Dellali A *et al.* (2023)
Notulae Scientia Biologicae
Volume 15, Issue 4, Article number 11634
DOI:10.15835/nsb15411634
Research Article

Characterization of lipases and preliminary purification of tributyrinesterases from lactic acid bacteria strains belonging to the *Enterococcus* and *Lactococcus* genera

Amina DELLALI[1,2*], Faiza BOUBLENZA[1], Halima ZADI-KARAM[1], Nour-Eddine KARAM[1]

[1]*University of Oran 1 Ahmed Ben Bella, Faculty of Nature and Life Sciences, Department of Biotechnology, Laboratory of Biology of Microorganisms and Biotechnology, Oran 31000, Algeria; nek1948@yahoo.fr; karam_halima@yahoo.fr; f.boublenza@yahoo.fr*
[2]*University of Sciences and Technology of Oran (Mohamed Boudiaf), Faculty of Nature and Life Sciences, Department of Living and Environment, Oran 31000, Algeria; amina.dellali@univ-usto.dz (*corresponding author)*

Abstract

The objective of this study is to characterize and attempt to purify lipolytic enzymes derived from strains of *Enterococcus* and *Lactococcus* genera isolated from various environments. For this purpose, ten strains of lactic acid bacteria were studied for their lipolytic and esterasic activities on an MRS (Man, Rogosa, and Sharpe) medium containing natural and/or artificial lipid substrates. Among them, two strains: *Lactococcus lactis subsp lactis* and *Lactococcus lactis subsp cremoris* showed maximum extracellular lipolytic activity in the presence of 1% olive oil. It was observed that both strains exhibited higher lipolytic activity at pH 7 and 8, with an optimal temperature of 30 °C and 37 °C, respectively. Glucose was found to be the best carbon source for both tested strains. The study of growth kinetics and fatty acid production over time revealed that fatty acid production begins during the exponential phase and reaches its maximum during the stationary phase. The purification of tributyrinesterases was carried out on two strains of *Enterococcus* genus: *Enterococcus feacium* and *Enterococcus durans* through ammonium sulfate precipitation and Sephadex G-100 gel filtration chromatography allowed the estimation of the molecular weight of the tributyrinesterases as 32.09 kDa and 38.49 kDa for both strains, respectively.

Keywords: *Enterococcus*; esterasic activity; growth kinetics; *Lactococcus*; lipolytic activity; olive oil; purification; tributyrin esterase

Introduction

Lactic acid bacteria, although present in numerous biotopes, exhibit relatively low lipolytic activity compared to other bacterial species (Brennan *et al*., 2002). This activity has no impact on bacterial growth or nutritional role (Nardi *et al*., 2002). However, in cheeses, microbial lipases present at higher concentrations and specific times contribute to the release of fatty acids responsible for the final taste, which is important for the production of new foods and dietary supplements (García-Cano *et al*., 2019).

Received: 25 Jul 2023. Received in revised form: 30 Oct 2023. Accepted: 13 Nov 2023. Published online: 15 Nov 2023.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

It is important to note that enzymatic activity can vary not only between different biotopes but also within the same biotope due to the diversity of bacterial strains present. Therefore, in-depth studies are necessary to evaluate the lipolytic activity of lactic acid bacteria in different biotopes and to understand the factors that influence these activities. Such research can contribute to a better understanding of the metabolic capacities of lactic acid bacteria and their exploitation in various industrial applications, such as the production of food enzymes and food fermentation.

Lactic acid bacteria, which play an essential role in food maturation and charcuterie production, are also known for their probiotic potential and their ability to produce conjugated fatty acids through the hydrolysis of triacylglycerols thanks to their lipolytic activity (Kuhl *et al*., 2017).

Some esterases previously isolated from lactic acid bacteria show a preference for the degradation of para-nitrophenyl or beta-naphthyl derivatives of C4 or C6 fatty acids, and they show good activity in the presence of tributyrin. However, their esterase activity decreases significantly as the fatty acid chain lengthens (Corrieu and Luquet, 2008). Meanwhile, lipases are active against long-chain and water-insoluble substrates (Ramakrishnan *et al*., 2012).

Several esterases and lipases from lactic acid bacteria, such as *Lactococcus lactis, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus helveticus, Lactobacillus rhamnosus, Streptococcus thermophilus,* and *Enterococcus faecium*, have already been characterized. These enzymes exhibit optimal activity within a temperature range of 30 °C to 45 °C, depending on the strains, and they are active at neutral or slightly alkaline pH (Gupta *et al*., 2004).

The purification of microbial lipases involves different steps depending on the intracellular or extracellular localization of the enzyme. In both cases, the produced proteins are precipitated using ammonium sulfate (Yang *et al*., 2016). The precipitated protein is then subjected to dialysis and chromatography techniques. The choice of chromatography techniques varies depending on the source of microorganisms, the size and the charge of proteins (Unni *et al*., 2016; Zhou *et al*., 2012).

Therefore, our objective is to study the extracellular lipolytic activity of *Lactococci* and *Enterococci* isolated from different biotopes. Our study will aim to highlight enzymatic activity using various natural and artificial lipid substrates, investigate the growth kinetics and lipase production over time, physicochemical characterization of the enzymes in question, and finally, attempt the purification of lipolytic and/or esterasic enzymes, whether they are intracellular or extracellular.

Materials and Methods

Bacterial strains

Ten strains of lactic acid bacteria belonging to the genera *Lactococcus* and *Enterococcus* were isolated from camel milk or raw cow's milk, olive brine, and fresh meat collected in Algeria and Mauritania (Tab1), using a series of dilutions on MRS medium (De Man *et al*., 1960). The bacterial cultures were stored at a temperature of -20 °C in MRS broth containing 20% glycerol (v/v). For subsequent experiments, the cultures were prepared by performing two successive subcultures in MRS broth and cultivating them at a temperature of 30 °C for 18 hours.

Table 1. Bacterial strains used in this study

Origin	Code of strain	Species
Camel milk (Mauritania)	CAM18	*Enterococcus faecium*
Camel milk (Timimoun, Algeria)	CAT13, CAT18	*Enterococcus faecium*
Cow's Milk (Oran El-Kerma, Algeria)	LKV11	*Enterococcus durans*
Olive brine (Sig, Algeria)	OV5	*Lactococcuslactissspdiacetylactis*
Fresh beef meat (Mostaganem, Algeria)	V6-2, V11,V18	*Lactococcus lactis ssp lactis*
	V9	*Lactococcus lactis ssp cremoris*
Fresh sheep meat (Relizane, Algeria)	VO19	*Enterococcus faecium*

Detection of extracellular lipolytic activity

50 µl of each supernatant obtained after centrifugation of 18-hour pre-cultures were deposited in wells previously made on pH 7 buffered agar (phosphate buffer, 0.1M) supplemented with 1% olive oil, natural butter, butyric acid, or oleic acid. The medium was opacified with 0.5% calcium carbonate ($CaCO_3$) if necessary to visualize any clarification around the wells, indicating degradation of the lipid substrates. The results were obtained after 72 hours of incubation at 37 °C.

The strains, as well as the lipid substrate showing maximum activity, were selected for further work.

Optimal conditions to obtain maximum enzymatic activity

These physicochemical conditions were determined to achieve maximum enzymatic activity. The MRS medium without Tween 80 was supplemented with the best lipid source that allows good enzymatic activity (previously obtained).

Search for optimal temperature

The pH 7 buffered MRS medium (phosphate buffer, 0.1M) was inoculated with a 1% inoculum of a young 18-hour culture. Incubation was performed at different temperatures (20 °C, 25 °C, 30 °C, 37 °C, 40 °C, or 45 °C) for 72 hours under agitation to promote contact between the lipid substrate and bacterial cells. 50µL of the culture supernatant was deposited on agar supplemented with the same lipid source to visualize any enzymatic activity. $CaCO_3$ was added, if necessary, at a concentration of 0.5%, and incubation was carried out at the same temperatures as previously described.

Search for optimal pH

To determine the optimal pH, lipolytic activity was measured in the pH range of 4 to 9 using 0.1M buffers. The buffers used acetic acid buffer (pH 3 to 5), sodium phosphate buffer (pH 6 to 7), Tris-HCl buffer (pH 8), and glycine-NaOH buffer (pH 9). To visualize any enzymatic activity, agar was prepared in the same pH range.

Determination of the best carbon source

The MRS medium was prepared without sugar and buffered at the optimal pH. This medium was supplemented with 20 g (the same amount of sugar as indicated in the composition of MRS medium) of one of the following different sugars: galactose, fructose, glucose, xylose, sucrose, lactose, maltose, and starch. The revelation of enzymatic activity was carried out in the same manner as described above.

Concurrently, the lipolytic activity of the three parameters investigated (pH, temperature, and carbon source) was determined by measuring the released fatty acids over time using the method described by Ginalska *et al*. (2004). The results are expressed in µmol of fatty acids/ml of sample (Sokolovska *et al*., 1998).

Kinetics of growth and release of fatty acids as a function of time

Bacterial growth was determined by measuring the absorbance at 600 nm every 4 hours. Simultaneously, 0.5 ml of enzymatic extract was mixed with 1 ml of pH 7 phosphate buffer and 1.5 ml of lipid substrate. After 60 minutes of incubation at 37 °C with stirring to promote lipid-lipase interaction, the fatty acid assay was performed using the method described by Ginalska *et al.* (2004).

Lipase purification assay

Prior to any purification step, the culture supernatant of the enzymatic extract was concentrated by dialysis to ¼ from the initial volume. The results of enzymatic activity are obtained by using a medium enriched with a lipid substrate where the activity is at its maximum (as previously obtained)., and the total protein content was determined using the Bradford technique.

Ammonium sulfate precipitation

Ammonium sulfate was added to a known volume of the enzymatic solution to achieve 70% or 90% saturation (w/v) at 4 °C. The centrifugation is then performed at 12,000 rpm for 15 minutes, following the method described by Shu *et al.* (2006). In addition, a dialysis against water is subsequently performed at 4 °C overnight to remove the ammonium sulfate bound to the proteins.

Sephadex-G100 gel filtration chromatography

The Sephadex G-100 column (Pharmacia) was prepared according to the method described by Jakoby (1971) and Sukhacheva *et al.* (2004). To determine the molecular weight of the tested lipases, two proteins of known molecular weights, bovine serum albumin (MW = 69.32 kDa) and lysozyme (MW = 16.65 kDa), were eluted under the same conditions.

Results and Discussion

Demonstration of extracellular lipolytic activity

The obtained results are presented in Figure 1, and an example of the result is shown in Figure 2.

The tested strains exhibited higher lipase activity in the presence of olive oil compared to other tested substrates (Figure 1).

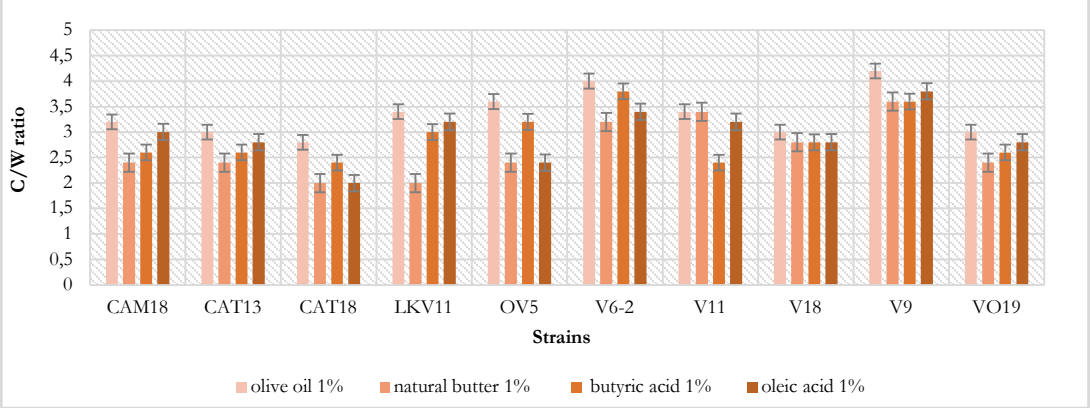

Figure 1. Lipolytic activity on artificial and natural lipid substrates in lactic strains (C: clarification area, W: well diameter).

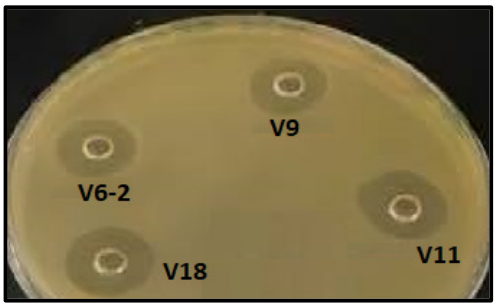

Figure 2. Lipolytic activity in the presence of 1% olive oil

This can be attributed mainly to the composition of olive oil, which is considered a carbon source rich in triglycerides linked to fatty acids (saturated, monounsaturated, or polyunsaturated) (Moftah *et al*., 2013). These fatty acids include palmitic acid (C16), palmitoleic acid (C16:1), stearic acid (C18), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20), and behenic acid (C22), which are major components of olive oil (Ollivier *et al*., 2006).

Moreover, several studies have shown that olive oil is the best inducer and carbon source for bacterial growth and lipase synthesis (Brozzoli *et al*., 2009; Salihu *et al*., 2011; Adetunji and Olaniran, 2018).

Zarevúcka (2012) also highlighted that lipid sources, such as natural oils, have been demonstrated to stimulate lipase production. Olive oil is one of the most effective inducers of lipase production.

A maximum lipase activity was observed in the presence of 20 g/L of olive oil in strains of *L. fermentum* (Foruzan *et al*., 2022). Acu (2022) also demonstrated that 10% olive oil allows for the maximum extracellular lipase production in *Enterococcus faecium* E68.

Natural butter also exhibited significant lipolytic activity (Figure 1). Microbial lipases are widely used in the dairy industry for the hydrolysis of milk fats, and current applications include accelerating cheese ripening, butter, fat, and cream processing (Aravindan *et al*., 2007). Several microorganisms have shown lipolytic activity in the presence of butter, particularly strains of *Lactococcus lactis* (Meyers *et al*., 1996). Furthermore, various lipolytic microorganisms are involved in the interesterification of butter fat.

Butyric acid is often detected as a fatty acid released during the degradation of dairy products' fat and plays an important role in the ripening of cheeses, especially FETA cheese originating from Greece.

In order to demonstrate lipolytic and/or esterasic activity, butyric acid is frequently used in the form of p-nitrophenyl butyrate (pNPB). pNPB has shown significant activity in 22 strains of Halobacteria (Bhatnagar *et al*., 2005). Similarly, pNPB has revealed esterasic activity in *Haloarculamarismortui* (Camacho *et al*., 2009), and hydrolytic activity is also clearly visible in strains of *Candida Antarctica* and *Yarrowialipolytica* (Pliego *et al*., 2015).

The obtained results obtained show good enzymatic activity, especially in *Lactococcus* strains V9 and V6-2, where maximum activity was observed (Figure 1). Similar results were also observed in the presence of oleic acid. Indeed, Csutak *et al*. (2018), in a study on the effect of fatty acids on lipase production by the yeast *Rhodotorulaglutinis* CMGB-RG5, demonstrated that butyric acid and oleic acid are inducers of lipase production, while the presence of palmitic acid inhibits lipase synthesis.

Also, these results allowed us to select strains V9 (*Lactococcus lactis ssp cremoris*) and V6-2 (*Lactococcus lactis ssp lactis*) as the most lipolytic for the remaining work, particularly in the presence of 1% olive oil (Figure 1).

Determination of the optimal temperature for lipase production

The maximum temperature for fatty acid production is 37 °C and 30 °C for strains V9 and V6-2, respectively (Figure 3).

This finding is consistent with the results obtained by most authors and for the tested strains. Generally, for optimal lipase production, the temperature is around 30 to 35 °C. However, other studies have shown that bacteria can secrete lipases at low or high temperatures (Hassan *et al*., 2018). According to the literature, strains *Lysinibacillus* PL33 and *Bacillus* FW2 exhibited an optimal temperature range of 30 °C to 40 °C for lipase production.

Other research studies have identified temperatures of 50 °C and 55 °C as optimal for efficient lipolytic activity production (Amoozegar *et al*., 2008; Bora and Bora, 2012). Some *Bacillus* species have been found to produce lipases at 60-80 °C (Faiz *et al*., 2007; Khan *et al*., 2017; Febriani *et al*., 2019). The lipase from *Janibacter sp*. R02 has an optimum temperature of 90 °C (Castilla *et al*., 2017).

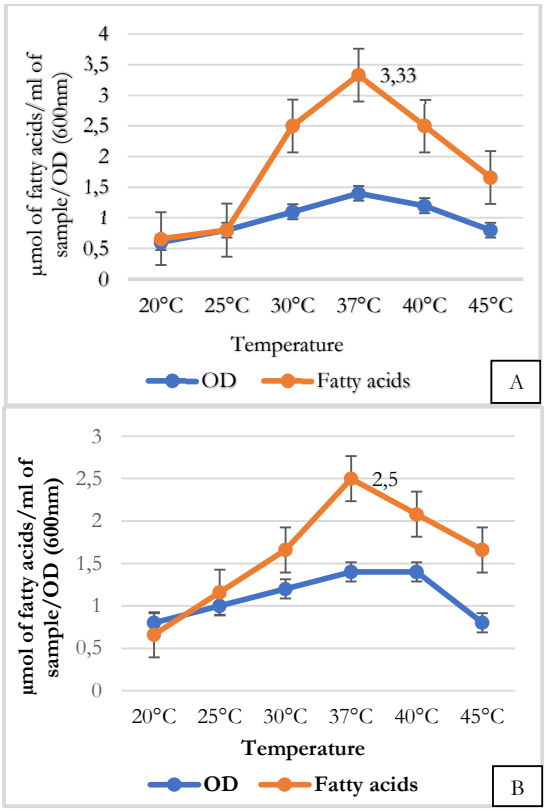

Figure 3. Search for the optimal temperature (A: V9: B: V6-2)

Temperature also influenced the enzymatic activity differently in the two yeast strains producing lipase. *W. californica* 1639 demonstrated significant lipolytic activity at 20 °C, while a temperature of 30 °C allowed for better lipase production in *S. cerevisiae* 15 25. High enzymatic activity was also observed at 36 °C in strains isolated from contaminated soils (Khan, 2010). Similarly, Gayatriben *et al*. (2021) determined an optimal temperature of 40 °C for *Nocardiopsis alba*.

Determination of the optimal pH

A pH of 8 was found to be optimal for strain V6-2, while strain V9 showed maximum production at pH 7 (Figure 4).

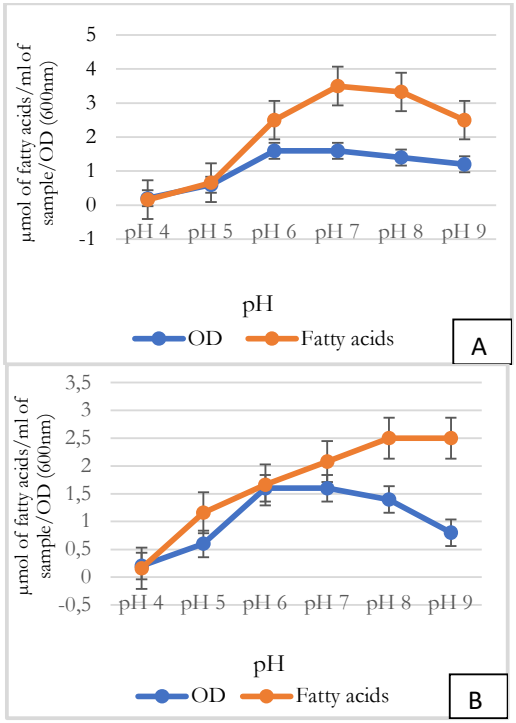

Figure 4. Search for the *optimal pH* (A: V9: B: V6-2)

Many microbial lipases exhibit optimal activity in a pH range of 7 to 9 (Mahmoud *et al.*, 2015), which confirms the obtained results.

Pham *et al.* (2021) revealed that most bacterial strains tested produced lipase at pH 5, except for *Paenibacillus* PL2 and *Stenotrophomonas* N-PL7, which could not survive at pH 5. The lipase yield increased from pH 6, was optimal at pH 7, and gradually decreased at pH 8.

According to Syed *et al.* (2010), the maximum lipase activity of *Pseudomonas aeruginosa* BN-1 at 37 °C was achieved using a medium at pH 6.5. Similarly, *Pseudomonas spp*. BWS-5 exhibited optimal pH at 6.5 (Sooch and Kauldhar, 2013), while *Pseudomonas fluorescens* SIK W1 showed an optimal acidic pH of 4.8 (Mobarak-Qamsari *et al.*, 2011).

The production of lipases in *Bacillus sp*. LBN2 was found to be optimal at an alkaline pH of 8.0 to 10.0, with maximum production achieved when the initial pH of the medium was 9.0 (Bora and Bora, 2012). Different pH values allowing better lipase production were also observed, particularly in *Staphylococcus aureus* at pH 6.5 (Paiva *et al.*, 2000), *Bacillus sp*. RSJ1 at pH 8 (Sharma *et al.*, 2001), and *Fusarium oxyporum* at pH 7.0 (Prazeres and *al.*, 2006). Thus, most *Bacillus sp*. strains exhibit an optimal pH range between 5 and 8 (Gupta *et al.*, 2004)

Determination of the optimal carbon source

The search for the carbon source that promotes the highest enzymatic activity was conducted under the previously optimized conditions for the two tested strains.

The results showed maximum lipase activity in the presence of glucose, followed by lactose (Figure 5).

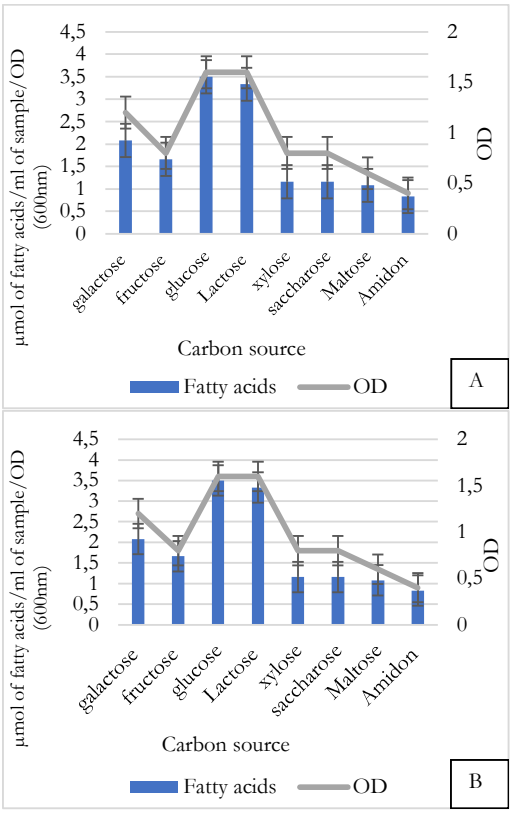

Figure 5. Search for the *optimal carbon source* (A: V9: B: V6-2)

This can be explained by the fact that lactic acid bacteria are known to have a transport system that favors the utilization of glucose as an essential carbon source for cell maintenance and metabolism. The presence of lactose stimulates B-galactosidase, allowing the cells to release glucose into the medium (De Roissart and Luquet, 1994).

Several studies have been conducted to identify the carbon source that induces lipase production. The major factor influencing lipase activity has always been the carbon source, as lipases are largely inducible enzymes and are significantly influenced by various carbon sources such as sugars, sugar alcohols, polysaccharides, whey, amino acids, and other complexes (Rashid *et al.*, 2001).

Nwachukwu *et al.* (2017) demonstrated that glucose had the highest value for stimulating lipase production in *Serratia marcescens*. Similarly, significant lipolytic activity was observed in the presence of glucose and sucrose in *Candida sp*. KKU-PH2-15 (Boonchaidung *et al.*, 2013), while lipase activity appeared to be considerable in *Bacillus coagulans* VKL1 when supplemented with maltose and sucrose (Gowthami *et al.*, 2015). In *Bacillus sp*., sucrose and maltose were also found to be good inducing substrates for lipase production among the tested sugars (Bora and Bora, 2012).

Study of growth kinetics, lipase production, and release of fatty acids over time

The graphs below depict the evolution of growth and the release of fatty acids over time. The results obtained indicate an 8-hour latency period, followed by an exponential phase characterized by continuous lipase production until growth reaches its stationary phase. Lipid degradation peaks during the decline phase, which occurs after 96 hours of incubation. At this stage, it is estimated that the amount of released fatty acids is 3.5 µmol/ml for strain V9 and 2.91 µmol/ml for strain V6-2 (Figure 6). An example of the results is illustrated in Figure 7.

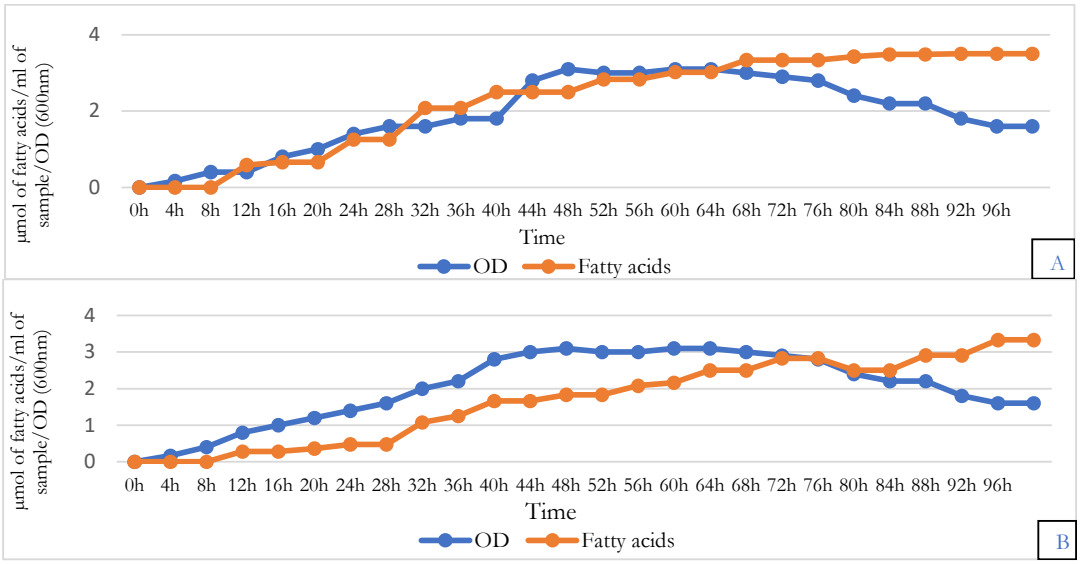

Figure 6. Growth kinetics and fatty acid production as a function of time
A: V9; B: V6-2

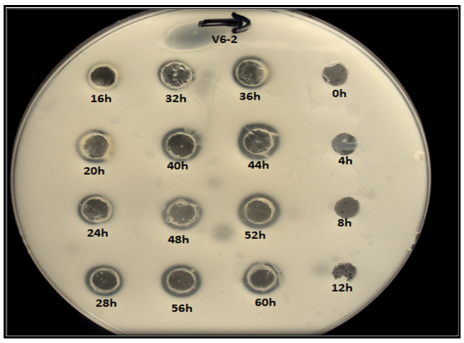

Figure 7. Fatty acid production as a function of time for strain V6-2

Lima *et al.* (2019) demonstrated that after 48 hours of *Aspergillus niger* incubation, there was a slight decrease in dry biomass concentration, suggesting the depletion of the main carbon source. Some microbial cells may have undergone autolysis, resulting in the release of intracellular lipase and increasing its concentration in the enzymatic extract. The highest specific activity was obtained at 72 hours of incubation.

Purification of lipolytic enzymes

In comparison with the results obtained by Dellali *et al.* (2020), which showed that the intracellular enzymatic activity obtained is higher than that found in the extracellular activity, purification was performed on the tributyrinesterases of both *Enterococcus faecium* (CAM18) and *Enterococcus durans* (LKV11) strains. The purification was carried out under the optimal conditions previously determined (pH, temperature, activating ions) by Dellali *et al.* (2020). Similar results have been observed in several studies, revealing that intracellular lipase activity is higher than extracellular activity. This has been confirmed by the study of Meyers *et al.* (1996), which showed that lipases from *Lactococcus lactis subsp. hordniae 2181, Lactococcuslactis subsp. lactis 1835, and Streptococcus thermophilus 821* are present in the intracellular content. Katz *et al.* (2002) also revealed that lipases and esterases are predominantly intracellular, particularly in strains of *Enterococcus faecium* (0426 and 0174).

Semi-purification with ammonium sulfate

The dialysis step aims to concentrate the intracellular content obtained by cell lysis, and it is performed before all the purification steps. The results show significant enzymatic activity at 60% saturation for both tested strains (LKV11 and CAM18) compared to the p70 fraction, where a slight decrease in esterase activity is observed. However, a considerable decrease is detected at 90% and 80% saturation for both strains. This can be explained by the inhibitory effect of ammonium sulfate on lipases, highlighting the need for a better desalting process.

Gasparin *et al*. (2020) observed a significant increase in esterase activity at 60% saturation of ammonium sulfate, but a decrease in enzymatic activity was shown at 80% saturation. Similarly, another study found that 60% saturation was sufficient for better enzymatic activity, while the activity decreased when the degree of ammonium sulfate saturation was increased from 60% to 80% (Abigor *et al*., 2002).

Sephadex-G100 gel filtration chromatography

The results of size exclusion chromatography on Sephadex G100 for the two strains are shown in Figure 9. Vitamin B12 and blue dextran are used to delineate the fractions. Vitamin B12 exhibits maximum absorbance at the 18th fraction, with a total volume of VT=38 ml, while blue dextran is used to determine the void volume (V0) corresponding to a volume of 10 ml. An example of the results is shown in Figure 8.

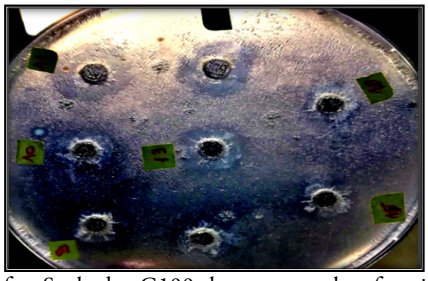

Figure 8. Fractions obtained after Sephadex-G100 chromatography of strain LKV11

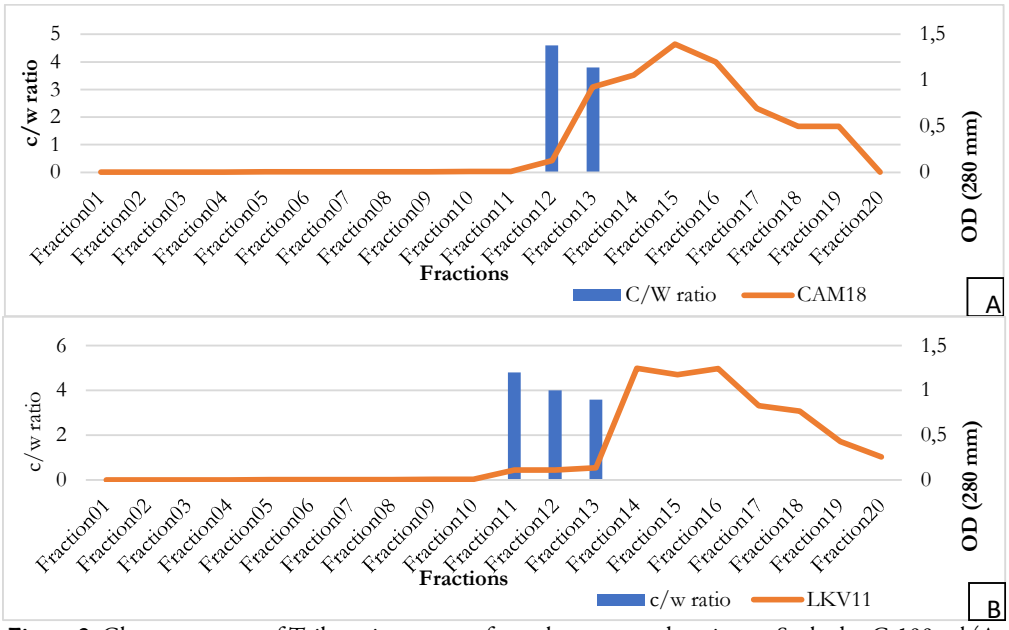

Figure 9. Chromatograms of Tributyrin-esterases from the two tested strains on Sephadex G-100 gel (A: CAM18, B: LKV11), (C: clarification area, W: well diameter)

In order to approximate the molecular weight of the semi-purified proteins obtained from the chromatography fractions where the enzymatic activity is maximal, two proteins (BSA and lysozyme) are eluted under the same conditions. Their elution volumes are used to plot a logarithmic curve of molecular weight as a function of elution volume (Figure 10), with the known molecular weights of BSA and lysozyme being 66.5 kDa and 14.3 kDa, respectively.

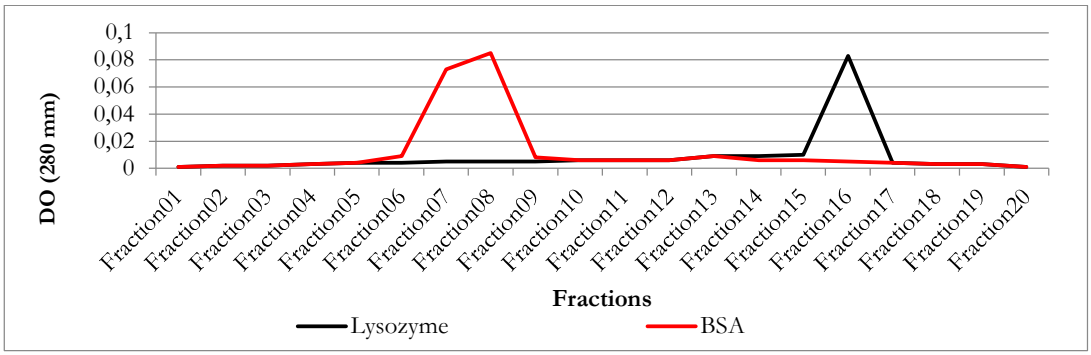

Figure 10. Chromatograms of the two proteins (BSA and Lysozyme) on Sephadex G-100 gel

Table 2 represents the estimated molecular weights of the two intracellular semi-purified tributyrin-esterases obtained by projecting them onto the standard curve of log (MM) versus elution volume of the two previous proteins (BSA and lysozyme) (Figure 11).

Table 2. Estimated molecular weight of the tributyrin-esterases from the two tested strains

Tributyrin-esterases	CAM18	LKV11
Log (MM)	1.5065	1.5854
MM (kDa)	32.09	38.49

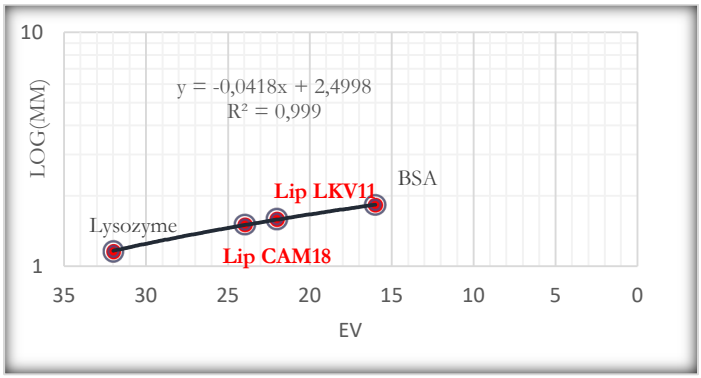

Figure 11. Standard curve of log (MM) as a function of elution volume

According to the literature, the molecular weight of lipases can vary between 11 and 67 kDa (Saxena *et al*., 2003). Our results are consistent with several studies conducted on lactic acid bacteria. For example, lipases from *Lactobacillus sp*. G5 initially purified by ion exchange chromatography have a molecular weight of 27 kDa (Park *et al*., 2007), lipases from *Enterococcus feacium* are estimated to be 19.172 kDa (Ramakrishnan *et al*., 2016), tributyrinesterases from *Lactobacillus plantarum* strains purified and analyzed by SDS-PAGE electrophoresis are 31 kDa (Esteban-Torres *et al*., 2014), and *Propionibacterium freudenreichii* exhibits intracellular esterases of 52 kDa (Kakariari *et al*., 2000).

Several other studies on different strains have shown that bacterial lipases can have molecular weights ranging from 19 to 60 kDa (Sharma *et al*., 2001).

Total protein estimation using the Bradford method

In this method, proteins form a coloured complex with Coomassie Blue G250, which exhibits maximum absorption at 595 nm. The analysis of proteins is based on the colour change of the dye in response to different protein concentrations. A calibration curve is created using a solution of bovine serum albumin (BSA) prepared at 1 mg/ml in distilled water.

Table 3 encompasses the measurements of all the previously obtained results from each purification step, estimating the total proteins in different samples. A standard curve was generated to determine the protein concentration.

Table 3. Protein concentration at each purification step for the two tested strains

Strains / Purification stages (mg/ml)	CAM18	LKV11
Supernatant of crude culture	0.006	0.005
Concentration by dialysis	0.008	0.009
Ammonium sulfate precipitation	0.011	0.014
Gel filtration chromatography on Sephadex G-100	0.003	0.004

These results show that the protein content increases during the two steps of dialysis and precipitation due to the concentration of proteins in the tested strains. Indeed, proteins precipitate in the presence of ammonium sulfate, which allows the removal of other molecules, explaining the increase in protein content during the precipitation step. However, a significant decrease is observed during chromatography due to sample dilution by the mobile phase and protein separation

Conclusions

In conclusion, our study revealed that two *Lactococcus* strains exhibited maximum extracellular lipolytic activity when cultured in a medium supplemented with 1% olive oil. These strains demonstrated optimal lipase production at neutral to slightly alkaline pH levels and temperatures of 30 °C and 37 °C, with glucose serving as a crucial carbon source. The production of extracellular lipases appeared to be closely linked to bacterial growth kinetics, peaking during the stationary phase. Additionally, our successful purification of intracellular tributyrinesterases from Enterococcus strains yielded molecular weight estimates consistent with existing literature. The observed decrease in protein content during the purification process highlights the need for further refinement.

These findings shed light on the enzymatic capabilities of lactic acid bacteria and offer insights into their potential applications in various industries. Moving forward, research in this area could explore additional aspects of enzyme characterization and optimization, paving the way for enhanced industrial applications and bioprocessing.

Authors' Contributions

Conceptualization: AD, HZK, NK; Data curation: AD; Formal analysis: AD; Investigation: AD; Methodology: AD, HZK, NK; Resources: AD, FB; Supervision: HZK, NK; Validation: AD; Writing - original draft: AD; Writing - review and editing: AD, HZK, NK. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

The authors expressed their gratitude to the Algerian Ministry of Higher Education and Scientific Research (CNEPRU F01820090065) and the Directorate General for Scientific Research and Technological Development (LBMB 02/2000) for providing financial support.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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