Extracellular lipase production by local isolate of *Penicillium citrinum*

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ABSTRACT:

This study investigated the screening, production and optimization of extracellular lipase from a fungus isolated from different samples in Egypt. The isolated fungi were screened on tributyrin agar for exogenous lipolytic activity. A promising lipase producing isolate of SH5 *Penicillium citrinum* was selected and identified by the isolate SH5 of *Penicillium citrinum* that was identified as the highest lipase-producing strain. Molecular identification was carried out by amplifications of ITS-1, 5.8S and ITS-2 regions. The effects of incubation time, inducers, pH, temperature, carbon and nitrogen sources were varied for optimal lipase production using one factor at a time approach. Maximum lipase production was observed on the third day, pH 7.0 and at 30°C. Olive oil, maltose and ammonium sulphate were the best inducers, carbon and nitrogen sources were respectively for lipase production.

Keywords: Lipase; Extracellular enzyme; Optimization; Fermentation; *Penicillium citrinum*.

INTRODUCTION

Lipases (E.C. 3.1.1.3) are biotechnologically imperative group of enzymes which act on the ester bonds present in triacylglycerol and liberate fatty acids and glycerol (Abrunhosa *et al*., 2013). Lipases do not only catalyze hydrolysis reaction but they are also capable of catalyzing range of reactions such as esterification, trans-esterification (alcoholysis, aminolysis, acidolysis, inter-esterification). They catalyze hydrolytic reaction in aqueous environment and other reactions (esterification, trans-esterification) in non-aqueous environment (Charles and James, 2011). Lipases are utilized broadly in food and dairy industry for the hydrolysis of milk fat, ripening of cheese, flavor development and hydrolysis of butterfat and cream (Ray, 2012). These are also utilized as additives in detergent industry, for removal of oil stains, and enhancing cloth absorbency in the fabric industry (Shivika and Shamsher, 2014). In addition, they are utilized as a catalyst for the synthesis of diverse products utilized in the cosmetic industry, pulp and paper, production of biodiesel, degreasing of leather and resolution of racemic mixtures in the pharmaceutical industry (Ferreira *et al*., 2013). Lipases are also utilized in wastewater treatment for removal of lipid clogged drains and in the medical industry for estimation of blood lipid contents (Verma and Prakash, 2014). Lipases are produced from a variety of living organisms such as plants, animals, bacteria, fungi, yeasts and actinomycetes. Amongst all, microbial sources of lipase have gained industrial attention in past few years because of unique properties and chemical stability (Andualema and Gessesse, 2012). Fungal lipase production has been reported in different species of *Geotrichum*, *Candida*, *Aspergillus*, *Humicola*, *Mucor*, *Penicillium* and *Rhizopus* genus (Gopinath *et al*., 2002; Prabhakar *et al*., 2012).

Costa and Peralta (1999) reported highest activity of lipase by *Penicillium wortmanii* strains using olive oil. Lakshmi and Ram (2021) showed that, the *Penicillium citrinum* maximum lipase production when grown in submerged fermentation. Nwuche and Ogbonna, (2011) reported production of lipase by twelve fungal isolates belonging to genera *Penicillium* using submerged fermentation. (Sztajer *et al.,* 1988) Whereas Lipase producing microbes can be found in different habitats such as industrial effluents, edible oil processing industries, dairies, soil contaminated with edible oil, diesel oil, oilseeds, and decaying food. They are greatly influenced by physico-chemical and nutritional factors such as; pH, temperature, nitrogen and carbon sources as well as presence of lipids, dissolved oxygen, agitation, and inorganic salts (Maria, 2005).

In this work, filamentous fungi were isolated and screened for lipolytic activities. The most active fungal isolate was further studied for lipase production. The effect of carbon, nitrogen sources, pH, growth temperature and incubation period on lipase production by isolated lipase-producing fungal strain was examined.

MATERIALS AND METHODS

Samples

Samples were collected from the rhizosphere of some oil plants i.e., cotton, sesame, olive, maize grown in the area of Kafr El-Sheikh, El- Gharbia, El- Monufia and El-Sharqia, Governorates in Egypt in 2018. The extracellular lipase producing fungi were isolated from soil contaminated with oil seeds by dilution plate method.

Isolation and purification of filamentous fungi:

Fungi were isolated from serial dilutions of the collected samples on Potato dextrose agar (PDA) supplemented with streptomycin and incubated at 28 °C for 5-7 days. Well-separated fungal colonies were picked and streaked onto PDA-streptomycin plates where purity checks were confirmed by repeated streaking on PDA plates (Pandey, *et al*., 2015). Pure isolates were maintained on PDA at 4°C.

Screening fungal isolates for lipase production

The isolated fungi were examined for lipase production on Tributyrin agar (TBA) medium containing (g l^{-1}): peptone, 5; yeast extract 3; agar, 15; tributyrin, 10 and pH was adjusted to 7.0 (Freire, 1996). The isolates were individually inoculated on TBA plates and kept at 28°C for 5 days. Inoculated TBA plates were observed for halo zone around fungal colonies (Pandey *et al.*, 2015). The radius (r) of the colonies and the radius (R) of the clear hydrolytic halos around the cultivated fungal isolates were measured.

Morphological identification of the selected fungal isolates:

The selected fungal isolate (SH5) was characterized and identified on the basis of their colonial and morphological characteristics according to Gilman, (1957). Mycelia morphology colonial characteristics such as surface appearance, texture, reverse and pigmentation were determined on the PDA plates after incubation at 28°C for 7 days. Microscopic characterization, spore shape, color and conidiophores shape were observed under light microscope.

Molecular identification of lipase producing fungal isolate (SH5):

The selected fungal isolate SH5 was identified by molecular techniques.

Internal Transcribed Spacer (ITS) analysis

DNA isolation:

Firstly, the DNA was isolated from SH5 isolate by CTAB method according to (Sambrook, *et al.,* 1989)

PCR Reactions:

The PCR amplification was performed in a total volume of 50 ul, containing 1X reaction buffer, 1.5 mM $MgCl_2$, 1U *Taq* DNA polymerase (promega), 2.5mM dNTPs, 30 poml of each primer and 30 ng genomic DNA (White *et al*., 1990).

Primer Code	Sequence	Product Size
(ITS-1) F	5′- TCCGTAGGTGAACCTGCGG -3′	600bp
(ITS-4) R	5′- TCCTCCGCTTATTGATATGC-3′	

Thermo-cycling PCR program

PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 30 sec., an annealing step at 45ºC for 30 sec. and an elongation step at 72ºC for 1 min. The primer extension segment was extended to 7 min at 72ºC in the final cycle (Attallah *et al*., 2019).

Detection of the PCR Products

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 ug/ml) in 1X TBE buffer at 95 volts. A 100 bp DNA ladder was used as a molecular size standard. PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD 2000) (Attallah *et al*., 2019).

Purification of PCR Products

Amplified products for all PCR were purified using EZ-10 spin column PCR products purification PCR reaction mixture that was transferred to 1.5 ml microfuge tube and three volumes was added to binding buffer 1 then the mixture solution was transferred to the EZ-10 column and let stand at room temperature for 2 minutes after that centrifuge, 750 ul of wash solution was added to the column and centrifuge at 10.000 rpm for two minutes, repeated washing, 10.000 rpm was spine for an additional minute to remove any residual wash solution. The column was transferred into a clean 1.5 ml microfuge tube and adds 50 ul of elution buffer, incubated at room temperature for 2 minutes and when

store purified DNA at -20 °C (Attallah *et al*., 2019).

ITS sequencing analysis

The sequencing of the PCR product was carried out using an automatic sequencer ABI PRISM 3730XL Analyzer using Big Dye TM Terminator Cycle Sequencing Kits following the protocols supplied by the manufacturer. Single-pass sequencing was performed on each template using Rbcl Forward primer. The fluorescent-labeled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples were resuspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Microgen Company). (Attallah *et al*., 2019).

Computational analysis (BLASTn) ITS.

The sequences were analyzed using BLAST program (http://www.ncbi.nlm.nih.gov/BLAST). Sequences were aligned using Align Sequences Nucleotide BLAST.

Preparation of fungal inoculum:

Fungal inocula spore suspensions were prepared from malt extract medium (ME) cultures incubated at 28°C for 7 days **(Lima *et al*., 2003).** One ml aliquots of the fungal spore suspension was used for inoculating the submerged fermentation process.

Fungal lipase production in submerged Fermentation:

Lipases production in submerged fermentation by the selected fungal isolates was examined in a medium contained (g/l): ($NaHPO_4$ 12.0g, KH_2PO_4 2.0g, $MgSO_4$. $7H_2O$ 0.3g, $CaCl_2$ 0.25g, $FeSO_4$. $7H_2O$ 0.005g, $MnSO_4$. $7H_2O$ 0.015g, $ZnSO_4$. $7H_2O$ 0.030g) (Akyil and Cihangir, 2018). The initial pH was adjusted at 5.5. Olive oil, as the sole carbon source, was added to 100 ml fermentation broth in 250 ml Erlenmeyer flasks to reach an oil concentration of 1%. The flasks were inoculated with 1ml spore suspension and incubated on a rotary shaker incubator at 150 rpm and 28°C for 3 days. Cultural filtrate was used to measure lipase activity and protein content.

Lipase Assay

Substrate emulsion was prepared with a composition of (g/100 ml) 70 ml of Emulsification reagent (Na Cl 1.79 g; KH_2PO_4 0.04 g; Glycerol 54ml; Gum Arabic 1.0 g) mixed with 30 ml olive oil. 0.2 M Potassium phosphate buffer (pH 7.0) was prepared by mixing 6.96 g of K_2HPO_4 in 200 ml of distilled water and 5.44 g of KH_2PO_4 in 200 ml of distilled water. In a 250 ml conical flask, 1ml of substrate emulsion, 0.8 ml of 0.2 M Potassium phosphate buffer and 200 μl of sample were mixed. The flask was placed in a water bath at 55°C for 30 minutes. This reaction was terminated by adding 1 ml of acetone, 1 ml of ethanol (1:1, v/v) and 4 drops of phenolphthalein blue indicator. Fatty acids liberated were titrated against 0.01 N NaOH (Adinarayana *et al.,* 2003).

Lipase activity (U/ml) = Final burette reading X Normality of NaoH X Time of Incubation X Dilution factor/ Volume of the enzyme added **Dilution factor =** V2/V1

V1 = Volume before added of enzyme.

V2 = Volume after added of enzyme.

Specific activity (U/mg) = lipase activity/protein content

Total protein assay:

All submerged culture fermentation brews were filtrated through Whattman No. 1 filter paper and the total proteins were determined adopting the methods described by Lowry *et al.,* (1951) in the culture filtrate.

Optimization of lipase production by the fungal isolate SH5:

Effect of different carbon sources:

Different carbon sources *i.e.*, maltose, fructose, lactose, sucrose, glucose and galactose were studied in the fungal mineral medium containing 1% of the examined carbon source. Lipase activity was measured as U/ml after 3 days at 28°C.

Effect of different nitrogen sources:

The effect of different nitrogen sources on enzyme production was investigated by replacing the source of nitrogen in fungal mineral medium containing 0.5% with different organic nitrogen source such as, beef extract, peptone, urea, yeast extract, casein and inorganic nitrogen source such as, ammonium sulphate and sodium nitrate. The lipase activity was determined after 3 days at 28°C.

Effect of various mineral salts:

Olive oil is a carbon source (1% v/v) and ammonium sulfate as a N-source as well as one of the mineral salts *i.e.* $CaCl_2$, $MgSo_4$, $CuSo_4$, $ZnSo_4$, $MnSo_4$, $FeSo_4$ or NaCl at 0.2% concentration were added to 1000 ml distilled water where the pH was adjust to 5.5. The mixture was then autoclaved and used for submerged cultivation of the fungal isolate

SH5 on a rotary shaker incubator at 150 rpm for 28°C for 3 days. Lipase activity and the total protein were determined (Lowry *et al.,* 1951) in culture filtrates and the specific lipase activity was calculated.

Effect of pH values:

To determine the optimum pH for lipase production by the tested fungal isolate SH5, the mineral medium was adjusted at different initial pH values i.e., 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 and inoculated with the fungal spore inoculum. Cultures were incubated at the rotary shaker at 150 rpm and 28 °C for 3 days. The lipase activity and total protein content were determined in culture filtrates.

***Effect of incubation temperatures degree*:**

The effect of different temperature on lipase production by the fungal isolate was studied by cultivation of the fungal isolate SH5, in the mineral medium at different incubation temperatures i.e., 20, 25, 30, 35, and 40 °C on the rotary shaker at 150 rpm where lipase activity and total protein were determined after 3 days.

Effect of incubation periods:

The effect of incubation period on lipase enzymes production by selected fungal isolate SH5 was carried out by adding 1 ml of fungal inoculum in mineral medium and incubated for different periods 1, 2, 3, 4, 5 and 6 days at 28 ºC. The lipase activity was determined after each incubation time.

Lipase production using different oils sources:

To evaluate the effect of different oils on the production of lipase by the fungal isolate SH5, cultures were grown in a medium with the addition of 1.0 % of either olive oil, mustard oil, castor oil, sesame oil, ginger oil or coconut oil (El-Batal *et al*., 2016). Cultures with different oils were incubated on a rotary shaker (150 rpm) at 30 ºC for 3 days where lipase activities and total proteins were measured in the cultural filtrates.

RESULTS AND DISCUSSIONS

Isolation and purification of soil fungi:

In this work, a total of 12 fungal isolates were isolated from the collected samples on Potato dextrose agar (PDA) containing Streptomycin. Previous investigations referred to the fact that oil seeds wastes, dairy product industries, soils contaminated with oils, and spoiled foods are among the habitats colonized with lipolytic fungi (Sharma, *et al.,* 2001). Also, lipolytic fungi were isolated by (Choudhary, 2017) from oil mill soil samples. Among these, selected positive lipolytic isolates were subjected to quantitative screening by titrimetric method.

Screening fungal isolates for lipase production:

The twelve isolates of filamentous fungi from the collected samples were subjected to screening on TBA medium. Ten isolates demonstrated a zone of lipolytic activities on TBA medium, indicating their extracellular lipase activities. Among these, the fungal isolate SH5 showed the largest clear zone diameter to colony diameter ratio, 2.33 mm (figure 1). The isolate SH5 was selected as a superior lipase producing isolate with the largest lipolytic hydrolysis zone diameter of 35 mm. In a previous work by Sharma, *et al.,* (2001) among the lipase-producing fungi, the genus *Penicillium* was the best producer, which is in agreement with the findings in our study. Colla *et al*., (2010) obsearved that the maximum lipolytic activities produced by the *Penicillium* used in submerged fermentation. Kumar *et al*., (2012) found that a lipolytic fungal strain was isolated from common city. Tthe fungal strains were garbage using tributyrin agar. Veerapagu, *et al.,* (2014) stated that, the utilization of tributyrin agar medium for screening of lipase producers.

Morphological identification of the lipolytic fungal isolate (SH5):

The morphological characteristics were studied to identify the selected fungal isolate SH5. The fungal isolate (SH5) colonies were irregular and yellow to green. The colony reversed yellow and entire margin. Fungal species such as *Penicillum* was considered to be the chief producers of lipase (Gilman, 1957& Wadia and Kumar, 2017).

Molecular identification of the lipolytic isolate SH5:

Fragments obtained from the sequenced ITS area were compared to available Genbank database sequences. Although results did not provide significant variation to distinguish between the sequences from *Penicillium* isolates under investigation and sequences from Genbank, ITS area showed strong evidence that our isolated fungus should be classified under *Penicillium* gender. Comparison between isolates under investigation and GenBank database sequences revealed 83.6% similarity, which highly

recommends that isolates under investigation affiliated with *Penicellium citrinum* (Fig. 2).

Production of lipase by submerged fermentation:

Data illustrated by Table (1) show lipase activities by the examined fungal *Penicillium citrinum*. The selected strain *Penicillium citrinum* (SH5) exhibited a lipase activity of 13.50 U/ml with a specific activity of 24.91 U/mg. This is in concordance with the previous findings by Shafei and Allam (2010) who recorded that *Penicillium chrysogenum* produced an extracellular lipase during growth on a medium containing olive oil as the sole carbon source. Jayaprakash and Ebenezer, (2012) Studied the production of an extracellular lipase from *Penicillium citrinum* using Submerged fermentation. Lakshmi and Ram (2021) showed the *Penicillium citrinum* KU613360 maximum lipase production when grown in submerged fermentation.

Optimization of lipase production by the examined strain *Penicillium citrinum* SH5 in submerged fermentation:

Several chemical and physical factors are known to affect the production of lipase by selective strain SH5 of *Penicillium citrinum* in submerged fermentation process. The most notable among these are carbon sources, nitrogen sources, metal salts, pH and incubation temperature. Optimization of these parameters is one of the most important factors used for improving lipase production to meet the growing industrial demands.

***Carbon sources**:*

As depicted in Table (2), the carbon source in the medium has considerably effect on the synthesis of lipolytic enzymes by selective strain *Penicillium citrinum* (SH5) in liquid culture. The fungal basal medium was supplemented with different carbon sources at 1% concentration. Improved production of lipase by strain SH5 of *Penicillium citrinum* was achieved in the basal medium containing maltose as the sole carbon source with lipase activity, which reached 16.50 U/ml, followed by galactose, fructose, lactose and sucrose. Whereas minimum lipase production was achieved in the medium containing glucose i.e., 12.75 U/ml. The obtained results were in concordance with those obtained by Amrane *et al*., (2003) who detected little lipase activity when glucose was used as carbon source in submerged fermentation by *Penicillium camembertii*. Rehman *et al.,* (2011) reported that the maltose in the growth medium enhanced lipase production by *Penicillium notatum*. Jayaprakash and Ebenezer, (2012) also reported forward lipase activity by *Penicillium citrinum* using sucrose as source of carbon.

***Nitrogen sources**:*

Nitrogen sources play an important role in the biosynthesis of lipase by microorganisms (Dheeman *et al.,* 2011). Both organic and inorganic nitrogen relieve catabolite repression and induce hydrolase synthesis. Inorganic nitrogen is rapidly consumed by microorganisms, while organic sources provide amino acids and growth factors. Data in Table (3) show that ammonium sulphate was the most appropriate nitrogen source for lipase production by *Penicillium citrinum*. As a result of using such N-source in the fermentation medium the examined strain *Penicillium citrinum* produced a maximum lipase activity of up to 11.25U/ml. followed by casein and beef extract as N-sources as well supported higher levels of affected by lipase production. This was in concordance with the findings by Amin and Bhatti, (2014) who demonstrated that ammonium sulfate induced lipase production in other *Penicillium* strains. Boratynski, *et al.,* (2018) found that the Ammonium sulphate enhanced lipase by *Penicillium camemberti*. Lakshmi and Ram (2021) improved lipase activity by novel isolate of *Penicillium citrinum* when medium was supplemented with ammonium sulphate.

Mineral salts:

The effects of mineral salts were studied at initial concentration 0.2 %. Table (4) shows the effect of various metals on lipase productivity by the examined strain (SH5) of *Penicillium citrinum* . High production of lipase was recorded in the basal media containing; NaCl with lipase activity 10.05 U/ml, followed by $ZnSo_4$, $FeSo_4$, $CaCl_2$, $MgSo_4$ and $MnSo_4$ which reached (8.25, 7.95,7.80,7.65 and 7.50 U/ml, respectively). The obtained results were in agreement with those obtained by Mase *et al.,* (1995) Who reported that lipase from *Penicillium roqueforti* strain displayed high tolerance toward NaCl. Bancerz *et al.,* (2005) demonstrated that the Ca^{2+} and Mn^{2+} ions enhanced lipase production by *Penicillium chrysogenum*. Lakshmi and Ram (2021) improve lipase activity by *Penicillium citrinum that* was achieved in a medium containing Mn^{2+}.

PH values:

The pH of the growth medium is an imperative factor, which greatly affects the

microbial growth and enzyme production during solid state fermentation. Each microorganism possesses a unique optimum pH, and a pH range for its growth and activity (Rehman *et al.,* 2011). Data in Table (5) showed that the increased enzyme production by strain SH5 of *Penicillium citrinum* was observed at pH 7.0 (13.50 U/ml), followed by pH 6.0 (12.90 U/ml), pH 8.0 (12.30 U/ml) and pH 4.0 (11.25 U/ml). Minimum production of the lipase enzymes was obtained in the basal medium with initial pH 10.0 which reached 8.25 U/ml. The results obtained were in agreement with those reported by Jayaprakash and Ebenezer, (2012) who reported that the highest activity of lipase by *Penicillium citrinum* at pH 7.0. Lakshmi and Ram (2021) reported increased production of lipase by *Penicillium citrinum* when cultivated at pH 7.5.

Incubation temperatures:

The effect of the various incubation temperatures on the enzyme production of selective strain SH5 of *Penicillium citrinum* was studied to determine the optimum temperature for lipase production. The results in Table (6) showed the optimum temperature for lipase production by strain SH5 of *Penicillium citrinum* (7.60U/ml). They were obtained at 30 ºC followed by 25ºC (6.45 U/ml). The obtained results were in agreement with those obtained by Lima, *et al.,* (2003) Who investigated production of lipase by *Penicillium aurantiogriseum* under different incubation temperatures (26-32 ºC). Lipase production was highest at 28-30 ºC. Bancerz *et al.,* (2005) investigated lipase production by *Penicillium chrysogenum* at various incubation temperatures. Highest activity was obtained at 28-30 °C. Jayaprakash and Ebenezer, (2012) reported that the highest activity of lipase was obtained when the culture of *Penicillium citrinum* was incubated at 35 °C.

Different incubation periods:

Lipase production by microbial strain was based on the specific growth rate; incubation time influences the growth rate of the culture and its enzyme synthesis ability. Results in Table (7) show the effect of varying incubation period on lipase productivity by strain (SH5) of *Penicillium citrinum*. The obtained results found that the rate of enzyme production significantly increased with the increase in the fermentation period, and reached its maximum activity after 3 days by strain (SH5) of *Penicillium citrinum* which were 9.30 U/ml. The incubation periods above and below the optimum incubation periods resulted in a significant inhibition of lipase production. These results were similar to the previous results reported by Maliszewska and Mastalerz (1992) who found that the highest activity of lipase at 4 days of incubation by a novel strain of *Penicillium citrinum* in submerged fermentation, followed by decrease in activity with the increase of incubation period. Gutarra, *et al.,* (2009) reported that highest yield of lipase by *Penicillium simplicissimum* was when it was cultured in submerged fermentation for 3 days.

Different oils sources:

Microbial lipases are mostly inducible. Upon induction, they secrete extracellular enzymes into the surrounding environment. Such inducible extracellular lipases are produced in the presence of inducers such as fatty acids, oils, triacylglycerol, tween, bile salts and glycerol (Yang *et al.,* 2005) although the requirement for sugar as a carbon source in addition to lipids varies with the microorganism. Inducers in the form of lipid substrates (olive oil, mustard oil, castor oil, sesame oil, ginger oil and coconut oil) were studied for their effects on lipase production by *Penicillium citrinum* (SH5). Our findings showed that in Table 8 olive oil induced highest lipase production when compared with other seeds oils by SH5 strain of *Penicillium citrinum* (9.00 U/ml). The results obtained were in concordance with those obtained by pervious investigators. Annibale *et al*., (2006) reported that the strain of *Penicillium citrinum* had maximum growth in effluent of the olive oil industry. Jayaprakash and Ebenezer, (2012) recorded that the maximum lipolytic activity was in the presence of olive oil by *Penicillium citrinum*. Zainab, *et al*., (2017) reported that olive oil is considered as the best inducer of lipase production.

CONCLUSIONS:

The strain SH5 of *Penicillium citrinum* has great potential for extracellular lipase production at initial pH of 7.0, maltose carbon source, ammonium sulphate nitrogen source, olive oil inducer, for 3 days at 30C°. This potential can be further enhanced several times through the use of above physical parameters at different condition.

REFRENCES

Abrunhosa, L., Oliveira, F., Dantas, D., Goncalves, C., Belo, I. 2013: Lipase production by *Aspergillus ibericus* using olive mill wastewater. Bioproc Biosyst Engin 36: 285-291.

Adinarayana, K., Raju, K., Zargar, M., Devi, R., Lakshmi, P., Ellaiah, P. 2003: Optimization of process parameters for production of lipase in solid state fermentation by newly isolated *Aspergillus* species. Int. J. Biotechnol 3: 65-69.

Akyil, M., Cihangir, N. 2018: Detection of lipase production from newly Isolated *Trichoderma Citrinoviride*. Hacettepe J. Biol. & Chem. 46 (2), 229–235.

Amin, M., Bhatti, H. 2014: Effect of physicochemical parameters on lipase production by *Penicillium fellutanum* using canola seed oil cake as substrate. Int. J. Agric. Biol. 16, 118–124.

Amrane, A., Pestel, V., Prigent, Y. 2003: Submerged cultures of *Geotrichum candidum* and *Penicillium camembertii* on amino acids and glucose. The Journal of General and Applied Microbiology. 49(4): 251-255.

Andualema, B., Gessesse, A. 2012: Microbial lipases and their industrial applications: review. Biotechnology. 11: 100-118.

Annibale, A., Sermanni, G., Federici, F., Petruccioli, M. 2006: Olive-mill wastewaters: a promising substrate for microbial lipase production. Bioresour. Technol. (97): 1828 – 1833.

Attallah, A., Sahab, A., Ziedan, E. 2019: Molecular identification and pathogenicity of *fusarium* fungi causing root rot disease of cucumber under plastic greenhouses in egypt. Plant Archives. 19 (1): 1301-1307.

Bancerz, R., Ginalska, G., Fiedurek, J., Gromada, A. 2005: Cultivation conditions and properties of extracellular crude lipase from the psychrotrophic fungus *Penicillium chrysogenum*. J. Int. Microbiol Biotechnol. 32: 253 – 260.

Boratynski, F., Szczepanska, E., Grudniewska, A., Gniłka, R., Olejniczak, T. 2018: Improving of hydrolases biosynthesis by solid-state fermentation of *Penicillium camemberti* on rapeseed cake. Scientific Reports. 8:10-157.

Charles, O., James, C. 2011: Isolation of lipase producing fungi from palm oil Mill effluent (POME) dump sites at Nsukka. Braz Arch Biol Technol. 54: 113-116.

Choudhary, R. 2017: Isolation and screening of lipase producing bacteria from oil mill effluent. International Journal of Science and Research. 13(2): 192-194.

Colla, L., Primaz, A., Benedetti, S., Loss, R., Lima, M., Reinehr, C., Bertolin, T., Costa, J. 2010: Selection of lipase-producing microorganisms through submerged fermentation. Z. Naturforsch. (65):483 – 488.

Costa, M., Peralta, R. 1999: Production of lipase by soil fungi and partial characterization of lipase from a selected strain (*Penicillium wortmanii*). J. Basic Microbiol. 39(1): 11-15.

Dheeman, D., Frias, J., Henehan, G. 2011: Purification and characterization of an extracellular lipase from a novel strain *Penicillium* sp. DS-39.J. Mol. Catal. B Enzym. 72(3-4):256-262.

El-Batal, A., Farrag, A., Elsayed, A., El-Khawaga, M. 2016: Effect of environmental and nutritional parameters on the extracellular lipase production by *Aspergillus niger*. 60: 18-29.

Ferreira, S., Georgina, S., Francisco, P., Francisco, V. 2013: The potential use of lipases in the production of fatty acid derivatives for the food and nutraceutical industries. Electronic J. Biotechnol. 16.

Freire, D. 1996: Selection of lipolytic microorganisms and study of lipase production by *Penicillium restrictum*. Ph D Thesis, University Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

Gilman, J. 1957: Amanual of soil fungi. Lowa State Uni. Press, Ames, lowa U. S. A.

Gopinath, S., Hilda, A., Priya, T., Annadurai, G. 2002: Purification of lipase from Cunninghamella verticillata and optimization of enzyme activity using response surface methodology. World J Microbiol Biotech 18: 449-458.

Gutarra, M., Godoy, M., Maugeri, F., Rodrigues, M., Freire, D., Castilho, L. 2009: Production of an acidic and thermostable lipase of the mesophilic fungus *Penicillium simplicissimum* by solid-state fermentation. Bioresour Technol. 100(21): 5249-5254.

Jayaprakash, A., Ebenezer, P. 2012: Extracellular lipase production by *Penicillium citrinum* isolated from petroleum contaminated soil. *J. Acad. Indus. Res.* Vol. 1(2):77-80.

Kumar, D., Kumar, L., Nagar, S., Raina, C., Prashad, R., Gupta, V. 2012: Screening, isolation and production of lipase/esterase producing *Bacillus* sp. strain and its potential evaluation in esterification and resolution reactions. Archives of Applied Science Research, 4(4): 1763-1770.

Lakshmi, L., Ram, M. 2021: Production, purification and characterization of extracellular lipase from a mutated strain of *Penicillium Citrinum* KU613360. International Journal of Scientific Research in Science and Technology.8(1):111-120.

Lima, V., Krieger, N., Sarquis, M., Mitchell, D., Ramos, L., Fontana, J. 2003: Effect of nitrogen and carbon sources on lipase production by *Penicillium aurantiogriseum*. Food Technol Biotechnol; 41(2): 105-110.

Lowry, O., Rosenbrough, N., Farr, L., Randall, A. 1951: Protein measurement with the folin phenol reagent. J. Biol Chem.193: 265-275.

Maliszewska, I., Mastalerz, P. 1992: Production and some properties of lipase from *Penicillium citrinum*. Enzyme Microb. Technol.14:190-193.

Maria, P. 2005: Biotechnological applications of Candida antarctica lipase A: state-of-theart, Journal of molecular catalysis b: enzymatic. 37: 36-46.

Mase, T., Matsumiya, Y., Matsuura, A. 1995: Purification and characterization of *Penicillium roqueforti* lAM 7268 lipase. Biosci. Biotech. Biochem. 59 (2): 329-330.

Nwuche C., Ogbonna J. 2011: Isolation of lipase producing fungi from palm oil mill effluent (POME) dump sites at nsukka. Braz. Arch. Biol. Technol. 54(1): 113-116.

Pandey, H., Kestwal, A., Chauhan, D., Kumari, S., Dhalwal, V., Singh, G., Singh, P., Mann, P., Sharma, A., Saxena, G., Kapoor, A., Giri, B. 2015: Isolation and screening of potential fungi and standardization of a process for the production of extracellular lipase. J. of Undergraduate Research and Innovation. 1(1): 116-123.

Prabhakar, T., Bhogavalli, P., Vallem, P., Srirangam, V. 2012: Studies on optimization of extracellular lipase from potential fungal strains isolated from oil contaminated soil. J. Microbiol Biotechnol Res. 2: 418-425.

Ray, A. 2012: Application of lipase in industry. Asian J. Pharm Tech 2: 33-37.

Rehman, S., Nawaz, B., Ahmad, B., Asgher, M. 2011: Optimization of process parameters for enhanced production of lipase by *Penicillium notatum* using agricultural wastes. African Journal of Biotechnology. 10 (84):19580-19589.

Samboork, J., Fritch, E., Maniatis, T. 1989: Molecular cloning; A laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory Press.pp.,1659.

Shafei, M., Allam, R. 2010: Production and immobilization of partially purified lipase from *Penicillium chrysogenum.* Malaysian Journal of Microbiology. 6 (2):196-202.

Sharma, R., Chisti, Y., Banerjee, U. 2001: Production, purification, characterization, and applications of lipases. Biotechnology Advances, 19, 627–662.

Shivika, S., Shamsher, S. 2014: Organic solvent tolerant lipases and applications. Sci World J. 1-15.

Singh, M., Chandraveer, M., Tripathi, A. 2017: Isolation and screening of Lipase Producing Microorganisms from Natural Sources. Indian Journal of Ecology. 44(1): 19-23.

Sztajer, H., Maliszewska, I., Wieczorek, J. 1988: Production of exogenous lipase by bacteria, fungi and actinomycetes. Enzyme Microb Technol 10: 492-497.

Veerapagu, M., Sankara, A., Jeya, K., Alagendran, S. 2014: Isolation and identification of a novel lipase producing bacteria from oil spilled soil. International Journal of Innovative Research in Science, Engineering and Technology. 3(12): 18122- 18129.

Verma, S., Prakash, S. 2014: Isolation, identification and characterization of lipase producing microorganisms from environment. Asian J Pharm Clin Res. 7: 219-222.

Wadia, T., Kumar, S. 2017: Isolation, screening and identification of lipase producing fungi from oil contaminated soil of Shani Mandir Ujjain. Int.J.Curr.Microbiol.App.Sci. 6 (7): 1872-1878.

White, T., Bruns, T., Lee, S., Taylor, J. 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications (Innis MA, Gelfand DH, Sninsky JJ and White TJ, eds.). Academic Press Inc., New York, 315-322.

Yang, X., Wang, B., Cui, F., Tan, T. 2005: Production of lipase by repeated batch fermentation with immobilized *Rhizopus arrhizus.* Process Biochemistry. 40:2095–2103.

Zainab, A., Ademakinwa, A., Agboola, F. 2017: Studies on the optimization of lipase production by *Rhizopus* sp. zac3 isolated from the contaminated soil of a palm oil processing shed. Journal of Applied Biology & Biotechnology. 5 (2): 30-37.

Lima, V., Krieger, N., Sarquiz, M., Mitchell, D., Ranos, L., Fontana, J. 2003: Effect of nitrogen and carbon sources on lipase production by *Penicillium, aurantiogriseum.* Food Technology and Biotechnology. 41: 105-110.

Table 1: Lipase activity, total proteins and specific lipase activity by the strain SH5 of *Penicillium citrinum* in submerged fermentation.

Lipase activity (U/ml)	13.500
Total protein (mg/ml)	0.542
Specific activity (U/mg)	24.910

Table 2: Lipase production by *Penicillium citrinum* (SH5) on different carbon sources

Carbon sources	Lipase activity (U/ml)	Total protein (mg/ml)	Specific activity (U/mg)
Glucose	12.75	0.831	15.34
Galactose	15.30	0.399	38.35
Fructose	14.70	0.424	34.67
Maltose	16.50	0.383	43.08
Sucrose	13.50	0.524	25.76
Lactose	14.25	0.458	31.11

Table 3: Lipase production by *Penicillium citrinum* SH5 on different nitrogen sources:

Nitrogen sources	Lipase activity (U/ml)	Total protein (mg/ml)	Specific activity (U/mg)
Ammonium sulphate	11.25	0.66	17.07
Sodium nitrate	8.40	1.08	7.79
Yeast extract	9.15	0.93	9.84
Casein	11.10	0.77	14.40
peptone	9.00	0.97	9.32
Beef extract	10.05	0.76	13.29
Urea	9.45	0.85	11.14

Table 4: Lipase production by *Penicillium citrinum* SH5 on various mineral ions on the production of lipase

Metal salts	Lipase activity (U/ml)	Total protein (mg/ml)	Specific activity (U/mg)
NaCl	10.05	0.948	10.60
$Cacl_2$	7.80	0.895	8.72
$MgSo_4$	7.65	0.99	7.73
$MnSo_4$	7.50	1.394	5.38
$ZnSo_4$	8.25	0.856	9.64
$FeSo_4$	7.95	0.987	8.05
$CuSo_4$	6.00	1.186	5.06

Table 5: Lipase production by *Penicillium citrinum* (SH5) at different pH values

pH	Lipase activity (U/ml)	Total protein (mg/ml)	Specific activity (U/mg)
4	11.25	1.008	11.16
5	12.00	1.061	11.31
6	12.90	0.862	14.97
7	13.50	0.869	15.54
8	12.30	1.028	11.96
9	9.75	1.092	8.93
10	8.25	1.200	6.88

Table 6: Lipase production by *Penicillium citrinum* (SH5) at incubation temperatures

Temperatures C°	Lipase activity (U/ml)	Total protein (mg/ml)	Specific activity (U/mg)
20	6.00	1.04	5.75
25	6.45	0.89	7.24
30	7.60	1.01	7.52
35	6.30	1.02	6.16
40	5.40	0.99	5.46

Table 7: Lipase production by *Penicillium citrinum* (SH5) at different incubation periods

Incubation periods (days)	Lipase activity (U/ml)	Total protein (mg/ml)	Specific activity (U/mg)
1	6.00	1.15	5.22
2	7.50	0.71	10.56
3	9.30	0.54	17.22
4	7.80	0.55	14.18
5	7.50	0.80	9.38
6	7.20	1.03	6.99

Table 8: Lipase production by *Penicillium citrinum* (SH5) using different oils sources

different Oils	Lipase activity (U/ml)	Total protein (mg/ml)	Specific activity (U/mg)
olive oil	9.00	0.60	14.98
mustard oil	7.50	0.62	12.16
castor oil	7.65	0.62	12.28
sesame oil	8.25	0.60	13.82
ginger oil	7.05	0.67	10.49
coconut oil	7.50	0.62	12.20

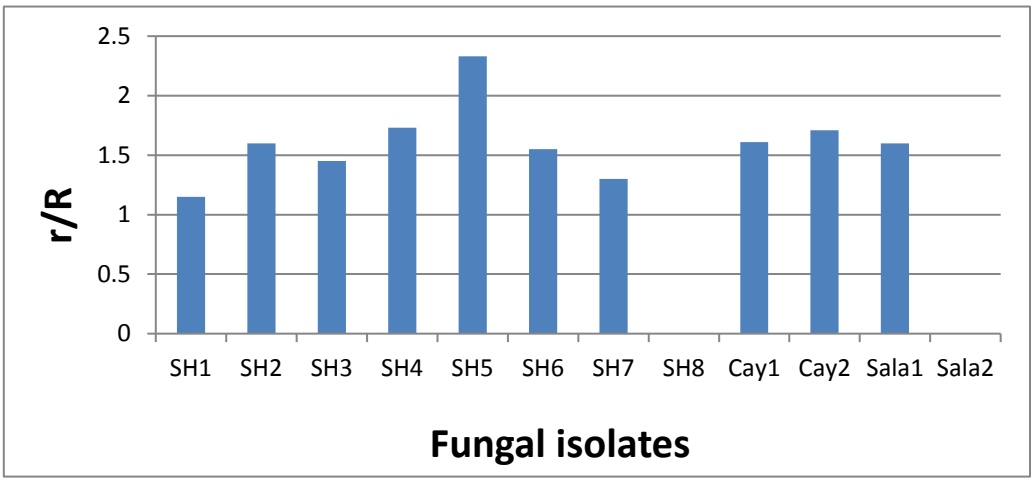

Figure 1: Screening fungal isolates for lipase enzyme production on TBA medium

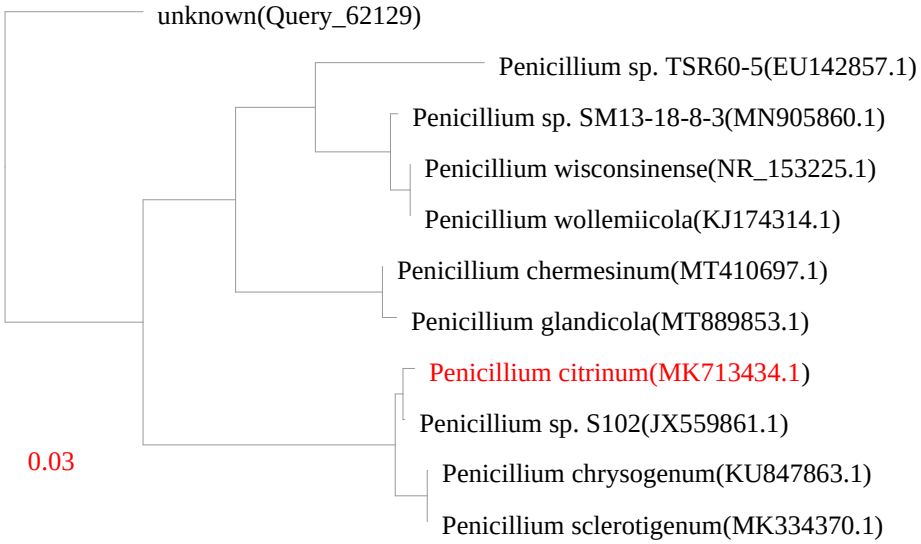

Figure 2: Dendrogram generated using Un- weighted pair group method with arithmetic average (UPGMA) analysis showing relationships *Penicillium citrinum* strain using ITS.

إنتاج الليبيز خارجيا بواسطة *Penicillium citrinum* المعزول محليا

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الملخص العربي:

تهدف هذه الدراسة الى الكشف عن قدرة العزلات الفطريـــة على إنتاج إنزيم الليبيز باستخدام الوسط الغذائي المحتوى على مادة Tributyrin حيث تم اختيار سلالة الفط SH5 التابعة ل *Penicillium citrinum* ذات النشاط الإنزيمي الأفضل وأظهرت دراسة الظروف المثلى لإنتاج إنزيم الليبيز (مصادر الكربون – مصادر النتروجين - الأملاح المعدنية - الرقم الهيدروجيني- درجة حرارة التحضين - المحفزات - فترات التحضين) المختلفة للسلالة SH5 التابعة ل*Penicillium citrinum* وقد أظهرت النتائج أن أقصى إنتاج لإنزيم الليبيز يكون بإضافة المالتوز كباعتباره صدراً للكربون ومع استخدام كبريتات الأمونيوم كمصدر للنيتروجين وعلى فترة تحضين ثلاثة أيام على درجة حرارة 30° م ورقم هيدروجيني 7 ومع استخدام زيت الزيتون كأفضل محفز لإنتاج إنزيم الليبيز.

الكلمات الاسترشادية: الليبيز, الانزيمات الخارجية, الظروف المثلى, التخمر, بنيسليوم ستريم.