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Lipase and esterase activities of lactic acid bacteria isolated from different biotopes

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The lipolytic and esterase activities of fifteen strains of lactic acid bacteria isolated from different biotopes of Algeria and Mauritania were tested on MRS medium supplemented with lipidic substrates. Five of them showed maximum activity in the presence of tributyrin; the activity is therefore a tributyrin esterase. These strains were identified by MALDI-TOF in *Enterococcus faecium* and *Enterococcus durans*. The study of growth kinetics as a function of time shows a start of fatty acid production during the exponential phase to reach its maximum in the stationary phase. A better esterase activity is observed at between pH 6 and 9 and at an optimal temperature of 30 to 40°C for the five strains. The influence of metal and additive ions on the esterase activity varies between bacteria but generally, total inhibition was observed in all strains tested in the presence of SDS, NaN_3, $CuCl_2$, EDTA, $AgNO_3$ and $HgCl_2$.

Key words: Lipolytic activity, esterase activity, tributyrin, tributyrin esterase, MALDI-TOF, *Enterococcus*, growth kinetics, metal ions.

INTRODUCTION

Lipases (EC 3.1.1.3) and esterases (EC 3.1.1.1) are carboxyl esterases that hydrolyze the carboxylic ester linkages of triacylglycerols to release diglycerides, monoglycerides, free fatty acids and glycerol. The lipolytic and/or esterase activity of lactic acid bacteria contributes to the production of new foods or food supplements (García-Cano et al., 2019). These bacteria with probiotic potential can also produce conjugated fatty acids by hydrolyzing triacylglycerol thanks to their lipase activity (Kuhl et al., 2016). It is generally recognized that lactic acid bacteria play an important role in quality, flavor, and maturation in cured meat production (Dinçer and Kıvanç, 2018).

Carboxyl esterases act only in ester-water interface and are of considerable physiological and industrial importance (Martinelle et al., 1995). The water activity in the reaction medium controls the balance of the reactions (Borrelli and Trono, 2015). In case of low water activity, lipases catalyze other reactions (esterification, interesterification, acidolysis, alcoholysis, and aminolysis reactions) (Joseph et al., 2008; Bajaj et al., 2010).

Generally, esterases hydrolyze only short chain fatty acid triglycerides while lipases are active on water insoluble substrates and hydrolyze long chains to fatty acids (Kilcawley et al., 1998).

Microbial lipases are widespread in bacteria, especially

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Table 1. Bacterial strains used in this study.

Origin	Code of strain	Species
Camel's milk (Illizi, Algeria)	BH21	*Lactobacillus plantarum*
Camel's milk (Nouakchott, Mauritania)	CAM18	*Enterococcus* sp.
Camel's milk (Timimoun, Algeria)	CAT13; CAT18	*Enterococcus* sp.
Camel's milk (Béchar, Algeria)	CHBK320	*Leuconostoc mesenteroides ssp dextranicum*
Camel's milk (Tindouf, Algeria)	CHTD27	*Lactobacillus brevis*
Cow's Milk (Oran El-Kerma, Algeria)	LKV11	*Enterococcus* sp.
Olive brine (Sig, Algeria)	OV5	*Lactococcus lactis* ssp *diacetylactis*
Fresh beef meat (Mostaganem, Algeria)	V6-2 ; V17 ; V18	*Lactococcus lactis* ssp *lactis*
	V9	*Lactococcus lactis* ssp *cremoris*
Fresh sheep meat (Relizane, Algeria)	VO19	*Enterococcus* sp.

in Gram+ (Fickers et al., 2008). Among them are lactic acid bacteria, which are considered slightly lipolytic in comparison with other bacterial species (Brennan et al., 2002). This activity does not influence bacterial growth. In fact, these enzymes do not show any nutritional role (Fernández et al., 2000; Nardi et al., 2002). However, their presence in cheeses, and at higher concentrations and at precise periods, leads to the release of fatty acids responsible for the final taste (Das et al., 2005). *Lactobacillus plantarum* CCFM12 shows good esterase activity responsible for a considerable improvement in the production of ethyl esters and which leads to the fruity taste of camembert cheese (Hong et al., 2018).

Esterases of lactic acid bacteria preferentially degrade para-nitrophenyl or beta-naphthyl derivatives of C4 or C6 fatty acids, and a good activity was also observed in the presence of tributyrin. Esterasic activity decreases considerably with lengthening of the chain (Corrieu and Luquet, 2008).

Lipases and esterases enzymes of lactic acid bacteria are either extracellular or intracellular (Katz et al., 1997; Meyers et al., 1996), hence in the second case the need for cell lysis to promote their access to the substrate.

Several esterases of lactic acid bacteria such as *Lactococcus lactis, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus helveticus, Lactobacillus rhamnosus, Streptococcus thermophilus and Enterococcus faecium,* were previously characterized. These enzymes have an optimal activity in a temperature range of 30 to 45°C depending on the strains considered, as well as a neutral or slightly alkaline pH. Their activators are Zn^{2+}, Mg^{2+}, Ca^{2+} ions as well as NaCl. Phenylmethylsulfonyl fluoride (PMSF), para-chloro-mercuribenzoate (PCMB), and Hg^{2+}, Cu^{2+}, Ag^{2+} ions inhibit esterase activity (Tsakalidou et al., 1994; Holland and Coolbear, 1996; Fernendez et al., 2000; Nardi et al., 2002; Chich et al., 1997; Fenster et al., 2000; Gobbetti et al., 1997a; Castillo et al., 1999; Choi et al., 2004; Liu et al., 2001; Mobarak-Qamsari et al., 2011; Salwoom et al., 2019).

The objective of this research was to highlight the lipase and esterase activity of lactic acid bacteria cultivated in the presence of different lipid substrates. Studies of the kinetics of growth and production of esterases and/or lipases depending of various physico-chemical parameters (pH, temperature, surfactants, metal ions and other additives) will be useful in order to optimize the use of these enzymes.

MATERIALS AND METHODS

Bacterial strains

The 15 lactic acid bacterial strains in the genus *Lactococcus*, *Lactobacillus*, *Enterococcus* and *Leuconostoc* have been isolated by serial dilution plating on MRS medium (De Man et al., 1960) from raw camel or cow milk, olive brine and fresh meat collected in Algeria and Mauritania (Table 1). The bacterial cultures were maintained at -20°C in MRS broth containing 20% glycerol (v/v). Working cultures were prepared by two consecutive transfers in MRS broth at 30ºC for 18 h.

Bacterial identification

The bacteria were streaked on MRS agar plates and incubated at 30°C for 24 to 48 h. Single isolates were identified by Bruker Daltonic's MALDI-TOF Biotyper – CM according to the manufacturer instructions. The identification is carried out by mass spectrometry analysis coupled with a source of laser ionization assisted by a MALDI (Matrix Assisted Laser Desorption Ionization) and a TOF (Time of Flight) analyser. For the MALDI-TOF MS analysis, the strains were grown on MRS medium for 24 h. Each colony was smeared on the target and then covered with 1 µl of formic acid and 1 µl of the matrix. It is then identified by the MALDI-TOF.

Detection of intracellular lipolytic and esterase activity

Bacterial preculture was grown on MRS broth at 30°C until it reaches OD600 ~1.2. Tubes of 10 ml of fresh MRS broth were inoculated with 0.1 ml of the bacterial preculture and incubated at 30°C for 18 h. The intracellular enzymes from the isolate were extracted by using glass bead stirring. After centrifugation at 8000

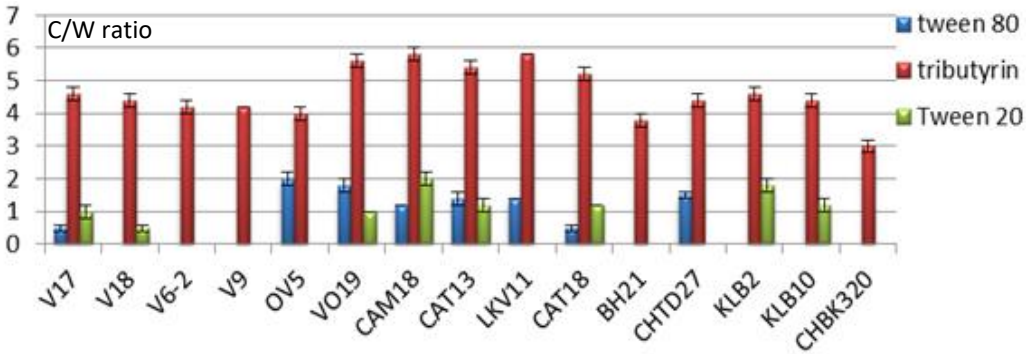

Figure 1. Lipolytic activity on artificial lipid substrates in lactic strains (C: clarification area, W: well diameter).

rpm, the supernatant is recovered. It constitutes the crude enzymatic extract.

The activity of enzymes was investigated according to the diffusion method in buffered agar at pH 7 (0.1 M phosphate buffer) containing various natural or artificial lipid substrates as follows:

1. 3% olive oil, almond oil, argan oil or oleic acid. The media are then opacified with $CaCO_3$ in order to visualize a possible lipase activity.
2. 0.25% of tributyrin is added and the emulsion is homogenized by sonication. In case degradation of tributyrin an observed clarification reflects the presence of esterases (Medina et al., 2004).
3. 3% Tween 80, 0.01% $CaCl_2$ and 0.5% NaCl. Under these conditions, the presence of lipases is manifested by opacity (Guiraud and Galzy, 1980).

Plates were incubated at 37°C for 72 h. The strains as well as the lipid substrate where the activity is maximal are selected for further work.

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

Tubes of 10 ml of fresh MRS broth were inoculated with 0.1 ml of the bacterial preculture as described above and incubated at 30°C. The kinetics of bacterial growth is determined by measuring OD600 nm every 4 h.

In parallel, 0.5 ml of enzymatic extract is mixed with 1 ml of pH 7 phosphate buffer and 1.5 ml of lipid substrate. After 60 min of incubation at 37°C with stirring, in order to promote lipid-lipase source contact, 1 ml of 95% ethanol is added to extract the free fatty acids. Titration with 0.05 M KOH solution in the presence of phenolphthalein was carried out to determine the concentration of free fatty acids. The control consists of the same reaction mixture but without enzymes (Ginalska et al., 2004). The results were expressed in µmol of fatty acid / ml of sample (Sokolovska et al., 1998).

pH and temperature determination for lipase activity

The lipase activity was measured in a pH range of 4 to 9. This pH range is obtained using 0.1 M buffer solutions of acetic acid-sodium acetate buffer (pH 3-5), or phosphate buffer sodium (pH 6-7), or Tris-HCl buffer (pH 8) or glycine-NaOH buffer (pH 9). The concentration of released fatty acids was carried out by titration in the same manner as previously described.

The optimal temperature for lipase activity was determined in the same way and in the presence of the optimal pH buffer obtained previously for each strain. To determine the optimal temperature for lipase activity, incubation was performed at 20, 30, 37, 40 and 45°C.

Effect of metal ions and additives on lipase activity

The enzymatic extract was incubated in the presence of 10 mM of various additives and metal ions for 1 h at a temperature of 37°C. The ions, additives and surfactants used are: Ascorbic acid, aspartic acid, folic acid, glutamic acid, nicotinic acid, serine, cysteine, riboflavin, EDTA, Triton X100, beta-mercaptoethanol, ammonium sulfate, barium sulfate, magnesium sulfate, lithium sulfate, sodium thiosulfate, SDS, copper chloride, silver nitrate, iron, calcium chloride, manganese chloride, mercury chloride, zinc chloride, urea, sodium azide, calcium carbonate, Tween 80, Tween 20, sodium molybdate and potassium permanganate. The reaction mixture was supplemented with lipid substrate and different buffers at optimal pH and then incubated for 60 min at optimal temperatures for each strain in order to obtain the released fatty acids.

RESULTS AND DISCUSSION

Intracellular lipolytic and esterase activities

The results of lipolysis obtained on medium supplemented with different lipid substrates are shown in Figures 1 and 2. An example of lipase effect on tributyrin is shown on Figure 3. The lactic acid bacterial strains degrade the lipid substrates differently with a maximum of activity when adding 0.25% tributyrin. This occurs especially in strains VO19, CAM18, CAT13, CAT18 and LKV11 of the genus Enterococcus (Table 1). Ginalska et al. (2004) and Gobbetti et al. (1997b) reported that the lipolytic activity is often observed in enterococci and it shows higher activity than strains of most other genera of lactic acid bacteria.

Our results agree with the work of Cardenas et al., (2001) who showed that bacterial lipases tend to reveal better hydrolytic activity on tributyrin, which is a

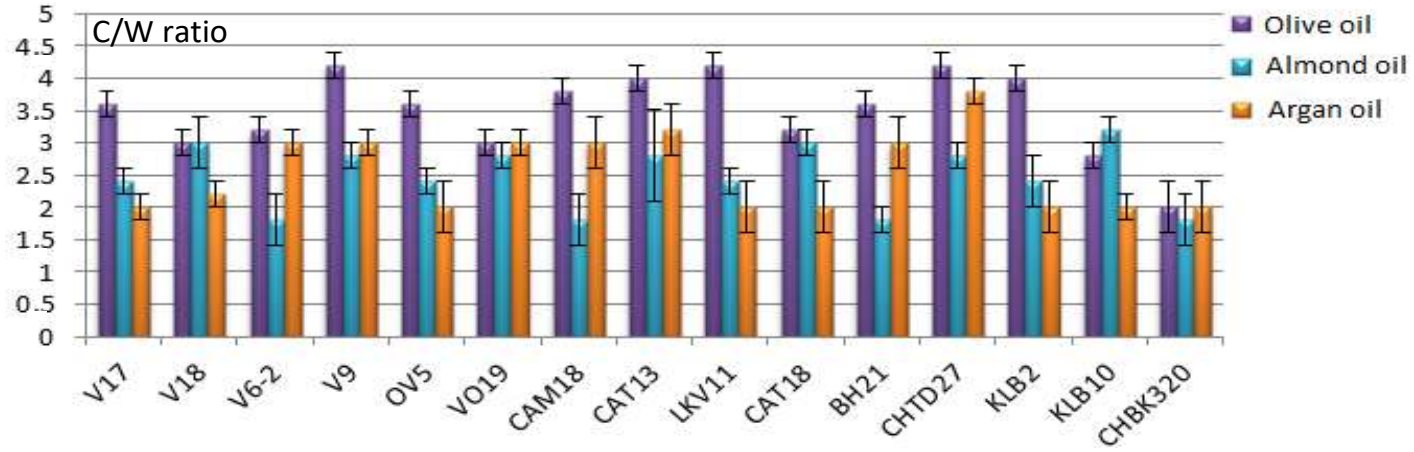

Figure 2. Lipolytic activity on natural lipid substrates in lactic strains (C: clarification area, W: well diameter).

Figure 3. Tributyrin esterase activity of lactic strains.

triglyceride composed of short chain fatty acid, namely butyric acid. Tributyrin can be easily broken down by esterases acting on short lipid chains (<10C). Likewise, the lipases of lactic acid bacteria have an optimal efficacy with tributyrin and lower with natural lipids (Talon and Montel, 1994; De Roissart and Luquet, 1994; Guiraud and Galzy, 1980), which has been observed in the presence of natural substrate, in particular olive oil. The lipase activity was less effective with olive oil where in comparison with tributyrin; on the other hand, the presence of olive oil leads to an increase in lipase activity compared to other oils supplemented. This is due to the presence of oleic acid in large quantities in olive oil (78%) because lipases are of the inductive type with a preference for monounsaturated long chain fatty acids. These results are consistent with several studies on microbial lipases showing a high production of lipase in the presence of olive oil among several oils tested (Feitosa et al., 2010; Sooch and Kauldhar, 2013; Iqbal and Rehman, 2015; Quian and Chun-Yun, 2009; Nwachukwu et al., 2017; Vishnupriya et al., 2010; Esakkiraj et al., 2010; Dandavate et al., 2009). An important lipolytic activity is also observed with 2% olive oil in other bacterial species, like *Pseudomonas aeruginosa* KM110 and *Bacillus* sp ZR-5 (Mobarak-Qamsari et al., 2011; Soleymani et al., 2017).

In the presence of Tween 80 and Tween 20, the lactic strains are weakly lipolytic (Figure 1), so the degradation of these two substrates differs depending on whether the bacteria hydrolyze the Tween 20 containing the lower chain lauric acid esters and the Tween 80 composed of oleic acid degraded respectively by esterases and lipases (Kumar et al., 2012). Therefore, the five strains of *Enterococcus* mentioned above therefore show better

Table 2. Identification of lactic strains by MALDI-TOF.

Analyte name	Organism (best match)	Score value
CAM18	*E. faecium*	2,306
CAT13	*E. faecium*	2,344
CAT18	*E. faecium*	2,333
LKV11	*E. durans*	2,151
VO19	*E. faecium*	2,177

degradation in the presence of tributyrin, the activity then turns out to be a tributyrin esterase.

These strains are retained for further work and are identified by MALDI-TOF; the results obtained are shown in the Table 2.

Kinetics of growth and release of fatty acids

The kinetics are carried out every 4 h by measuring the bacterial growth at 600 nm and assaying of freed fatty acids obtained by the action of intracellular tributyrin esterase on the tributyrin supplemented therefore 1 µmol of fatty acid released / ml / min corresponds to an enzyme unit (EU). The results obtained are shown on Figure 4a to e.

The results showed the presence of tributyrin-esterase in the intracellular content of the five strains tested from the exponential phase and with maximum production during the stationary phase. On the other hand, the enzymatic activity varies from one strain to another. In addition, according to the phases, a significant production is then observed in the CAT13 and VO19 strains during the exponential phase, which is maintained until the stationary phase. The production of lipases is therefore associated with cell growth, this agrees with studies showing that bacterial lipases are produced during the growth phase or late in the same phase (Papon and Talon, 1988; Makhzoum et al., 1995; Gupta et al., 2004). Other works on intracellular extracts of *Lactobacillus* species had shown that ester activity was present from the start of the exponential phase and then increased to reach a maximum value at the start of the stationary phase (El Soda et al., 1986; Khalid et al., 1990). Serio et al. (2010) also noted an ester activity on strains of *Enterococcus* in stationary phase; similar results are observed in our study where strains CAT18, LKV11 and CAM18 activity is moderate in the exponential phase with maximum production during the stationary phase.

Determination of optimum pH and temperature

Bacterial lipases are generally neutral (Dharmsthiti and Kuhasuntisuk, 1998; Dharmsthiti and Luchai, 1999; Lee et al., 1999), or slightly alkaline (Kanwar and Goswami, 2002; Schmidt-Dannert et al., 1994; Sidhu et al., 1998a, b; Sunna et al., 2002). These results are observed in five strains tested with an optimum pH between pH 6 and 9 (Figure 5b), which is consistent with the work of Esteban-Torres et al. (2014b) who reported a noticeable lipolytic activity at pH 7. Tributyrin esterase of *Lactobacillus plantarum* strain 2739 had an optimum pH of 7 (Gobbetti et al., 1996, 1997a), while *Lactobacillus plantarum* MF32 lipase shows maximum activity at a more alkaline pH (pH 9.3) (Andersen et al., 1995).

The bacteria studied in this work have an optimal temperature ranging from 30 to 40°C (Figure 5a), some work has shown that bacterial lipases have an optimal temperature of 30 to 60°C (Lesuisse et al., 1993; Wang et al., 1995; Dharmsthiti et al., 1998; Litthauer et al., 2002). This has been observed in lipases of *Lactobacillus plantarum* with an optimal temperature of 35°C (Andersen et al., 1995; Gobbetti et al., 1996; 1997a; Lopes et al., 2002), and in *Enterococcus faecium* where maximum activity occurs at 40°C (Ramakrishnan et al., 2016).

Effect of metal ions and additives on lipase activity

Several studies show the effect of metal and additive ions on lipase and esterase activity, despite their concentration and the mechanism of induction may vary from one species to another (Saxena et al., 1994). Supplemented metal ions and additives act differently on the esterase activity of the strains tested as shown in Figure 6. The activity is completely inhibited by SDS, sodium azide, EDTA, copper chloride, silver nitrate and mercury chloride in the five bacteria studied. Some studies showed a considerable decrease in activity in the presence of EDTA, which can influence the interfacial zone between substrate and lipase (Sztajer et al., 1992). The activity of lipase Lp_3562 is strongly inhibited by Hg^{2+}, Cu^{2+} and SDS (Esteban-Torres et al., 2014a). A significant inhibition is observed in Lp_1760 in the presence of Hg^{2+}, Zn^{2+}, Cu^{2+} and SDS (Esteban-Torres et al., 2014b). Significant tributyrin-esterase activity is detected when adding Tween 20 and barium sulfate in VO19 and cysteine in CAM18. It was reported that Tween 20, 40, 60 and Triton X-100 could activate lipases in

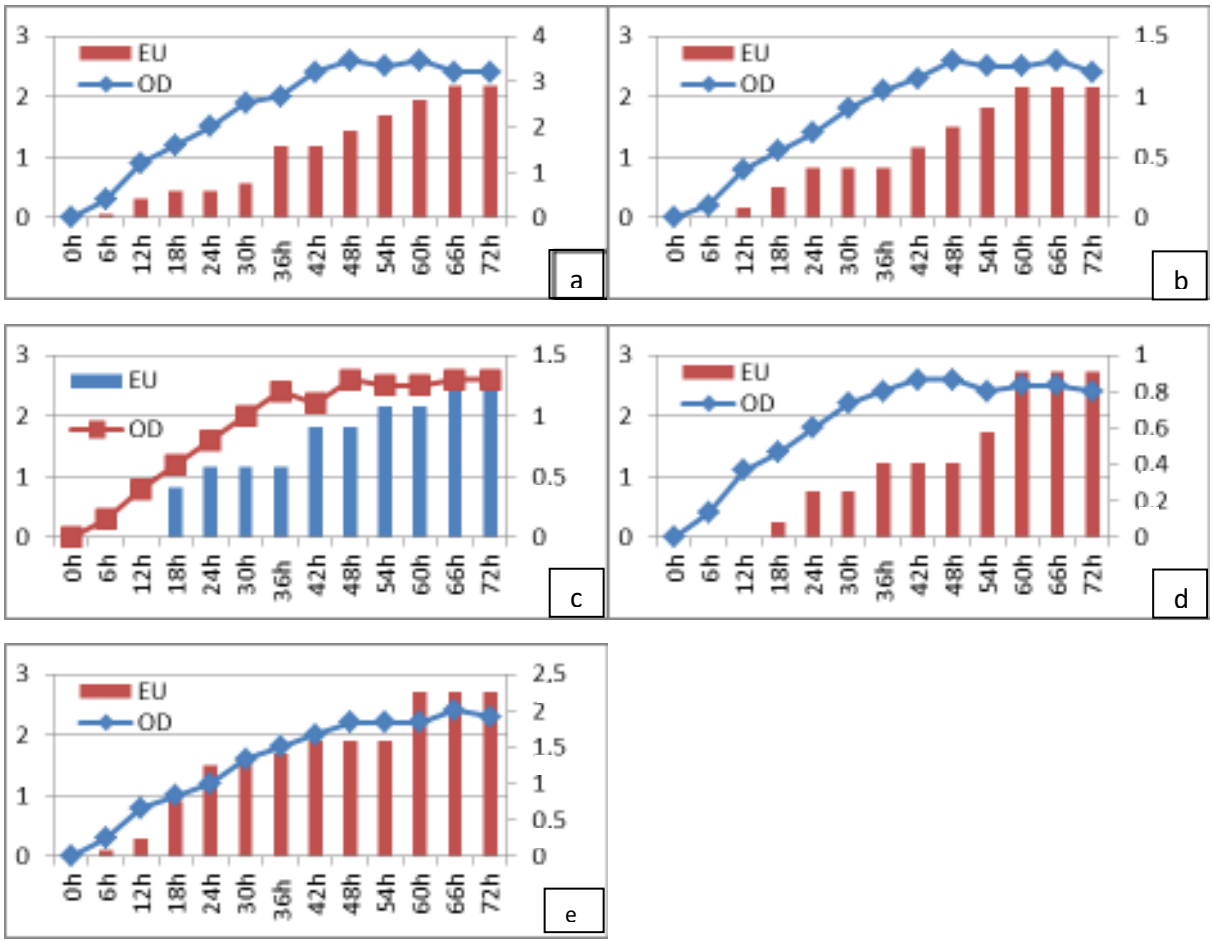

Figure 4. Growth kinetics and fatty acid production as a function of time. a: CAM18; b: LKV11; c: CAT13; d: CAT18; e: VO19. EU: Enzyme unit ; OD: Optical density.

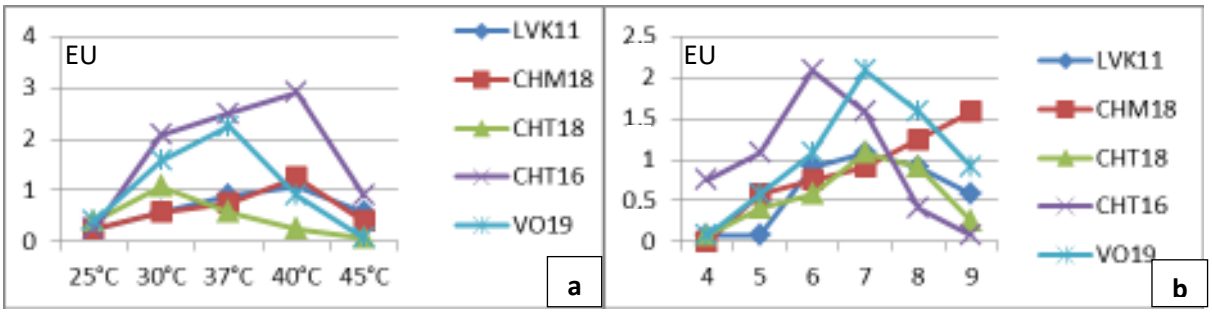

Figure 5. Study of optimal parameters (a: Optimal temperature, b: Optimal pH).

Cryptococcus sp. (Thirunavukarasu et al., 2008). Tween 20 also increases the production of *Bacillus altitudinis* AP-MSU esterases (Palanichamy et al., 2012), but inhibition of lipase activity is detected in the presence of surfactants (Tween-20, Tween-80 and Triton X-100) tested on Celite-immobilized commercial lipase (Lipolase 100 L) (Sharma et al., 2016). It was also noted that lipases produced by *Pseudomonas aeruginosa* HFE733 are activated by beta-mercaptoethanol and cysteine (Jun et al., 2018). Other ions and additives tested had little or no effect on enzyme activity with a slight decrease or sometimes activation of tributyrin esterases. In some

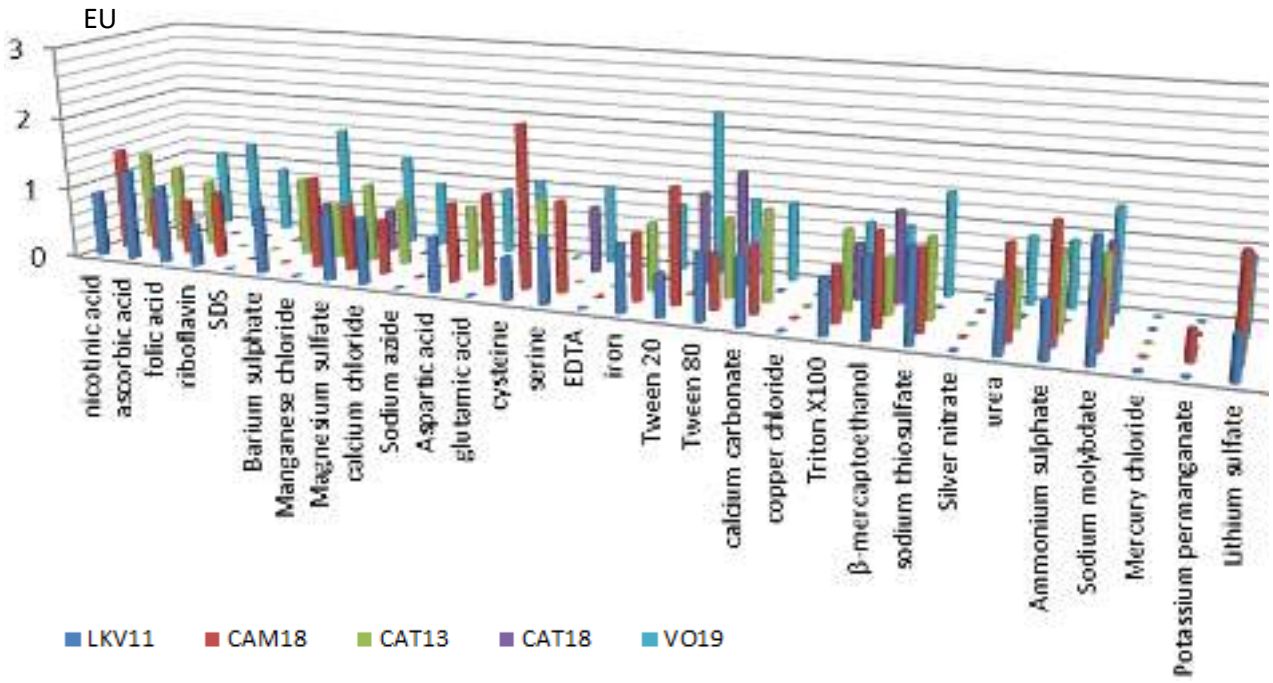

Figure 6. Effect of additives on the lipolytic activity of the five enterococcal strains.

cases the same ion or additive acts differently in the five strains tested by activating or inhibiting enzyme activity as shown in Figure 6.

Conclusion

Five enterococcal strains show a maximum esterase activity in medium supplemented of tributyrin. The production of intracellular tributyrin esterases can be related to bacterial growth or sometimes maximal during the stationary phase, requiring a neutral or slightly alkaline pH and an optimal temperature between 30 and 40°C. The tributyrin esterases act differently when metal ions and additives are added, while SDS, sodium azide, EDTA, copper chloride, silver nitrate and mercury chloride were found to inhibit enzyme activity.

CONFLICT OF INTERESTS

The authors declared no conflict of interests.

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REFERENCES

Andersen HJ, Ostdal H, Blom H (1995). Partial purification and characterisation of a lipase from *Lactobacillus plantarum* MF32. Food Chemistry 53:369-373.

Bajaj A, Lohan P, Jha PN, Mehrotra R (2010). Biodiesel production through lipase catalyzed transesterification: an overview. Journal of Molecular Catalysis B Enzymatic 62:9-14.

Borrelli GM, Trono D (2015). Recombinant lipases and phospholipases and their use as biocatalysts for industrial applications. International Journal of Molecular Sciences 16(9):20774-20840.

Brennan NM, Ward AC, Beresford TP, Fox PF, Goodfellow M, Cogan TM (2002). Biodiversity of the bacterial flora on the surface of a smear cheese. Applied and Environmental Microbiology 68(2):820-830.

Cardenas F, Alvarez E, Castro Alvarez MS, Sanchez- Montero JM, Valmaseda M, Elson SW, Sinisterra JV (2001). Screening and catalytic activity in organic synthesis of novel fungal and yeast lipases. Journal of Molecular Catalysis B Enzymatic 14:111-123.

Castillo I, Requena T, Fernandez de Palencia P, Fontecha J, Gobbetti M (1999). Isolation and characterization of an intracellular esterase from *Lactobacillus casei subsp.casei* IFPL731. Journal of Applied Microbiology 86:653-659.

Chich JF, Marchesseau K, Gripon JC (1997). Intracellular esterase from *Lactococcus lactis subsp. Lactis* NCDO 763: Purification and Characterization. International Dairy Journal 7:169- 174.

Choi YJ, Miguez CB, Lee BH (2004). Characterization and Heterologous Gene Expression of a Novel Esterase from *Lactobacillus casei* CL96. Applied and Environmental Microbiology 70(6):3213-3221

Corrieu G, Luquet FM (2008). Bactéries lactiques - De la génétique aux ferments. Lavoisier, Paris, pp. 849.

Dandavate V, Jinjala J, Keharia H, Madamwar D (2009). Production, partial purification and characterization of organic solvent tolerant lipase from *Burkholderia multivorans* V2 and its application for ester synthesis. Bioresources Technology 100(13):3374-3381.

Das S, Holland R, Crow VL, Bennett RJ, Manderson GJ (2005). Effect of yeast and bacterial adjuncts on the CLA content and flavour of a

washed-curd, dry-salted cheese. International Dairy Journal 15:807-815.
De Man JC, Rogosa M, Sharpe ME (1960). A medium for the cultivation of lactobacilli. Journal of Applied Bacteriology 23(1):130-135.
De Roissart H, Luquet FM (1994). Lactic acid bacteria, Vol. I et II, Edition Lorica, Uriage, France.
Dharmsthiti S, Kuhasuntisuk B (1998). Lipase from *Pseudomonas aeruginosa* LP602: biochemical properties and application for wastewater treatment. Journal of Industrial Microbiology and Biotechnology 21:75-80.
Dharmsthiti S, Luchai S (1999). Production, purification and characterization of thermophilic lipase from *Bacillus sp*. THL027. FEMS Microbiology Letters 179:241-246.
Dharmsthiti S, Pratuangdejkul J, Theeragool GT, Luchai S (1998). Lipase activity and gene cloning of *Acinetobacter calcoaceticus* LP009. The Journal of General and Applied Microbiology 44:139-145.
Dinçer E, Kıvanç M (2018). Lipolytic activity of lactic acid bacteria isolated from Turkish pastırma. Journal of Science and Technology C- Life Sciences and Biotechnology 7:1.
El Soda M, Fathallah S, Ezzat N, Desmazeaud MJ, Abou Donia S (1986).The esterolytic and lipolytic activities of lactobacilli. Detection of the esterase systems of *Lactobacillus casei, Lactobacillus plantarum, Lactobacillus brevis* and *Lactobacillus fermentum.* Sciences des Aliments 6:545-547.
Esakkiraj P, Rajikunar M, Palavesam A, Immanuel G (2010). Lipase production by *Staphylococcus epidermidis* CMSSTPI isolated from the gut of shrimp *Penaeus indicus*. Annals of Microbiology 60:37-42.
Esteban-Torres M, Mancheño JM, De Las Rivas B, Muñoz R (2014b). Production and characterization of a tributyrin esterase from *Lactobacillus plantarum* suitable for cheese lipolysis. Journal of Dairy Science 97:6737-6744.
Esteban-Torres M, Mancheño JM, De Las Rivas B, Muñoz R (2014a). Characterization of a halotolerant lipase from the lactic acid bacteria *Lactobacillus plantarum* useful in food fermentations. LWT - Food Science and Technology 60:246-252.
Feitosa IC, Barbosa JMP, Orellana SC, Lima AS, Soares CMF (2010). Lipase production by microorganisms isolated from soils with a history of oil contact. Acta Scientiarum - Technology 32:27-31.
Fenster KM, Parkin KL, Steele JL (2000). Characterization of an arylesterase from *Lactobacillus helveticus* CNRZ32. Journal of Applied Microbiology 88:572-583.
Fernández L, Beerthuyzen MM, Brown J, Siizen RJ, Coolbear T, Holland R, Kuipers OP (2000). Cloning, characterization, controlled overexpression, and inactivation of the Major Tributyrin Esterase Gene of *Lactococcus lactis*. Applied and Environmental Microbiology 66(4):8-1360.
Fickers P, Destain J, Thonart P (2008). Lipases are atypical hydrolases: main characteristics and applications. Biotechnologie, Agronomie, Société et Environnement 12:119-130.
García-Cano I, Rocha-Mendoza D, Ortega-Anaya J, Wang K, Kosmerl E, Jiménez-Flores R (2019). Lactic acid bacteria isolated from dairy products as potential producers of lipolytic, proteolytic and antibacterial proteins. Applied Microbiology and Biotechnology 103:5243-5257.
Ginalska G, Bancerz R, Korniffowicz-Kowalska T (2004). A thermostable lipase produced by a newly isolated Geotrichum-like strain, R59. Journal of Industrial Microbiology and Biotechnology 31:177-182.
Gobbetti M, Fox PF, Smacchi E, Stepaniak L, Damiani P (1996). Purification and characterisation of a lipase from *Lactobacillus plantarum* 2739. Journal of Food Biochemistry 20:227-246.
Gobbetti M, Fox PF, Stepaniak L (1997b). Isolation and characterisation of a tributyrin esterase from Lactobacillus plantarum 2739. Journal of Dairy Science 80:3099-3106.
Gobbetti M, Smacchi E, Corsetti A (1997a). Purification and characterisation of a cell surface-associated esterase from *Lactobacillus fermentum* DT41. International Dairy Journal 7:13-21.
Guiraud JY, Galzy P (1980). Microbiological analysis in the food industries. Edition de l'usine, p 39.
Gupta R, Gupta N, Rathi P (2004). Bacterial lipases: an overview of production, purification and biochemical properties. Applied Microbiology and Biotechnology 64 (6):763-781.
Holland R, Coolbear T (1996). Purification of Tributyrin Esterase from *Lactococcus lactis subsp cremoris* E8. Journal of Dairy Research 63:131-140.
Hong Q, Liu XM, Hang F, Zhao JX, Zhang H, Chen W (2018). Screening of adjunct cultures and their application in ester formation in Camembert-type cheese. Food Microbiology 70:33-41.
Iqbal AS, Rehman A (2015). Characterization of lipase from *Bacillus subtilis* I-4 and its potential use in oil contaminated wastewater. Brazilian Archives of Biology and Technology 58(5):789-797.
Joseph B, Ramteke PW, Thomas G (2008). Cold active microbial lipases: some hot issues and recent developments. Biotechnology Advances 26:457-470.
Kanwar L, Goswami P (2002). Isolation of a Pseudomonas lipase produced in pure hydrocarbon substrate and its applications in the synthesis of isoamyl acetate using membrane-immobilized lipase. Enzyme and Microbial Technology 31:727-735.
Katz M, Medina R, Gonzalez S, Oliver G (1997). Esterolytic and Lipolytic Activities of Lactic Acid Bacteria Isolated from Ewes Milk and Cheese. Journal of Food Protection 65:2002-2001.
Khalid NM, El Soda M, Marth EH (1990). Esterases of *Lactobacillus helveticus* and *Lactobacillus delbrueckii spp.bulgaricus*. Journal of Dairy Science 73:2711-2719.
Kilcawley KN, Wilkinson M, Fox PF (1998). Enzyme modified cheese. International Dairy Journal 8:1-10.
Kuhl GC, De J, Lindner D, Barron F (2016). Biohydrogenation of linoleic acid by lactic acid bacteria for the production of functional cultured dairy products: a review. Foods 5(1):13. doi: 10.3390/foods5010013.
Kumar D, Kumar L, Nagar S, Raina C, Parshad R, Gupta VK (2012). Screening, isolation and production of lipase/esterase producing *Bacillus sp*. strain DVL2 and its potential evaluation in esterification and resolution reactions. Archives of Applied Science Research 4:1763-1770.
Lee OW, Koh YS, Kim KJ, Kim BC, Choi HJ, Kim DS, Suhartono MT, Pyun YR (1999). Isolation and characterization of a thermophilic lipase from *Bacillus thermoleovorans* ID-1. FEMS Microbiology Letters 179:393-400.
Lesuisse E, Schanck K, Colson C (1993). Purification and preliminary characterization of the extracellular lipase of *Bacillus subtilis* 168, an extremely basic pH-tolerant enzyme. European Journal of Biochemistry 216:155-160.
Litthauer D, Ginster A, Skein E (2002). Pseudomonas luteola lipase: a new member of the 320-residue Pseudomonas lipase family. Enzyme and Microbial Technology 30:209-215
Liu SQ, Holland R, Crow VL (2001). Purification and properties of intracellular esterases from *Streptococcus thermophilus*. International Dairy Journal 11:27-35.
Lopes MF, Leitao AL, Regalla M, Figueiredo Marques JJ, Teixeira Carrondo MJ, Barreto Crespo MT (2002). Characterization of a highly thermostable extracellular lipase from *Lactobacillus plantarum*. International Journal of Food Microbiology 76:107-115
Makhzoum A, Knapp JS, Owusu RK (1995). Factor affecting growth and lipase production by *Pseudomonas fluorescens* 2D. Food Microbiology 12:277-290
Martinelle M, Holmquist M, Hult K (1995). On the interfacial activation of *Candida antarctica* lipase A and B as compared with *Humicola lanuginosa* lipase. Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism 1258:272-6.
Medina RB, Katz MB, González S, Oliver G (2004). Methods to Determination of Esterolytic and Lipolytic Activities of Lactic Acid Bacteria. Methods in molecular biology, public Health microbiology. Methods and protocols. John F. T. Spencer (Editor), Alicia L. Ragout de Spencer (Series Editor) pp:437-441.
Meyers SA, Cuppett SL, Hutkins RW (1996). Lipase Production by Lactic Acid Bacteria and Activity on Butter Oil. Food Microbiology 13(5):383-389.
Mobarak-Qamsari E, Kasra-Kermanshahi R, Moosavi-nejad Z (2011). Isolation and identification of a novel, lipase-producing bacterium, *Pseudomnas aeruginosa* KM110. Iranian Journal of Microbiology 3(2):92-98.
Nardi M, Fiez-Vandal C, Tailliez P, Monnet V (2002). The EstA esterase is responsible for the main capacity of *Lactococcus lactis* to synthesize short chain fatty acid esters in vitro. Journal of Applied

Microbiology 93:994-1002.
Nwachukwu E, Ejike EN, Ejike BU, Onyeanula EO, Chikezie-abba RO, Okorocha NA, Onukaogu UE (2017). Characterization and optimization of lipase production from soil microorganism (*Serratia marcescens*). International Journal of Current Microbiology and Applied Sciences 6(12):1215-1231.
Palanichamy E, Rajamony U, Arunachalam P, Grasian I (2012). Solid-state production of esterase using fish processing wastes by *Bacillus altitudinis* AP-MSU. Food and Bioproducts Processing 90:370-376.
Papon M, Talon R (1988). Factors affecting growth and lipase production by meat lactobacilli strains and Brochothrix thermosphacta. Journal of Applied Bacteriology 64:107-115
Ramakrishnan V, Goveas LC, Suralikerimath N, Jampani C, Halami PM, Narayan B (2016). Extraction and purification of lipase from *Enterococcus faecium* MTCC5695 by PEG/phosphate aqueous-two phase system (ATPS) and its biochemical characterization. Biocatalysis and Agricultural Biotechnology 6:19-27.
Salwoom L, Raja Abd Rahman RNZ, Salleh AB, Mohd Shariff F, Convey P, Pearce D, Mohamad Ali MS (2019). Isolation, Characterisation, and Lipase Production of a Cold-Adapted Bacterial Strain *Pseudomonas sp.* LSK25 Isolated from Signy Island, Antarctica. Molecules 24(4):715. doi: 10.3390/molecules24040715
Saxena P, Whang I, Lee J, Voziyanov Y, Mendoza V, Jayaram M (1994). Role of tyrosine phosphorylation-dephosphorylation in copy number control of the yeast plasmid 2 micron circle. Cellular and Molecular Biology Research 40(3):215-22.
Schmidt-Dannert C, Sztajer H, Stocklein W, Menge U, Schmid RD (1994). Screening purification and properties of a thermophiliclipase from *Bacillus thermocatenulatus*. Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism 1214:43-53
Serio A, Chaves-López C, Paparella A, Suzzi G (2010). Evaluation of metabolic activities of enterococci isolated from Pecorino Abruzzese cheese. International Dairy Journal 20:459-464.
Sharma S, Kanwar K, Kanwar SS (2016). Ascorbyl palmitate synthesis in an organic solvent system using a Celite-immobilized commercial lipase (Lipolase 100L). 3 Biotech 6:183.
Sidhu P, Sharma R, Soni SK, Gupta JK (1998a). Effect of cultural conditions on extracellular alkaline lipase production from *Bacillus sp*. RS-12 and its characterization. Indian Journal of Microbiology 38:9-14.
Sidhu P, Sharma R, Soni SK, Gupta JK (1998a). Production of extracellular alkaline lipase by a new thermophillic *Bacillus* sp. Folia Microbiologica 43:51-54.
Sokolovska I, Albasi C, Riba JP, Bales V (1998). Production of extracellular lipase by *Candida cylindracea* CBS 6330. Bioprocess Engineering 19:179-186.
Soleymani S, Alizadeh H, Mohammadian H, Rabbani E, Moazen F, Sadeghi HM, Shariat ZS, Etemadifar Z, Rabbani M (2017). Efficient Media for High Lipase Production: One Variable at a Time Approach. Avicenna Journal of Medical Biotechnology 9(2):82-86.
Sooch BS, Kauldhar BS (2013). Influence of multiple bioprocess parameters on production of lipase from *Pseudomonas sp*. BWS-5. Brazilian Archives of Biology and Technology 56(5):711-721.
Sunna A, Hunter L, Hutton CA, Bergquist PL (2002). Biochemical characterization of a recombinant thermoalkalophilic lipase and assessment of its substrate enantioselectivity. Enzyme and Microbial Technology 31:472-476.
Sztajer H, Lunsdorf H, Erdmann H, Menge U, Schmid R (1992). Purification and properties of lipase from *Penicillium simplicissimum*. Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism 1124(3):253- 261
Talon R, Montel MC (1994). Activités estérasiques et lipolytiques des bactéries lactiques. in: Luquet FM, De Roissart H, Bacteries lactiques, Vol. 1, editions Coquand, Grenoble, France, pp. 349-352.
Thirunavukarasu K, Edwinoliver NG, Anbarasan S, Gowthaman MK, Iefuji H, Kamini NR (2008). Removal of triglyceride soil from fabrics by a novel lipase from Cryptococcus sp.S-2. Process Biochemistry 43:701-6.
Tsakalidou E, Dalezios I, Kalantzopoulos G (1994). Isolation and partial characterization of an intracellular esterase from *Enterococcus faecium* ACA-DC 237. Journal of Biotechnology 37:201-208.
Vishnupriya B, Sundaramoorthi C, Kalaivani M, Selvam K (2010). Production of lipase from *Streptomyces griseus* and evaluation of bioparameters. International Journal of Chemtech Research 2(3):1380-1383.
Wang Y, Srivastava KC, Shen GJ, Wang HY (1995). Thermostable alkaline lipase from a newly isolated thermophilic Bacillus, strain A30-1 (ATCC 53841). Journal of Fermentation and Bioengineering 79:433-438.