

Efficiency of *Aspergillus* Species to Produce the Lipase Enzyme from Various Types of Oil Seeds

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Abstract

Filamentous fungi can easily degrade agroindustrial wastes in solid- state fermentation processes synthesizing many important commercial biocompounds such as lipolytic enzymes. Lipases have various applications in industry so the aim of this study to evaluate the effect of the composition of the solid culture medium on the production of lipolytic enzymes by various species of *Aspergillus*, so in this study we use different types of oil seeds to isolate different species of *Aspergillus* and study their effect on production of lipase enzyme by using of this different seeds as a substrate for culturing of this fungi and in this study *Aspergillus niger* that are isolated from *pistacia vera* seeds is more efficient for production of the lipase enzyme.

Keys word: *Pistacia vera* (pistachio), *Anacardium accidentae* (kazoo) ,*Prunus delicis*(almonds) ,*Arachis hypogavae*(pistachio slaves), *Juglanis regis*(walnut)

Introduction

The demand for microbial lipases is on the increase owing to their application in a wide variety of industrial process and it is used in food, dairy, cosmetic and pharmaceutical industries due to their hydrolytic reaction⁽⁷⁾.

Various types of microorganism, such as fungi are widely recognized as a good source of extracellular lipase⁽²⁾ *Aspergillus* is the best candidate because it can produce more enzymes than other microbes in microbial engineering research development related to very high lipase⁽³⁾. The best species of fungi to produce the lipase enzyme is *Aspergillus niger* because it is classified as GRAS (Generally Regarded A Safe) by the FDA (Food and Drug Administration of the United States of America)⁽⁴⁾. So that the lipase from this fungi can be used in various fields including food and pharmaceutical industry⁽⁵⁾. Solid state fermentation is a fermentation technique that allows the use of simple substrates such as oil seeds, marine water, solid waste of agroindustrial products that are induced by using oil such as olive oil⁽⁶⁾.

Lipase (triacylglycerol acyl-hydrolases EC 3.1.1.3) are enzymes capable of hydrolyzing the ester bands of insoluble substrates in water at the substrate – interface⁽⁷⁾.

Many researcher worldwide direct their activities to the screening of new lipase producing microorganism, and subsequently to the optimization of the medium composition to increase the efficiency of the microorganism to produce the lipase enzyme because the composition of the medium can effect on the characteristic and efficiency of the lipase enzyme and change its properties⁽⁸⁾.

Material and Method

I-Isolation and diagnosis of the *Aspergillus* from oil seeds

The fungi used in this study was isolated from oil seeds of (*pistacia vera*(pistachio), *Anacardium accidentae*(kazo), *Prunus delicis*(almond),*Arachis hypogavae*(pistachio slaves),*Juglanis regis*(walnut)),various *Aspergillus* species was isolated from there various oil seeds by washing these seeds well by distilled water and sterilized by adding of 1% sodium hydrochloride solution (Naocl) for one minutes and then this seeds cultured on potato dextrose agar (PDA) that sterilized by adding of streptomycin, and this plates was incubated at (28°C ± 1) for 7 days. The species of *Aspergillus* was identified by macro and micro-morphological analysis⁽⁹⁾. The macro morphological characteristics observed were color, relief and edges of the colonies.

II-Isolation of the *Aspergillus* spp. from the soil

The samples that are used for isolation *Aspergillus* spp. from the soil that taken from 2-5 cm deep and cultured on (PDA) agar with added chloramphenicol in 10µg/ ml with 3 dilution for each sample and incubated at 28°C for (2-8 days), then *Aspergillus* spp. colonies was isolated depending on macro- morphological characteristics.

III-Screening for extracellular lipase production

Modified basal salt medium (BSM) containing (g/L): NH_4NO_3 , (1) K_2HPO_4 (1.5) $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ (0.025) CaCl_2 (0.025) $\text{FSSO}_4 \cdot 7 \text{H}_2\text{O}$ (0.015) $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (0.005), plus distilled water 1000ml and PH must be adjusted to 7. Sterilization of the medium is done and then adding of 250 mg of streptomycin and 1% (VIV) sterilized tween 80 were added.

Then 1 ml of the *Aspergillus* colonies suspension was transferred to 30 ml of the previously prepared medium. The flask that contain the fungi cultures were incubated at 28°C for 5 days, under shaking (150 rpm) and then filtrate was centrifuged under cooling at 1000 xg for 30 min at 4°C, and the supernatants was subsequently collected for lipase activity determination. Lipase activity was determined by using ⁽¹⁰⁾.

IV-Screening of *Aspergillus* spp. for lipase production

Screening was carried out using mineral medium described by ⁽⁷⁾ with olive oil emulsion as the carbon source. Strains that showed by hydrolytic halos on this medium were selected for further lipase determination.

V- Extraction of pure lipase.

The production of lipase was studied in 250 ml flask containing 50 ml of mineral medium with olive oil as a carbon source, it also contain 3 gm of KNO_3 , 1 gm of KH_2PHO_4 , 1 gm of $\text{Mg So}_4 \cdot 7 \text{H}_2\text{o}$ and 2% of sterile olive oil, then the strain of isolated *Aspergillus* was inoculated and incubated at 28°C on a rotatory shaker at 120 rpm up to 7 days, and then the growth was filtered through Whitman No.1 filter paper. The clear culture filtrate then was assayed for lipase activity.

VI- Effect of initial PH.

The fungi can grow on culturing medium at different initial PH ranging from 2 – 12. The initial PH

was adjusted by 0.1 m HCL OR 0.1 M Na oH. After 6 days the fungi mass was grown and yield of lipase produced was determined.

VII- Effect of temperature on lipase activity and stability.

The effect of reaction temperature on the activity and stability of lipase was determined by incubation the enzyme at different range of temperature ranging from 30-70 °c. Immediately after incubation at each temperature, the enzyme activity was then determined at 30°C.

IX- Measuring of enzymes activity.

The enzymes activity was determined using the method standardized by ⁽¹¹⁾ which is based on titration with NaoH of fatty acids released by the action of lipase on the extract on the triacylglycerol of olive oil was added to 250 ml flasks, 2 ml buffer prepared, 5 ml of olive oil and then 1 ml of enzyme extract was added to this system and it was incubated for 30 min. at 30°C. After incubation the reaction was stopped by adding 15 ml of acetone: ethanol: water (1:1:1) and released fatty acids were titrated with a solution of 0.01 mol/2 NaoH using phenolphthalein as indicator.

One unit of activity was defined as the amount of enzyme that releases 1 mol of fatty and per minute per ml of enzyme extract of the submerged fermentation under the test condition.

Results:

In this study 3 species of *Aspergillus* was isolated from soil and different oil seeds included *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus* and each one of these species was tested for its ability for production of lipase enzyme, and activity of this enzymes also was studied. The frequency of isolation of *Aspergillus* spp. from different types of substrate. We found that the most common isolate was the *Aspergillus niger* that was isolated from all types of oil seeds that are used in this experience in addition to soil.

As was showed in the table (1) the most active species for secretion of active lipase was *Aspergillus niger* isolated from (*Pistacia vera*) pistachio while (*Pistacia vera*) *Aspergillus fumigates* that are isolated from pistachios show no lipase activity, while *Aspergillus flavus* isolated from pistachios (*Pistacia vera*) showed

moderate lipase activity. *Aspergillus flavus* that were isolated from walnut (*Jugans regis*) also showed – ve activity for lipase production. While all species isolated from soil and pistachios slaves showed activity of lipase enzymes in various values. from this study we noticed

that lipase enzymes can be produced by different species of *Aspergillus* and activity of these enzymes are depended on the substrate that are used for isolation of these enzymes, but the best one is *Aspergillus Niger* and the best substrate was the pistachios (*Pistacia vera*).

Table (1) Efficiency of enzyme lipases that are isolated from *Aspergillus* spp. according to types and substrates:

Types of <i>Aspergillus</i> spp	types of substrate	Efficiency of lipase
- <i>Aspergillus niger</i>	Soil	+++
- <i>Aspergillus flavus</i>	Soil	++
- <i>Aspergillus niger</i>	<i>Pistacia vera</i>	++++
- <i>Aspergillus flavus</i>	<i>Pistacia vera</i>	++
- <i>Aspergillus niger</i>	<i>Arachis hypogavae</i>	++
- <i>Aspergillusfumigatus</i>	<i>Seasum indicum</i>	++
- <i>Aspergillus niger</i>	<i>Seasum indicum</i>	++
- <i>Aspergillus niger</i>	<i>Anacardium accidentae</i>	+++
- <i>Aspergillus flavus</i>	<i>Anacardium accidentae</i>	+
- <i>Aspergillus niger</i>	<i>Prunus delcis</i>	+++
- <i>Aspergillus flavus</i>	<i>Juglans regis</i>	++
- <i>Aspergillus niger</i>	<i>Juglans regis</i>	-
- <i>Aspergillusfumigatus</i>	<i>Juglans regis</i>	+

Discussion

This study appears that the most common species that are isolated from soil and oil seeds were the *Aspergillus niger* and it was the most efficient producer of lipase enzyme by using of pistichios (*Pistacia vera*) as a substrate and this was gone with the study of Tim. ⁽¹⁾. The suitability of pistichios for lipase production may be attributed to the oil present in it. This oil may have induced the production of this enzyme without an additional of carbon source while supplementation of glycerol favored enzyme production ⁽¹²⁾.

Also there are many studies appear that the enzymatic activity of *Aspergillus niger* that are cultured

on solid state fermentation were higher than using other methods. As approved by studies done by ⁽¹³⁾.

This study approves that substrate type can effect efficiently on the types of *Aspergillus* species and their efficiency for production of lipase enzymes and also the activity of these enzymes, that are based on the analysis conducted on the unite of lipase and approved also by study ⁽⁶⁾.

Although the primary function of the substrate was to support the growth of *Aspergillus* but the lipid content in the substrate medium could increase level of lipase activity generated.

Study done by ⁽¹⁴⁾ agree with our study that using of solid lipid substrate that are served as the sole course of nutrient for lipase production may have a combined benefit of utilizing a low-value waste material while producing a commercially valuable product due to using the inexpensive culture media comparison and stability of the enzymes over longer period.

Study of ⁽⁷⁾ approved that the best species of *Aspergillus* to produce lipase enzyme was *Aspergillus*

niger, next to it was *Aspergillus fumigatus*, while *Aspergillus flavus* showed the least potential for lipase production.

We conclude from this study that oil seeds considered as a good substrate for lipase production because beside being a source of carbon and supporting the fungus has lipids that assist in the production of this enzyme. The addition of lipid substrate as oil is essential to obtain highest lipase production.

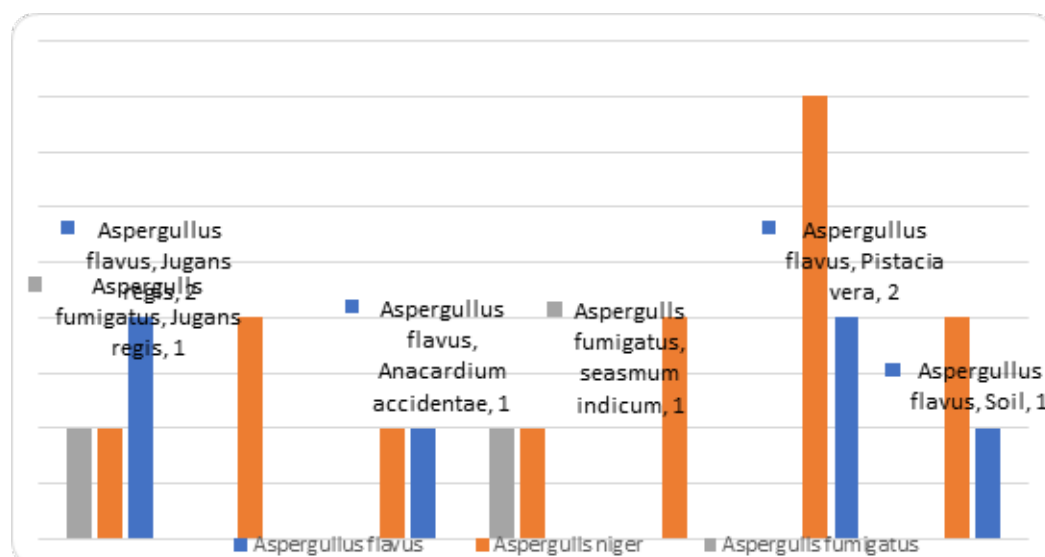


Figure (1) show frequency of isolation of *Aspergillus* from different source of substrate

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

- Brooks A A, Asamudo N U, "Lipase production by strains of *Aspergillus* species isolated from contaminated body creams". *Journal of Toxicology and Environmental Health Sciences*, , October 2011; 3 (11): 311-316.
- Monica CaramazTrichesDamaso, et al, "Utilization of Agro-industrial Residues for lipase production by solid state fermentation", *Brazilian Journal of Microbiology*, 2008 ; (39): 676-681 .
- Gwen Falong, et al. "Production of extracellular lipase from *Aspergillus niger* by solid state fermentation. *Food Technology Biotechnology*" 2006 ; (44): 235- 240.
- Koda A, Minetoki T, et al. "Translation efficiency mediated by the untranslated region greatly affects protein production in *Aspergillus oryzae*'s App. *Microbial Technology*.2004 ; 66 (3) : 291-296.
- NC Mhetras, et al. "Using fermentation processes for production of lipase by *Aspergillus niger*" 2nd international conference on Environment Kuala lumpur, Malaysia, 2013.
- Tania Surya Utami, Ines Hariyani, Gandhi Alamsyah , Heri Hermansyah . "Production of dry extract extracellular lipase from *Aspergillus niger* by solid state fermentation method to catalyze biodiesel synthesis", 4th International Conference on Energy and Environment Research, ICER 2017, 17-20 July 2017, Porto, Portugal .
- R Sharma, X Chisti, Y C Banerjee . "Production purification, characterization and Application of lipases" *Biotechnology Advances*, 2001 ; 19(8) : 627-662 .

8. R R Maldonado . Production purification and characterization of lipases of *Geotrichum candidum* obtained from industrial media, Universidad Estadual de Campinas-Unicamp, Campinas, Brazil, 2006.
9. T M Costa, K L Hermann, et al . “Lipase production by *Aspergillus niger* Grown in different AGVO-industrial wastes by solid- state fermentation”, Brazilian Journal of Chemical Engineering” 2017 ; 34(02) : 419-427,.
10. Licia M P, Mario M R, Guillerma R C. Catalytic properties of lipase extract from *Aspergillus niger*. Food Tech. Biotech. 2006; 44: 247-252.
11. J F M Burkent, Maugeri, M J Rodrigues . “Optimization of extracellular lipase production by *Geotrichum* sp. Using factorial design” Bioresource Technology, 2004 ; 91(1) : 77-84.
12. Levinson WE , Kurtzman C P, Kuo T M . Characterization of *yarrvia lipolytica* and related species for citric acid production from glycerol, Enzyme and Microbial Technology. 2007; 41 : 292-295.
13. Sundar W A, Kumarespaillai N . “Isolation purification and medium optimization of lipase enzyme producing strains of *Aspergillus niger* isolated from natural sources, International Journal of Pharmacy and Pharmaceutical Sciences, 2013; 5: 321-321.
14. AkhilaRajan A, Jayakumaran , “A comparative study on alkaline lipase production by a newly isolated *Aspergillusfumigatus*MTCC 19657 in submerged and solid- state fermentation using economically and industrially feasible substrate”, Turk J. Biol, 2009 ; 35: 569-576.