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Chicken fat and inorganic nitrogen source for lipase production by *Fusarium* sp. (*Gibberella fujikuroi* complex)

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In this work, we report the production of lipases by a *Fusarium* sp. isolate (FCLA-MA41) from Atlantic Forest, using chicken fat and association of organic and inorganic nitrogen sources in submerged fermentation to seek economically attractive bioprocess. A 2-level, 4-factor Central Composite Design (CCD) and response surface methodology (RSM) were used to study the influence of the concentrations of chicken fat, $(NH_4)_2SO_4$, Triton X-100 and yeast extract as a nutrient supplement in Vogel minimum salt medium. RSM defined the region with the best response as consisting of the following combination of variables: chicken fat 15.0 mL/L, Triton X-100 15.0 g/L, ammonium sulfate 4.5 g/L and yeast extract 1.0 g/L. A validation study was performed according to the described concentrations and produced an enzymatic activity of 4.22 ± 0.35 U/mL. Considering the cost estimates of the nutrient medium optimized for lipase production, the production cost was $US 518.00/million Units of lipase.

Key words: Fungal enzyme, central composite design, chicken fat, ammonium sulfate, Triton X-100.

INTRODUCTION

The interest of this work is the triacylglycerol lipases (E.C.3.1.1.3), which are enzymes, described as glycerol ester hydrolases acting on ester bonds present in acylglycerols, releasing fatty acids and glycerol (Jaeger et al., 1994). They constitute a special class among the carboxylic ester hydrolases (Egloff et al., 1995). The use of lipases has increased considerably, especially in the food, beverage, textile, pharmaceutical, cosmetic, bioenergetics, fine chemicals and pulp and paper industries. Currently, enzymes are produced naturally from plants, animals, fungi, yeasts and bacteria. When microorganisms are used, they can be inoculated in residues resulting from food processing, thereby reducing the production cost.

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Fungi of different genera proven to be good producers of lipases and enzymes have been previously studied (Singh and Mukhopadhyay, 2012). However, the costs of production of microbial lipases have limited the use of these enzymes. The potential of using low-cost nutrients such as agro-industrial residues in microbial fermentation for enzyme production is extremely important in dictating future uses of lipases. One critical factor in producing microbial lipases is the choice of carbon sources as enzyme inducers. Compounds such as plant seed-oils (triacylglycerols), free fatty acids, surfactants, bile salts and glycerol have been included in the nutrient medium to increase levels of lipase activity (Gupta et al., 2004). Corn oil has frequently been cited as an inducer of *Fusarium* lipases (Maia et al., 2001; Rifaat et al., 2010), however, vegetable oils are regarded as expensive fermentation substrates, and are mainly used as a food stock.

Animal fat has the advantage of wide availability and low cost, and waste product of meat processing. Chicken fat is waste product of poultry processing industry (Arnaud et al., 2004) which has been used to produce biodiesel. However, as shown in this work, it can also be used for obtaining bio-products with high added value such as enzymes. The average cost of the disposal is of $ 0.60/ L, 80% cheaper than olive oil, mostly used substrate for lipase production from microorganisms (Benjamin and Pandey, 2001; Hatzinikolaou et al., 1996; Long et al., 1996) and 50% cheaper than corn oil, which is frequently being cited as an inducer of *Fusarium* lipases (Maia et al., 2001; Rifaat et al., 2010; "USDA Economic Research Service," 2013). To date, no other work in the literature has reported the use chicken fat to induce production of lipases. The chicken fat used in this work was obtained from poultry slaughtering which was discarded as waste. The fatty acid composition of the chicken fat used for this work has 95% of long chain fatty acids and can thus serve as a source of carbon for inducing production of the enzyme.

Although, the carbon source is the key choice for lipase production, due to the inductive effect and also the costs associated with it, the N source occupies a prominent place especially for the consequences in upstream process (Keller et al., 2001). The organic sources often provide higher enzymatic activity in cultures with micro-organisms, but are costly in regard to inorganic salts and complicate the purification due to the complex composition that includes proteins and peptides. Thus, studies to minimize or even to avoid their use as a component in the culture medium are encouraged. Although not well studied, lipases from *Fusarium* spp. (filamentous fungi) are known to exhibit some interesting properties, such as their stability in polar organic solvents like ethanol, acetone and n-propanol (Camargo-de-Morais et al., 2003). Recently a strain of *Fusarium* sp. (*Gibberella fujikuroi* complex) FCLA-MA41 (Oliveira et al., 2013) was isolated from decaying plant matter in the Atlantic Forest of the state of São Paulo in Brazil. Cultivation of the strain in Submerged Fermentation, a medium containing crambe oil (17.5 mL/L), Triton X-100 (5 g/L), ammonium sulfate (5 g/L) and yeast extract (1 g/L) was proposed, resulting in a lipase titer of 3.0 ± 0.25 U/mL. Using Solid State Fermentation, the same fungus produced a maximum lipase titer of 5.0 ± 0.25 U/gds on crambe meal moistened with phosphate buffer.

In this work, we report on the production of lipases by a *Fusarium* sp. (GFC) isolate FCLA-MA41, using chicken fat and association of organic and inorganic nitrogen source in submerged fermentation to seek economically attractive bioprocess.

MATERIALS AND METHODS

Materials

The chicken fat was kindly supplied by Fricock Frigorificação Avicultura Indústria Comércio Ltda. (Rio Claro-SP, Brazil).

Microorganism

The fungal strain *Fusarium* sp. (*Gibberella fujikuroi* complex) FCLA-MA41 was isolated from decaying plant matter in the Atlantic Forest in the state of São Paulo (Brazil) and identified as described by Oliveira et al. (2013). The fungal isolate was maintained on MEA medium (malt extract agar) and stored at 4°C. For spore production, the fungal isolate was grown at 28°C for 5 to 7 days. A spore suspension was prepared at a concentration of 1 x 10^8 spores/mL and glycerol added to a final concentration of 200 mL/L. Aliquots of this preparation was transferred into cryovials for storage at -20°C. To ensure sterile growth of the fungal isolate, solid medium was autoclaved at 121°C for 20 min and used for propagation, inoculum and production purposes.

Enzyme production by submerged fermentation (SmF)

Optimization of enzyme production in SmF using factorial design

A 2-level, 4-factor Central Composite Design (CCD) and response surface methodology (RSM) were used to study the influence of the concentrations of chicken fat (X_1), $(NH_4)_2SO_4$(X_2), Triton X-100 (X_3) and yeast extract as a nutrient supplement (X_4) in Vogel minimum salt medium (VMSM) (Vogel, 1956). Table 1 shows the independent factors (X_i), their levels and the experimental design in terms of the coded (-α, -1, 0, 1 and +α) and the non-coded (actual value) variables. The analyses were performed using STATISTICA 8.0 software (Statsoft Inc.) to calculate the main effects of the variables and their interactions, and to perform the analysis of variance (ANOVA). The response of variables, *Y* (lipase activity, U/mL), may be approximated by the polynomial equations:

$$\text{CCD } 2^4\text{: } Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j$$

where: Y is the predicted response, β_0 is the offset term, β_i the linear effect, β_{ii} the squared effect and βij the interaction effect.

The inoculum was prepared by transferring three agar discs (0.25

cm diam.) colonized with mycelium to a 250 mL Erlenmeyer flask containing 50 mL of nutrient medium. The flasks were kept at 28°C and shaken at 180 rpm for 72 h (best fermentation time – data not show). After fermentation, the culture fluid was filtered through gauze cloth, used as the source of extracellular enzyme and the biomass was dried and determined by gravimetry. The tests were carried in triplicate.

Analytical methods

Lipase assay and protein determination

The lipase activity of the fermentation samples was measured by the hydrolysis of *p*-nitrophenyl palmitate (*p*NPP) as first described by Winkler and Stuckmann (1979), and modified by Lima et al. (2004). One unit of lipase activity is defined as the release of 1 mmol/min of *p*-nitrophenol (*p*NP). The molar extinction coefficient of *p*NP (1.5 x 10^4 mol/L x cm) was used to correlate the concentration of product from the absorbance readings.

Gas chromatography (GC) analysis of fatty acids

The fatty acid derived from chicken fat were identified at Technology Laboratory of Animal Products (UNESP – Jaboticabal/SP) by GC using a Shimadzu 14B Gas Chromatograph connected to a OMEGAWAX250 capillary column (30 m x 0.25 mm x .25 µm). The injector and detector temperatures were set at 250 and 280°C, respectively. The column temperature was initially maintained at 100°C for 2 min, increased to 220°C at 4°C/min, and finally held at 300°C for 25 min. A mix of fatty acids (SIGMA) was used as standard.

RESULTS

Fatty acid composition of chicken fat

The chicken fat oil used in this study was analyzed by GC to assess its fatty acid composition, verifying that it was composed of 24.61% palmitic acid (C-16:0), 9.39% palmitoleic acid (C-16:1), 4.84% estearic acid (C-18:0), 42.93% oleic acid (C-18:1), 13.63% linoleic acid (C-18:2) and 4.60% of othersas reported elsewhere (Arnaud et al., 2004).

Effect of concentration of chicken fat, Triton X-100, ammonium sulfate and yeast extract on lipase production using CCD matrix analysis

A 2^4 CCD matrix was used to evaluate the process variables adopted in this experiment. The variables included the concentration of chicken fat (X_1), Triton X-100 (X_2), ammonium sulfate (X_3) and yeast extract (X_4) in nutrient media containing VMSM. Time (72 h), orbital agitation (180 rpm) and fermentation temperature (28°C) were the fixed parameters in the experimental design. Table 1 presents the experimental matrix with the variables in their coded forms. The responses (lipase activity) are presented in U/mL. Highest lipase activities were observed in runs 14 and 24 with chicken fat resulting in activities of 8.33 and 8.04 U/mL, respectively.

ANOVA indicated that there was no interaction between the variables at a confidence level of p <0.05. However, when considered in isolation, variables X_2, X_3 and X_4 produced statistically significant results. Triton X-100 (X_2) and yeast extract (X_4) showed a positive influence on the response, and therefore increasing these variables led to an increase in lipase activity of 1.92 and 2.37 U/mL, respectively. On the other hand, increasing the concentration of ammonium sulfate (X_3) has a negative effect on the response, causing a decrease in lipase activity of 1.59 U/mL.

After testing the validity, the F test, $F_{calc}>F_{tab}$ (Table S1), indicated that the model was statistically significant (valid). An empirical mathematical model of lipase production can be described by Equation 1.

$$\boldsymbol{Y}\ (\text{U/mL}) = 5.1975 + 0.9600.(X_2) - 0.7975.(X_3) + 1.1841.(X_4) \quad (1)$$

Three-dimensional response surfaces obtained for the model are shown in Figure 1, the contour plots are shown in Figure S1.Thus, RSM defined the region with the best response as consisting of the following combination of variables: chicken fat 15.0 mL/L, Triton X-100 15.0 g/L, ammonium sulfate 4.5 g/L and yeast extract 1.0 g/L. This combination was predicted to produce a lipase activity of 9.11 ± 5.0 U/mL. A validation study was performed according to the described concentrations and produced lower values than expected, but still within the margin of error, with an enzymatic activity of 4.22 ± 0.35 U/mL.

Costs of lipase production by *Fusarium* sp. (GFC) FCLA-MA41

Considering the cost estimates of the nutrient medium optimized for lipase production by SmF containing 15.0 mL/L of chicken fat ($0.60/ L), 4.5 g/L of ammonium sulfate ($15.75/ kg), 1.0 g/L of yeast extract ($173.20/ kg) and 15.0 g/L of Triton X-100 ($80.00/ kg) in a solution containing Vogel minimum salts ($0.73/ L) used in this study, the production cost was $US 518.00/million Units of lipase.

DISCUSSION

Rapp (1995) described the addition of trimyristin, olive oil, oleic acid, and surfactant Span 85 inducing formation of extracellular lipolytic activity in cultures from *Fusarium oxysporum* sp. *vasinfectum*. *F. oxysporum* lipase was induced by rape seed oil (Tamerler and Keshavarz, 2000) and triolein (Camargo-de-Morais et al., 2003); sesame oil

Table 1. CCD matrix (factorial 2^4) containing four repetitions at the central point for the production of lipase by *Fusarium* sp. (GFC) FCLA-MA-41 in SmF.

Run	Variable in coded levels				Response			
					(*Y*, U/mL)		Biomass (g/L)	At/Biomass (U/g)
	X_1	X_2	X_3	X_4	Predicted	Observed	Observed	Observed
1	-1	-1	-1	-1	0.00	2.37	11.57	204.84
2	-1	-1	-1	+1	2.88	3.07	12.05	254.77
3	-1	-1	+1	-1	1.65	0.93	13.60	68.38
4	-1	-1	+1	+1	4.28	4.37	11.75	371.91
5	-1	+1	-1	-1	5.79	4.39	12.53	350.36
6	**-1**	**+1**	**-1**	**+1**	**8.04**	**6.87**	**8.95**	**767.60**
7	-1	+1	+1	-1	3.84	5.24	13.12	399.39
8	-1	+1	+1	+1	5.80	5.81	11.44	507.87
9	+1	-1	-1	-1	2.73	3.18	16.36	194.38
10	+1	-1	-1	+1	5.51	5.17	17.51	295.26
11	+1	-1	+1	-1	1.78	4.01	14.64	273.91
12	+1	-1	+1	+1	4.27	6.13	14.32	428.07
13	+1	+1	-1	-1	5.05	6.01	11.34	529.98
14	**+1**	**+1**	**-1**	**+1**	**7.16**	**8.33**	**18.27**	**455.94**
15	+1	+1	+1	-1	0.46	0.72	21.39	33.66
16	+1	+1	+1	+1	2.27	0.91	20.66	44.05
17	-α	0	0	0	2.98	3.35	7.53	444.89
18	+α	0	0	0	2.23	0.39	20.61	18.92
19	0	-α	0	0	2.45	0.13	18.78	6.92
20	0	+α	0	0	6.29	7.13	14.56	489.70
21	0	0	-α	0	4.87	4.50	12.36	364.078
22	**0**	**0**	**+α**	**0**	**1.68**	**0.57**	**20.62**	**27.64**
23	0	0	0	-α	2.75	0.73	13.90	52.52
24	**0**	**0**	**0**	**+α**	**7.49**	**8.04**	**13.77**	**583.88**
25[a]	0	0	0	0	5.19	5.95	14.78	402.57
26[a]	0	0	0	0	5.19	4.89	14.23	343.64
27[a]	0	0	0	0	5.19	1.80	15.43	116.66
28[a]	0	0	0	0	5.19	1.45	15.79	91.83

Factor	Real level				
	-α	-1	0	+1	+α
X_1 (Chicken fat, mL/L)	10.0	15.0	20.0	25.0	30.0
X_2 (Triton X-100, g/L)	0.0	5.0	10.0	15.0	20.0
X_3 ($(NH_4)_2SO_4$, g/L)	1.0	4.5	10.0	15.5	19.0
X_4 (Yeast extract, g/L)	0.00	0.25	0.50	0.75	1.00

α = 2.0; [a] Central point runs.

was used to induce lipase from *Fusarium solani* (Maia et al., 2001). The chicken fat used in this study, according to analysis, is composed of long-chain fatty acids, primarily oleic (18:1, 42.93%), palmitic (16:0, 24.61%) and linoleic (18:2, 13.63%). With this composition, this food-processing residue could induce lipase production from *Fusarium* sp. (GFC). In fact this was observed, because enzyme activity was not detected in runs conducted in the absence of chicken fat (data not show). When comparing the runs 17 and 18, it is clear, the importance of chicken fat for microorganism growth, but the increase in its concentration has little influence on the extracellular lipase activity.

In the literature it is common to justify the reduction of extracellular activity with increasing addition of oil due to the aeration difficulty of the culture medium, which affect cell metabolism (Li et al., 2006). However, even with the adding of surfactant Triton X-100 as a variable in this

Table S1. Analysis of variance of enzymatic activity (U/mL) obtained for the 2^4CCD matrix with chicken fat (X_1), Triton X-100 (X_2), ammonium sulfate (X_3) and yeast extract (X_4).

Factor	Ss	df	Ms	F_{calc}	p-Value
X_1 (L)	0.8438	1	0.84375	0,26904	0,612695
X_1 (Q)	10.0233	1	10.02334	3.19601	0.097140
X_2 (L)	**22.1184**	**1**	**22.11840**	**7.05261**	**0.019797**
X_2 (Q)	1.0209	1	1.02094	0.32553	0.578030
X_3 (L)	**15.2642**	**1**	**15.26415**	**4.86708**	**0.045975**
X_3 (Q)	5.5296	1	5.52960	1.76315	0.207076
X_4 (L)	**33.6540**	**1**	**33.65402**	**10.73082**	**0.006022**
X_4 (Q)	0.0074	1	0.00735	0.00234	0.962125
X_1 x X_2	12.3904	1	12.39040	3.95077	0.068333
X_1 x X_3	6.9696	1	6.96960	2.22231	0.159890
X_1 x X_4	0.0196	1	0.01960	0.00625	0.938193
X_2 x X_3	13.2860	1	13.28603	4.23634	0.060195
X_2 x X_4	0.4556	1	0.45562	0.14528	0.709245
X_3 x X_4	0.0870	1	0.08703	0.02775	0.870264
Error	40.7706	13	3.13620	-	-
Total SS	159.6361	27	-	-	-

Ss: Sum of squares;df: degrees of freedom; Ms: mean square; R^2 = 0.738; R = 0.859; F_{tab} (1;13; 0.05) = 4.67.

experiment, increasing the concentrations of chicken fat is not important for increasing the extracellular lipolytic activity.

On the other hand, the runs varying the concentration of Triton X-100 showed a significant effect for increasing the extracellular lipolytic activity, though at a reduced biomass (runs 19, 20 and 25). Rapp (1995) reported inducing effect of another surfactant, Span 85, in the formation of extracellular lipase from F. oxysporum sp. vasinfectum. Messias et al. (2009), Silva et al. (2005) and Mahadik et al. (2002) also reported increased production of lipase with different concentrations of Triton X-100 from Botriosphaeria ribis, Metarhizium anisopliae and Aspergillus niger, respectively. The mechanism for this effect, however, is not yet understood. The increased activity in cultures of Fusarium sp. (GFC) FCLA-MA41 with Triton X-100, reported in this study, is not due to the influence of the surfactant on the structure of the lipase. This statement is based on experimental observation after incubation of the lipase from *Fusarium* sp. (GFC) at different concentrations of Triton X-100 (0.0016 to 0.80 mM). It was observed that increase in activity of max 3% for concentrations below 0.22 mM, critical micelle concentration (CMC) for Triton X-100. Above the CMC, there was inhibition of activity at 0.5, 3.5, 9.5 and 13.5% for surfactant concentration of 0.32, 0.48, 0.64 and 0.80 mM, respectively.

Yeast extract is a complex nutrient, with peptides, amino acids and vitamins, being expected and its addition to the culture medium would provide more biomass. It is interesting to note, however, that the increase in the concentration of yeast extract favored the increase of extracellular lipase activity, but had no effect on biomass (runs 9, 10, 23 and 24). Ammonium sulfate, instead, favored growth but had no effect on enzyme production (runs 14 and 15). Rapp (1995) found the necessity of adding peptone for formation of extracellular lipase by F. oxysporum sp. vasinfectum. To biomass production there was no interaction between the variables at a confidence level of $p < 0.05$. However, when considered in isolation, chicken fat (X1) and ammonium sulfate (X3) showed a positive influence on the biomass production, and therefore increasing these variables led to an increase in biomass production of 5.47 and 2.40 g/L, respectively.

The production of enzyme has been associated, in the literature, with cell growth (Ghosh et al., 1996; Zarevúcka et al., 2005). However, runs 6 and 22 (Table 1), for example, show opposite relation of Lipase Activity/Biomass (767 and 27.6, respectively). As the factorial design was carried out at a fixed time of 72 h, to confirm the difference in activity was due to experimental condition and not because of the time of cultivation, runs 6 and 22 were repeated with analysis every 24 h (Figure 2). In both cases, the maximum activity was found in the stationary phase of cell growth. However, the results confirmed that despite higher biomass in condition 22, the extracellular lipolytic activity is lower compared to run 6 in t he condition in which growth is reduced at higher lipolytic activity. It was found that the lipase production from *Fusarium* sp. (GFC) FCLA-MA41 is not directly related to the increased number of cells, but due to the metabolic effect or transport (Table 2). Rapp (1995) found that

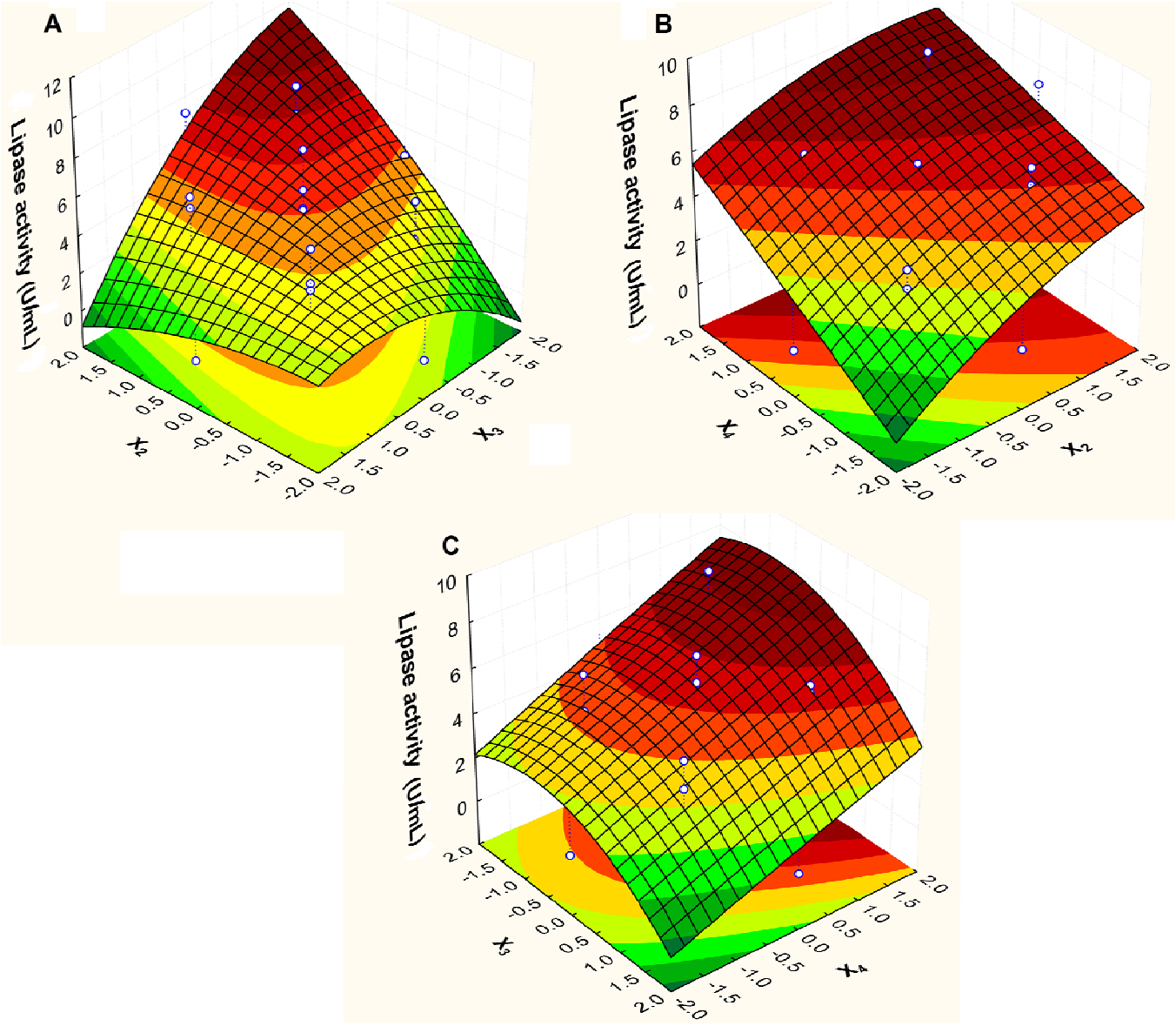

Figure 1. Three-dimensional response surface of lipase activity (U/mL) obtained by submerged fermentation of *Fusarium* sp. (GFC) FCLA-MA-41 in function of X_2 and X_3 (A), X_2 and X_4 (B) and X_3 and X_4 (C), on medium with various concentrations of chicken fat (X_1), Triton X-100 (X_2); ammonium sulfate (X_3) and yeast extract (X_4).

during growth of *F. oxysporum f.* sp. *vasinfectum* in shake flasks, a small amount of lipase activity was found to be associated with the mycelium.

The authors suggested that lipase activity is associated for some time with the cell wall in the course of its secretion into the culture medium. The assumption was made for the secretion of lipase activity from Geotrichum candidum (Tsujisaka et al., 1973), Serratia marcescens (Winkler and Stuckmann, 1979) and F. oxysporum f. sp. lini SUF.8 (Hoshino et al., 1991). Therefore, the increase in activity with the addition of Triton X-100, with no increase in biomass, may be due to the detergent effect by increasing the permeability of the cell wall, and consequently increasing the secretion of the enzyme by the cell.

Although, the cost of culture medium is an important parameter to determine the economic viability of a bioprocess, the upstream process are more expensive and may represent up to 80% of the total costs of enzyme production (Keller et al., 2001). Thus, the priorities are nutrients that contribute to increased production but preferably do not result in difficulties in recovering, purifying

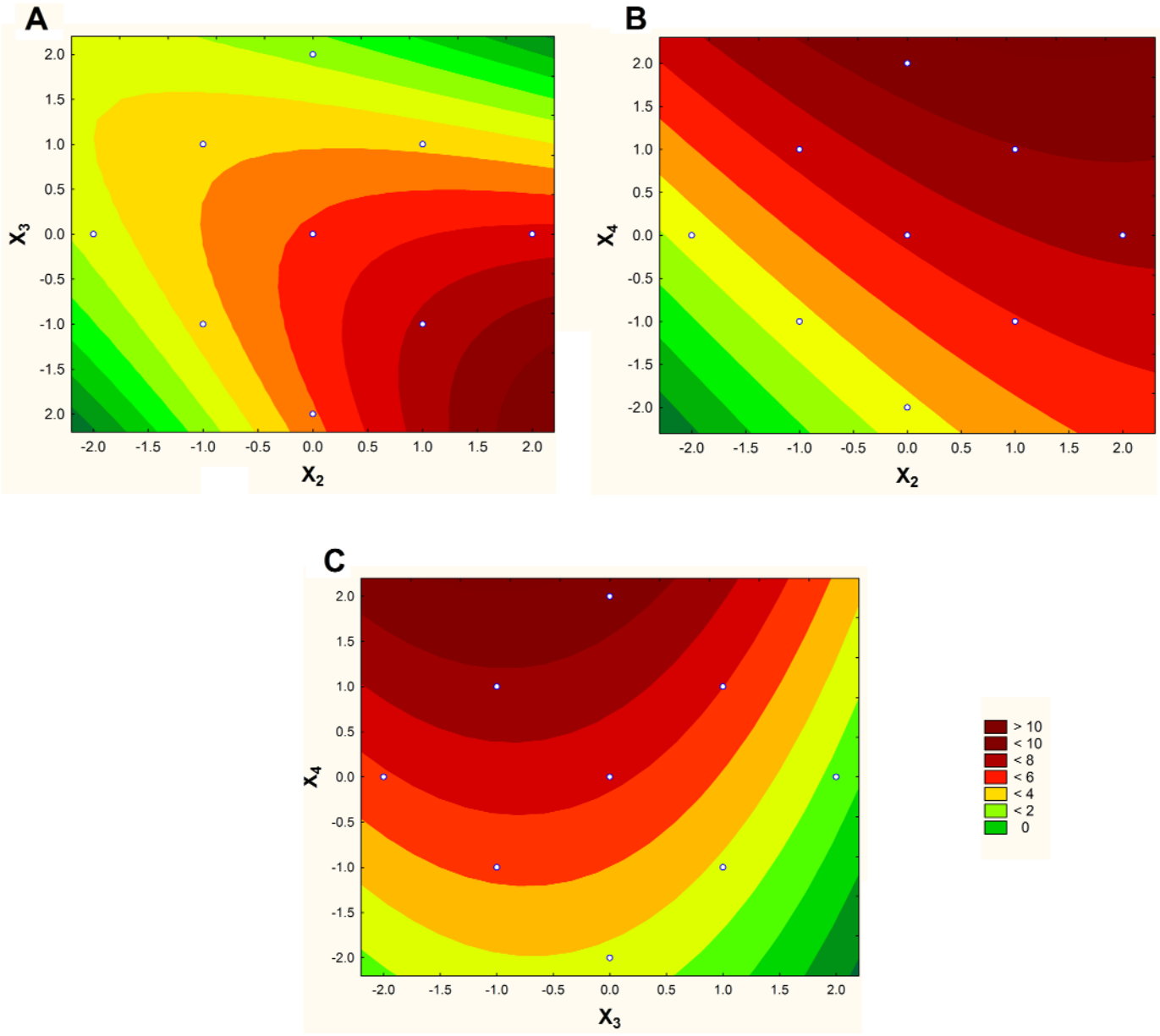

Figure S1: Contour plots of lipase activity (U/mL) obtained by submerged fermentation of *Fusarium* sp. (GFC) FCLA-MA-41 in function of X_2 and X_3 (A), X_2 and X_4 (B) and X_3 and X_4 (C), on medium with various concentrations of chicken fat (X_1), Triton X-100 (X_2); ammonium sulfate (X_3) and yeast extract (X_4).

and applying the enzyme by increasing the number of upstream process. In the present study, VSMS represents 33.46% of the production cost and it is essential for the production of lipase (activity is 98% lower in its absence – data not show), is comprised only of inorganic salts whose residues are easily separated at the end of fermentation. Yeast extract, in contrast, are very expensive and have a complex composition including proteins and peptides, which complicate the purification. The results are important because they demonstrate the possibility of combining organic and inorganic sources for the production of lipase from *Fusarium* sp. (GFC) FCLA-MA41 reducing the concentration of yeast extract. The production was optimized through the use of ammonium sulfate, this way; a cheaper ecologic culture medium for lipase production is feasible.

Previous study with the same *Fusarium* sp. (GFC) FCLA-MA41 strain in SmF with culture medium containing crambe oil (17.5 mL/L), Triton X-100 (5 g/L), ammonium sulfate (5 g/L) and yeast extract (1 g/L) was

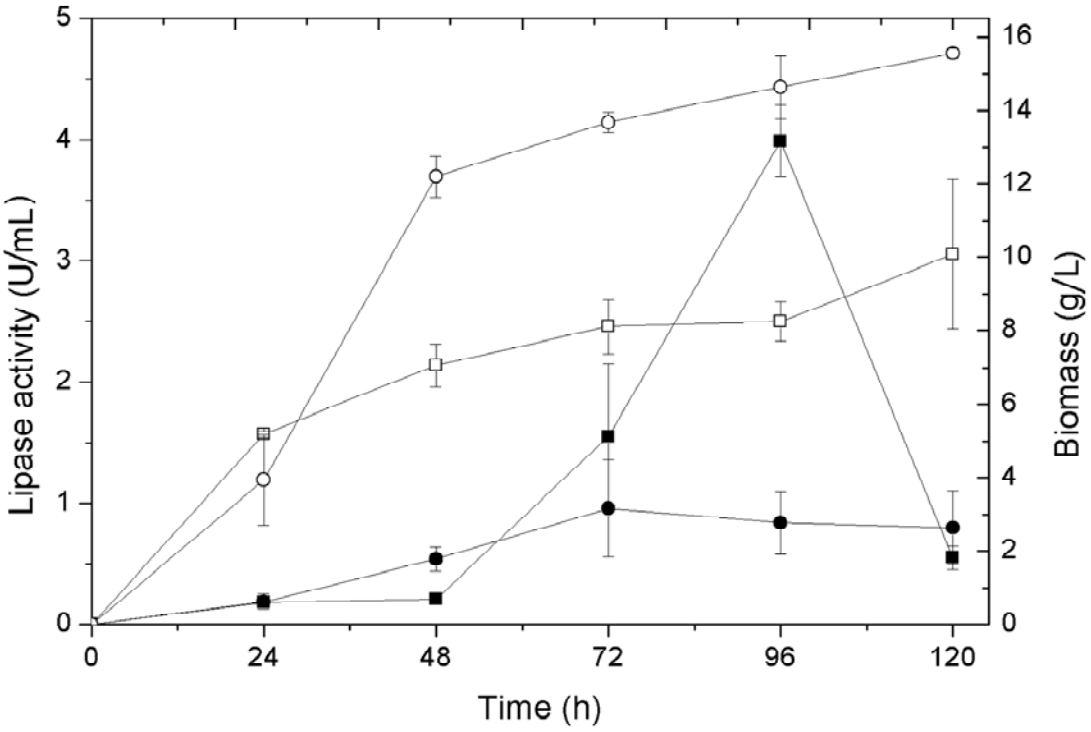

Figure 2. Curve of growth and extracellular lipase activity in cultures of *Fusarium* sp. (GFC) FCLA MA-41. Run 6: (■) Lipase activity, (□) Biomass. Chicken fat 15.0 mL/L, Triton X-100 15.0 g/L, ammonium sulfate 4.5 g/L and yeast extract 0.75 g/L. Run 22: (●) Lipase activity, (○) Biomass. Chicken fat 20.0 mL/L; Triton X-100 10.0 g/L, ammonium sulfate 19.0 g/L and yeast extract 0.5 g/L.

proposed, resulting in a lipase titer of 3.0 ± 0.25 U/mL (Oliveira et al., 2013). Although providethis provides lower activity than that obtained with chicken oil, the cost per unit of enzyme activity was also lower ($US 183.00 and $US 518.00/million Units of lipase with crambe oil and chicken fat, respectively). Still, the economic viability of the bioprocess is also related to other factors such as availability of raw materials. The crambe oil has the advantage of being a non-food oil seed, however, currently is cultivated as an oil-seed on a large scale in Mexico, New Zealand, Russia, United States, but most crambe oil processing occurs in Europe (Warwick and Gugel, 2003) for industrial uses. The poultry processing, by contrast, has a worldwide distribution and the oil/fat is an inexpensive residue.

Conclusion

The findings of this work have demonstrated good results of lipase production from *Fusarium* sp. (GFC) FCLA-MA41 by SmF. The fungal isolate was found to be a producer of lipase with market potential, as lipase activity was obtained on nutrient medium containing low-cost nutrients, such as ammonium sulfate and chicken fat. The lipase activity obtained was 4.22 U/mL and equated to a cost of $US518.00/million units of lipase. Moreover, the use of chicken oil as an inducer for lipase production is unprecedented.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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