academicJournals

Vol. 13(4), pp. 566-573, 22 January, 2014
DOI: 10.5897/AJB2013.13453
ISSN 1684-5315 ©2014 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Selection and optimization of extracellular lipase production using agro-industrial waste

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Accepted 16 January, 2014

The aim of this study was to isolate and select lipase-producing microorganisms originated from different substrates, as well as to optimize the production of microbial lipase by submerged fermentation under different nutrient conditions. Of the 40 microorganisms isolated, 39 showed a halo around the colonies and 4 were selected (*Burkholderia*, *Bacillus* sp., *Penicillium lanosum* and *Corynebac glutamyl*), where strains showing lipolytic halo Radius (R) / colony radius (r) ratio greater than 2.0 were selected. The results of submerged fermentation expressed as enzymatic activity revealed that the genera of microorganisms significantly influenced the enzymatic reaction, and lipase obtained from *Burkholderia cepacia* was the most promising, with activity of 0.0058 $U.mL^{-1}$. It was also observed in the optimization step of lipase production that the sodium nitrate content ($NaNO_3$) had a positive effect on enzyme production, and its increase was indicative of higher enzymatic activity. The addition of sources of organic nitrogen (corn steep liquor, p = 0.2398), carbon (soybean oil, p = 0.3379), magnesium $MgSO_4.7H_2O$ (p = 0.4189) and potassium KH_2PO_4 (p = 0.8562) had no significant effects on the lipase production and could result in decreased production of extracellular lipases.

Key words: *Burkholderia cepacia*, hydrolytic enzymes, residue, submerged fermentation.

INTRODUCTION

Lipases (triacylglycerol acylhydrolases, EC3.1.1.3) are water-soluble enzymes that catalyze the hydrolysis of ester bonds of insoluble triacylglycerols, releasing free fatty acids, mono-or diacylglycerolin the oil-water interface (Linko et al., 1998; Treichel et al., 2010). Lipases may be of animal, microbial or plant origin, with variations in their catalytic properties, and can be obtained by solid-state fermentation or by submerged fermentation (Annibale et al., 2006; Rigo et al., 2010; Papagora et al., 2013). These enzymes catalyze a wide range of reactions such as hydrolysis, esterification, trans-esterification, alcoholysis, acidolysis and aminolysis (Joseph et al., 2008). Lipases represent an important group of enzymes with various applications in the food, chemical and pharmaceutical industry (Salihu et al., 2012), besides having great interest for the treatment of effluents with high lipid content (Dharmsthiti and Kuhasuntisuk, 1998; Jung et al., 2002; Jeganathan et al., 2007; Ertugrul et al., 2007). However, the high cost of commercial enzymes makes treatment costly in the production of enzymes and in the search for new microorganisms. In this context, the use of agroindustrial waste contributes in the reduction of production costs (Leal et al., 2006; Salihu et al., 2012). In the production of lipases, studying the optimization of fermentation conditions, relating the carbon and nitrogen sources to temperature and pH conditions is of fundamental importance (Silva et al., 2005). Some studies (Nascimento et al., 2007; Kona et al., 2001) reported the

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use of some low-cost substrates in the production of enzymes such as corn steep liquor and soybean or olive oil, food industry by-products, as an alternative to reduce production costs, thus contributing to agro-industrial sustainability.

Corn steep liquor is a by-product rich in carbohydrates, amino acids, peptides, minerals, vitamins and phosphates and is thus considered as an excellent culture medium for enzyme production (Lee et al., 2003; Rivas et al., 2004).

Some studies have also shown that producing lipase by bacterial strains is more common because they offer higher enzymatic activity compared to fungi, tend to have optimal pH at neutrality or alkalinity, they are often thermostable and are mostly extracellular, facilitating their extraction from the fermented medium (Hasan et al., 2006; Li et al., 2010).

Thus, the aim of this study was to isolate and select lipase-producing microorganisms originated from different substrates (yeast extract, peptone and soybean oil), as well as to optimize the production of microbial lipase by submerged fermentation under different nutrient conditions of substrate (corn steep liquor, yeast extract, peptone, soybean oil), sources of sodium (Na_2HPO_4), magnesium ($MgSO_4.7H_2O$), potassium (KH_2PO_4) and nitrate ($NaNO_3$).

MATERIALS AND METHODS

Collection of effluent and microorganisms

Microorganisms were isolated from effluent collected in the "CicopalLtda" Potato chip industry, Senador Canedo, GO, and in the Sewage Treatment Plant (STP) in Goiânia-GO, Brazil. *Burkholderia cepacia* strain ATCC 25416 (BC25416) was obtained from the "Fundação Tropical André Tosello", Campinas-SP, Brazil, originated from the collection of tropical crops. Other organisms such as *Candida tropicallis* CCT 5846 (UCT), *Candida* sp. (UCS), *Zymomonas mobilis* CCT 4494 (UZM), *Escherichia coli* (UEC), *Kluveromyces marxianus* (UKM), *Kocuria rhizophila* (UKR), *Bacillus subtilis* NRRL 14819 (UBS14), *Corynebac glutamil* (UCG), *Bacillus* sp. NRRL 41094 (UBS41) and *Penicillium lanosum* NRRL 3442 (UPL) were obtained from strains provided by the Laboratory of Wastewater Treatment and Fermentation Processes (LARPF), Faculty of Food Engineering, State University of Campinas-SP, Brazil.

Isolation of microorganisms

The effluents collected were inoculated in Petri dishes in the following media: potato agar (PA), violet red bile lactose agar (VRBA) and standard count agar (PCA), according to methodology of the American Public Health Association (APHA, 2001). After incubation, they were analyzed for the development of microorganisms. The colonies were selected by visual analysis. For isolation, the selected colonies were inoculated in Petri dishes containing nutrient agar using the exhaustion technique and incubated at 30°C for 48 h in a bacteriological incubator. The microorganisms were maintained in test tubes containing nutrient agar pH 7.0 at 4°C.

Selection of lipase-producing microorganisms

The pre-selection of lipase-producing strains was performed by analyzing the formation of transparent halo around colonies on medium containing tributyrin, according to the methodology proposed by Freire (1997). A small fraction of each strain was inoculated in Petri dishes containing 0.5% peptone, 0.3% yeast extract, 2% agar and 0.1% tributyrin, pH 6.0 and incubated at 30°C for 48 h. Strains showing lipolytic halo radius (R)/colony radius (r) ratio greater than 2.0 were selected according to the study of Freire (1997) and Colen et al. (2005) choosing only the strains lipolytic that showed higher halos for further studies and submitted to submerged fermentation. The cultivation on solid medium was performed in a completely randomized design (CRD) with forty microorganisms and three replications, and the means were compared by the Tukey test at 5% probability.

Lipase production via submerged fermentation

The microorganisms previously selected were used in the liquid medium cultivation. In the preparation of inoculum, microorganisms were scraped with platinum loop and transferred to 125 ml Erlenmeyer flasks containing 50 ml medium cultive solution of (g L^{-1} of distilled water): yeast extract (5.0), peptone (5.0), and 1%$_{w/w}$ soybean oil in the proportion of 1:20 inoculum in relation to the culture medium, incubated at 30°C for 48 h under stirring in a water bath (Tecnal TE-184). After the incubation period, 10 ml of the inoculum was transferred to each reactor to carry out fermentations. Fermentations were carried out in Erlenmeyer flasks with capacity of 125 ml and incubated at 30°C for 72 h under stirring (100 rpm) in water bath. Fifty milliliter (50 ml) of medium cultive solution of (g L^{-1} of distilled water): yeast extract (5.0), peptone (5.0) and soybean oil (1%$_{w/w}$) were part of the culture medium. After fermentation, extracellular enzymes were extracted, where an aliquot of 5 ml of the reaction medium was centrifuged (TG16-WS Tabletop High Speed Centrifuge) at 2000 xg for 15 min. The supernatants were used as enzyme extract for analysis of the enzymatic activity.

Lipase activity determination

The enzymatic activity was determined by spectrophotometric method (Spectrophotometer BEL photonics), according to the methodology described by Parul et al. (2005). The Elisa plate well was added of 100 µL of solution containing sodium phosphate buffer pH 7 (0.1 M), 0.9% Triton X-100 and 0.27 M sodium chloride, as well as 100 µL of sample and 20 µL p-nitrophenyl phosphate substrate (pNPP). The mixture was incubated for 30 min in water bath at 37°C and readings were made in a spectrophotometer at 405 nm. The enzymatic activity unit was defined as 1 µmol. min^{-1} of p-nitrophenol released from the substrate.

Optimization of the production of lipases

To optimize the production of lipases from microorganism with the highest lipolytic potential, a 2^{6-2} fractional factorial exploratory design was initially performed, which evaluated the effect of the corn steep liquor (5 to 30 gL^{-1}), soybean oil (4 to 12%) sources of sodium (0 to 4 gL^{-1} Na_2HPO_4), magnesium (0.1 to 0.3 g L^{-1} $MgSO_4.7H_2O$), potassium (2 to 4 g L^{-1} KH_2PO_4) and nitrate (0 and 6 gL^{-1} $NaNO_3$). Peptone and yeast extract were used at concentration of 1 gL^{-1}. Each effect varied in three levels (-1, 0, +1), with duplicates of the central point, totaling 18 assays. Fermentations were performed at 30°C for 72 h under stirring in water bath. The two nutrients that showed the greatest effect on the enzyme activity were chosen and evaluated by means of a central composite design. Each effect varied in five levels (-1.42, -1, 0, +1, +1.42) with triplicate of the central point, totaling 11 assays. The model validation was performed in triplicate.

RESULTS AND DISCUSSION

Isolation and selection of microorganisms

Overall, 29 microorganisms were isolated, 24 from the Potato chip industry and 5 from the sewage treatment plant (STP). The coding for microorganisms isolated from industrial effluents was: IEA1, IEA2, IEB1, IEB2, IEB3, IEB4, IEV1, IEV2, ITEA1, ITEA2, ITEB1, ITEB2 , ITEB3, ITEB4, ITEV1, ITEV2, ITA1, ITA2, ITB1, ITB2, ITB3, ITB4, TV1, TV2, and for isolates from the STP: ETDB1, ETDB2, ETDB3, ETDB4 and ETDB5. Of the 40 microorganisms used for the lipase activity evaluation (29 isolates from the effluents and 11 from research institutes), 39 showed a halo around the colonies, indicating the presence of degradation reaction of tributyrin by extracellular enzymes, inducing lipase production. Of these 39 microorganisms, 4 showed lipolytic halo radius (R) / colony radius (r) ratio greater than 2.0 (Table 1), among them are *B*. *cepacia* ATCC 25416, *Bacillus* sp. NRRL 41094, *P*. *lanosum* NRRL 3442 and *C*. *glutamil*. These results showed that there was a variation of 23.1% in the hydrolysis halos, where strain BC25416 was the one showing the highest R/r ratio, and this difference in intensity between halos is due to the amount of extracellular lipase secreted by microorganism (Cardenas et al., 2001). Similar results were found by Griebeler et al. (2009), who studied the production capacity of lipolytic enzymes from different microorganisms on solid medium containing tributyrin, and obtained for strain *Penicillium* sp. and *B*. *cepacia*, the greatest hydrolysis halo (radius) of 9.35 and 9.1 mm, respectively, suggesting a probable lipolytic potential of these microorganisms.

Table 1 shows that although none of the organisms isolated from effluents have been pre-selected for submerged fermentation, this confirmed that lipase-producing microorganisms can be found in various habitats, especially those containing lipids (Sharma et al., 2001).

Submerged fermentation

The results of the submerged fermentation expressed in enzymatic activity of the 4 previously selected microorganisms are shown in Table 2. The results obtained reveal that the pre-selected microorganisms significantly influenced ($p \leq 0.05$) the enzymatic degradation reaction of p-nitrophenyl palmitate. Among the microorganisms that differ from one another, the lowest activity was 0.0022 $U.mL^{-1}$, referring to lipase from *C*. *glutamil* (UCG), which showed 62% less activity than lipase from *B*. *cepacia* ATCC 25416 (BC25416), with activity of 0.0058 $U.mL^{-1}$. The enzymatic activities of microorganisms *Bacillus* sp. NRRL 41094 (UBS41) of 0.0032 $U.mL^{-1}$ and *P*. *lanosum* NRRL 3442 (UPL) of 0.0027 $U.mL^{-1}$ were 45 and 53% less than the maximum activity found in the present study. These genera of microorganisms *Burkholderia*, *Bacillus* and *Penicillium* have been extensively studied for the production of extracellular lipases in order to optimize the production of enzymes for various applications, where there is great variation between the results, probably due to the different cultivation media, methods for determining the enzymatic activity and microorganisms are used.

Wolski et al. (2009) compared the production of lipase from *Penicillium* sp. in solid and submerged fermentation and found greater lipase production and activity at 42°C of 15.17 $U.mL^{-1}$. Annamalai et al. (2011) studied the production of thermostable lipase from *Bacillus licheniformis* isolated from the marine environment, using peanut oil as substrate and reported hydrolytic activity of 730 $U.mL^{-1}$ after 48 h of incubation at 55°C.

Optimization of the lipase production

Strain *B*. *cepacia* ATCC 25416 was selected to study the optimization of the production of extracellular lipase, where the effects of different nutrients on the lipase production were assessed. The results are shown in Table 3, where the enzymatic activity values ranged from 0 to 0.0181 $U.mL^{-1}$. Table 4 shows the results of the effects of variables on the enzymatic activity response. It was observed that the sodium nitrate concentration ($NaNO_3$) had a positive effect on the enzymatic activity, and the increase in the concentration of this salt in the reaction medium from 0 to 6 gL^{-1} resulted in increased response, indicating increased production of extracellular lipase from *B*. *cepacia* ATCC 25416, and consequently, higher enzyme activity. Generally, microorganisms provide high lipase yields when organic nitrogen sources are used (Supakdamrongkul et al., 2010); however, in this experiment, the high yield was due to inorganic sources. Na^+ ion has positive and significant effect only when sodium nitrate was used. For sodium phosphate monobasic (Na_2HPO_4), a positive effect was found, although not significant ($p = 0.2352$), and only sodium nitrate was statistically significant ($p = 0.0463$). The addition of sources of organic nitrogen (corn steep liquor, $p = 0.2398$), carbon (soybean oil, $p = 0.3379$), magnesium $MgSO_4.7H_2O$ ($p = 0.4189$) and potassium KH_2PO_4 ($p = 0.8562$) had no significant effects on the lipase production. However, these effects had a negative sign, indicating that increases in the concentration of these nutrients showed little tendency to lower the enzymatic activity and could result in decreased production of extra-cellular lipases.

Some studies have revealed that vegetable oils such as soybean, corn, sunflower, olive, palm and cotton are referred to as inducers of lipase production, being used by microorganisms as carbon source in the reaction medium (Paques and Macedo, 2006). However, this phenomenon was not observed in this study with results obtained by varying the soybean oil concentration, being not statistically significant in the production of extracellular lipases from *B*. *cepacia* ATCC 25416. The induction of lipase production appears to be related not only to the length of the carbon chains of fatty acids in triacylglycerols and to the number of instaurations, but also to the concentration of the substrate used (Lima et al., 2003).

Table 1. Measurements of the radius of colonies (r), lipolysis halos (R) and R / r ratio in solid culture medium.

	Radius (cm)		
Code	**Radius of colonie (r)**	**Lipolysis halo (R)**	**R/r**
UCT	0.25 ± 0.10	0.29 ± 0.04	1.15
UCS	0.29 ± 0.09	0.34 ± 0.04	1.17
UZM	1.06 ± 0.05	0.18 ± 0.02	0.16
UEC	0.50 ± 0.07	0.09 ± 0.04	0.18
UKM	0.25 ± 0.03	0.29 ± 0.04	1.15
UKR	0.25 ± 0.06	0.35 ± 0.14	1.40
UBS14	0.38 ± 0.12	0.65 ± 0.06	1.70
UCG	0.33 ± 0.08	0.70 ± 0.13	2.10
UBS41	0.33 ± 0.05	0.76 ± 0.08	2.30
UPL	0.34 ± 0.05	0.72 ± 0.07	2.15
EA1	0.30 ± 0.08	0.41 ± 0.02	1.33
EA2	0.45 ± 0.03	0.10 ± 0.04	0.22
EB1	0.27 ± 0.06	0.50 ± 0.11	1.87
EB2	0.25 ± 0.02	0.47 ± 0.04	1.86
EB3	0.44 ± 0.04	0.48 ± 0.07	1.09
EB4	0.38 ± 0.05	0.11 ± 0.04	0.30
TEB1	0.29 ± 0.07	0.29 ± 0.04	1.00
TEB2	0.35 ± 0.11	0.41 ± 0.09	1.18
TEB3	0.25 ± 0.00	0.47 ± 0.06	1.86
TEB4	0.30 ± 0.07	0.31 ± 0.04	1.04
TEV1	0.30 ± 0.07	0.47 ± 0.08	1.53
TEV2	0.43 ± 0.16	0.46 ± 0.04	1.09
BC25416	0.24 ± 0.05	0.91 ± 0.05	3.76
TA1	0.28 ± 0.03	0.02 ± 0.01	0.08
TA2	0.30 ± 0.13	0.41 ± 0.04	1.38
TDB1	0.24 ± 0.05	0.10 ± 0.02	0.42
TDB2	0.40 ± 0.03	0.46 ± 0.04	1.16
TDB3	0.41 ± 0.18	0.19 ± 0.04	0.45
TDB4	0.38 ± 0.06	0.19 ± 0.07	0.50
TDB5	0.23 ± 0.07	0.34 ± 0.04	1.50
TV1	0.34 ± 0.04	0.41 ± 0.04	1.22
TV2	0.26 ± 0.11	0.36 ± 0.04	1.38
EV1	0.29 ± 0.04	0.28 ± 0.07	0.96
EV2	0.25 ± 0.04	0.31 ± 0.07	1.25
TB1	0.25 ± 0.06	0.35 ± 0.07	1.40
TB2	0.35 ± 0.11	0.68 ± 0.10	1.95
TB3	0.60 ± 0.08	0.23 ± 0.07	0.38
TB4	0.34 ± 0.12	0.29 ± 0.04	0.85
TEA1	0.26 ± 0.14	0.26 ± 0.04	1.00
TEA2	0.55 ± 0.00	0.00 ± 0.00	0.00

UCT: *Candida tropicallis* CCT 5846; UCS: *Candida* sp.; UZM: *Zymomonas mobilis* CCT 4494; UEC: *Escherichia coli*; UKM: *Kluveromyces marxianus*; UKR: *Kocuria rhizophila*; UBS14: *Bacillus subtilis* NRRL 14819; UCG: *Corynebac glutamil*; UBS41: *Bacillus* sp NRRL 41094; UPL: *Penicillium lanosum* NRRL 3442; BC 25416: *Burkholderia cepacia* ATCC 25416; Industrial efluent: IEA1, IEA2, IEB1, IEB2, IEB3, IEB4, IEV1, IEV2, ITEA1, ITEA2, ITEB1, ITEB2, ITEB3, ITEB4, ITEV1, ITEV2, ITA1,ITA2, ITB1, ITB2, ITB3 e ITB4, TV1 e TV2; sewage treatement plant: ETDB1, ETDB2, ETDB3, ETDB4 e ETDB5.

These results show that the reduced lipase production at higher olive oil concentrations may be explained by the lower oxygen transfer to the medium, since lower oxygen supply could decrease the metabolism of microorganisms and consequently the lipase production (Elibol and Özer, 2000). From the results previously obtained and all the substances tested, only sodium nitrate was significant for lipase production; however, another variable was selec-

Table 2. Enzymatic activity of pre-selected microorganisms after submerged fermentation.

Microorganism	Enzymatic activity (U mL^{-1})
Corynebac glutamil	0.0022[d]
Penicillium lanosum NRRL 3442	0.0027[c]
Bacillus sp. NRRL 41094	0.0032[b]
Burkholderia cepacia ATCC 25416	0.0058[a]

Same letters indicate no significant difference (p> 0.05) in the production of lipase from microorganisms under study.

Table 3. Matrix of the 2^{6-2} fractional factorial exploratory design used to determine the enzymatic activity of lipases from *Burkholderia cepacia* ATCC 25416 with different nutrients.

Experiment	CST (g L^{-1})	Na_2HPO_4 (g L^{-1})	OS (g L^{-1})	$MgSO_4.7H_2O$ (g L^{-1})	KH_2PO_4 (g L^{-1})	$NaNO_3$ (g L^{-1})	Enzymatic activity (U.mL^{-1})
1	5	0	4	0.1	2	0	0.0029
2	30	0	4	0.1	4	0	0.0023
3	5	4	4	0.1	4	6	0.0181
4	30	4	4	0.1	2	6	0.0090
5	5	0	12	0.1	4	6	0.0089
6	30	0	12	0.1	2	6	0.0022
7	5	4	12	0.1	2	0	0.0000
8	30	4	12	0.1	4	0	0.0080
9	5	0	4	0.3	2	6	0.0113
10	30	0	4	0.3	4	6	0.0017
11	5	4	4	0.3	4	0	0.0012
12	30	4	4	0.3	2	0	0.0063
13	5	0	12	0.3	4	0	0.0011
14	30	0	12	0.3	2	0	0.0025
15	5	4	12	0.3	2	6	0.0114
16	30	4	12	0.3	4	6	0.0011
17	15	2	8	0.2	3	3	0.0036
18	15	2	8	0.2	3	3	0.0034

CST, Corn Steep Liquor; Na_2HPO_4, sodium phosphate monobasic; SO, soybea noil; $MgSO_4.7H_2O$, magnesium sulfate heptahydrate; KH_2PO_4, potassium phosphate monobasic; $NaNO_3$, sodium nitrate.

Table 4. Estimate of the effect of each nutrient added to the culture medium on the enzymatic activity of lipase from *Burkholderia cepacia* ATCC 25416.

Sources of variation	Effect	Standard deviation	t (11)	p-value	Conf. Lim. -95%	Conf. Lim. +95%
CST	-0.00273	0.002	-1.243	0.23975	-0.00756	0.00210
Na_2HPO_4	0.00276	0.002	1.256	0.23517	-0.00207	0.00758
OS	-0.00220	0.002	-1.002	0.33794	-0.00703	0.00263
$MgSO_4$	-0.00184	0.002	-0.840	0.41890	-0.00667	0.00299
KH_2PO_4	-0.0004	0.002	-0.186	0.85619	-0.00523	0.00442
$NaNO_3$	0.00493	0.002	2.245	0.04632*	0.00010	0.00975

* Significant at 5% probability. CST, corn steep liquor; Na_2HPO_4, sodium phosphate monobasic; SO, soybean.

ted, corn steep liquor, because it is a by-product from the food industry rich in nutrients such as carbohydrates, amino acids, peptides, vitamins and minerals, reducing the culture medium costs. The results obtained in the optimization stage are shown in Table 5, which are expressed as enzymatic activity response as a function of

Table 5. Matrix of the central composite design used to determine the enzymatic activity of lipase from *Burkholderia cepacia* ATCC 25416 due to the enrichment of the medium with corn steep liquor (CST) and sodium nitrate ($NaNO_3$).

Experiment	CST (g L^{-1})	$NaNO_3$ (g L^{-1})	Enzymatic activity (U.mL^{-1})
1	10	1	0.0068
2	30	1	0.0063
3	10	5	0.0025
4	30	5	0.0034
5	20	3	0.0110
6	20	3	0.0103
7	20	3	0.0089
8	5.858	3	0.0056
9	20	5.828	0.0044
10	34.142	3	0.0024
11	20	0.172	0.0039

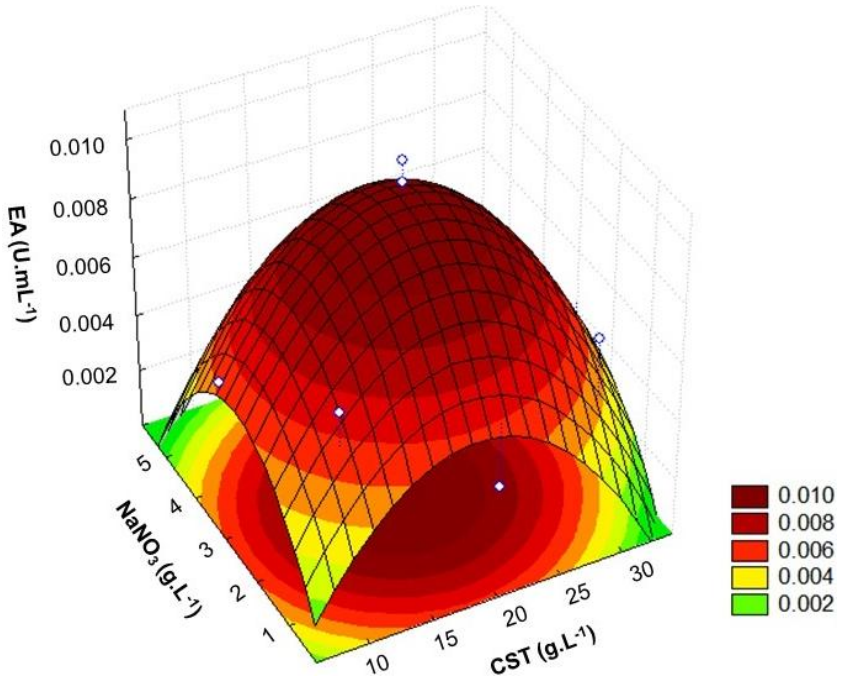

Figure 1. Response surface for the activity of lipase from *Burkholderia cepacia* ATCC 25416 under different corn steep liquor (AMM) and sodium nitrate concentrations.

the corn steep liquor and sodium nitrate contents, which ranged from 0.0024 to 0.0110 U.mL^{-1}. These results also show that both nutrients produced a negative effect on the enzymatic activity response, that is, the increase in the sodium nitrate and corn steep liquor concentration decreased the production of extracellular enzymes by *B. cepacia* ATCC 25416. This significant effect of the concentration of these nutrients was quadratic, indicating that there was an increase in the production of enzymes up to a maximum value, declining from this point on.

From the data obtained for the effect of each nutrient, a second-order mathematical model was built. Equation 1 represents the model representing the enzymatic activity (EA) versus STP (X_1) and $NaNO_3$ concentration (X_2). Words in bold refer to statistically significant variables ($p \leq 0.05$). According to these results and Figure 1, it was found that the optimization of the production process was achieved, and the optimum production was achieved for concentrations from 13.8 to 26.2 gL^{-1} of corn steep liquor and from 1.5 to 3.9 g L^{-1} of sodium nitrate, with maximum response for the enzymatic activity of 0.0100 U.mL^{-1}, which allows reduction of the culture medium costs in this process.

$$EA = 0.001007 - 0.002864X_1^2 - 0.000826X_2 - 0.002806X_2^2) \quad (1)$$

These results also showed that the lowest activity found (0.0040 U.mL^{-1}) by the model had response 60% lower than the maximum activity obtained, found when sodium nitrate concentrations higher than 5 g L^{-1} and lower than 0.17 gL^{-1} were used. Nitrogen is an essential nutrient for the production of enzymes, since these are composed of amino acids, which have nitrogen in their structure (Rodeva et al., 2010). Thus, sodium nitrate corn steep liquor both supplied nitrogen to microorganisms to produce lipases. These results also show that the fact that intermediate concentrations of these compounds provided high lipase production was probably a result of the balance between carbon and nitrogen concentrations in the medium, and usually, the carbon concentration must be greater than that of nitrogen in order to favor the physiological needs of microorganisms for lipase biosynthesis (Freire et al., 1997). Complex nitrogen sources have been traditionally used in the production of lipases (Lima et al., 2003). Roveda et al. (2010) evaluated the production of lipases from microorganisms isolated from dairy effluents using sodium nitrate as nitrogen source at fixed concentration of 0.1% and obtained satisfactory lipase activity for fungus of the genus *Aspergillus*. The validation of the model was performed by testing the enzymatic activity of the lipase enzyme extract of *B*. *cepacia* ATCC 25416 in culture medium containing 20 g L^{-1} corn steep liquor and 3 g L^{-1} sodium nitrate.

The results were a response estimated by the model of 0.0100 U.mL^{-1}, and the average response experimentally obtained (EA) was 0.0110 U.mL^{-1}, indicating good fit of the experimental points. This enzymatic activity is equivalent with almost 6.2% of the enzymatic commercial enzyme lipase calculated under the same experimental conditions.

Conclusion

According to the experimental results, in the step of preselection of microorganisms with hydrolytic capacity performed with 40 microorganisms, 39 showed halo around the colonies, indicating degradation reaction of tributyrin by extracellular enzymes, inducing the production of lipases. It was possible to isolate microorganisms from agroindustrial waste. In the submerged fermentation stage, the results expressed as enzymatic activity showed that the highest activity observed was for strain *B*. *cepacia* ATCC 25416. The optimization step for the production of extracellular lipase allowed establishing appropriate conditions for the production of lipases when using corn steep liquor and sodium nitrate in the culture medium.

ACKNOWLEDGMENTS

Special thanks go to CAPES - (Coordination of Improvement of Higher Education Personnel), CNPq - National Council for Scientific and Technological Development – for the research productivity and technological innovation fellowship, to the local Sewage Treatment Plant and to “CicopalLtda” for supplying the samples.

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