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(Original Article)

Isolation and Identification of Phenylalanine Ammonia Lyase (PAL) Enzyme–Producing Microorganisms in Assiut Governorate, Egypt

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Abstract

This study aimed to isolate microorganisms producing phenylalanine ammonia-lyase (PAL) from various locations in Assiut Governorate, Egypt. The results demonstrated that 14 fungal isolates gave positive results for PAL production during primary screening. These isolates were belonging to *Aspergillus niger* (2 isolates), *A. oryzae* (1), *Fusarium proliferatum* (1), *Fusarium solani* (3), *A. flavus* (2), *A. fumigatus* (1), *Cladosporium cladosporioides* (1), *Geotrichum candidum* (1), *Fusarium oxysporum* (1), and *A. terreus* (1). Additionally, four bacterial isolates coded as YA2, YE2, YB1 and YC1 tested positive for PAL production, while no yeast isolates were PAL-positive. Results also showed that *Fusarium solani* NO.1 was the highest PAL producer among the tested fungal isolates (the enzyme activity was 0.224 U/min/ml of cell suspension). Moreover, YA2 isolate was the highest bacterial enzyme producer with volume activity of 0.112 U/min/ml of cell suspension. These results demonstrate the diversity of PAL-producing microorganisms, which can be used in industrial enzyme synthesis.

Keywords: *Bacteria, Fungi, Isolation, Phenylalanine ammonia lyase, Yeast.*

Introduction

L-Phenylalanine, an aromatic essential amino acid, is a substrate for many enzymes (Liu *et al.,* 2014). Phenylalanine ammonia-lyase (PAL, EC 4.3.1.24) is one of these enzymes; which catalyzes the deamination of L-phenylalanine to produce *trans*-cinnamic acid and ammonia (Koukol and Conn, 1961). PAL is specific for L-Phenylalanine, and to a lesser degree, for L-Tyrosine. Other prevalent L-amino acids are not deaminated; D-Phenylalanine and D-Tyrosine act as competitive inhibitors of PAL but do not function as substrates for it (Kyndt *et al.,* 2002). PAL enzyme belongs to the ammonia lyase family, which breaks down carbon nitrogen bonds. Similar to other lyases, PAL requires two substrates for the reverse reaction but only one for the forward reaction. It is believed to share mechanistic similarities with histidine ammonia-lyase (Schwede *et al.,* 1999).

PAL has been identified in numerous plant species and algae. It was initially shown to exist in microorganisms such as filamentous fungi (*Nectria cinnabarina*, *Ustilago maydis*, *Aspergillus niger* and *A. flavus*); yeasts (*Rhodotorula glutinis and R. rubra*) and

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bacteria (*Streptomyces maritimus* and *S. verticillatus*) as previously recorded in several studies (Ogata *et al*., 1966; Evans *et al.,* 1987; Hyun *et al*., 2011; Cui *et al.,* 2014; Kong, 2015).

In microorganisms, PAL contributes to the biosynthesis of protective or competitive metabolites. Due to its vital role in both primary and secondary metabolism, PAL has garnered a significant interest in the fields of microbial metabolism and plant biology and is highly significant in numerous clinical, industrial and biotechnological applications (Cui *et al.,* 2014).

PAL has several potential clinical and industrial applications, including the production of L-phenylalanine, quantitation of serum L-phenylalanine, treatment of phenylketonuria and in the preparation of low L-phenylalanine diets (Cui *et al.,* 2014). However, the relatively low specific activity and instability of PAL during the applications are still a challenge for researchers. There is growing interest for searching of new sources of this enzyme. So, this study aimed to isolate microorganisms (fungi, bacteria and yeasts) which can produce phenylalanine ammonia-lyase (PAL) enzyme from various locations in Assiut Governorate, Egypt.

Materials and Methods

1. Collection of samples

Both soil and leaf samples were used for microorganism isolation. These samples were collected from the Research Farm of Faculty of Agriculture, Assiut University, Egypt as well as from local farms in Al-Qusiyah, Assiut, Egypt as shown in Table (1). Soil samples were collected from various farms using a trowel to dig to a depth of 20 cm. The soil was then placed into double polyethylene bags (Akinyanju and Fadayomi, 1989). To ensure representative sampling, material from four random spots in each area was combined. Samples were stored at 4°C until use (Sagova-Mareckova *et al.,* 2008). Leaves samples were collected from plants by cutting it using sterilized scissors and then put into doubled polyethylene bags. Samples kept at 4°C for not more than 24 hours (Bills, 1996).

Table 1. Samples used in the study and their collection sources.

Sample Code	Source of isolation
A	Grape growing soil from the Research Farm of Faculty of Agriculture, Assiut University, Egypt.
B	Pomegranate growing soil from the Research Farm of Faculty of Agriculture, Assiut University, Egypt.
C	Peanut growing soil from a farm in Al-Qusiyah, Assiut, Egypt.
D	Soil around a gas station in Al-Qusiyah, Assiut, Egypt.
E	Soybean growing soil from a farm in Al-Qusiyah, Assiut, Egypt.
L	Grape and pomegranate leaves from the Research Farm of the Faculty of Agriculture, Assiut University. Peanut and soybean leaves from a farm in Al-Qusiyah, Assiut, Egypt.

2. Media used

- Sucrose-free Czapek's medium (g/L)

Czapek's medium consists of: $NaNO_3$ (2), sucrose (10), KH_2PO_4 (1), $MgSO_4.7H_2O$ (0.5), KCl (0.5), $CuSO_4$ (0.005), $ZnSO_4$ (0.01) and agar-agar (20) (Smith and Onions, 1994). Sucrose-free Czapek's medium used in this study as selective medium for isolation of PAL producing fungi and also as cultivation broth medium for determination of PAL activity by fungal isolates. In this medium sucrose was re-pleased only by L-phenylalanine (10 g/L).

- Phenylalanine selective medium (g/L)

This medium used for isolation of PAL producing bacteria and yeasts, also used as cultivation broth medium for determination of PAL activity by bacterial and yeast isolates. It consists of (g/L) KH_2PO_4 (4), Na_2HPO_4 (2), $MgSO_4.7H_20$ (1.2), NH_4CI (2), yeast extract (0.22), $CaC1_2.6H_20$ (0.08), $FeCI_3.6H_20$ (0.08), $ZnSO_4.7H_20$ (0.001), NaCl (0.5), agar-agar (20) and L-phenylalanine (15) (Evans *et al.*, 1987).

- Yeast extract medium (YEM)

YEM medium utilized for preservation and activation of the yeast cultures, as recommended by Wickerham (1951) with some modifications**.** The medium consists of (g/L): Yeast extract, 3; peptone, 5; glucose, 10 and agar-agar, 20.

- Potato dextrose agar (PDA) medium

PDA medium utilized for preservation and sporulation of the fungal cultures, as described by Ragab (1989). The medium consists of (g/L): Potato extract from 200 g potatoes; glucose, 20 and agar-agar, 20.

All media were prepared by dissolving all components in distilled water, then autoclaved at 121°C for 20 minutes.

3. Isolation of microorganisms

Microorganisms were isolated from soil samples using the dilution plate method according to Tournas *et al.* (2006). Sucrose-free Czapek's medium was used for fungal isolation, and phenylalanine selective medium was used for yeast and bacteria isolation. A homogeneous suspension was made by adding 10 grams of the sample to 90 ml of sterile distilled water then shaking for 10 minutes. The suspension was then diluted through a series of standard volumes of sterile diluent and one ml of the third dilution (1/1000) was pipetted into a sterilized Petri dish. After melting and cooling, 20 mL of the sterilized medium was added to each Petri dish, mixed, and then left to solidify. Three replicates were prepared from this dilution.

Isolation of microorganisms from leaves was carried out by washing them with sterile distilled water, then cutting the sample into approximately 1 cm^2 pieces, which were then aseptically transferred to the surface of solidified medium in Petri dishes (Bills, 1996). The plates were incubated at 28±2°C for 7 days for fungi and 3 days for yeasts, and at 37±2°C for 2 days for bacteria. After the incubation period, colonies were counted and isolated separately.

4. Identification of the isolated fungi

Fungi were isolated and cultured at the Department of Food Science and Technology, Faculty of Agriculture, Assiut University. The isolates were identified at the Department of Botany and Microbiology, Faculty of Science, Assiut University using taxonomic methods based on both macroscopic and microscopic morphological characteristics according to Ainsworth (1971); Ellis and Hyphomycetes (1971); Pitt (1979); Klich and Pitt (1988); Moubasher (1993) and Pitt and Hocking (2009).

5. Collection of yeast and bacterial isolates

Yeast and bacterial isolates recovered on the phenylalanine selective medium from different sources under study were collected for detecting their ability to produce PAL.

6. Preparation of inoculum

After inoculating slant agar of PDA medium with a young fungal culture, the medium was incubated for five days at 28±2°C. Five milliliters of sterilized distilled water were added to each slant to prepare the spore suspension. An inoculating loop was used to scrape the spores, and spore suspension was then collected in a sterilized Erlenmeyer flask. The same procedure was repeated for bacteria using phenylalanine selective medium and incubating for two days at 37±2°C.

7. Screening for enzyme production

Sucrose-free Czapek's medium supplemented with 5 mL/L of 2% phenol red stock solution in ethanol was prepared. The pH was adjusted to 6.7. Slants were prepared by adding 10 ml of sucrose-free Czapek's medium into test tubes (15 mL) Slants were sterilized and inoculated with isolated prepared fungal inoculate and incubated for 5 days at 28±2°C.The appearance of pink color beneath the colonies was an indicator that these colonies might be PAL producers (Wakayama *et al.,* 2005). The same procedure was repeated for yeasts and bacteria using phenylalanine selective medium (Evans *et al.*, 1987). The tested isolates were divided into three categories: high, moderate, and low enzyme producers based on the intensity of the pink zone beneath the growing colonies.

8. Determination of enzyme activity

- Cultivation of microbial isolates

This was achieved based on method described by Evans *et al.* (1987) with some modification, 50 ml of phenylalanine selective medium broth for bacteria and sucrose-free Czapek's medium broth for fungi were prepared in 250 ml flasks (each flask had 50 ml medium). Each flask was inoculated with 2 mL of spore suspension. Fungal cultures were incubated in a shaking incubator (100 rpm at 28±2°C for 7 days), while bacterial cultures were incubated at 37±2°C for 2 days.

- Preparation of cell suspension

Bacterial cells were harvested by centrifugation at 10,000 × g for 10 minutes, while fungal cells were harvested by filtration. The harvested cells were washed twice with 0.9% NaCl, then re-suspended in 6 mL of phosphate buffer (pH 7.0) (Evans *et al.*, 1987).

- Enzyme assay

Enzyme assay was performed according to method described by Yamada *et al.* (1981) with some modifications. The reaction mixture contained one aliquot of cell suspension and an equal volume of substrate solution (1% L-phenylalanine and 0.05% cetylpyridinium chloride in phosphate buffer pH 7.5). After five hours at 30° C, the reaction was centrifuged at 10,000×g for 10 minutes.

Trans-cinnamic acid concentration, as the enzymatic reaction product, was measured at 290 nm using C-7200 spectrophotometer (Marusich *et al.,* 1981). Standard curve was prepared using different concentrations of Trans-cinnamic acid in ethanol (Chadchan *et al.,* 2017) and the absorbance was measured at 290 nm (Figure, 1).

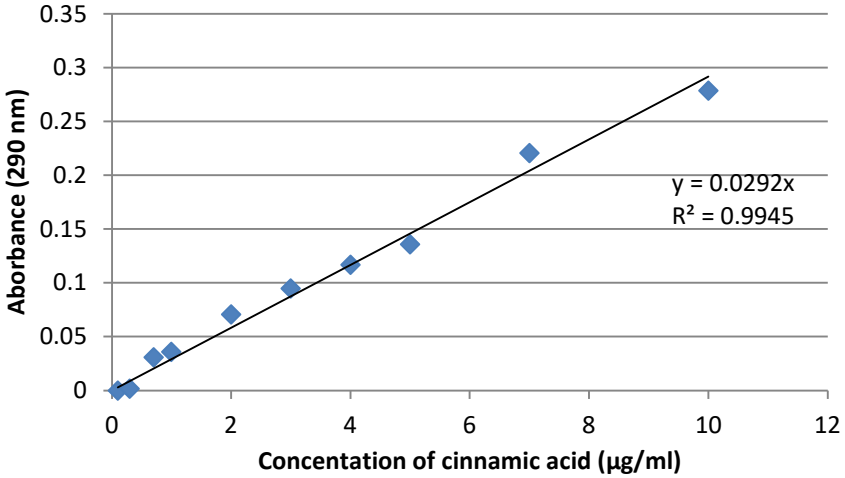

Figure 1. Standard curve of Trans-cinnamic acid at 290 nm

Determination of protein concentration

Protein concentration was determined using the method of **Lowry *et al.* (1951)**. Standard curve was prepared by dissolving varying albumin concentrations in water, and the absorbance was measured at 750 nm using C-7200 spectrophotometer (Figure, 2).

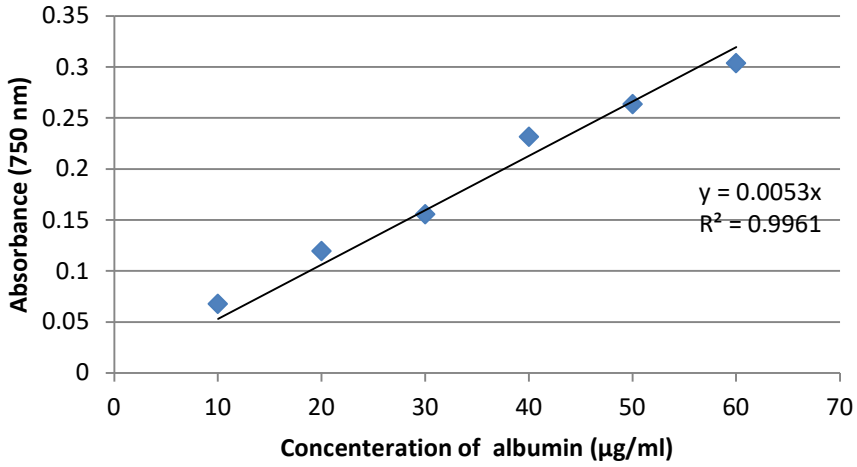

Figure 2. Standard curve of albumin at 750 nm

- Microbial dry weight determination

Microbial dry weight was estimated as the residual mass after cell separation by filtration and drying at 85°C for 24 hours.

Results and Discussion

1. Isolation and identification of microorganisms

Twenty- seven isolates of growing fungi on sucrose-free Czapek's medium were obtained in addition to 9 isolates of growing bacteria and two isolates of growing yeasts on Phenylalanine selective medium were obtained as shown in Tables (2 - 5). Data in Table (2) showed that 11 fungal species belonging to five genera were isolated and identified from the studied samples. One species of *Aspergillus* (*A. niger*) was isolated from sample A, while five species belonging to three genera were isolated from sample B (*Aspergillus niger, A. oryzae, Fusarium proliferatum, F. solani* and *Penicillium chrysogenum).* For sample C, three species belonging to one genus were isolated: *Aspergillus flavus, A. fumigatus* and *A. ochraceus.* Three species belonging to three genera were isolated from sample D: *Aspergillus niger, Fusarium solani* and *Cladosporium cladosporioide*. Regarding sample E only *Fusarium oxysporum* and *Geotrichum candidum* were isolated, while *Aspergillus niger* was isolated from sample L.

Data in Table (2) also showed that the most common fungal isolate from all samples was *A. niger*. The largest count of fungal isolates was obtained from sample D (15667 CFUs/10 g), while the lowest one (2666 CFUs/10 g) was from sample E.

Data in Table (3) revealed that 1500 CFUs/10 g of yeasts were isolated from sample D, while no yeast strains were isolated from other samples. For bacteria, the counts of isolates were 2667, 1500, 1000 and 2500 CFUs/10 g for samples A, B, C and E, respectively. However, no bacterial isolates were obtained from sample L.

Table 2. Total count of fungal genera and species isolated from soil (per 10 g) and leaves (per 1 cm^2) on sucrose-free Czapek's medium at 28 ± 2 ° C.

Genera and species	Sample Code						TC	D%	NCI	F%
	A	B	C	D	E	L				
Aspergillus	6000	7667	11666	2500		4333	32166	58.13	5	83.33
Aspergillus niger	6000	5667		2500		4333	18500	33.43	4	66.67
Aspergillus oryzae		2000					2000	3.61	1	16.67
Aspergillus flavus			6333				6333	11.45	1	16.67
Aspergillus fumigatus			1333				1333	2.41	1	16.67
Aspergillus ochraceus			4000				4000	7.23	1	16.67
Fusarium		5333		9667	1333		16333	29.52	3	50.00
Fusarium proliferatum		3000					3000	5.42	1	16.67
Fusarium solani		2333		9667			12000	21.69	2	33.33
Fusarium oxysporum					1333		1333	2.41	1	16.67
Penicillium chrysogenum		2000					2000	3.61	1	16.67
Cladosporium cladosporioides				3500			3500	6.33	1	16.67
Geotrichum candidum					1333		1333	2.41	1	16.67
Total count (TC)	6000	15000	11666	15667	2666	4333	55332			
No. of genera (5)	1	3	1	3	2	1	5			
No. of species (11)	1	5	3	3	2	1	11			

(TC) total count, (D %) density, (NCI) number of cases of isolation, (F %) frequency

Table 3. Total count of yeasts and bacteria strains isolated from soil (per 10 g) and leaves (per 1 cm^2) on Phenylalanine selective medium at 28±2° C for yeasts and 37±2°C for bacteria.

Microorganism	Sample Code						TC
	A	B	C	D	E	L	
Yeasts	--	--	--	1500	--	1500	
Bacteria	2667	1500	1000	--	2500	--	7667

2. Primary screening of isolated microorganisms for PAL production

Results showed that 14 fungal isolates tested positive for PAL production using the phenol red test (Figure, 3). These isolates were belonging to *Aspergillus niger* (2 isolates), *A. oryzae* (1), *Fusarium proliferatum* (1), *Fusarium solani* (3), *A. flavus* (2), *A. fumigatus* (1), *Cladosporium cladosporioides* (1), *Geotrichum candidum* (1), *Fusarium oxysporum* (1), and *A. terreus* (1) as shown in Table (4) and illustrated in Figure (3). These results align with Hyun *et al.* (2011) who reported *A. flavus, A. fumigatus, A. niger, A. oryzae* and *F. oxysporum* as established PAL producers in multiple studies. Seshime *et al.* (2005) and Rao *et al.* (2005) also explained existence of genes encoding PAL enzyme in *A. oryzae.*

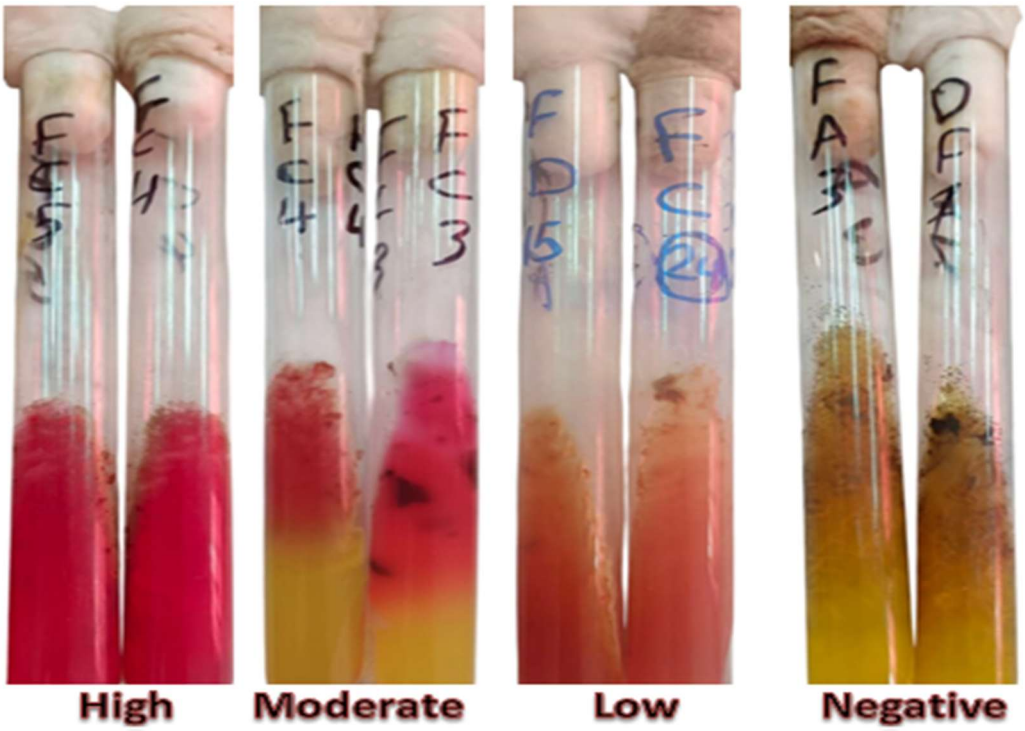

Figure 3. Primary screening of PAL activity of some isolates (High = ++++; Moderate = +++ and ++; Low = +)

From tested isolates of bacteria and yeasts, only four bacterial isolates (YA2, YE2, YB1 and YC1) showed positive results for PAL production using the phenol red test, whereas no yeast isolates tested positive (Table 5). Williams *et al.* (2005) reported that *Photorhabdus luminescens* and *Escherichia coli* produce PAL when cultivated at 28 °C and 37 °C, respectively, in either LB broth or on LB agar. Shahzad *et al.* (2019) reported

the production of PAL by *Bacillus subtilis* GCB-31 using solid-state fermentation on agro industrial wastes.

Table 4. Primary screening of isolated fungi for PAL production.

Fungal strain	Rapid assay
Aspergillus flavus 1	+
Aspergillus flavus 2	++
Aspergillus fumigatus	++++
Aspergillus niger 1	-
Aspergillus niger 2	++
Aspergillus niger 3	-
Aspergillus niger 4	-
Aspergillus niger 5	-
Aspergillus niger 6	+
Aspergillus niger 7	-
Aspergillus niger 8	-
Aspergillus niger 9	-
Aspergillus niger 10	-
Aspergillus niger 11	-
Aspergillus niger 12	-
Aspergillus ochraceus 1	-
Aspergillus ochraceus 2	-
Aspergillus oryzae	+++
Aspergillus terreus	++++
Cladosporium cladosporioides	+
Fusarium oxysporum	++++
Fusarium proliferatum	++++
Fusarium solani 1	++++
Fusarium solani 2	++++
Fusarium solani 3	++++
Geotrichum candidum	++++
Penicillium chrysogenum	-

Table 5. Primary screening for isolated yeasts and bacteria for PAL production.

Microorganism		Rapid assay
Yeasts	Bacteria	
	YA2	+++
	YA5	-
	YA6	-
	YA9	-
	YC1	++
	YB1	+
YD2		-
YD4		-
	YE1	-
	YE2	++
	YE3	-

3. Determination of PAL Activity

The following experiments were conducted on microbial isolates that tested positive using the phenol red test for detecting the volumetric and specific activities of PAL. PAL activity was measured according to Yamada *et al.* (1981). For fungi, PAL volumetric activities were ranged from 0.224 U/min/ml of cell suspension by *Fusarium solani* 1 to 0.050 U/min/ml of cell suspension by *Fusarium solani* 2, while the specific activities of PAL were ranged from 0.565 U/min/mg protein by *Aspergillus oryzae* to 0.115 by *Fusarium solani* 2. On the other hand, fungal biomass was ranged from 0.249

g/50 ml of medium by *Cladosporium cladosporioides* to 0.133 g/50 ml of medium by *Aspergillus fumigatus,* as shown in Table (6) and Figure (4). Nearly similar results were obtained by Roura Padrosa *et al.* (2023). They found that the specific activity of AvPAL enzyme which expressed in *Escherichia coli* was 0.1 ± 0.02 U/min/mg protein. Also, Goldson-Barnaby and Scaman (2013) determined PAL activity that produced by *Trichosporon cutaneum* as 1.6 µmol/h/g wet weight.

Table 6. Secondary screening of PAL activity of the highly PAL fungal producers.

Strain	Biomass (g/50 ml of medium)	PAL activity (U/min/ml of cell suspension)	Soluble protein (mg/ml)	specific activity (U/min/mg protein)
***Aspergillus flavus* 1**	0.154	0.078	0.368	0.212
***Aspergillus flavus* 2**	0.182	0.093	0.331	0.282
Aspergillus fumigatus	0.133	0.092	0.294	0.314
***Aspergillus niger* 2**	0.176	0.221	0.508	0.434
***Aspergillus niger* 6**	0.161	0.185	0.491	0.377
Aspergillus oryzae	0.201	0.196	0.348	0.565
Aspergillus terreus	0.192	0.102	0.421	0.242
Cladosporium cladosporioides	0.249	0.071	0.532	0.134
Fusarium oxysporum	0.196	0.076	0.376	0.202
Fusarium proliferatum	0.18	0.110	0.269	0.408
***Fusarium solani* 1**	0.178	0.224	0.459	0.489
***Fusarium solani* 2**	0.241	0.050	0.435	0.115
***Fusarium solani* 3**	0.213	0.127	0.449	0.283
Geotrichum candidum	0.183	0.146	0.542	0.269

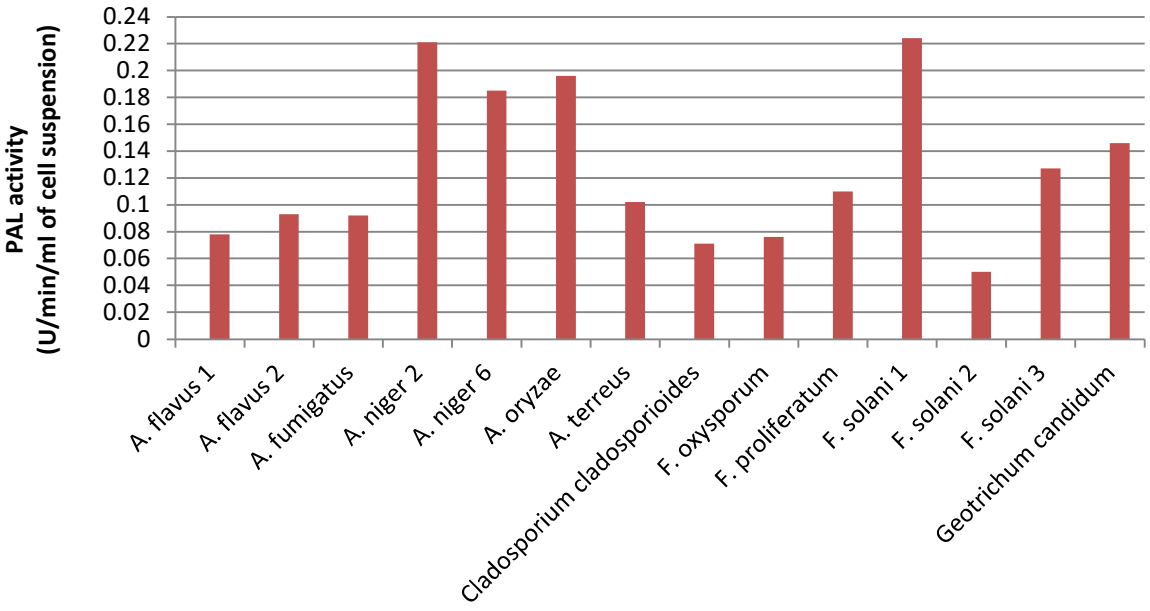

Figure 4. PAL activity (U/min/ml of cell suspension) for positive fungal isolates.

Regarding bacteria, PAL volumetric activities ranged from 0.112 U/min/ml of cell suspension by isolate YA2 to 0.072 U/min/ml of cell suspension by isolate YE2, while the specific activities of PAL ranged from 0.276 U/min/mg protein by isolate YE2 to

0.192 by isolate YB1. The bacterial biomass was ranged from 0.113 g/50 ml of medium by isolate YA2 to 0.083 g/50 ml of medium by isolate YB1, as shown in Table (7) and Figure (5). In this respect, Aydaş *et al.* (2013) determined PAL specific activity that produced by *Leptolyngbya* sp. BASO700 when it was cultivated in BG11 medium at 25°C as 1.5 ± 0.5 U/mg protein. Zhu *et al.* (2013) recorded PAL specific activity that produced by *E. coli* when it was grown in LB medium at 37°C as 4.2 U/mg.

Table 7. Secondary screening of PAL activity of the highly PAL bacterial producers.

Strain	Biomass (g/50 ml of medium)	PAL activity (U/min/ml of cell suspension)	Soluble protein (mg/ml)	Specific activity (U/min/mg protein)
YA2	0.113	0.112	0.423	0.264
YE2	0.1	0.072	0.260	0.276
YC1	0.104	0.093	0.381	0.243
YB1	0.083	0.081	0.421	0.192

YA2, YE2, YC1 and YB1 are PAL producer bacterial isolates.

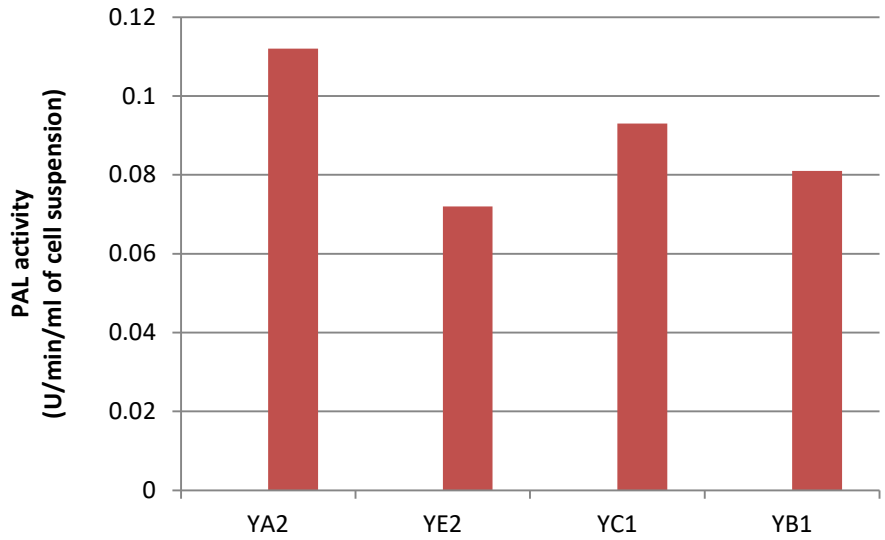

Fig. 5. PAL activity (U/min/ml of cell suspension) for positive bacterial isolates. YA2, YE2, YC1 and YB1 are PAL producer bacterial isolates.

Conclusion

This study introduced several new sources of PAL from fungal and bacterial isolates isolated from various locations in Assiut Governorate, Egypt. The highest volumetric activity of PAL produced by one isolate of *Fusarium solani* reached to 0.224 U/min/ml of cell suspension, while 0.565 U/min/mg protein was the highest specific activity of the enzyme and produced by one isolate of *Aspergillus oryzae*. In cases of bacterial tested isolates, the highest PAL volumetric and specific activities were 0.112 U/min/ml of cell suspension and 0.276 U/min/mg protein recorded for enzyme produced by isolates YA2 and YE2, respectively. Therefore, this study opens the way for the possibility of production of this important enzyme in large quantity and low cost. Further studies should be focused on improving microbial PAL activity, purification, characterization and stability by some efficient biotechnological production process for industrial and medical applications.

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عزل وتعريف الكائنات الحية الدقيقة المنتجة لإنزيم فينيل ألانين أمونيا لياز (PAL) في محافظة أسيوط، مصر

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الملخص

هدفت هذه الدراسة إلى عزل الكائنات الحية الدقيقة المنتجة لإنزيم فينيل ألانين أمونيا لياز (PAL) من مواقع مختلفة في محافظة أسيوط، مصر. أظهرت النتائج أن 14عزلة فطرية ثبت انها مُنتجة لإنزيم فينيل ألانين أمونيا لياز. تنتمي هذه العزلات إلى فطريات *Aspergillus niger* (عزلتان)، *A. oryzae* (عزلة واحدة)، و*Fusarium proliferatum* (عزلة واحدة)، و*Fusarium solani* (عزلتان)، و *A. flavus* (عزلتان)*fumigatus* . (عزلة واحدة) ، و*Cladosporium cladosporioides* (عزلة واحدة) ، و*Geotrichum candidum* (عزلة واحدة) ، و*Fusarium oxysporum* (عزلة واحدة) ، و*A. terreus* (عزلة واحدة). بالإضافة إلى ذلك، أظهرت أربع عزلات بكتيرية مُرمزة بالرموز YA2، YE2، YB1، YC1 نتائج إيجابية لإنتاج إنزيم فينيل ألانين أمونيا لياز، بينما لم تظهر أي عزلة من الخميرة نتائج إيجابية لإنتاج إنزيم PAL. وأظهرت النتائج أيضًا أن 1 *Fusarium solani* هو أعلى منتج للإنزيم من بين جميع العزلات الفطرية المختبرة (كان نشاط الإنزيم 0.224 وحدة / دقيقة / مل من معلق الخلايا). ومن ناحيةً أخرى، فقد كانت العزلة YA2 أعلى العزلات البكتيرية إنتاجاً للإنزيم (كان نشاط الأنزيم 0.112 وحدة / دقيقة / مل من معلق الخلايا). تظهر هذه النتائج مدى تنوع الكائنات الحية الدقيقة المنتجة للـPAL ، والتي يمكن استخدامها في تخليق الإنزيمات صناعياً.

الكلمات المفتاحية: *بكتريا، خميرة ، عزل ،فطريات ، فينيل ألانين أمونيا لياز*