JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

NEW LIPASE-PRODUCERS MICROORGANISMS FROM PERUVIAN AMAZONIA WHICH HYDROLYZE PALM OIL AND DERIVATIVES

Roxana Trujillo[1], *Pedro Peláez**[2], *Josep-Vicent Sinisterra*[3]

Address(es): Dr. Pedro Peláez,
[1] National University of San Martin, Agro-industrial Engineering Faculty, Tarapoto, Perú.
[2] National Agrarian University of the Jungle, Faculty of Food Engineering, Av. Universitaria Km. 1.5, Tingo María, Perú.
[3] Universidad Complutense de Madrid, Faculty of Pharmacy, Biotransformations Group, 28040 Madrid. Spain.

*Corresponding author: pedro.pelaez@unas.edu.pe

ARTICLE INFO

Received 4. 2. 2014
Revised 6. 3. 2014
Accepted 10. 3. 2014
Published 1. 4. 2014

Regular article

OPEN ACCESS

ABSTRACT

Two yeasts: *Cryptococcus uchicensis* TMY9 and *Pichia uchicensis* TMY10 and one fungus *Verticillium tingalensis* TMFMB are described for the first time as lipase producer microorganisms. The strains have been isolated after an ecological screening in a palm oil industry. The yeasts- *C. uchicensis* and *Pichia uchicensis* - mainly produce extracellular lipases as active as those produced by traditional lipase producing microorganisms. The extracellular lipases are active in the hydrolysis of crude palm oil and its industrial derivatives. Contrarily in the isolated fungus, the lipase mainly remains bonded to biomass. In all cases, greater hydrolytic activities are observed in the hydrolysis of palm olein and super-olein than with saturated substrates as stearine. *P. uchicensis* lipase shows moderated selectivity versus saturated acid triglycerides compared to substrates with high proportion of oleic acid (olein or superolein). The opposite behavior is observed with *C. uchicensis* and fungal lipases. *P. uchicensis* produces a more active crude lipase than *C. uchicensis* with lower biomass production. The kinetic runs performed with crude yeast lipases suggest a three steps mechanism where the high penetration of lipase in the fat gouts favors the hydrolysis.

Keywords: Lipases, palm oil hydrolysis, *Verticillium sp.*, *Cryptococcus sp.*, *Pichia sp.*

INTRODUCTION

Palm oil is intensively produced in the Peruvian Amazon. The palm tree (*Elaeis guineensis*) culture covers 57,752 Ha with a production of 595,944 Tm RFF/year. Unfortunately, this vegetal oil has a high percentage in saturated fatty acids triglycerides. Indeed, the standard fatty acid composition shows high percentages of saturated fatty acids: 44% palmitic (C 16:0), 1.1% lauric (C12:0), 1.1 % myristic (C14:0) and 4.5% stearic (C18:0) *versus* 39.2% oleic acid (C18:1) and 10.1% linoleic acid (C18:2) (**Noor *et al.*, 2003; Sellami *et al.*, 2011**). As consequence, the ingestion of palm oil by humans is not recommended due to the large quantities of cholesterol that could be synthesised from saturated fatty acids. In addition, to prepare the oil for a continue human consumption the red colour associated to the presence of trienols and polyols must be removed by absorption and then a distillation at vacuum is performed to obtain palm olein (PO) or super-olein (PSO) with higher concentration in oleic acid triglycerides than palm oil (CPO) and with a nice yellowish colour. After this manipulation, near 70 % of PO is obtained and stearine is the second product (≈ 30 %) with low price and a reduced market. So, the transformation of stearine to high price substrates is an industrial objective for the palm oil factories.

An environmental sustainable alternative to the conventional refining methodology would be –after decolouration- the selective lipase-catalyzed hydrolysis of saturated fatty acid triglycerides. The lipase-catalyzed hydrolysis of palm oil triglycerides has been described using commercial lipases (**Sellami *et al.*, 2011; Kaewthong *et al.*, 2005; Ribas *et al.*, 2013; Gonçalves *et al.*, 2012; Voll *et al.*, 2012**). With the aim to develop more selective and cleaner processes avoiding the bacterial contamination, several lipase producer thermophile strains have been tested. *Bacillus thermoleovorans* (**Lee *et al.*, 2001**), thermophilic *Rhizopus oryzae* isolated from Cameroonian palm oil fruit (**Hiol *et al.*, 2000**) and *Acinetobacter baylyi* isolated from marine sludge in Angsila (**Uttatree *et al.*, 2010**) are three examples of this topic**.** Another interesting sustainable process is the concentration and isolation of tocopherols and tocotrienols (1 % until 15 % depending of sources) from palm oil distillate by means of selective lipase-catalyzed hydrolysis of their esters (**Chu *et al.*, 2002**).

Taking into account the great Biodiversity in the Peruvian Amazon, we have carried out an ecological screening of lipase-producing microorganisms with the aim to obtain interesting lipase producer strains for these processes, especially for the hydrolysis of saturated fatty acid triglycerides as palm stearine.

MATERIAL AND METHODS

Microorganisms and chemicals

The microorganisms were obtained from different zones of one Peruvian palm oil factory (*Industrias del Espino S.A.* (Santa Lucia, San Martín, Perú)). The reagents were from Merck. Crude palm oil (CPO), palm olein (PO), palm super-olein (PSO), palm stearine (PST), palm refined deodorized stearine (RDST) and crude palmist oil (PMO) were kindly supplied by *Industrias del Espino S.A.* (Perú). RDST is a decolorized stearine with similar qualitative composition than PST. In Table 1, the relative proportions of the major fatty acids of the substrate is shown. Palmitic acid (C16:0) is the main fatty acid in the palm oil derivatives. The refining process carrying to PO and PSO has the objective to increase the proportion of unsaturated fatty acids as oleic (C18:1) and linoleic (C18:2) acids.

Table 1 Relative fatty acid composition of the substrates

Fatty acid	Structure	Crude Palm oil (CPO)*	Palm Stearine (PST) **	Palmiste oil (PMO) **	Palm Olein (PO)*	Palm Superolein (PSO)*
Myristic	C14:0	0.961	1.1-1.7	14.3-16.8	0.849	0.5-1.5
Palmitic	C16:0	42.465	49.8-68.1	6.5-8.9	36.768	30-39
Stearic	C18:0	0.395	3.9-5.6	1.6-2.6	-	2.8-4.5
Oleic	C18:1	44.616	20.4-34.4	13.2-16.4	49.482	43-50
Linoleic	C18:2	10.372	5.0-8.9	2.2-3.4	11.745	10.5-15.0
I_2 index		50.4-53.7**	27.8-45.1	-	56.0-59.1 **	>60
Saponification Index		194-205 **	193-205	243-249	194.0-202.0**	180-205

* Eqbal *et al*. (2011), ** MPOB (2013).

The higher the I_2-index value is, the higher the percentage of unsaturated fatty acid is. The high I_2-index of crude palm oil is related to the presence of trienols and polyols (≈1-5 % w) that give the red colour to CPO. These polyols are not present in the palm oil derivatives (PST, PMO, PO and PSO). The higher insaturation degree in PO and PSO compared to PST and PMO should be related to greater proportion of unsaturated fatty acid (C=C bonds). Finally, the similar saponification index in all tested substrates indicates to us that similar free fatty acid amounts are present in all cases.

Culture media

The microorganisms were isolated from the solid or liquid samples using two culture media: i) yeasts, YED (**Cárdenas *et al.*, 2001**): glucose=20g/L; yeast extract = 10g/L; agar (15 g/L) and ii) fungi: Bengal pink agar (30 g/L) *(10)*, T= 28ºC.

Then the isolated microorganisms were cultured in the presence of crude palm oil to discriminate the best lipase-producer microorganisms. BYPO medium (**Cárdenas *et al.*, 2001**): peptone 10 g/L; yeast extract=3 g/L; meet extract=5 g/L; NaCl=5 g/L; KH_2PO_4 =7g/L; Agar=15 g/L (adjusted to pH=7 before sterilizing) supplied with crude palm oil (25mL/L) was used.

The selection of microorganisms with high production of lipase was performed using a minimal medium previously described *(11, 12)*: NH_4Cl = 1 g/L; KH_2PO_4 =1g/L; K_2HPO_4=2g/L; KCl=0.1 g/L; $MgSO_4.7H_2O$=0.5g/L; $CaCl_2$=0.01 g/L; $FeSO_4.7H_2O$= 0.012 g/L supplied with crude palm oil (20mL/L) as the carbon source. The fermenter conditions were: T=30 ºC, 150 r.p.m. We used 50 mL of culture medium in 250 mL Erlenmeyer.

The concentration of yeast cells (10^6cells/mL) was measured by turbidimetry at 660 nm using as reference equation [1]. *S. cerevisiae* culture was used as the reference and the cell concentration was determined using a Neubauer chamber (0.1mm deep and 0.022 mm^2 surface).

$$Abs = 0.011\,[cell] - 0.0023 \qquad [1]$$
$$R2 = 0.9829, n = 9$$

In the case of fungi, biomass production was measured as d.c.w. after calcinations of rinsed biomass at 120ºC till constant weight.

Hydrolysis of palm oil - Screening conditions

The hydrolysis of crude palm oil was performed using the whole cells cultured in 50 mL of minimal medium (pH=7) supplied with crude palm oil (2%). The culture flasks (250 mL) were stirred at 150 r.p.m., T=30 ºC. When the cells arrive to stationary phase (48 hrs. yeasts or 72 hrs. fungi). The hydrolysis reactions were followed by titration of generated free acids in 1mL of the reaction medium. The samples were picked-up every 2 hours.

Determination of extra-cellular and cell bond lipase activities

Different substrates were used: i) solid substrates such as PST, PMO, CPO and RDST were emulsified with *i*-rOH (1:3 (v/v)). The liquid substrates such as PSO and PO were emulsified with gum arabic (10%) solution (1:1(v/v)).

After the centrifugation of fermentation broth, the supernatant and the biomass were separated to explore the extracellular (**Molnarova *et al.*, 2013; Stathopoulou *et al.*, 2013**) and biomass-bonded activities (30), respectively**.** The total activity of the culture broth was measured with the liquid plus the biomass produced (**Cárdenas *et al.*, 2001**) in 50mL of culture broth using minimal medium with 2% palm oil. The cell concentration was measured using the absorption at 660 nm as described above. The enzymatic activity was measured as µmol acid/(mL x h). The total volume was 50 mL in all cases.

The supernatant lipase-activity was measured with 1mL of supernatant. One gout of EtOH was added to kill the picked-up cells in the supernatant sample. Then, 0.1mL of gum arabic (10%), 1.2 mL of water and 1.0 mL of the substrate and the pH was fitted to 7.0 with phosphate buffer. The solution was stirred for 1hr and the acid produced titrated with 0.05N NaOH in the presence of phenolphthalein. The enzymatic activity was measured as µmol of acid /(mL x h) (**Godia, 1998; Hlavsova *et al.*, 2009; Darvishi *et al.*, 2011**).

The lipase activity associated to the biomass was measured after centrifugation of biomass produced – 48hrs yeasts or 72 hrs. fungi. The solid biomass was suspended in 5 mL of water and the palm oil was hydrolyzed as above.

Kinetic runs

The kinetic runs performed in the same experimental conditions as above. The substrate concentrations were lower than 0.3 mmol/mL. The lipase activity was defined as the µmol of acid liberated per hour and the productivity as the µmol of acid liberated per hour and millions of cells (yeasts) or gram (d.c.w.) (fungi).

RESULTS AND DISCUSSION

Screening

An ecological screening was performed with samples obtained from different zones of one palm oil industry (*Industrias del Espino S.A.* (Santa Lucia, San Martin, Perú)). Different samples were taken from palm fruit, palm fruit waste, culture earths, solid and liquid residues etc. In addition, some samples were obtained from liquid water wastes from the plant (40ºC < T < 50ºC), looking for thermotolerant microorganisms. From these samples, 28 microorganisms were isolated using YED medium for the isolation of yeasts and pink Bengal agar for the isolation of fungi. Then, the strains were cultured in BYPO medium supplied with a 2% of crude palm oil. Only 15 strains grew in this medium with moderated or high lipase-activities. These microorganisms were mainly isolated from palm fruit residues, hot water wastes and of solid wastes, probably because in these wastes exist residual triglycerides from palm oil processing.

These 15 microorganisms were cultured in a minimal medium where the only carbon source was crude palm oil (2%). After this step, only 10 yeasts grew and 2 filamentous fungi were selected as lipase producers according to two criteria: i) easy growing conditions and ii) high lipolytic activity (Tab 2). The yeasts were identified in Colección Española de Cultivos Tipo (Universidad de Valencia, Spain). The fungi were identified in Glaxo-SmithKline laboratories (Tres Cantos, Madrid, Spain). The lipolytic activities were evaluated using cells in stationary growing conditions.

Table 2 Selected microorganism lipase producers using palm oil as the substrate. Microorganisms cultured in BYPO with crude palm oil (20mL/l).T=30ºC, 150 r.p.m

Microroganism	Colony colour	Origin of the microorganism	Reaction agaisnt scualene	Time (hr) [a]	Biomass (10^6 cells/mL)[a]	Lipolytic activity µmol/(mL x h)
Yeast						
Candida sp. TMY4a	Milky whitish	Palm fruit residues	Bluish	32	1,187	149
Candida albicans TMY4b	Whitish	Palm fruit residues	Green-bluish	34	996	127
Candida tropicalis TMY4b	Whitish	Palm fruit residues	Blue-greenish	21	647	55
Candida ciferrii TMY4b	Milky yellowish	Palm fruit residues	Black	13	426	57
Candida boidinii TMY7	Yellowish	Palm fruit residues	Blue	25	2,609	145
Candida albicans TMY7	Yellowish	Palm fruit residues	Blue	15	1,300	142
Crytococcus uchicensis TMY9	Strong yellow	Culture earth	Black	20	1,179	153
Candida lypolítica TMY10	Yellowish	Solid waste	Yellow-greenish	23	1,433	152
Pichia uchicensis. TMY10	Yellowish	Solid waste	Black	12	786	69
Candida albicans TMY11	Milky yellowish	Liquid waste (40ºC)	Black	15	1,384	174
Fungi						
Verticillium tingalensis TMFMB	White fungus	Solid waste		58	-	152
Aspergillus sp. TMFMV	Green fungus	Solid waste		58	-	148

[a] Stationary growing conditions.

Three new lipase-producer microorganisms have been identified, two yeasts *Cryptococcus uchicensis* TMY9, *Pichia uchicensis* TMY10 and one fungus *Verticillium tingalensis* TMFMB. *C. uchicensis* lipase (CUL) shows higher activity (µmol/(mL x h)), than that displayed by *P. uchicensis* lipase (PUL) and similar to that displayed by other traditional lipase producers such as *Candida* genus (Tab 2). The low lipase activity of *P. uchicensis* must be carefully considered due to low biomass production. This datum could indicate a high specific lipase activity. It should be noted that *V. tingalensis* shows a similar lipolytic activity than traditional lipase-fungus producers such as *Aspergillus sp* (Tab 2). The enzymatic activity values achieved with these new strains are similar to that described in the literature for lipase SP398 (Novo Nordisk A/S Denmark) (**Sellami *et al.*, 2011**) or for extra-cellular lipase from *Bacillus thermoleovorans* IDI (**Lee, *et al.*, 2001**) using palm oil.

Extra-cellular and membrane bonded lipase activities

To explore the future applications in palm oil industry, the lipases obtained from the new microorganisms were tested with different substrates (Tab 3). The lipase activities were compared in the same experimental conditions with three *Candida* lipases – the yeasts - and with *Aspergillus sp* as well-known lipase producer microorganisms.

The maximum lipolytic activity values were statistically evaluated by a factorial 6 x 6 complete hazardous analysis using *Estat Program* ®. The results are mean of three runs. This analysis indicated to us that the yeasts and fungi show significant different activities versus each substrate.

In the yeasts, the extra-cellular lipase activity is greater than that which remains bounded to the cell wall. Other workers with bacteria, actinomycetes or yeasts (**Cárdenas *et al*., 2001a; Dominguez de Maria *et al*.; Cárdenas *et al*., 2001b; Dominguez de María, 1999; Hlavsova *et al*., 2009; Darvishi *et al*., 2011**) described the same behaviour. Therefore, the study focused on yeast extra-cellular lipases by technical reasons and with the aim to purify the enzymes.

The observed extracellular lipase activity values of *C. uchicensis* with PO (figure 1) is similar to that described with crude lipase extract of *C. rugosa* in the hydrolysis of olive oil (**Dominguez de Maria *et al.*, 2006**). Therefore, the specific lipase activity could be improved by semipurification (**Moreno *et al.*, 1997**) or by the control in the fermenter conditions (**De la Casa *et al.*, 2006**; **Dominguez de Maria *et al.*, 2005**).

Both isolated fungi showed lower extracellular lipase activity than that bonded to that biomass (Tab 3). This finding could be a problem to scale up the process unless cloning and expression in another host should be done. The extra-cellular lipase activity of the fungi was greater than those of the lipases from *Ovadendrum sulphureo-ochraceum* or of *Fusarium oxysporum* (**Hiol *et al.*, 2000; Cárdenas *et al.*, 2001a**).

CUL shows a clear selectivity *versus* substrates with high percentage of unsaturated acids (PO and PSO) (figure 1 and Tab 3). Contrarily, *PUL* shows a moderated selectivity *versus* RDST, PMO and PST (substrates with high palmitic acid percentage) and a low biomass production. Otherwise, extracellular crude lipases from the other yeasts showed a moderated selectivity *versus* substrates with high percentage in oleic acid (PO and PSO) as expected in *Candida genus*.

As conclusion, we can say that yeasts are more interesting than fungi to scale up the production of lipases because they show opposite selectivity one to another and produce a high percentage of extracellular lipase.

Hydrolysis kinetic runs of extracellular lipases

The hydrolysis of the substrates was performed with different amounts of emulsified substrates (1.0, 2.0, 3.0, 4.0 y 5.0 mL). In these experiments, the percentage of hydrolysis was <20% to avoid secondary or reverse reactions The lipase-activity was measured as µmol acid liberated / (mL x h)) (**Godia, 1998**). The obtained results for *Cryptococcus uchicensis* TMY9 and *Verticillium tingalensis* TMF1, as examples, are shown in Figures 1 and 2, respectively. In some cases, the kinetic data could be fitted to a formal "*Michaelis Menten*" mechanism but in other case a *pseudo*-first order kinetic model was found. Therefore, we could think that a "*real no-michaelian"* mechanism is present in all cases, in accordance with the literature where a *pin-pog bi-bi* or other complexes mechanisms are proposed (**Dominguez de María *et al.*, 2006; Marwan *et al.*, 2007; Kulschewski *et al.*, 2013**). In our conditions, we could say that the extra-cellular lipase concentration should be low because we are working with *crude lipases*.

In accordance to the literature (**Ribas *et al.*, 2013; Tsai, 1993; Knezevic *et al.*, 1998; Mukataka *et al.*, 1986**) a conventional interfacial three steps mechanism was proposed. According to this mechanistic model, the complex equation could be fitted to a formal Michaelis-Menten equation [2] that is useful to obtain Vmax and an apparent Km (Tab 4).

$$r = \frac{K_{cat}\, E\, [S]}{K_e\left[\frac{K_d}{(K_p\, a^2)+1}\right] + [S]} = \frac{V_{max}\, [S]}{K_m + [S]} \quad [2]$$

Where

$$K_e = (K_{cat} + K_1)/K_1$$

$$K_m = K_e\left[\frac{K_d}{(K_p\, a^2)+1}\right] \quad [3]$$

If we assume that the affinity for the substrates is low, Km >>> [S] and the equation [2] is transformed in a *pseudo*-first order kinetic equation [4].

$$r = K_{ap}[S] = \frac{K_{cat}\left[\left(K_p a^2\right)+1\right]}{K_e K_d [S]}\ [S] \quad [4]$$

Table 3 Comparative lipase activities observed in the extra- cellular broth and as wall cell bounded. Substrates

Strain	Substrates	Activity	
		Extracellular lipase (μmol/mL x h)	Cell wall bonded lipase (μmol/mL x h)
Candida sp. TMY41	Palm stearine (PST)	153	55
	Crude palmist oil (PMO)	163	70
	Palm refined deodorized stearine (RDST)	152	52
	Crude palm oil (CPO)	149	59
	Palm super-olein (PSO)	167	58
	Palm olein (PO)	168	61
Candida albicans TMY72	Palm stearine (PST)	137	46
	Crude palmist oil (PMO)	144	61
	Palm refined deodorized stearine (RDST)	147.5	48
	Crude palm oil (CPO)	142.5	51
	Palm super-olein (PSO)	144	60
	Palm olein (PO)	147.5	61
Cryptococcus uchicensis TMY9	Palm stearine (PST)	158	60
	Crude palmist oil (PMO)	188	88
	Palm refined deodorized stearine (RDST)	158	64
	Crude palm oil (CPO)	153	66
	Palm super-olein (PSO)	167	66
	Palm olein (PO)	143	75
Pichia uchicensis TMY10	Palm stearine (PST)	158	47
	Crude palmist oil (PMO)	162	61
	Palm refined deodorized stearine (RDST)	158	51
	Crude palm oil (CPO)	152.5	54
	Palm super-olein (PSO)	149	61
	Palm olein (PO)	153	68
Candida albicans TMY11	Palm stearine (PST)	176	47
	Crude palmist oil (PMO)	192.5	66
	Palm refined deodorized stearine (RDST)	178	45
	Crude palm oil (CPO)	174	51
	Palm super-olein (PSO)	179	55
	Palm olein (PO)	177.5	57.5
Verticillum tingalensis TMFMB	Palm stearine (PST)	65.0	80.0
	Crude palmist oil (PMO)	90.8	121.7
	Palm refined deodorized stearine (RDST)	66.7	73.3
	Crude palm oil (CPO)	101.7	131.7
	Palm super-olein (PSO)	121.7	145.0
	Palm olein (PO)	123.3	148.3
Aspergillus sp TMFMV	Palm stearine (PST)	65.0	108.3
	Crude palmist oil (PMO)	88.3	176.7
	Palm refined deodorized stearine (RDST)	63.3	113.3
	Crude palm oil (CPO)	71.7	138.3
	Palm super-olein (PSO)	76.7	191.7
	Palm olein (PO)	76.7	176.7

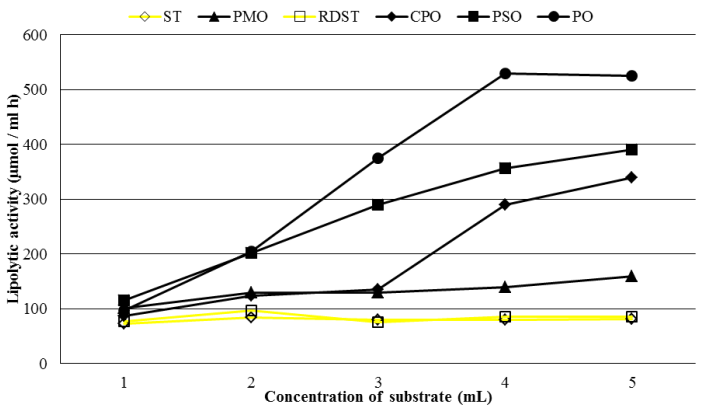

Figure 1 Lipolytic activity of the lipase from *Cryptococcus uchicensis* TMY9 with different substrates

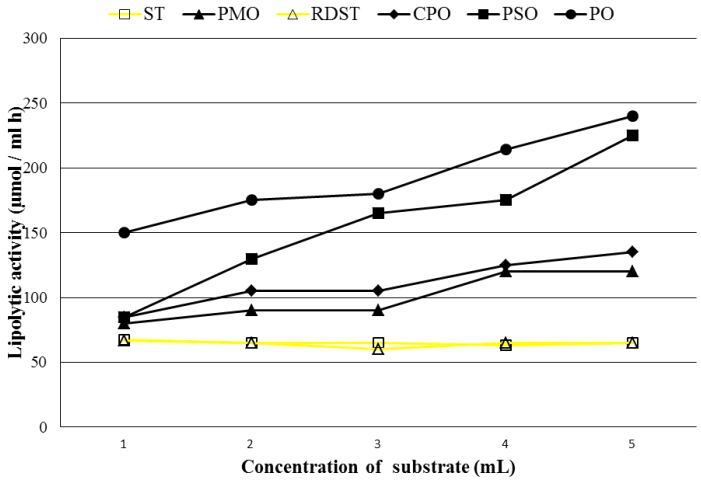

Figure 2 Lipase activity of the lipase from *Verticillium tingalensis* TMF1 at different substrate concentrations

The kinetic data fitted to the best model, are present in Tables 4, 5 and 6.

CUL kinetic data could be fitted to a *pseudo- michaelian* model. From Table 4 we can deduce that the greatest Vmax is observed with unsaturated substrates, PO and PSO, but the apparent Km is the largest, too. In the other substrates, with large proportion of saturated acids (CPO, PST, and PMO (Tab 1)), Vmax and Km are low. Probably these results are related to the better penetration of lipase trough the liquid substrate gouts (PO and PSO) compared to the solid fats (PST, PMO, etc.). In this case, the penetration constant, k_p, and k_{cat} would be very large and so, the apparent Km and Vmax would be high.

Contrarily, *PUL* (Tab 5) gave sigmoid curves indicating a complex mechanism (Figure not shown). Only the fitting to a *pseudo*-first order reaction was possible. The k_{ap} was greater with PO and PSO (Tab 5). As in the case of the lipase of *C. uchicensis* the greatest activities are obtained with the unsaturated substrates PO and PSO. According to [4], these results could be associated to high values of k_{cat} or/and of k_p. Taking into account the Km expression [3], the different kinetic equations observed in the case of both selected yeasts may be explained by a higher specific activity (k_{cat}) in the case of *PUL* compared to *CUL*.

Finally the kinetic behaviour of the lipase from the fungus *V. tingalensis*, is difficult to explain (Tab 6) because michaelian equations or *pseudo*-first order kinetics were obtained as the best models depending on the substrate.

Table 4 Kinetic runs in the hydrolysis of different substrates using lipase from *C. uchicensis* TMY9. [Substrate] < 0.3 mmol/mL

Substrate	Equation	R^2	Vmax x 10^3 (mmol/L min)	Km x 10^3 (mmol/mL)
Palm olein (PO)	1/V = 0.434 (1/[S]) + 32.4	0.974	309	13.40
Palm super-olein (PSO)	1/V = 2.9 (1/[S]) + 67.7	0.983	148	43.00
Crude palm oil (CPO)	1/V = 0.696 (1/[S]) + 465	0.943	22	1.50
Palm refined deodorized stearine (RDST)	1/V = 0.576 (1/[S]) + 616	0.912	16	0.90
Crude palmist oil (PMO)	1/V = 0.638 (1/[S]) + 433	0.971	23	1.50
Palm stearine (PST)	1/V = 0.4101 (1/[S]) + 945	0.940	11	0.40

Table 5 Kinetic runs in the hydrolysis of different substrates using lipase from *Pichia uchicensis* TMY10. [Substrate]< 0.3 mmol/mL

Substrate	Equation	R^2	k_{ap} ((mmol /(l x min))/ (mL/g subst.))
Palm olein (PO)	$V = 0.354 [S] + 3\ 10^{-6}$	0.840	0.3540
Palm super-olein (PSO)	$V = 0.276 [S] - 9\ 10^{-4}$	0.999	0.2760
Crude palm oil (CPO)	$V = 0.0883 [S] + 9\ 10^{-4}$	0.996	0.0833
Palm refined deodorized stearine (RDST)	$V = 0.0147 [S] + 8\ 10^{-4}$	0.900	0.143
Crude palmist oil (PMO)	$V = 0.0001 [S] + 7\ 10^{-4}$	0.928	0.0001
Palm stearine (PST)	$V = 0.0093 [S] + 8\ 10^{-4}$	0.942	0.0093

Table 6 Kinetic runs in the hydrolysis of different substrates using lipase from *Verticillium tingalensis* TMFMB

Substrate	Kinetic model	Equation	R^2	Vmax .10^3 (mmol/(L.min)	Km. 10^3 (g/mL)	k_{ap} ((mmol(L.min))/ (mL/g subst.))
Palm olein (PO)	Michaelian	1/V = 11,6 (1/[S]) + 447	0.999	2.2	25.20	
Palm super-olein (PSO)	Michaelian	1/V = 9.64 (1/[S]) +703	0.998	1.4	13.70	
Crude palm oil (CPO)	Difusión	$V= 10.9\ 10^{-3}[S] +3\ 10^{-4}$	0.965			0.011
Palm refined deodorized stearine (RDST)	Michaelian	1/V =0.810 (1/[S]) + 3495	0.999	0.3	0.24	
Crude palmist oil (PMO)	Difusión	$V=9\ 10^{-3} [S] + 3\ 10^{-4}$	0.88			0.009
Palm stearine (PST)	Michaelian	1/V = 0.781 (1/[S]) + 3474	0.98	0.3	0.23	

CONCLUSION

Two yeasts *C. uchicensis* and *P. uchicensis* and one fungus *V. tingalensis* have been described by the first time as lipase producers. They have been isolated after an ecological screening of one palm oil industry of Peruvian Amazonian. The yeasts show higher extracellular lipase activity than biomass associated lipase activity. The fungus shows the opposite behaviour. The enzymatic activities of crude lipases are similar to others described in the literature. As conclusion, we can say that yeasts are more interesting than fungi to scale up the production of lipases because they show opposite selectivity one to another and produce a high percentage of extracellular lipase. Nowadays the purification and the induction of the lipases from yeasts are in progress.

Acknowledgments: The authors would like to thank AECI the financial support for the sojourns of Dr. Sinisterra in Universidad Nacional Agraria de la Selva (Tingo Maria, Huánuco, Perú) for the supervision of the work.

REFERENCES

ALCÁNTARA, A. R. 2002. Heptyl oleate syntheses as useful tool to discriminate between lipases, proteases and other hydrolases in crude preparation. *Enzyme Microb.Technol.*, 31(3), 283-288.

CÁRDENAS, F., ALVAREZ, E., ELSON, S., SÁNCHEZ-MONTERO, J.M., SINISTERRA, J.V. 2000. Influence of the chemical structure of the inducer in the production of extracellular lipase by a *Pseudomonas cepacia* strain in *Lipase & Lipids: Structure, function and Biotechnological applications.* Kokotos G & Kokotos C. Crete University Press Heraklio, Greece. 153-168. ISBN-13: 978-960-524-111-7.

CÁRDENAS, F., ÁLVAREZ, E., DE CASTRO, M. S., SÁNCHEZ-MONTERO, J.M., VALMASEDA, M., ELSON, S., SINISTERRA, J.V. 2001a. Screening and catalytic activity in organic synthesis of novel fungal and yeasts crude lipases. *J. Mol. Catal. B: Enzymatic*, 14(4–6), 111–123.

CARDENAS, F., ALVAREZ, E., DE CASTRO-ALVAREZ, M.S., SÁNCHEZ-MONTERO, J.M., VALMASEDA, M., ELSON, S., SINISTERRA, J.V. 2001b. Three new lipases from actinomycetes and its use in organic reactions. *Biocatalysis & Biotransformations*, 19(4), 315-329.

CHU, B. S., BAHARIN, B. S., QUEK, S. Y. 2002. Factors affecting pre-concentration of tococpherols and tocotrieneol from palm fatty acid distillate by lipase-catalyzed hydrolysis. *Food Chemistry*, 79(1), 55-59.

DARVISHI, F., DESTAIN, J., NAHVI, I., THONART, P., ZARKESH-ESFAHANI, H. 2011. High-level production of extracellular lipase by Yarrowia lipolytica mutants from methyl oleate. *New Biotechnology*, 28(6), 755-760.

DE LA CASA, R.M., SINISTERRA, J.V., SANCHEZ-MONTERO, J.M. 2006. Characterization and catalytic properties of a new crude lipase from Candida rugosa. *Enzyme Microb. Technol.*, 38(5), 599-609.

DOMINGUEZ DE MARÍA, P., SINISTERRA, J. V. 1999. Causes of unreproducibility of Candida rugosa lipase-catalysed reactions in slightly hydrated organic media. *Tetrahedron.*, 55(28), 8555-8566.

DOMÍNGUEZ DE MARIA, P., MARTÍNEZ-ALZAMORA, F., RUA, M., SÁNCHEZ-MONTERO, J. M., SINISTERRA, J. V.,

DOMÍNGUEZ DE MARIA, P., ALCÁNTARA, VALERO, F., SINISTERRA, J.V. 2005. Rational strategy for the production of new crude lipase from C. rugosa. *Biotechnol. Lett.*, 27(7), 499-503.

DOMÍNGUEZ DE MARÍA, P., ALCÁNTARA, A., CARBALLEIRA, J.D., DE LA CASA, R.M., GARCÍA-BURGOS, C.A., SANCHEZ-MONTERO, J.M., SINISTERRA, J.V. 2006. Candida rugosa lipase: A traditional and complex biocatalyst. *Current Org.Chem.*, 10(10), 10053-1066.

EQBAL M. A. DAUQAN, HALIMAH ABDULLAH SANI, AMINAH ABDULLAH, ZALIFAH MOHD KASIM. 2011. 2011 2nd International Conference on Chemistry and Chemical Engineering. IPCBEE vol.14 (2011) © (2011) IACSIT Press, Singapore.

GODIA CASABLANCAS, F., LOPEZ-SANTIN, J.L. 1998. Ingeniería Bioquímica. Madrid – España, Editorial Síntesis, S.A. pp 127-160. ISBN: 978-84-7738-611-7.

GONÇALVES KM, SUTILI FK, LEITE SG, DE SOUZA RO, LEAL IC. 2012. Palm oil hydrolysis catalyzed by lipases under ultrasound irradiation--the use of experimental design as a tool for variables evaluation. *Ultrason Sonochem. Mar*., 19(2), 232-6.

HIOL, A., JONZO, M. D., RUGANI, N., DRUET, D., SARDA, L., COMEAU, L. C. 2000. Purification and characterization of an extracelllular lipase from a thermophilic *Rhizopus oryzae* strain isolated from palm fruit. *Enzyme Microb. Technol*., 26(5-6), 421-430.

HLAVSOVA, K., ZAREVUCK, M., WIMMER, Z., MACKOV, M., SOVOVA, H. 2009. *Geotrichum candidum* 4013: Extracellular lipase *versus* cell-bound lipase from the single strain. *Journal of Molecular Catalysis B: Enzymatic*, 61(3-4), 188–193.

KAEWTHONG, W., SIRISANSANEEYAKUL, S., PRASERTAN, P., H-KITTIKUN, A. 2005. Continuous production of monoacylglycerols by glycerolysis of palm olein with immobilized lipase. *Proc. Biochem.*, 40, 1525 - 1530.

KNEZEVIC, Z. D., SILVER-MARINKOVIC, S. S., MOJOVIC, L. V. 1998. Kinetic of lipase-catalysed hydrolysis of palm oil in lecithin/izooctane reverse micelles. *Appl. Microbial. Botechnol*., 49(3), 267-271.

KULSCHEWSKI, T., SASSO, F., SECUNDO, F., LOTTI, M., PLEISS, J. 2013. Molecular mechanism of deactivation of *C. antarctica* lipase B by methanol. *J Biotechnol.*, 168(4), 462-9.

LEE, D.W., KIM, H.W., LEE, K.W., KIM, B-CH., CHOE, E.A., LEE, H.S., KIM, D.S., PYUN, Y.R. 2001. Purification and characterization of two distinct thermostable lipases fromm the gram-positive thermophilic bacterium *Bacillus thermoleovorans* ID-1. *Enzyme. Microb. Technol.*, 29(6-7), 363-371.

MARWAN M., SHAMEL, K.B., RAMACHANDRAN, MASITAH HASAN, SULAIMAN AL-ZUHAIR. 2007. Hydrolysis of palm and olive oils by immobilised lipase using hollow fibre reactor. *Biochemical Engineering Journal*, 34(3), 228–235.

MOLNAROVA, J., VADKERTIOVA, R., STRATILOVA, E. 2013. Extracellular enzymatic activities and physiological profiles of yeasts colonizing fruit trees. *J. Basic Microbiol*., Jun 7. doi: 10.1002/jobm.201300072.

MORENO, J. M., HERNAÍZ M. J., SÁNCHEZ-MONTERO, J. M., SINISTERRA, J. V., BUSTOS, M. T., SÁNCHEZ, M. E., BELLO, J. F. 1997. Covalent immobilization of pure lipases A and B from Candida rugosa. *J. Mol. Catal. B Enzymatic.*, 2(4), 177-183.

MPOB. 2013. Official palm oil information source. [En línea]: http://www.palmoilworld.org/about_malaysian-industry.html.

MUKATAKA, S., TETSUO, K., JOJI, T. 1986. Kinetics of enzymatic hydrolysis of lipids in biphasic organic-aqueous systems. *J. Ferment.Technol*., 63(5), 461-466.

NOOR, I.M., HASAN, M., RAMACHANDRAN, K.B. 2003. Effect of operating variables on the hydrolysis rate of palm oil by lipase. *Proc. Biochem*., 39(1), 13-20.

RIBAS, M. C., DA SILVA, C., FILHO, L. C., DA SILVA, E. A. 2013. Enzymatic catalyzed palm oil hydrolysis under ultrasound irradiation: diacylglycerol synthesis. *Ultrason Sonochem. Jul*., 20(4), 1002-1007.

SÁNCHEZ, A., DE LA CASA, R., SINISTERRA, J. V., VALERO, F., SÁNCHEZ-MONTERO, J. M. 1999. Effect of fermentation conditions in the enzymatic activity and stereoselectivity of crude lipase from C. rugosa. *Appl. Biochem. Biotechnol.*, 80(1), 65-75.

SKIPPER, H.D., MUELLER, J.G., WARD, V.L., WAGNER, S.C. 1986. Microbial degradation of herbicides. In Research Methods in Weeds Science. N.D. Camper (Ed.). Southern Weed Science Society, Campaign, Il, USA. 457-475.

STATHOPOULOU, P. M., SAVVIDES, A. L., KARAGOUNI, A. D., HATZINIKOLAOU, D. G. 2013. Unraveling the lipolytic activity of thermophilic bacteria isolated from a volcanic environment. *Biomed Res Int*., 2013:703130. doi: 10.1155/2013/703130. Epub 2013 May 8.

TSAI, S. W., CHANG, C. S. 1993. Kinetics of lipase-catalysed hydrolysis of lipids in biphasic organic aqueous systems. *J.Chem. Tech. Biotech.*, 57(2), 147-154.

UTTATREE, S., WINAYANUWATTIKUN, P., CHAROENPANICH, JITTIMA. 2010. Isolation and characterization of a novel thermophilic-organic solvent stable lipase from *Acinetobacter baylyi*. Applied biochemistry and biotechnology. *Appl Biochem Biotechnol. Nov*., 162(5), 1362-76.

VOLL, F. A., ZANETTE, A. F., CABRAL, V. F., DARIVA, C., SOUZA, R. O., CARDOZO FILHO, L., CORAZZA, M. L. 2012. Kinetic modeling of solvent-free lipase-catalyzed partial hydrolysis of palm oil. *Appl Biochem Biotechnol. Nov*., 168(5), 1121-42.