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The application of agro-food agriculture in lipase production by *Aspergillus tubingensis* strain: Inductive effect of olive-pomace

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ABSTRACT

Aims: The aim of this study was to cultivate the fungus *Aspergillus tubingensis* (MO503), which was isolated and identified from Algerian soil using submerged fermentation. The focus was on the production of lipase, achieved through utilizing a minimal medium from agro-food industries. Specifically, the study investigated the potential of three waste sources – olive-pomace, *Pistacia lentiscus* fruit remains and olive mill wastewater as substrates for enhancing lipase production.
Methodology and results: The three aforementioned wastes were chosen to ascertain their value and determine the most cost-effective option. Factorial analysis of variance (ANOVA) was employed to evaluate the waste with a significant effect on lipase production, as this enables determining differences among the various waste sources. Growth monitoring revealed a maximum lipase activity of 1030 ± 0,039 U at pH 5.4 for olive pomace. A series of biochemical purification techniques displayed a visible band on the polyacrylamide gel obtained through SDS-PAGE. Lipolytic activity was evidenced by zymography in the presence of olive oil. The antibacterial activities of purified lipase exhibited a high sensitivity against Gram-negative bacteria compared to Gram-positive bacteria.
Conclusion, significance and impact of study: In addition to its activity against Gram-negative bacteria, the lipid degradation facilitated by these lipases in olive oil offers promising applications in the textile and therapeutic industries.

Keywords: ANOVA test, *Aspergillus tubingensis*, lipase, production, purification

INTRODUCTION

Lipases are enzymes that catalyze the hydrolysis of lipids; they perform essential roles in lipids' biochemical metabolism (Svendsen, 2000). These enzymes are serine hydrolases (triacylglycerol acyl hydrolase EC 3.1.1.3). They are considered among the largest enzyme groups, including proteases and carbohydrases (Soorej *et al.*, 2011). Lipases can be used in biotechnology applications, like wastewater treatment, health and agro-food processes, by changing the taste of food and cheese ripening (Kavitha, 2016). This metabolite is responsible for cleaning the oily contaminants in the detergent application (Sahay and Chouhan, 2018). These enzymes can be of plant, animal or microbial origin. Those produced from microorganisms have important applicability in biotransformations since microorganisms are the best source of lipases (Ismail *et al.*, 2021).

The fungal lipases have been the subject of several research projects. For example, the lipase from *Rhizomucor miehei* and that of *Aspergillus oryzae* were produced commercially (Ilmi *et al.*, 2017). Generally, fungal lipase production depends on several factors, such as pH and substrate concentration (Duarte *et al.*, 2021). In this kind of biosynthesis, carbon and nitrogen become necessary sources for microorganisms' growth and synthesis of the biomolecule (Tacin *et al.*, 2019). Many studies have demonstrated that lipases produced by various microorganisms can be induced by lipid or waste lipid substrates (Çağatay and Aksu, 2021). In this context, to develop an inexpensive process by valorizing agroindustrial wastes, olive-pomace, *P. lentiscus* fruit remains and olive mill wastewater were chosen as constituents of basic non-supplemented-culture media for fungus lipase production. This study aimed to determine the effect of each of the three wastes on lipase production

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by *A. tubingensis*. Analysis of variance (ANOVA) was applied to select the best culture medium. This approach will help us understand the nutritional needs of the strain for extracellular lipase production, which is important in biotechnological processes.

MATERIALS AND METHODS

Products and reagents

All products were obtained from Sigma-Aldrich (Germany). All reagents used were of analytical-reagent grade and solutions were prepared using Milli-Q water (Purelab option Q, France).

Microorganism

The detailed method of isolation of *A. tubingensis* (MO503) (Conidia France codification) has been reported in a previous study (Benlounissi *et al.*, 2012). In this study, we used lyophilized strain resuspended in sterile physiological water and incubated for 15 days at 25 °C, then seeded on Sabouraud medium at 25 °C for short-term storage. The inoculum was prepared at a rate of 50 mL in a 250 mL flask on potato-dextrose-agar (PDA) medium. After incubation (6 days, 25 °C), spores were suspended after adding 50 mL of sterile water and agitation with a sterile magnetic stirrer, counted in a Malassez cell and stored at 4 °C.

Halo assay

The lipolytic microorganisms can be screened on solid agar media-rich substrates that play the role of colored indicators like egg yolk. It is a rapid qualitative method of screening lipase-producing microorganisms (Bharathi and Rajalakshmi, 2019). The lipolytic activity of *A. tubingensis* was tested using egg yolk agar (EYA) (McClung and Toabe, 1947) for the isolation of organisms producing lecithinase and lipase because egg yolk contains lecithin, triglycerides and lipoprotein. The lecithinase activity is evidenced by the presence of a white opaque zone around colonies, but a clear area on the edges of colonies identifies the lipolytic activity. However, proteolytic activity, which means that enzymes have activity on lipoproteins, is identified by a clear zone surrounding colonies.

Culture media

The wastes used in this study were purchased from artisanal oil producers (JiJel, Algeria). Determination of the chemical composition of wastes gave the following values (g/L): crude fat: 1.16, 2.62 and 0.63; total proteins: 4.27, 5.99 and 4.25; total sugar: 4.23, 5.59 and 2.57; for olive pomace, *P. lentiscus* fruit remains and olive mill wastewater, respectively. The wastes were air dried for about 10 days and then crushed until obtaining a powder that was stored in sealed boxes at 4 °C. Ten percent (w/v) of the suspension of each waste was used after dilution with 0.1 M phosphate buffer, pH 6.0, like a basal medium. Moreover, three culture media formed were marked Medium I, Medium II and Medium III for olive pomace, *P. lentiscus* fruit remains and olive mill wastewater, respectively.

Inoculum preparation

After 8 days of incubation, *A. tubingensis* (MO503) was suspended with 20 mL of sterile water under agitation and then it was counted in the Malassez cell to determine the required density of spores as the master suspension (Mechakra *et al.*, 1999).

Fermentation conditions

As previously described, *A. tubingensis* strain (MO503) was cultivated on 50 mL of growth medium in 250 mL flasks. After cooling the flasks containing culture media autoclaved at 121 °C (250 °F) (15 psi) for 20 min, they were inoculated with (107 spore/mL) of the spore suspension and incubated at 25 °C for 8 days under 160 rpm (MaxQ 420HP Thermo Scientific, France). After growth, the flask content was filtered to separate mycelium from the crude extract that was used for different assays.

Statistical approach

A factorial ANOVA was used to evaluate variables (factors) that significantly positively or negatively affect biomass, protein rates and enzyme activity. This statistical test enables us to analyze the effect of the different interactions between factors and deduce the better waste for enzyme production. In doing so, SPSS software was used.

Proteins

Proteins of samples were quantified according to the Lowry method (Lowry *et al.*, 1951) using bovine serum albumin (Sigma-Aldrich) as standard.

Enzyme assay

Lipase activity was determined using an emulsion of 10% olive oil and gum Arabic (v/v). The reaction was carried at 37 °C in the presence of ethanol. The titration of hydrolysis products with sodium hydroxide (NaOH) allows for identifying the amount of the released oleic acid. One unit (U) of lipase activity corresponds to the amount of enzyme catalyzing the formation of one micro equivalent of oleic acid in 2 h at 37 °C and pH 7.5 (Adham and Ahmed, 2009).

Biomass

At the end of microorganism growth, the content of the flasks was filtered on Whatman paper No. 1. The filtered Biomass was washed with distilled water and dried at 105

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°C for 20 h.

Enzyme extraction and purification

At the end of the incubation period, supernatants crude obtained by filtration were used for the different steps of purification. The enzymes were precipitated in crude extracts with ammonium sulfate (80% saturation) under agitation (VELP Scientifica, Italy) for 4 h at 4 °C. The mixture was centrifuged in a centrifuge (Sigma) at 10000× *g* for 15 min and the precipitates were dissolved in phosphate buffer 0.1 M, pH 6.0. After 24 h dialyzing against the same buffer, the enzyme solution was loaded on analytical Sephadex G50 (Sigma-Aldrich) columns equilibrated and eluted with phosphate buffer (0.1 M, pH 6.0), the eluent was sampled, and the enzyme activity was measured. After exclusion chromatography, fractions exhibiting lipase activity were purified using column exchange ions of DEAE-Sepharose (Sigma-Aldrich). The column was washed and equilibrated with Bis-Tris buffer (20 mM, pH 8.3) and bound proteins were eluted with Tris-HCl and 0-0.5 M NaCl (20 mM, pH 8.3). The fractions showing lipase activity were applied to SDS-PAGE electrophoresis. Before this experiment, the fraction from exchange ions chromatography purification was concentrated on the Corning Costar Spin-X Centrifuge Tube (Fisher Scientific, USA) at 14000× *g* for 10 min.

Zymography

Under non-denaturing conditions, lipase activity was observed by zymography (Castro-Ochoa *et al.*, 2005). After electrophoresis, the gel was washed in a washing buffer to eliminate the SDS. Then, it was placed in a Petri plate containing 0.01% phenol red, 1% olive oil, 10 mM $CaCl_2$ and 2% agar, and the pH was adjusted to 7.4. A change in color from red to yellow indicates lipolytic activity (Ghamari *et al.*, 2015).

Antibacterial activity

Disk diffusion methodology on Muller-Hilton agar allows evaluation of the antibacterial activity of microorganisms. The antibacterial activity of the purified extracellular enzyme from *A. tubingensis* (MO503), produced on three different wastes with lipase activity of about 7.5U/mL in each disk, was investigated against five pathogenic bacteria *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 700603) and *Staphylococcus aureus* (ATCC 25923). The observation of non-growth viewed from the back of the plates and the diameters of the inhibition zone (DIZ) were measured (Tamiljothi *et al.*, 2011).

RESULTS

Halo assay

The tested lipolytic activity of *A. tubingensis*, using an EYA (Figure 1), showed that the mold could generate a lipolytic zone surrounding the growing region with a 16 mm diameter after 72 h of culture. This observation demonstrates that the fungus can hydrolyze egg yolk and that the enzyme responsible for this hydrolysis was secreted by the mold in its neighboring medium.

Reaction on EYA was used to identify *A. tubingensis* lipolytic activity, a positive reaction resulting in the formation of a transparent zone around the culture. The transparent zone in the medium surrounding growth extending away from the fungus growth suggests a positive lipoprotein lipase reaction.

Statistical approach

SPSS, a statistical tool, was employed to realize ANOVA two factors at a confidence level of 95%. The effect of each factor was carried out with a response table. Three different media were used to test lipase production. (Figure 1) shows that the maximum lipase activity was obtained by applying medium I, giving enzyme activity reached 1030 ± 0,039 U after 4 days of incubation at pH 5.4, followed by Medium II and III with 1011 ± 0,009 U after 4 days and 724 ± 0,087 U after 5 days, respectively. The conducted analysis of variance (ANOVA) presented in (Table 1) explains the significance of the effects of medium type in time. As shown in Table 1, the type of

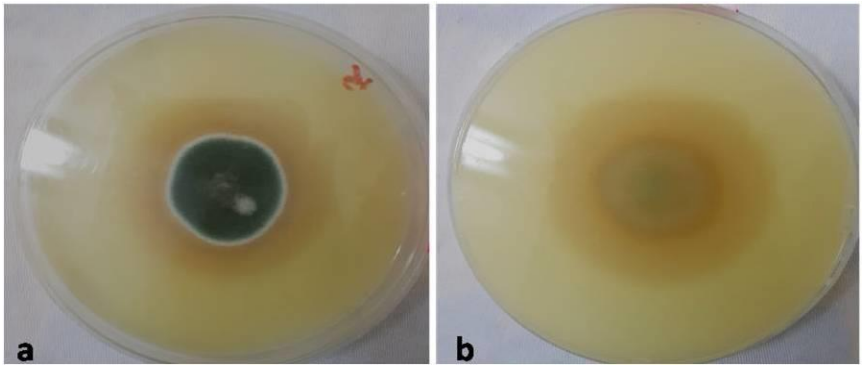

Figure 1: Macroscopic observation of the reaction *of A. tubingensis* strain on EYA. a: Upper side, b: Reverse side.

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Table 1: Two-factor ANOVA analysis for lipase production by *A. tubingensis* strain (MO503) in different media at different time courses.

Source of variation	F	*p*-value	F crit
Waste	64,219	0.000000	5.59
Days	137,160	0.000000	5.59
Interaction	2,406	0.008433	4.49

A *p*-value is a number, calculated to help decide whether to reject the null hypothesis. The F critical is a specific value, used to reject the null. The F-value is a variation between sample means/variation within the samples.

Table 2: Summary of lipase purification steps from *A. tubingensis* (MO503) on A: Medium I, B: Medium II and C: Medium III.

Purification step	Volume (mL)	Total activity (U.)	Total proteins (mg)	Specific activity (U./mg protein)	Purification fold	Yield (%)
A						
Crude extract	400	392000	1796	218.26	1	100
Ammonium sulfate	100	72000	320	225	1.03	18.37
Dialysis	90	69300	286.2	242.14	1.11	17.68
Sephadex G-50	20	25620	95	269.68	1.24	6.54
DEAE-Sepharose	20	39920	97.2	410.70	1.88	10.18
B						
Crude extract	380	427500	1869.6	228.66	1	100
Ammonium sulfate	135	132435	630.45	210.06	0.92	30.98
Dialysis	100	97600	462	211.26	0.92	22.83
Sephadex G-50	14	20500	98.6	207.91	0.91	4.80
DEAE-Sepharose	10	34520	97.8	352.97	1.54	8.07
C						
Crude extract	400	268000	1860	144.09	1	100
Ammonium sulfate	100	64500	434	148.62	1.03	24.07
Dialysis	95	60705	410.4	147.92	1.03	22.65
Sephadex G-50	10	11020	90.2	122.17	.85	4.11
DEAE-Sepharose	8	13920	92	151.30	1.05	5.19

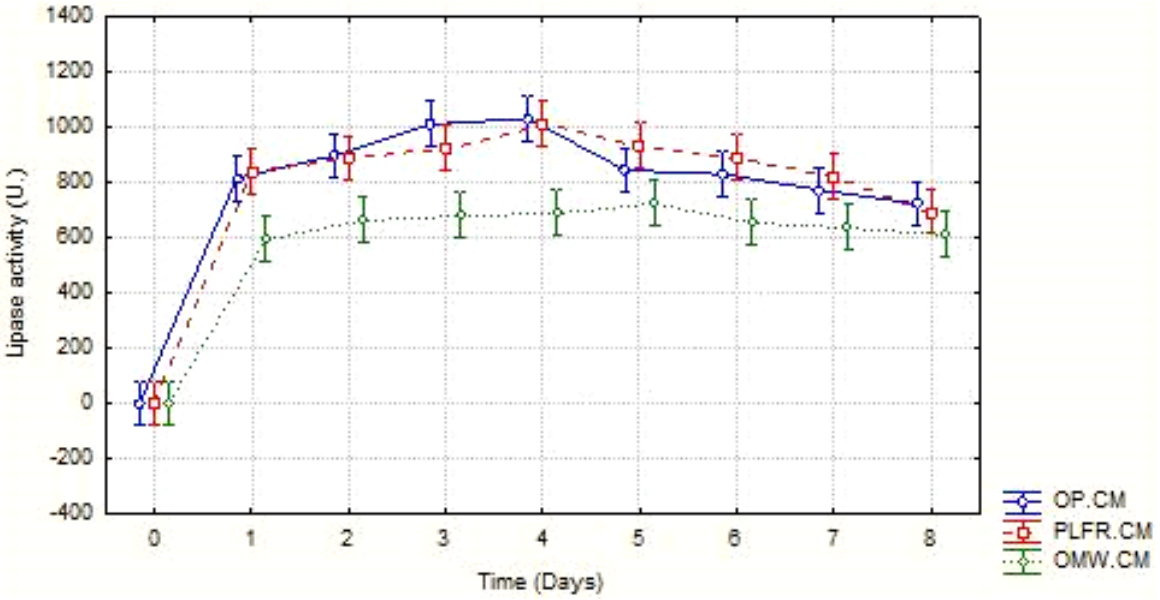

Figure 2: Production of lipase by *A. tubingensis* strain (MO503) on different fermentation media. OP.CM: Olive-Pomace Culture Medium, PLFR.CM: *P. lentiscus* Fruit Remains Culture Medium and OMW.CM: Olive Mill Wastewater Culture Medium.

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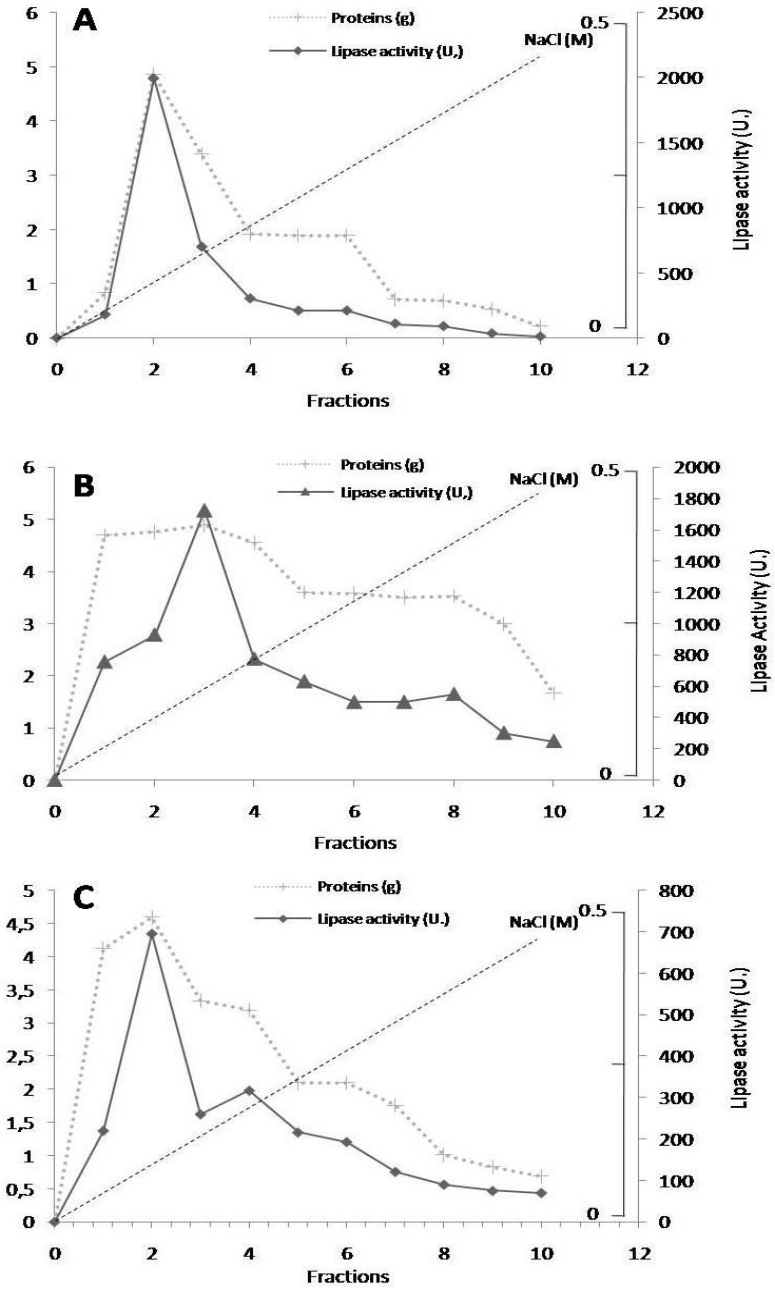

Figure 3: DEAE-Sepharose chromatography. A: Lipase from Medium I, B: Lipase from Medium II and C: Lipase from Medium III.

medium and incubation time course were of a highly significant role (*p*-value 5, 329070 10^{-15} and <0.01, respectively) and there was a significant interaction between them (*p*-value 0, 00843) on lipase production. Knowing that the chemical composition of the three media does not differ significantly, increased enzyme yield in a Medium I (Figure 2) was justified by the presence of more magnesium and higher hydrometric title supporting the lipase production.

Purification of the produced lipase from *A. tubingensis* strain (MO503)

As can be seen in (Table 2), lipase precipitation using ammonium sulfate (80%) shows an activity loss of about 18%, 31% and 24% for the three wastes: olive-pomace, *P. lentiscus* fruit remains and olive mill wastewater, respectively.

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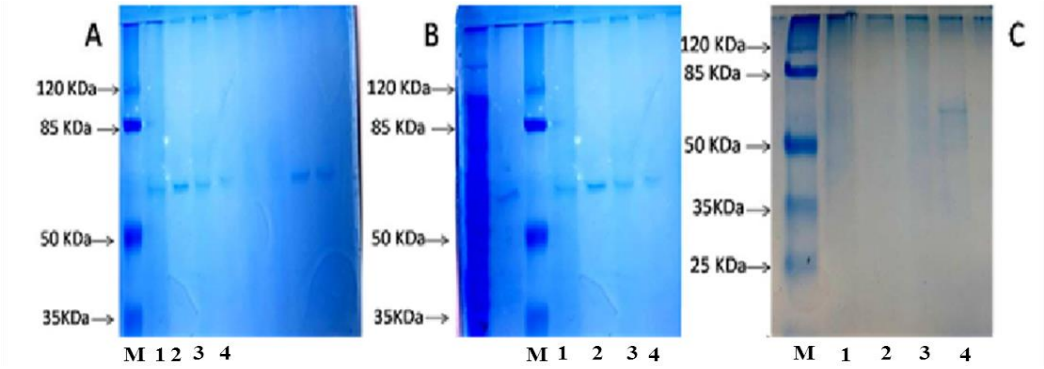

Figure 4: SDS-PAGE gel of lipase enzyme from *A. tubingensis* (MO503). A: Medium I, M: Standard molecular weight marker, Line 1: Crude extract, Line 2: Purified lipase using dialysis, Line 3: Purified lipase utilizing Sephadex G-50 column, Line 4: Lipase through DEAE-Sepharose column; B: Medium II, M: Standard molecular weight marker, Line 1: Crude extract, Line 2: Purified lipase using dialysis, Line 3: Purified lipase using Sephadex G-50 column, Line 4: Lipase utilizing DEAE-Sepharose column; C: Medium III, M: Standard molecular weight marker, Line 1: Crude extract, Line 2: Purified lipase using dialysis, Line 3: Purified lipase using Sephadex G-50 column, Line 4: Lipase using DEAE-Sepharose column.

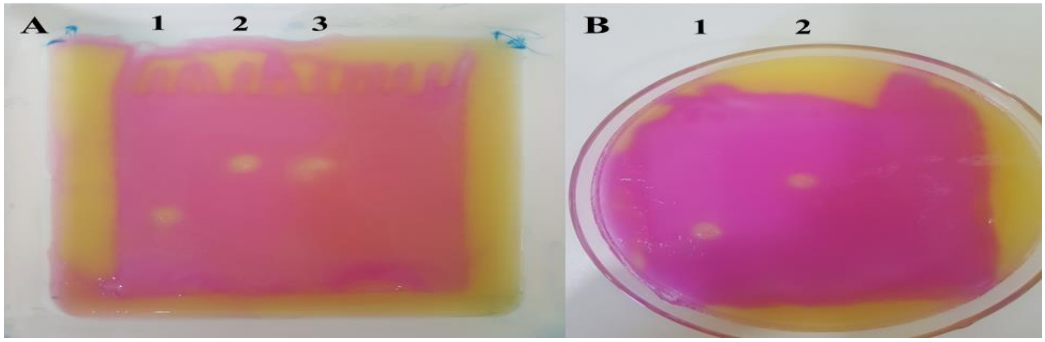

Figure 5: Zymogram in phenol red (A/B). 1(A/B): Industrial lipase (as control) 8,5U/mL, 2(A): Lipase from Medium II 7,5 U/mL, 2(B): Lipase from Medium III 7,5 U/mL, 3(A): Lipase from the Medium I 7,5U/mL.

After steps of Sephadex gel filtration and DEAE-Sepharose chromatography, the lipase produced on culture Medium I showed the best purification fold, particularly 2-fold and a yield of 10.18 as given in Table 2. The application of 4 purification steps did not promote enzyme purification. The peak of lipase activities emerged at 0.1 M NaCl for all media (Figure 3). Fractions containing lipase activity were gathered and applied on SDS-PAGE (Figure 4). Plotting log 10 of the molecular weights by the distance the standards migrated allowed us to determine the molecular weights of unknown bands. The molecular weight of the *A. tubingensis* lipase produced on three media was 64.28 kDa, 65.51 kDa and 62.97 kDa on Medium I, Medium II and Medium III, respectively.

Zymography

In this study, zymography revealed a yellow band on the red gel, indicating that the lowered pH caused the release of fatty acids, demonstrating that the purified enzyme had lipase activity (Figure 5).

Antibacterial activity

A clear zone of inhibition was formed around pathogenic bacteria in the presence of enzymes (Table 3). The results showed that the enzyme produced on the three wastes had antibacterial effects on some tested bacteria (Figure 6). The Gram-positive bacterium *B. subtilis* had no sensibility against the produced enzyme on the three culture media compared to the Gram-negative bacterium *E. coli,* which possessed a high sensitivity, with 20 mm of diameter inhibition zone, when the enzyme was produced on Medium II and III.

DISCUSSION

This study aimed to produce and purify lipase from *A. tubingensis* strain (MO503) on minimum culture media based on three different wastes. The tested lipolytic activity of *A. tubingensis*, using an EYA, has shown that the mold could secrete lipase in a neighboring medium to hydrolyze the egg yolk. On minimal agar medium in the presence of two inducers involved in lipase production

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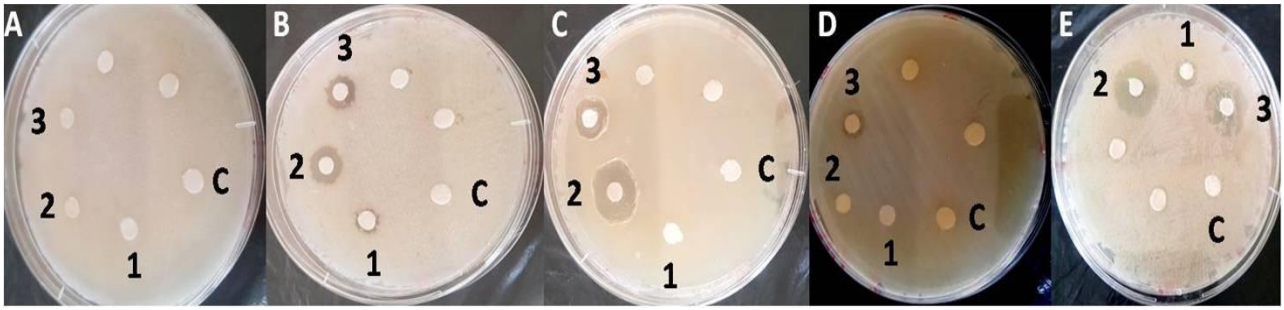

Figure 6: Disk diffusion on Muller-Hilton agar. A: *B. subtilis*, B: *P. aeruginosa*, C: *E. coli*, D: *K. pneumoniae*, E: *S. aureus*, 1: Lipase from Medium I, 2: Lipase from Medium II, 3: Lipase from Medium III.

Table 3: Inhibition zone diameter (mm) of the three lipases produced on the three media.

Bacteria	Inhibition zone diameter (mm)		
	Lipase from Medium I	Lipase from Medium II	Lipase from Medium III
Bacillus subtilis	0	0	0
Pseudomonas aeruginosa	9 ± 0.67 mm	15 ± 0.67 mm	12 ± 1.33 mm
Escherchia coli	0	20 ± 0.67 mm	17 ± 1.33 mm
Klebsiella pneumoniae	0	0	5 ± 0.44 mm
Staphylococcus aureus	9 ± 1 mm	19 ± 0.67 mm	15 ± 1.33 mm

(olive oil and Tween 80) for 72 h at 37 °C. The observation of a transparent zone of hydrolysis of substrates by strains considered as highly producing lipase, which is *B. subtilis*, *B. cereus*, *Geobacillus*, *P. fluorescens* and *B. licheniformis* (Larbi Daouadji, 2016). These strains exhibited hydrolysis zones with diameters from 20 to 34 mm and maximum lipolytic activity. It reported that the *Aspergillus niger* strain presented a halo on solid agar plates culture medium expressing lipolytic activity on olive oil as substrate (Salgado *et al.*, 2014). The absence of an opaque white area around the colony means a negative lecithinase reaction. Another study demonstrated a positive lecithinase reaction of *Streptomyces* sp. on egg yolk with the formation of a white opaque zone of precipitation surrounding bacteria, which means that lipase produced by *A. tubingensis* is not a lecitinase (El-Naggar *et al.*, 2011). The analysis of the ANOVA result, which is known to be statistically significant (Vishwanatha *et al.*, 2021), revealed that culture media have different effects on enzyme production and that these effects depend on the kind of waste used for microorganism culture (He and Tan, 2006) and the incubation time (Soleymani *et al.*, 2017). The conducted analysis of variance (ANOVA) showed that the maximum lipase activity was obtained by applying Medium I. Compared to other culture media, this one had a slightly high rate of magnesium and a hydrometric title of 2000 TH that can probably support the lipase production. Another study reported that in the presence of magnesium at level +1, the lipase activity was 24.12 U/mL compared to 1.36 U/mL when it was at level -1 with a *p*-value=0.007 (Aziz *et al.*, 2020). The supplementation with various inorganic sources showed good lipase production by *Aspergillus sydowii* on olive oil substrate (Bindiya and Ramana, 2014). Another study reported magnesium sulfate enhanced lipase production by *Staphylococcus epidermidis* (Esakkiraj *et al.*, 2010). However, when the culture medium was supplemented with nutrients, lipase production depended on an inducer's presence in the fermentation medium (Lotti *et al.*, 1998).

Nitrogen source is essential for microbial lipase production. The three wastes used in this study, not being supplemented with a nitrogen source, did not allow the strain to consume a lot of protein during its growth. A good rate of lipase production was observed when peptone combined with other nitrogen extract was used for lipase production by *Aspergillus* sp. (Colonia *et al.*, 2019). In the current study, lipase purification steps show a loss of proteins and low purification folds, but the enzyme could be visualized on an SDS-PAGE gel. During the valorization of oils wastes to produce lipase by *Aspergillus* sp., a significant activity loss (>50%) after ammonium sulfate followed by dialysis was observed. A lipase from *A. niger* purified among others by ammonium sulfate, showed a yield of 33%, but the enzyme was purified 73 times on supplemented culture medium (Shu *et al.*, 2007). On the other hand, it has been shown that *Spirulina platensis* lipase may be purified around 375-fold using DEAE-Sepharose chromatography and Sephadex gel filtration chromatography, with a yield of 29.35% and a molecular weight of 45 kDa on an SDS-PAGE gel (Demir and Tükel, 2010). The *B. sudeticus* lipase produced and purified with ammonium sulfate, size exclusion column chromatography and SDS-PAGE electrophoresis showed an estimated weight of 120 kDa (Yong *et al.*, 2016). In the case of bacterial lipase produced from *P. aeruginosa*, a molecular weight of 29 kDa was reported (He and Tan, 2006). Nevertheless, lipase was also present with a weight of 46 kDa in yeast (Kumar *et al.*, 2014). On their side, some researchers demonstrated that lipase from

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Geobacillus stearothermophilus produced on fish waste-based culture medium showed 63 kDa molecular weight on SDS-PAGE electrophoresis (Abol-Fotouh *et al.*, 2021). This study used a quick plate method by zymography on red phenol agar containing olive oil to demonstrate that the purified enzyme had lipase activity. The appearance of a yellow hydrolysis zone indicated that the purified enzyme had lipolytic activity (Ghamari *et al*., 2015). The test of the antibacterial activity of lipase produced from *A. tubingensis* strain (MO503) on the three selected wastes appears a clear zone of inhibition around some tested pathogenic bacteria. It was reported that this can be related to the absence of peptidoglycan layers in the Gram-negative cell wall compared to Gram-positive bacteria (Li and Liu, 2017). On the other side, it has been demonstrated that the lipase enzyme of essential oil from *Serapias orientalis* has antimicrobial activity against seven species of microorganisms, and lipase extracted with water was more susceptible to Gram-positive bacteria with inhibition zones that can reach 20 mm (Erik *et al.*, 2020). However, it was reported that the extract of *Aspergillus flavus* showed antibacterial activity against *Streptococcus pyogenes* and *P. aeruginosa*; on the other hand, there was a weak significant growth inhibition against *S. aureus* and *E. coli* (Almanaa *et al.*, 2021).

CONCLUSION

The current investigation demonstrates that when compared to other media, olive pomace (Medium I) can stimulate the synthesis of lipase by the *A. tubingensis* strain (MO503). The purified lipase showed good activity on Medium I with 1030 ± 0.039 U and showed a better purification fold, about 2 and a yield of 10.18 with 94 59% protein loss. The antibacterial activity of lipase was tested on 5 strains of bacteria and showed that Gram-positive bacterium had no sensibility against the produced enzyme compared to Gram-negative bacterium. Despite using an unsupplemented culture medium, the results are encouraging and can be further advanced by adding external nitrogen and carbon sources. The isolate's ability to produce this active enzyme can be taken advantage of for a variety of industrial uses as well as to lessen the danger these wastes provide to the environment.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

Abol-Fotouh, D., AlHagar, O. E. A. and Hassan, M. A. (2021). Optimization, purification, and biochemical characterization of thermoalkaliphilic lipase from a novel *Geobacillus stearothermophilus* FMR12 for detergent formulations. *International Journal of Biological Macromolecules* **181, 125-135.**

Adham, N. Z. and Ahmed, E. M. (2009). Extracellular lipase of *Aspergillus niger* NRRL3; Production, partial purification and properties. *Indian Journal of Microbiology* **49(1), 77-83.**

Almanaa, T. N., Rabie, G., El-Mekkawy, R. M., Yassin, M. A., Saleh, N. and EL-Gazzar, N. (2021). Antioxidant, antimicrobial and antiproliferative activities of fungal metabolite produced by *Aspergillus flavus* on *in vitro* study. *Food Science and Technology* **42, e01421.**

Aziz, M. M. A., Elgammal, E. W. and Ghitas, R. G. (2020). Comparative study on modeling by neural networks and response surface methodology for better prediction and optimization of fermentation parameters: Application on thermo-alkaline lipase production by *Nocardiopsis* sp. strain NRC/WN5. *Biocatalysis and Agricultural Biotechnology* **25, 101619.**

Benlounissi, A., Mechakra-Maza, A., Gheribi, Z., Mahfouz, M., Blum, L. J. and Marquette, C. A. (2012). Milk-clotting fungus enzymes production: A sustainable development approach based on whey recycling. *African Journal of Biotechnology* **11(8), 1997-2005.**

Bharathi, D. and Rajalakshmi, G. (2019). Microbial lipases: An overview of screening, production and purification. *Biocatalysis and Agricultural Biotechnology* **22, 101368.**

Bindiya, P. and Ramana, T. (2014). Optimization of lipase production from an indigenously isolated marine *Aspergillus sydowii* of Bay of Bengal. *Journal of Biochemical Technology* **3(5), 198-202.**

Çağatay, Ş. and Aksu, Z. (2021). Use of different kinds of wastes for lipase production: Inductive effect of waste cooking oil on activity. *Journal of Bioscience and Bioengineering* **132(3), 234-240.**

Castro-Ochoa, L. D., Rodríguez-Gómez, C., Valerio-Alfaro, G. and Oliart Ros, R. O. (2005). Screening, purification and characterization of the thermoalkalophilic lipase produced by *Bacillus thermoleovorans* CCR11. *Enzyme and Microbial Technology* **37(6), 648-654.**

Colonia, B. S. O., Woiciechowski, A. L., Malanski, R., Letti, L. A. J. and Soccol, C. R. (2019). Pulp improvement of oil palm empty fruit bunches associated to solid-state biopulping and biobleaching with xylanase and lignin peroxidase cocktail produced by *Aspergillus* sp. LPB-5. *Bioresource Technology* **285, 121361.**

Demir, B. S. and Tükel, S. S. (2010). Purification and characterization of lipase from *Spirulina platensis*. *Journal of Molecular Catalysis B: Enzymatic* **64, 123-128.**

Duarte, A. W. F., Bonugli-Santos, R. C., Duarte, A. L. F., Gomes, E. and Sette, L. D. (2021). Statistical experimental design applied to extracellular lipase production by the marine Antarctic yeast *Leucosporidium scottii* CRM 728. *Biocatalysis and Agricultural Biotechnology* **32, 101954.**

El-Naggar, N. E., Sherief, A. A. and Hamza, S. S. (2011). *Streptomyces aegyptia* NEAE 102, a novel

cellulolytic streptomycete isolated from soil in Egypt. *African Journal of Microbiology Research* **5(29), 5308-5315.**

Erik, İ., KiliÇ, G., Öztürk, E., Karaoğlu, Ş. A. and Yayli, N. (2020). Chemical composition, antimicrobial, and lipase enzyme activity of essential oil and solvent extracts from *Serapias orientalis* subsp. *orientalis*. *Turkish Journal of Chemistry* **44(6), 1655-1662.**

Esakkiraj, P., Rajkumarbharathi, M., Palavesam, A. and Immanuel, G. (2010). Lipase production by *Staphylococcus epidermidis* CMST-Pi 1 isolated from the gut of shrimp *Penaeus indicus*. *Annals of Microbiology* **60(1), 37-42.**

Ghamari, M., Alemzadeh, I., Tabatabaei Yazdi, F., Vossoughi, M. and Varidi, M. (2015). Purification and zymography of lipase from *Aspergillus niger* PTCC5010. *International Journal of Engineering* **28(8), 1117-1123.**

He, Y. Q. and Tan, T. W. (2006). Use of response surface methodology to optimize culture medium for production of lipase with *Candida* sp. 99-125. *Journal of Molecular Catalysis B: Enzymatic* **43(1-4), 9-14.**

Ilmi, M., Hidayat, C., Hastuti, P., Heeres, H. J. and van der Maarel, M. J. E. C. (2017). Utilisation of Jatropha press cake as substrate in biomass and lipase production from *Aspergillus niger* 65I6 and *Rhizomucor miehei* CBS 360.62. *Biocatalysis and Agricultural Biotechnology* **9, 103-107.**

Ismail, A. R., Kashtoh, H. and and Baek, K. H. (2021). Temperature-resistant and solvent-tolerant lipases as industrial biocatalysts: Biotechnological approaches and applications. *International Journal of Biological Macromolecules* **187, 127-142.**

Kavitha, M. (2016). Cold active lipases – An update. *Frontiers in Life Science* **9(3), 226-238.**

Kumar, K. D., Usha, K. Y., Satyanarayana, S. V. and Sailaja, V. (2014). Characterization of partially purified lipase from *Saccharomyces cerevisiae*. *International Journal of Pharmacy and Pharmaceutical Sciences* **6(8), 514-517.**

Larbi Daouadji, K. (2016). Isolement et caractérisation des souches productrices de lipase. Ph.D. Thesis. Université Djillali Liabès, Algeria.

Li, J. and Liu, X. (2017). Identification and characterization of a novel thermophilic, organic solvent stable lipase of *Bacillus* from a hot spring. *Lipids* **52(7), 619-627.**

Lotti, M., Monticelli, S., Montesinos, J. L., Brocca, S., Valero, F. and Lafuente, J. (1998). Physiological control on the expression and secretion of *Candida rugosa* lipase. *Chemistry and Physics of Lipids* **93(1-2), 143-148.**

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193(1), 265-275.**

McClung, L. S. and Toabe, R. (1947). The egg yolk plate reaction for the presumptive diagnosis of *Clostridium sporogenes* and certain species of the gangrene and botulinum groups. *Journal of Bacteriology* **53(2), 139-147.**

Mechakra, A., Auberger, B., Remeuf, F. and Lenoir, J. (1999). Optimization of a culture medium for acid proteolytic enzymes production by *Penicillium camemberti*. *Sciences des Aliments* **19(6), 663-675.**

Sahay, S. and Chouhan, D. (2018). Study on the potential of cold-active lipases from psychrotrophic fungi for detergent formulation. *Journal of Genetic Engineering and Biotechnology* **16(2), 319-325.**

Salgado, J. M., Abrunhosa, L., Venâncio, A., Domínguez, J. M. and Belo, I. (2014). Integrated use of residues from olive mill and winery for lipase production by solid state fermentation with *Aspergillus* sp. *Applied Biochemistry and Biotechnology* **172(4), 1832-1845.**

Shu, Z., Yang, J. and Yan, Y. (2007). Purification and characterization of a lipase from *Aspergillus niger* F044. *Chinese Journal of Biotechnology* **23(1), 96-101.**

Soleymani, S., Alizadeh, H., Mohammadian, H., Rabbani, E., Moazen, F., Sadeghi, H. M. *et al.* (2017). Efficient media for high lipase production: One variable at a time approach. *Avicenna Journal of Medical Biotechnology* **9(2), 82-86.**

Soorej, M., basheer, Chellappan, S., Beena, P. S., Sukumaran, R. K., Elyas, K. K. and Chandrasekaran, M. (2011). Lipase from marine Aspergillus awamori BTMFW032: Production, partial purification and application in oil effluent treatment. *New Biotechnology* **28(6), 627-638.**

Svendsen, A. (2000). Lipase protein engineering. *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology* **1543(2), 223-238.**

Tacin, M. V., Massi, F. P., Fungaro, M. H. P., Teixeira, M. F. S., de Paula, A. V. and de Carvalho Santos-Ebinuma, V. (2019). Biotechnological valorization of oils from agro-industrial wastes to produce lipase using *Aspergillus* sp. from Amazon. *Biocatalysis and Agricultural Biotechnology* **17, 369-378.**

Tamiljothi, E., Ravichandiran, V., Chandrasekhar, N. and Suba, V. (2011). Pharmacognostic and preliminary phytochemical screening of leaves of *Tecomaria capensis*. *Asian Journal of Plant Science and Research* **1(3), 34-40.**

Vishwanatha, J. S., Hebbale, A. M., Kumar, N., Srinath, M. S. and Badiger, R. I. (2021). ANOVA studies and control factors effect analysis of cobalt based microwave clad. *Materials Today: Proceedings* **46, 2409-2413.**

Yong, S. K., Lim, B. H., Saleh, S. and Tey, L. (2016). Optimisation, purification and characterisation of extracellular lipase from *Botryococcus sudeticus* (UTEX 2629). *Journal of Molecular Catalysis B: Enzymatic* **126, 99-105.**

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