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Screening of *Aspergillus Niger* for Lipase Production

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ABSTRACT

The current study was intended to identify *Aspergillus niger* isolated from different sources oil, peanut (pistachios, ground nut, walnuts, almonds and cashew), dried milk and factory waste water. Traditional methods were used to investigate the ability of *Aspergillus niger* for lipase production using T80 and T80 with phenol red agar and calculate *Aspergillus niger* efficiency in this enzyme production. The results revealed that all isolates were able to produce lipase, and the efficiency of lipase production ranged between (0.90-2.21) mm. A50 isolate showed the highest efficiency in lipase production, which was isolated from factory waste water factory.

KEYWORDS: Bi PbSr CaCuO; Zn; XRD; VSM.

الخلاصة

هدفت الدراسة الحالية إلى تشخيص والتعرف على فطر *Aspergillus niger* المعزول من مصادر مختلفة : التربة والفول السوداني (الفستق والجوز واللوز والكاجو) والحليب المجفف ومياه الصرف الصحي في المصنع بالطرق التقليدية لدراسة قدرة *Aspergillus niger* على إنتاج الليبيز باستخدام أجار T80 و T80 مع صبغة الفينول الحمراء و من خلال حساب كفاءة *A. niger* في إنتاج الليبيز. أظهرت النتائج أن جميع عزلات *A. niger* لها القدره على إنتاج الليبيز ، وتراوحت كفاءة إنتاج هذا الانزيم بين (0.90-2.21) ملم. بينما كانت العزلة A50 الاعلى كفاءة في إنتاجه التي تم عزلها من مياه الصرف الصحي للمصنع.

INTRODUCTION

The interest in lipase production has increased over the past several years due to its excellent catalytic properties [1,2] and diverse industrial applications including detergents, esterification and pharmaceuticals [3,4]. There are many studies have proven that soil contaminated with waste oils or dairy and agricultural products harbours many lipase-producing fungi [5,6]. Rich sources of fat are essential to obtain a high yield of lipase [7]. In addition, several studies showed the production of extracellular lipase enzyme in different strains of *Aspergillus niger* (*A. niger*) [8].

Lipase is an important enzyme because it can catalyze: hydrolysis, aminoglycolysis and esterification [9]. Lipase is produced by many microorganisms alone or in combination with other members of the hydrolase group, like esterases [10]. Fungi are among the most important organisms as preferred sources of lipase on a large scale because they produce enzymes outside the cell [11]. In addition, it is easy to recover enzyme from fermentation broth. *A. niger* is one of the most important organisms used in biotechnology. It has been used for decades in the production of many extracellular enzymes that are considered safe according to the (FDA) (Food and Drug Administration of the United States of America) [12]. It was found that the crude extracts obtained from *A. niger* fermentation broth contain many lipase and esterase enzymes that showed bindings with the target substrate [13,14]. Therefore, The aim of this study is to obtain the best natural and economically inexpensive source of the important lipase enzyme in the industrial and medical fields.

MATERIAL AND METHODOLOGIY

Culture Media

Potato Dextrose Agar

PDA were prepared according to the manufacturer instructions, and adjusted to pH 5

then sterilized and poured into sterilized plates (90mm in diameter were filled with 20ml medium), after cooling to 45 °C and supplemented with 100mg /l of chloramphenicol [15].

T80 Agar Plates

1.5 % of peptone, 0.5 % of sodium chloride, agar 1.5 %, and 0.1 % of calcium chloride was dissolved in 1L of distilled water and sterilized at 121 °C at 15 psi for 20 minutes, after it was cooled to 50 °C then adding 1 % Tween 80, and adjusted to pH 7 [16].

T80 Agar Plates with Phenol Red

1.5 % peptone, 0.5 % NaCl, 0.1 % calcium chloride, 1.5 % agar, and 0.01% phenol red were dissolved in 1 L of distilled water and sterilized at 121 °C at 15 psi for 20 min, Cool to 50°C, add 1 % Tween 80, and adjust to pH 7 [16].

Samples Collection

Soil Samples

Ten soil samples (4-5 cm depth) were collected from different places in the gardens of the University of Baghdad and then placed inside sterile polythene bags.

Seeds Samples

Forty-five samples of seeds (pistachios10, Ground nut10, walnuts10, Almonds10 and cashew5) were collected from different Baghdad markets at rate (50 g), transferred in sterile packets to the laboratory and kept in a cool place (3 - 5°C).

Dried Milk Samples

Eighteen samples of instant full cream milk powder (Almudhish, Anchor, Diallaak) were collected from markets (500 g).

Factory of Waste Water

Five samples of wastewater were obtained from batteries factory in Baghdad. The samples were kept in dry, sterile polypropylene bottles and stored in the refrigerator (4°C) till the isolation of fungi.

Isolation and Identification A. Niger from Different Sources

1. Soil and Dried Milk Samples

Series dilutions method (up to 10-6) was used for isolations of fungi from soil and dried milk by taken 5g from each soil and dried milk samples individually, and put into flask (100ml) contain 50 ml sterile distilled water and shaken for making suspensions and dilutions, after that, 1ml from dilution (of each soil and dried milk) was taken and spread on PDA plates and incubated at 28±2 °C. After 3 days, the growing colonies of fungi isolates were inoculated into PDA plates individually and incubated at 28 ± 2 °C for 5 days for identification [17].

2. Seeds

Fifty seeds: pistachios, Ground nut, walnuts, Almonds and cashew (from each sample) were taken and superficially sterilized with 1% sodium hypochlorite solution for one minute and then rinsed in sterile distilled water three times. Five seeds were placed on the surface of agar plates containing potato dextrose agar. Then all dishes were incubated for 3-7 days at 28±2°C, after 3 days the growing colonies of fungi isolates were re-cultured then individually placed on PDA plates and incubated at 28 ± 2 °C for 3-5 days [18]. All of the growing fungi were subjected to identification by studying colony morphology and microscopic observation (Lacto Phenol cotton blue staining). The identified *A. niger* was sub-cultured multiple times until pure culture obtained [19].

3. Factory Waste Water

Fungi were isolated from factory wastewater by a series of dilution. (up to 10-6) were taken 5ml from collected samples and put into flask contain 50 ml sterile distilled water and shaken for making suspensions, After that, 1ml from dilution was taken and spread on PDA plates then incubated at 28 ± 2 °C for 3 days. Colonies of isolates of fungi were selected on PDA plates.

Identification:

The *A. niger* isolates were identified based on macroscopic and microscopic characteristics (Lacto phenol cotton blue stain), and confirmed by microscopic examination and study of colony morphology, characterization, spores, shape and color according to [17].

Screening for Lipase Production Using T80 Agar and T80 Agar with Phenol Red Plates

The ability of *A. niger* isolates to produce lipase was tested by taking mycelia plug 5mm in diameter (were cut from margin of colony) of 3

days old cultures of the *A. niger*, T80 agar, phenol red was added individually in the center of a Petri dish and incubated for 4 days at 28 ± 2 °C [16].

After incubation, the isolates were observed for zones of hydrolysis around the colonies. Lipase activity was screened by following formula [20]:

$$Efficiency\ fungus\ product\ Lipase = \frac{Diameter\ Zone\ of\ hydrolysis}{Diameter\ of\ colony}$$

RESULTS AND DISCUSSION

Fifty isolates of *A. niger* were isolated from 78 samples from different sources (10 soil, 45 peanut, 18 dried milk, 5 factory waste water (Table 1). The percentage of isolation from each source is 100% from soil, 73 % from peanut, 22% from Powdered Milk, and 100% from factory waste water.

Table 1. *A. niger* isolates from different source

Isolate No.	Sources
A1-A10	Soil
A11-A20	Ground nut
A21-A25	pistachios
A26-A32	walnuts
A33-A35	Cashew
A36-A41	Almonds
A42-A 45	Powdered milk
A46-A50	factory wastewater

Screening of *A. niger* for Lipase Production

All isolates of *A. niger* cultured on T80 agar and T80 with phenol red agar media produced lipase enzyme by observing white clear zone around the colonies on T80 agar due to the formation of calcium salt of oleic acid [21]. While on T80 with phenol red agar, the color of media was changed from red to yellow. The change in the color of media due to change in pH from basic to acidic (from pH 7.2 to below 6.5) condition because of the release of fatty acids by hydrolysis of Tween 80. In addition, to the screening methods proposed by [21], phenol red is used as an indicator for the preparation of assay media for lipase-producing fungi that gave a good result for lipase production in the screening medium, see Figure 1.

The results in Table 2 showed that all the isolates of *A. niger* under the study which able to produce lipase on indicator medium as indicated by the diameter of the zone (mm). The maximum *A. niger* efficiency in the production of the Lipase on indicator medium after 4 days was 2.21 and 2.19 for A50 (isolate from factory wastewater), while the lowest was 0.89 and 0.90 for A35 (isolate from Cashew). This result was closer to the finding of [22] who reported the maximum production lipase on indicator medium after 4 days was 1.65 from seeds.

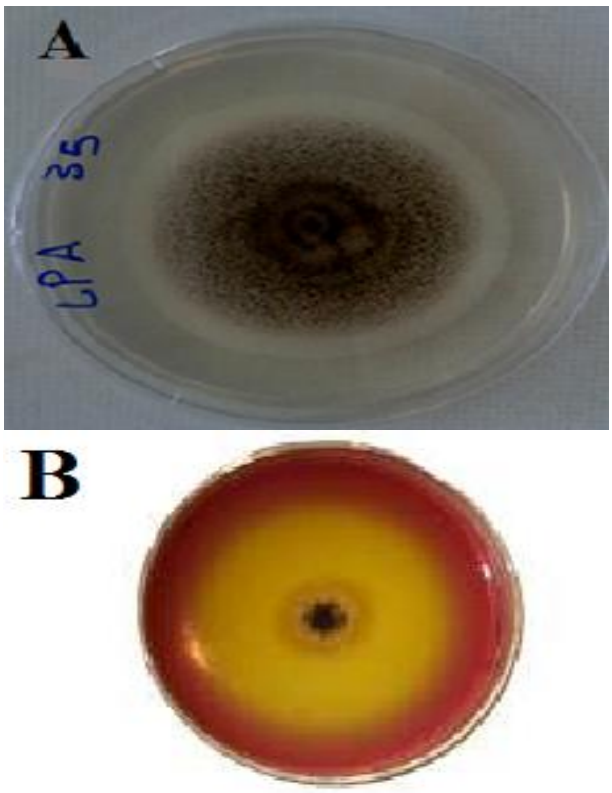

Figure 1. Lipase production by *A. niger* on (A)T80 agar medium and (B) T80 agar with Phenol red medium at 28± 2 °C after 4 days incubation.

Aspergillus species have been known to produce lipase. The selection of suitable isolates for lipase production depends on a number of factors such as the nature of the substrate and environmental conditions. It was found that the attempt to isolate the lipase produced by *A. niger* was successful. The present study bias in the isolation process targeted *A. niger* for lipase production and the basis of platelet morphology and microscopic observation was identified [20].

The screening method involved screen the plates using Tween 80 plates with calcium as an ingredient. Also, the examination methods proposed by Mehta et.al [23] using phenol red as an indicator for preparing the screening medium

for the fungi producing lipase enzyme gave a good result in the screening medium.

Table 2. Efficiency of *A. niger* isolates in production of lipase in T80 agar and T80 agar with phenol red medium

Isolate No.	***A. niger* efficiency in the production of the Lipase (diameter in mm)** Each number represents the average of three replication		Isolate No.	***A. niger* efficiency in the production of the Lipase (diameter in mm)** Each number represents the average of three replication	
	T80	**T80 with phenol reed**		**T80**	**T80 with phenol reed**
A1	1.72±0.02 A	1.71±0.1 A	A26	1.24±0.03 C	1.27±0.02 C
A2	1.32±0.06 B	1.33±0.02 B	A27	1.27±0.04 C	1.29±0.02 C
A3	1.17±0.02 C	1.16±0.01 C	A28	1.38±0.04 B	1.39±0.01 B
A4	1.23±0.08 C	1.22±0.01 C	A29	1.18±0.09 C	1.12±0.02 C
A5	1.37±0.03 B	1.34±0.01 B	A30	1.23±0.08 C	1.25±0.01 C
A6	1.40±0.02 B	1.40±0.02 B	A31	1.32±0.06 B	1.36±0.01 B
A7	1.30±0.06 B	1.32±0.02 B	A32	1.64±0.02 B	1.66±0.01 B
A8	1.47±0.09 B	1.48±0.02 B	A33	1.36±0.04 B	1.39±0.01 B
A9	1.11±0.03 C	1.10±0.01 C	A34	1.35±0.03 B	1.29±0.02 B
A10	1.35±0.04 B	1.33±0.03 B	A35	0.90±0.04 A	0.89±0.01 A
A11	1.82±0.04 A	1.80±0.02 A	A36	1.99±0.02 A	2.01±0.02 A
A12	1.23±0.02 C	1.20±0.01 C	A37	1.75±0.02 A	1.77±0.02 A
A13	2.02±0.02 C	2.07±0.02 C	A38	1.65±0.05 B	1.67±0.03 B
A14	1.88±0.02 A	1.89±0.02 A	A39	1.71±0.03 A	1.73±0.01 A
A15	1.80±0.03 A	1.79±0.01 A	A40	1.47±0.02 B	1.40±0.01 B
A16	1.58±0.02 B	1.58±0.03 B	A41	1.60±0.06 B	1.63±0.01 B
A17	1.40±0.04 B	1.41±0.02 B	A42	1.60±0.04 B	1.64±0.02 B
A18	1.47±0.01 B	1.50±0.01 B	A43	1.20±0.02 C	1.22±0.02 C
A19	1.33±0.02 B	1.35±0.01 B	A44	1.59±0.03 B	1.62±0.01 B
A20	1.23±0.01 C	1.23±0.02 C	A45	1.70±0.02 A	1.74±0.01 A
A21	1.20±0.05 C	1.18±0.03C	A46	2.01±0.01 A	2.09±0.02 A
A22	1.10±0.03 C	1.08±0.01C	A47	1.89±0.02 A	1.91±0.01 A
A23	1.64±0.07 B	1.66±0.01 C	A48	1.88±0.04 A	1.87±0.01 A
A24	1.05±0.02 C	1.09±0.2C	A49	1.96±0.08 A	2.01±0.02 A
A25	1.29±0.02 C	1.30±0.1 C	A50	2.21±0.02 A	2.19±0.01 A

C: Diameter Zone of hydrolysis (12-18) mm
B: Diameter Zone of hydrolysis (20-30) mm
A: Diameter Zone of hydrolysis over 30 mm
Statistical analysis: The results showed significant differences. using a complete randomized design (CRD) with three replicates per treatment. All data were analyzed using analysis of variance (ANOVA), and means separated using (LSD) tests and alpha values of 0.05.

CONCLUSIONS

This study showed that the *A. niger* is a good product of the lipase enzyme, but it depends on the environmental conditions of the fungus. The isolates of *A. niger* obtained from waste water gave the highest percentage of Lipase enzyme production.

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