheat straw(WS), corncob(CC) and sawdust(SD) supplemented with 25% wheat bran(W) and 2% calcium carbonate (CaCo3) on mycelium growth, yield and biological efficiency (BE) of local oyster mushroom which comparison with Chinese strain *pleurotus ostreatus*121 and *pleurotus eryngii* 080. The highest growth average of local *P. ostretus* on the SD substrate was 1.50 cm, and *P.eryngii* was recorded at 1.18 cm. The substrate CC was the most suitable for the yield of mushroom local *P. ostreatus* and *P. ostreatus*121(485.40 and 418.50 g/bag, respectively), while local *P. eryngii* recorded 470.40 in the same substrate. It was also found that the WS substrate recorded the highest BE for each local isolate and Chinese strain.

Keywords*:* Iraqi strains, *Pleurotus spp*., agricultural wastes, wild agricultural mushrooms

Introduction

Pleurotus spp. (oyster mushroom) belongs to the family Pleurotaceae, identified by their color and habitat. They are commonly domesticated as fungal saprotrophic and rarely parasites on the root of herbaceous plants like P.eryngii [1, 2, 3]. The public often cultivates oyster mushrooms on different lignoselleose waste because it requires nutrients such as lignin cellulose, hemicelluloses, and protein in wood and agricultural waste [3, 4, 5]. Mushrooms, in general, produce a variety of lignocellulose enzymes, both intracellular and extracellular, especially the enzymatic system of Pleurotus spp. (white–rot fungi) which can mineralize lignin fibers in the substrate to CO_2 and water [6, 4, 7]. Pleurotus spp. Like other mushrooms, they are widely known to be thermo sensitive to temperatures over 23C°, restricting their cultivation [8, 9]. Despite the geography of Iraq's tropical climate, which gives a hot summer accompanied by low humidity levels, there

are many kinds of Pleurotus spp. isolates were Suitable for growth in the Iraqi environment. So, the estimation of genetic diversity between local Species is crucial for the improvement of isolates and preserving this vital wealth [10, 11, 12]. The goal of this study was to identify by using morphological and molecular techniques of two local isolates and to grow them in the indoor environment to Know the production conditions and obtain the highest productivity.

Methods and material

Morphological and genetic identification

Isolates of two wild mushrooms were collected from Baghdad city (Latitude 33.3152° N Longitude 44.3661° E) from the natural habitats. Genetic characterization of genomic DNA from pure isolates mycelia was the detection of ITS gene using primer for amplification.ITS1 forward prime(5-TCCGTAGGTGAACCTGCGG-3) and the ITS4 reverseprimer (5-TCCTCCGCTTATTGATATGC-3) [10] . PCR products were separated by 1.5% agarose gel at 5 volts/ cm, photographed using a UV (302 nm) and delivered to Microgen Inc. (Seoul, Korea) for sequencing. ITS sequence of two isolates analyzed by using the Basic Local Alignment Search Tool (Blast) program, which is available at the National Center Biotechnology Information (NCBI) online at http://www.ncbi.nlm.nih.gov.

Average of daily mycelium growth

The daily growth mycelium of local isolates Pleurotus ostreatus (L1) and P. eryngii (L2)compared with growth of Chinese species P. ostreatus 121(F1) and P. eryngii 080 (F2) obtained from University Of Fujian Agriculture and Forestry /JUNCAO Research Institute, for evaluation of daily growth on four the substrate wheat straw (WS), Corn cobs (CC), Sawdust (SD), and Albizia tree waste(AL) supplemented with 25%Bran Wheat (W) and 2% calcium carbonate ($CaCo_3$) which filled into a test tube (20 cm long and 3cm wide. The tubs were plugged with cotton wool and sterilized in an autoclave at 121 C for one hour. Inoculation with Plugs of 5 mm diameter was cut from the actively growing mycelial colony. The tubs incubated at 25Cº in the dark, and the mycelium elongation position was indicated as the beginning point according to [17]. The daily growth rate determined as [13, 14] the mycelium growth rate (cm) \ number of days for growth.

Yield Performance of Oyster Mushroom

The substrates mentioned in 2-2 were chopped into small pieces of 1-2 cm except sawdust and wetted to reach 60-70 % and filled into a polypropylene bag (20 ×40 cm), autoclaved at temperature 121Cº for one hour. Inoculated with 30gm of the spawn of Pleurotus spp separately (prepared through tissue culture technique in National Center For Organic Farming \ Ministry of Agriculture), the dry weight of the substrates was recorded before wetting. After mycelium colonization, the bag was transferred to the production room. The relative humidity and temperature were under a controlled system, and the yield parameters were recorded as [15]:

Production yield (total weight of fruiting body taken from three Replicators and considered as total yield production)

Biological efficiency (BE%) using the formula[16]

$$BE\% = \frac{\text{yield of fresh mushroom in gram}}{\text{total weight of dry substrate in gram}} \times 100$$

Data were collected, and analysis of variance (ANOVA) was conducted using SPSS; the mean value and standard error of each parameter were separated by using LSD at a 5% level of significance.

Results

1 Morphological and genetic characterization of isolates fungi

Description of Pleurotus ostreatus (L1) Cap expanded to broadly convex, eventually flat and even in age. The diameter was 5-15 cm. The color of the fruit body is brown to grey—figure 1 (A). The cap margin is smooth to undulating like an Oyster shell, and the stem is typically eccentrically attached to the cap and short; Lamellae are whitish to gray and It collected in winter and autumn at temperatures between 15 -20 Cº. Figure 1 (B) shows a local isolate of Pleurotus eryngii. The diameter of the cap was 3-12cm at first, convex expanding with age, becoming funnel-shaped with a margin typically inrolled, stem 3-10cm in length, central, thick tapering downwards.

Figure 1. *Pleurotus* Isolates collected from natural habitats of Baghdad. A (*Pleurotus ostretus*), B (*Pleurotus eryngii*).

The results of the genetic characterization of selected isolates (Figure 4) indicated that PCR products from the primers ITS1 and ITS4 have number scores of 1511 and 1464 bits for Pleurotus ostretus and Pleurotus eryngii, respectively. The Blast results for both collected isolates were analyzed with a database in Gene bank(Figures 2 and 3), produced sequence similarity percentage (99%) against ITS sequence of deposited species Pleurotus ostretus sequence (accession number MW276128.1), and similarity percentage (100%) against ITS sequence of deposited speciesPleurotus eryngii sequence (accession number JX429941.1).

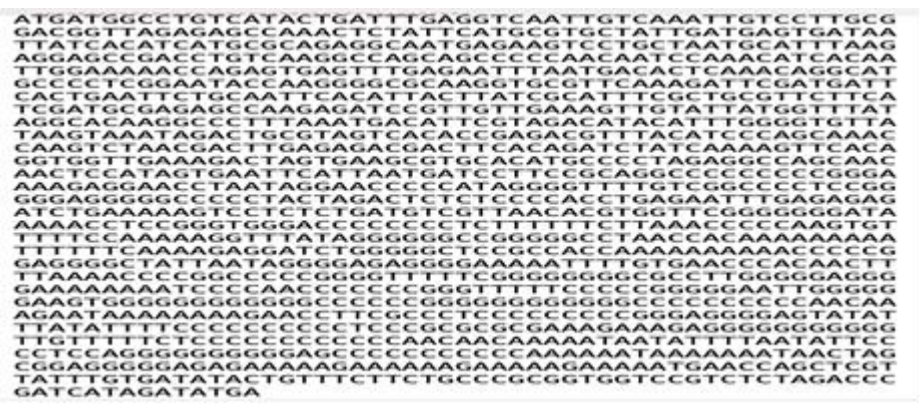

Figure 2. Nucleotide sequence of *Pleurotus ostretus*.

GAATGCTTAAGCCTATTAAATTCCAATTGAGGTCAATTGTCAAATTGTCC
TTGCGGACGATTAGAGAGCTGGACTCTATTCATGCGTGCTATTGATGAGT
GATAATTATCACATCATGCGCAGAGGCAATGAGAAGTCCTGCTAATGCAT
TTAAGAGGAGCCGACCTGTCAAGGCCAGCAGCCCCCAACAATCCAAACAT
CACAATTGGAAAGAAACCAAAGTGAGTTTGAGAATTTAATGACACTCAAA
CAGGCATGCCCCTCGGAATACCAAGGGGCGCAAGGTGCGTTCAAAGATTC
GATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCG
TTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTTGAAAGTTGTATTAT
GGTTTAAAGGCACAAGGCCCATTAAATGACATTCGTAGACATACATTTGG
GGTGTGTAAGTAAATAGACTGCGTAGTCACACCGAGACGTTTAAATCCCA
GCAACCAAGTCTGACGACTTGAGAGACGACTTCACAGATCTATCAAAAGT
TCACAGGTGGTTGAAAGACTAGTGAAGCGTGCACATGCCCCTAGAGGCCA
GCAACAACTCCATAGTGAATTCATTAATGATCCTTCCGCAGGTCACCCCT
TCCGGAAAGAGGGCCCCCTGAAGAACCCAACAGAGAGGTGGCGGGCGGCT
CTGGGGGGTGTGGGCCCCCTACCTAGTCTTTCCGCCACCTGGTCAACTTTG
GATAAAATCTGTAAAGTCTCCCTCACGTGGTCAAAACACGTTAGCTGGCG
GAGTAAAACAACGGGGTGGTGCACCAACTCTGTTTACTTACCCACTCAAC
GTTAATTCTATGATATACAATAGAGGAGCCGGGGGGCTTAATCCAAAAAA
ACCTTTTATACAACGAATTTTGCGGCCTGGGCTCTTTAATACATAAACAC
GAGTGAGCATCGAAACAGGAATGGGGGAAAAAAATTGGGGACCCCCCGAC
TTTTTGTTGACCCCCTCGCCCCCCTGTCTGACGTAACGGGGGGGGCCCCCT
TTGGGTTTTCGGGAAGATAAACAAACCCCTTCCCTGTTTTTTTCTCACTT
CCCAGAGAAATAAGGTAGTATGTGTTGGGTGGGCCCTGCCCGCTGCCGTG
TTGGTGTTCCCTCCCGTGTAATAAGATAAAATAGAAGAGAGACTCGATCT
CCCCCCCTGCGCATGAGAGAATGAAAAAAAAAATATAATTAACTATATAAC
TAATGGAAAAAAGAAAAGGTTATTTTTTTTTTCCCCTCCCATTAGGCGA
CGGGTTGTTTTATTGTATCTAGTATCGGACTGAATTCTACTCCCAACACA
GAGACTGTTTTTTCTTCTTAAAGAAGAAAAAAAAAAAAAAAATATATGAA
TATACACGCCCCGAATTAATACTATATTAATTTTTATAACGAGTAAAATA
TAAAAAAAATATTATTCACCTTTGGATAGGCCGCTTTTTATTTTTTATAA
TTTAGATTGTT

Figure 3. Nucleotide sequence of *Pleurotus eryngii*.

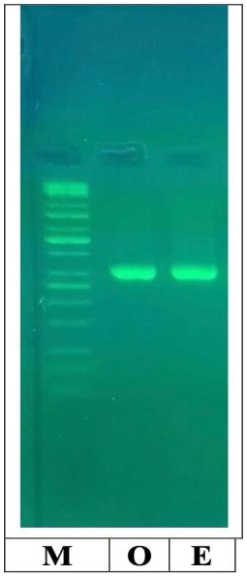

Figure 4. Agarose gel electrophoreses of PCR amplified product of *Pluerotus ostreatus* and *Pleurotus eryngii*: M (DNA marker), O(*Pluerotus ostreatus*), E (*Pleurotus eryngii*).

2 -Impact of substrate on growth and production of oyster mushroom

Mycelia growth on four different substrates (corn cobs, wheat straw, sawdust and albizia tree waste)differenced significantly; L1 and F1on SD substrate showed faster mycelia growth at 1.50 and 1,60 cm, followed by CC (1.28 and 1.43 cm)respectively (Figure 5)while L2 and F2 recorded 1.18 cm,1.22 cm on SD substrate respectively. However, mycelia growth of L2 did not differ significantly between SD and CC. Similarly, the slowest mycelia growth was observed in the AL substrate. (Table 1)

L2	F2	L1	F1	Substrate
0.700	0.623	0.933	0.987	WS
1.180	1.073	1.283	1.437	CC
1.180	1.220	1.503	1.600	SD

0.193	0.253	0.210	0.190	AL
0.168		0.207		LSD

Table 1. Vertical mycelium growth on different waste.

The values are presented as the mean of three triplicates: L1(Isolate pleurotus ostratus), L2(Isolate pleurotus eryngii), F1(pleurotus ostratus), F2 (pleurotus eryngii), WS(Wheat straw), CC (Corn cobs), SD (Saw dust) AL(Albisia tree waste).

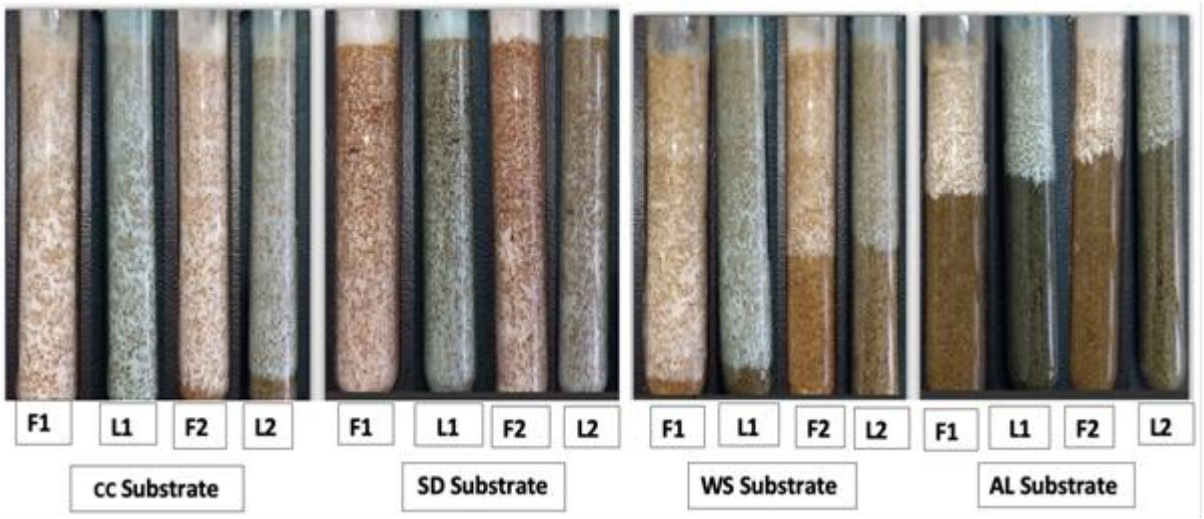

Figure 5. Vertical mycelium growth of isolates and strains of Pleurotus in substrate formulated with different waste, SD(Sawdust), CC (Corn cob), WS(Wheat straw), AL(albizia tree waste).

Biological efficiency and economic yield

The highest yield of L1 recorded on CC was 458.40 and g/bag. In contrast, L2 and F2 were recorded at 470.40 and 339.6g/bag, respectively, followed by WS (235.90 g/bag) and SD (235.50g/bag), L2 and F2 gave the lowest yield on 235.50 and 213.50g/bag respectively on the substrate SD The results recorded were very close to [21] who reported that the highest yield was (146.1g) taken from corn cobs substrate which was much higher than rest substrate in cultivation Pleurotus ostreatus.

Biological efficiency				Yield				Sub.
L2	F2	L1	F1	L2	F2	L1	F1	
88.02	91.66	87.69	97.82	235.90	242.80	232.40	259.20	WS
54.38	48.19	65.02	60.21	470.40	339.6	458.40	418.50	CC
49.58	44.94	67.53	60.82	235.50	213.50	320.80	288.90	SD
2.85		2.69		20.64		8.39		LSD

Table 2. Biological efficiency and economic yield of Pleurotus isolates and strains on different substrate.

The value are presented as mean of three triplicates,L1 (Isolate*pleurotusostratus*), L2 (Isolate*pleurotus*eryngii), F1(*pleurotusostratus*), F2 (*pleurotus*eryngii), WS (Wheat straw), CC (Corn cobs) , SD(Saw dust).

In terms of the Biological efficiency of the substrate, define the ability of the particular strain to grow on that substrate [16]—the highest biological efficiency of mushroomL1 and F1 were 87.69 and 97.82%, respectively, on WS substrate

table 2. At the same time, L2 and F2 were observed to have the highest BE from the WS substrate (88.02 and 91.66%, respectively). The present study agrees with Harunet. Al, 2016 mentioned that the BE of oyster mushrooms (Pleurotus florida) on several SD substrates ranges from 189.9% to 212.8%. [22] also found that the range of BE for P. ostreatus mushroom was between 51.3 and 125.6% on rice and wheat straw substrate enhanced with cotton seed hull. The highest yield and biological efficiency were recorded on local isolates L1 and L2 due to the distinctive high diameter of the fruiting body and thickness of the cup and stalk—figure 6.

Figure 6. fruiting body of isolates oyster mushroom grown on CC substrate, A (Pleurotus ostreatus), B (Pleurotus eryngii), CC (CornCob).

Discussion

Gill is relatively distant, thin, grayish, and decurrent. Spores are white and—growing individual or in small groups [3, 18]. However, the C/N ratio, total N, total C, pH, chemical composition and mineral content are critical factors for mycelium growth [5, 19].[20] observed that mycelium growth rate at the beginning depends on C\N ratio, primordial formation favored C\N ratio about 22-30:1the lower C\N ratio the faster mycelium growth due to nitrogen content is high, but too much of nitrogen prevents mycelium growth.

Conclusion

Iraqi environment, which is considered ecologically diverse, made it suitable for mushroom cultivation. Two isolates of Pleurotus mushrooms were recorded through the available Gen Bank database: Pleurotus ostreatus with accession number (MW276128.1), and Pleurotus eryngii with accession number (JX429941.1). The molecular identification of mushrooms matches the morphological definition up to the species level. Growing Isolates on corncob substrate was remarkably dense with high yield, which scored 438.50 and 470.40 g/bag for Pleurotus ostreatus and Pleurotus eryngii, respectively. Bioconversion of agricultural waste into high-protein mushrooms is an excellent alternative for easy and inexpensive recycling.

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