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Assesment the efficiency of some local fungal isolates in the production of Laccase enzyme

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ABSTRACT:

In this study (34) samples of fungal from infected fruits and vegetables were isolated related to the following species:*Alternariaalternata*,*Aspergillusflavus*,*A. niger*,*Fusarium sp*. and *Penicillium sp*. Their ability to produce Laccase enzyme was studied and the study showed the ability of (11) fungal isolates to produce this enzyme and the *A. alternata* is the strongest one to produce the Laccase enzyme reaching (1.35 U/ml). Optimization of some nutritional and physical factors in the basal medium in order to intensify the production from *A. alternata*. The optimization studies revealed that the maximum Laccase production was achieved when the production medium was at the following conditions: pH 5.5 sucrose as a carbon source, yeast extract as nitrogen source was (4.01 u/ ml).

KEYWORDS: Laccase enzyme,guaiacol indicator, *Alternariaalternata*, *Fusarium sp.*

1. INTRODUCTION

Laccase is oxidizing phenolic compounds that use oxygen as an electron acceptor. This enzyme include fungal Laccase and prokaryotic Laccase like enzymes that typically have multiple copper (Cu) atoms at the reaction centre (Souza *etal*., 2002). This enzyme is classified as a protein that catalyses the oxidation. A wide variety of organic and inorganic compounds by using molecular oxygen as an electronacceptor (Ardon*etal*.,1996;Kahraman& Gurdal,2002). Laccase is widely distributed among fungi, plant, insect and bacteria (Thuston, 1994). Laccases were studied intensively for many environmental and industrial application(Nandal*etal*., 2013).They play an important role in the bioremediation of contaminated soil production of medical agents (Slomczynski*et al*.,1995). Industrially Laccases are attractive significant enzymes for pulp delignification organic synthesis(Sirak*etal*., 2010).

In general there are a number of approaches to improve production of an enzyme like, strain selection optimization of production media and growth conditions. However various media have been investigated for production ofLaccase by the culture of fungal (Souza-Ticlo*etal*.,2006).This enzyme interesting for commercial biotechnology and environmental application such as paper and pulp mills, textiles and dye-making industries.

Alcohol distilleries and leather industries are some of the industries that discharge highly colure effluents. The paper and pulp industry release large volumes of intensely. Including chlorolignins, chlorophenols and chloroaliphatics(Kurtz&Champe,1982) as well as biotransformation of environmental pollutants biosensor, biofuel and organic synthesis applications(Ali &Sreekrishnaan, 2001).

Therefor in this study we aimed to find suitable supplies that can be incorporate in the culture medium and determine the favourable conditions for production of Laccase enzyme.

2. MATERIAL AND METHOD

2.1/Fungal isolate

For the collection of raw vegetables and fruits to identify dominating microbial fungal communities the main infected fruits and vegetables from markets of Mosul city / Iraq various samples were taken like potato, orange, strawberry, tomato, apple and onion, to isolate fungi from the samples depended on the method ofAl-Inzy, 2012. These samples were cultured on (PDA) media and incubated at 25±2C°. After 7 days all fungal colonies wereobtained in pure culture and all of them were visually examined for morphological characters and microscopic structures(Cauto&Iterrer, 2006).

2.2/ Basal medium used for the qualitative production of Laccase

The based medium used for optimizing the enzyme production had following composition in g/L at pH(6,0):

Glucose 10,peptone 3, KH2PO4 0.6, ZnSO4 0.001, K2HPO4 0.4, FeSO4 0.005, MnSO4 0.5, MgSO4 0.5, Guaiacol 200 µl (Khanna, 2013).

2.3/ Quantitative production of laccase

The medium used to produce Laccaseenzyme contain: KH2PO4 O.2 g, MgSO4.7H2O 0.1g, NH4Cl2 0.3g, CaCO3 1.0g in 1L distilled water, pH (5.5). Were placed in (250ml) flasks and autoclave at 121Cº for 20 min. The fungal spore'ssuspensions were prepared by adding 2ml of sterile distilled water to these freshly (7 days) grown slants of the above cultures. These suspensions were used to inoculate in (50ml) of different media into (250ml) flasks used in the present study.The flasks were incubated on a shaker with shaking at (200 rpm) at (30Cº± 1).

At desired intervals, (250ml) flasks with a growing culture of *A. alternata* (4) isolates, *Penicilliumsp*., *Fusariumsp*.,

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(two flasks for each isolate).Then the flasks were incubated for 10 days at (30±1), and flasks without inoculation were used as a control.

Laccase activity was determined spectrophotometrically depended on (Singh and Abraham,2013)

2.4/ Effect of pH on enzyme production

The effect of pH on Laccase production was determined within a pH range of (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0), by preparing the production medium at different pH then the culture medium was inoculated with the *A. alternata* isolated and incubated at 30Cº for 7 days.

2,5/ Effect of carbon source on enzyme production

Different sources of carbon (glucose, Lactose, Maltose and sucrose) were used at the concentration of (1%) w/v. After inoculating with *A.alternata* the medium was incubated at optimum pH for (5.5), and 7 days at 30C.

2.6/ Effect of different nitrogen source on enzyme production.

The Laccase production medium containing optimum sucrose of carbon and supplied with 0.1%(w/v) of different nitrogen sources (peptone, yeast extract, calcium nitrate, sodium nitrate). The medium was inoculated with the strain and incubated at optimum pH(5.5) for 7 days. In order to find the suitable nitrogen source for the maximum production of Laccase by *A. alternata*.

3/RESULTS AND DISSECTION

3.1/Fungal isolate:

different techniques were used for isolation and characterization the fungi and done with help morphology. The total number of isolated fungi were (34) strainshowed in the table (1). All isolates were identified using morphological and microscopic characterization (Cauto&Iterrer, 2006).

These results have agreement withAbdulkareem, 2012, when he used *Penicillium spp.* fungi to produce Laccase enzyme.

Table (1): The types of different fungi found to be present on fruit and vegetables

NO.	Fungi name		Results
1	*Alternariaalternata*	Tomato	+
2	*A. alternate*	Tomato	+
3	*A. alternate*	Potato	+
4	*A. alternate*	Tomato	+
5	*A. alternate*	Tomato	+
6	*A. alternate*	Tomato	+
7	*A. alternate*	Tomato	+
8	*A. alternate*	Tomato	-
13	*A. alternate*	Tomato	-
15	*A. alternate*	Tomato	-
17	*A. alternate*	Tomato	-
18	*A. alternate*	Tomato	-
23	*Aspergillus sp.*	Cucumber	-
24	*Aspergillus sp.*	Potato	-
20	*Aspergillus sp.*	Strawberry	-
26	*Aspergillusflavus*	apple	-
27	*A. Flavus*	Apple	-
11	*A. Flavus*	orange	-
28	*A. Flavus*	Potato	-
30	*A. Flavus*	Potato	-
19	*A. Flavus*	strawberry	-
9	*A. Flavus*	Tomato	-
10	*A. Flavus*	Tomato	-
25	*A.niger*	onions	-
31	*A. Niger*	strawberry	-
22	*Fusarium sp.*	strawberry	+
12	*Penicillum sp.*	Apple	-
21	*Penicillum sp.*	cucumber	-
29	*Penicillum sp.*	cucumber	-
16	*Penicillum sp.*	onions	+
32	*Penicillum sp.*	orange	+
33	*Penicillum sp.*	Potato	-
34	*Penicillum sp.*	Tomato	+
14	*Penicillum sp.*	Tomato	-

3.2/ Basal medium used for the qualitative production of Laccase

In order to find Laccase producing fungi from the fungi isolated from various environment samples a simple screening method was followed using solid media containing indicator compound guaiacol. A total of (32) species were screened and among these. (11)IsolateswereLaccase positive (Table 1). From these Laccase positive fungi *A. alternata* strain No.(1, 2, 3, 4, 5,6,7), *Penicillium sp.* (16,32,34) and *Fusarium sp*. were selected for further studies of guaiacol to form reddish brown zones in the medium was high in these (11) organisms compared to other fungi (table 1). This results as the same results (Cunha *etal*., 2003;Wood, 2016).

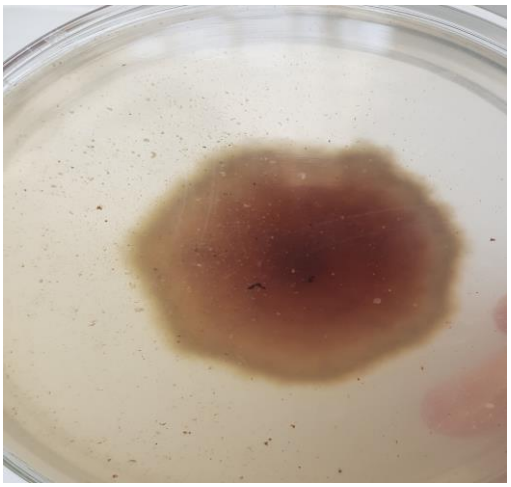

Figure (1):screening of Laccase producing by*A.alternata* usingguaiacolindicator

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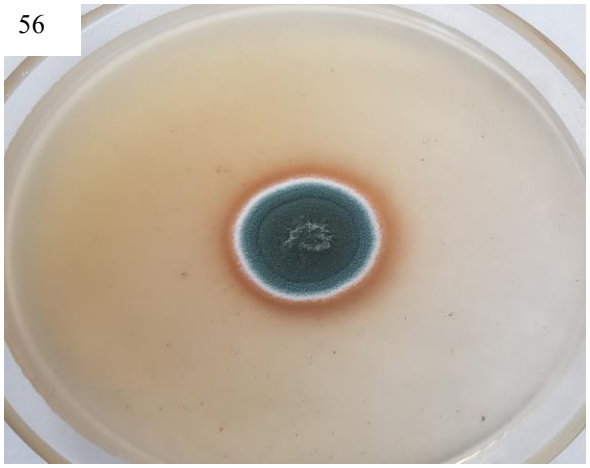

Figure (2): screening of Laccase producing by*Penicillium sp.* using guaiacolindicator

3.3/ Quantitative production of laccase:

Laccaseproduction from (11) fungi isolates. Results in table (2) indicated that the highest level of Laccase production was obtained from *A. alternate* no. (4).Laccase activity was (1.35) U/ml.Other fungal isolates seen to be as a weak laccase activity were: 0.39,0.22 U/ml from*Penicillium sp., Fusarium sp.* respectively. So in this study we used the *A. alternata*no.(4) for Laccase produce.

Table (2): Laccase production from different fungal isolates

NO.	Fungal isolated	Laccase activity U/ml
1.	*Alternariaalternata (1)*	0.82
2.	*A. alternata (2)*	0.60
3.	*A. alternata (3)*	0.80
4.	*A. alternata (4)*	1.35
5.	*A. alternata (5)*	1.01
6.	*A. alternata (6)*	0.39
7.	*A. alternata (7)*	0.72
8.	*Fusariumspp*	0.22
9.	*Penicillium spp.(16)*	0.62
10	*Penicillium spp.(32)*	0.47
11	*Penicillium spp. (34)*	0.39

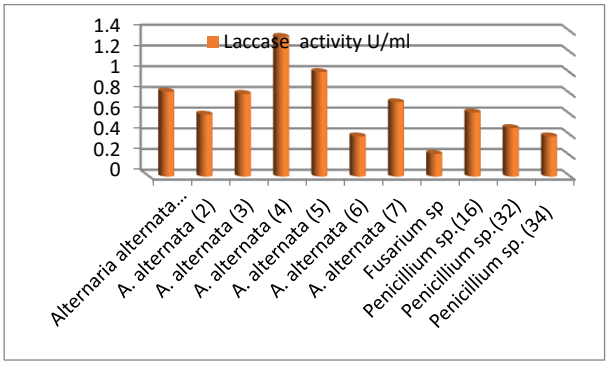

Figure (3): Effect of different fungal on Laccase production

3.4/ Effect of pH on enzyme production

Higher Laccase activity (2.01) U/ml was obtained at pH (5.5) of *A.alternata* no. (4) As showed intable(3). The pH of the culture significantly influence many enzymatic process and transport of the compounds across the cell membrane(Arora& Gill, 2013) who reported that the maximum Laccase activity of *A. alternata* was at pH 5.5

Table (3): Effect of pH on Laccase production from *A. alternata* (4)

pH	Laccase activity U/ml
4.0	0.10
4.5	0.34
5.0	0.60
5.5	2.01
6.0	0.59
6.5	0.42
7.0	0.13

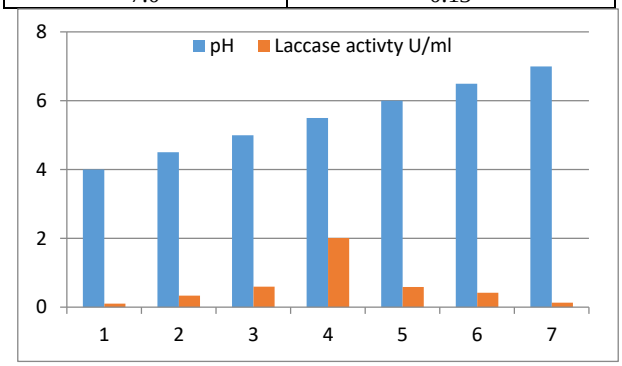

Figure (4): Effect of different (pH) on Laccase production from *A. alternata* (4).

3.5/ Effect of carbon source on enzyme production

Laccase production was detected in the presence of different Carbone sources incorporated in the basal production medium with concentration of (1%). As can be noticed in table (4). The Laccase activity was significantly increased in the culture supplemented withSucrose(2.93) U/ml compared with Galactose containing medium which was 0.65 U/ml. The six different carbon sources supported good growth and Laccase production.

Table (4):effect of different carbone sources on Laccase production from *A. alternata*at pH 5.5

Carbon sources	Laccase activity U/ml
Maltose	1.72
Sucrose	2.93
Lactose	2.05
Fructose	1.00
Glucose	0.93
Galactose	0.65

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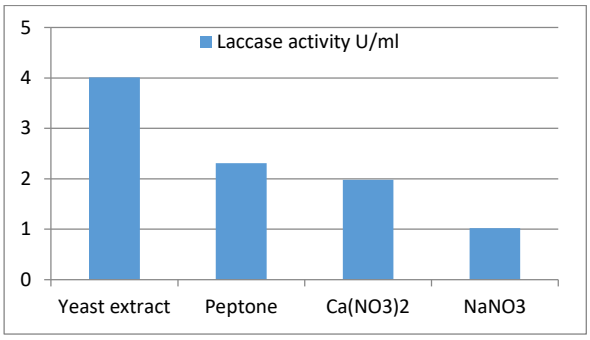

Figure (5): Effect of different carbon sources on Laccase production from *A. alternata* (4).

3.6/ Effect of different nitrogen source of the enzyme production

The effect of different nitrogen sources was evaluated at optimum pH and carbon source. Based on the result. The Laccase activity was significantly increased to (4.01) U/ml in the culture contained (Yeast extract) compared with other nitrogen sources which had an inhibitory effect on the Laccase production table (5). The yeast extract test source of nitrogen that supported the growth and production of an enzyme (Bazaraa& Al-Dagl, 2016) observed that addition of inorganic nitrogen source in the production medium resulted in low enzyme production. The type of nitrogen sources plays an important role in the production of Laccase. (Imran*etal*., 2012)

Table (5): Effect of different nitrogen sources on Laccase production from *Alternariaalternata*at pH 5.5 at sucrose as a carbon.

Nitrogen sources	Laccase activity U/ml
Yeast extract	4.01
Peptone	2.31
Ca(NO3)2	1.98
NaNO3	1.02

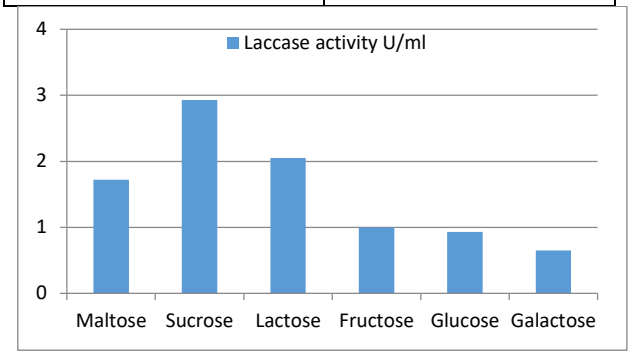

Figure (6): Effect of different nitrogen sources on Laccase production from *Alternariaalternata*

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