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BIOTECHNOLOGY

Medium composition and Amazonian oils for lipase production by *Candida viswanathii*

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ABSTRACT. Lipases have been successfully produced under submerged cultivation conditions using olive oil as carbon source and complex culture media. The aims of this study were to evaluate lipase production by *Candida viswanathii* in submerged cultivation with different culture medium compositions, replace olive oil with plant oils from the Amazonian region, and assess the effect of nitrogen sources. By using olive oil as the sole carbon source, *C. viswanathii* produced high levels of biomass and lipase with minimal nutritional requirements (potassium, magnesium, and yeast extract). Plant oils from the Amazonian region supported cell growth, and pequi oil was the best inducer for lipase production (1.66 U mL^{-1}). In comparison with inorganic sources, organic nitrogen sources induced the highest levels of lipase production. Typical microbial growth and lipase production were observed using pequi oil and peptone as a nitrogen source. The selected conditions resulted in good cell growth ($Y_{X/S}$ = 1.25 g g^{-1}) and lipase production ($Y_{P/S}$ = 118.10 U g^{-1}) after 60 hours of cultivation. In conclusion, the fermentation parameters indicated that Amazonian oils are promising for lipase production by *C. viswanathii*, and oils modified by this enzyme can be used in the food and cosmetic industries.

Keywords: carbon sources; enzyme production; fermentation parameters.

Composição do meio de cultivo e óleos amazônicos para a produção de lipase por *Candida viswanathii*

RESUMO. As lipases são produzidas com sucesso em cultivo submerso utilizando azeite de oliva como fontes de carbono, e meios de cultura complexos. Os objetivos deste estudo foram avaliar a produção de lipase por *Candida viswanathii* em cultivo submerso com diferentes composições de meio de cultura, substituir o azeite de oliva por óleos vegetais da região amazônica e avaliar o efeito de fontes de nitrogênio. Ao utilizar o azeite como única fonte de carbono, *C. viswanathii* produziu altos níveis de biomassa e lipase com requerimentos nutricionais mínimos (potássio, magnésio e extrato de levedura). Os óleos vegetais da região amazônica foram capazes de promover o crescimento celular e o óleo de pequi foi o melhor indutor para a produção de lipases (1,66 U mL^{-1}). Em comparação com fontes inorgânicas, as fontes de nitrogênio orgânicas induziram os níveis mais elevados de produção de lipase. O crescimento microbiano típico e a produção de lipase foram observados utilizando óleo de pequi e peptona como fonte de nitrogênio. As condições selecionadas resultaram num bom crescimento celular ($Y_{X/S}$ = 1,25 g g^{-1}) e produção de lipase ($Y_{P/S}$ = 118,10 U g^{-1}) após 60h de cultivo. Em conclusão, os parâmetros de fermentação indicaram que os óleos da Amazônia são promissores para a produção de lipases por *C. viswanathii*, e óleos modificados por esta enzima podem ser utilizados nas indústrias de alimentos e cosméticos.

Palavras-chave: fonte de carbono; produção de enzimas; parâmetros fermentação.

Introduction

Lipases (E.C. 3.1.1.3, triacylglycerol acyl hydrolase) hydrolyze carboxylic ester bonds, releasing carboxylic acids and alcohols. Under low water conditions, however, reactions known as esterification or transesterification reactions may occur. Their capacity of catalyzing such reactions with high efficiency and stability, their chemo-, regio- and enantioselectivity, as well as not requiring cofactors and being active in organic solvents render lipases very attractive and versatile enzymes from the industrial point of view (Daiha, Angeli, Oliveira, & Almeida, 2015). Esterases and cutinases may also cleavage fatty acid ester bonds in triacylglycerol in aqueous systems; however, lipases are mainly active against water-insoluble substrates, such as

triglycerides composed by long-chain fatty acids, whereas esterases preferentially hydrolyze 'simple' esters and usually only triglycerides composed by fatty acids shorter than C6 (Lopes, Fraga, Fleuri, & Macedo, 2011).

Lipases are distinguished by a broad substrate scope and excellent stability in various media and have participated in a wide range of industrial applications in the oleochemical, polymer, textile, biodiesel, detergent industry and others. With their excellent enantioselectivity, they belong to the most important biocatalysts for the synthesis of fine chemicals (Kourist, Hollmann, & Nguyen, 2014). So, these enzymes are considered to have great potential as biocatalysts in various industrial processes including the synthesis of food ingredients, detergents, drugs, and other refined products (Adrio & Demain, 2014; Barriuso, Vaquero, Prieto, & Martínez, 2016).

Approximately 90% of all industrial biocatalysts are produced by submerged fermentation, frequently using specifically optimized media and genetically manipulated microorganisms. Many studies have been undertaken to define the optimal culture and nutritional requirements for lipase production (Bandiya & Ramana, 2012). Medium composition is still one of the most critically investigated phenomenon that is carried out before any large-scale metabolite production and possess many challenges too. An increase in productivity reduces the overall cost of the product, as well as the production cost; hence, it is one of the important topics for the research (Singh et al., 2017).

Microbial lipases are mostly extracellular, and their production is greatly influenced by medium composition besides physicochemical factors such as temperature, pH, and dissolved oxygen. The major factor for the expression of lipase activity has always been reported as the carbon source, since lipases are inducible enzymes. These enzymes are generally produced in the presence of a lipid such as oil or triacylglycerol or any other inductor, such as fatty acids, hydrolysable esters, Tweens, bile salts, and glycerol. Lipidic carbon sources seem to be essential for obtaining a high lipase yield (Zarevúcka, 2012). Olive oil is composed of 80% oleic acid and is considered as a standard carbon source for lipase production (Almeida, Tauk-Tornisielo, & Carmona, 2012). Alternative carbon sources for lipase production include palm oil, crambe oil, sesame oil, corn oil, beef tallow, and oil cakes from agroindustrial wastes. Plant oils from the Amazonian region have high levels of monounsaturated fatty acids, tocopherols, and carotenoids; they have functional importance, such as antioxidant, anti-inflammatory, and antimicrobial activities, and exhibit good digestibility (Silva et al., 2009; Parfene, Horincar, Tyagi, Malik, & Bahrim, 2013). The use of whole-cell biocatalysts is an attractive alternative for the modification of Amazonian oils for potential applications in the pharmaceutical and cosmetic industries. In this study, lipase production by *Candida viswanathii* was evaluated with different medium compositions using plant oils from the Amazonian region as sole carbon sources. The effect of nitrogen sources and fermentation parameters were also evaluated.

Material and methods

Microorganism and inoculum

The *C. viswanathii* strain was obtained from the Laboratory of Biotechnology, Food Analysis, and Products Purification, Habite – Biotechnology-Based Company Incubator, Federal University of Tocantins, Gurupi, state Tocantins, Brazil. For inoculum preparation, *C. viswanathii* was cultivated on malt extract agar (MEA) for 3 days at 28°C. Submerged cultivation was performed using 1.0 mL suspension containing 1.0×10^7 cells mL^{-1}.

Submerged cultivation and selection of culture medium

Cells were cultured in Erlenmeyer flasks (125 mL) containing 20 mL of culture medium supplemented with olive oil (2%, w v^{-1}) as an initial carbon/inducer source for lipase production and cell growth. Six culture medium formulations were used for *C. viswanathii* growth and lipase production: medium 1: NaH_2PO_4 (12.0 g L^{-1}), KH_2PO_4 (2.0 g L^{-1}), yeast extract (10.0 g L^{-1}), $CaCl_2{\cdot}2H_2O$ (0.03 g L^{-1}), $ZnSO_4{\cdot}7H_2O$ (0.03 g L^{-1}), $FeSO_4{\cdot}7H_2O$ (0.005 g L^{-1}) (Ülker, Özel, Çolak, & Karaoğlu, 2011); medium 2: yeast extract (1.0 g L^{-1}), meat peptone (20.0 g L^{-1}), NaCl (5.0 g L^{-1}) (Freire, Teles, Bon, & Sant'anna Jr., 1997); medium 3: K_2HPO_4 (4.0 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (1.0 g L^{-1}), yeast extract (5.0 g L^{-1}), peptone (10.0 g L^{-1}) (Papagora, Roukas, & Kotzekidou, 2013); medium 4: K_2HPO_4 (5.5 g L^{-1}), $KHPO_4$ (15.0 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (0.5 g L^{-1}), yeast extract (10.0 g L^{-1}) (Açikel, Ersana, & Açikel, 2010); medium 5: K_2HPO_4 (5.5 g L^{-1}), $KHPO_4$ (15.0 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (0.5 g L^{-1}), NaCl (0.1 g L^{-1}), $CaCl_2$ (0.1 g L^{-1}), $(NH_4)_2SO_4$ (5.0 g L^{-1}), yeast extract (10.0 g L^{-1}), micronutrient solution (1 mL) (Dalmau, Montesinos, Lotti, & Casas, 2000); medium 6: Na_2HPO_4 (6.0 g L^{-1}), $KHPO_4$

(15.0 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (1.0 g L^{-1}), $(NH_4)_2SO_4$ (6.0 g L^{-1}), $FeCl_3 \cdot 6H_2O$ (0.010 g L^{-1}), urea (4.0 g L^{-1}), biotin (0.5 mL), micronutrient solution (1 mL) (Wei, Zhang, & Song, 2004). Flasks containing the liquid cultures were autoclaved at 121°C for 20 min. The cultures were inoculated with 5% (v v^{-1}) of the inoculum (10^7 cells mL^{-1}) and incubated (200 rpm, 28°C, pH 6.0) for 72 hours. Micronutrient solution: $C_6H_8O_7$ (5.0 g), $ZnSO_4 \cdot 7H_2O$ (5.0 g), $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (1.0 g), $CuSO_4 \cdot 5H_2O$ (0.25 g), $MnSO_4 \cdot H_2O$ (0.005 g), H_3BO_3 (0.005 g), $NaMo_4 \cdot 2H_2O$ (0.005 g), distilled water (100 mL).

Selection of vegetable oil

Five vegetable oils including andiroba (*Carapa guianensis*), buriti (*Mauritia flexuosa*), castor (*Ricinus communis*), pequi (*Caryocar brasiliense*), and tucum (*Bactris setosa*) were evaluated for lipase production as substitutes for olive oil. The cultures were prepared in Erlenmeyer flasks (125 mL) containing 20 mL of the previously selected medium supplemented with 2.0% (w v^{-1}) vegetable oil and pH-adjusted to 6.0. The cultures were incubated (200 rpm, 28°C) for 72 hours, and all cultivations were performed in triplicate.

Selection of nitrogen source

Nitrogen sources [meat peptone, yeast extract, soy protein, casein, urea, NH_4Cl, or $(NH_4)_2SO_4$] were added to the culture media at 1.0 % (w v^{-1}). The cultures were prepared in Erlenmeyer flasks (125 mL) containing 20 mL of the previously selected medium supplemented with 2.0% (w v^{-1}) pequi oil and pH-adjusted to 6.0. The cultures were incubated (200 rpm, 28°C) for 72 hours, and all cultivations were performed in triplicate.

Separation of biomass and protein extract

The biomass was separated from the fermentation broth by filtration (membrane cellulose acetate, 0.45 μm) and dried at 105°C until a constant weight was obtained. Cell-free broth was used for lipase activity assays.

Determination of residual olive oil

The culture supernatant was transferred to Erlenmeyer flasks (125 mL) and acidified to pH 1.0 with concentrated sulfuric acid. Then, 10 mL of hexane was added to the samples, which were vigorously agitated and maintained in a separation funnel until the organic and aqueous phases were separated. The organic phase was oven-dried at 40°C, and the mass was measured with an analytical balance.

Lipase activity assay

Lipase activity was assayed using p-nitrophenyl-palmitate (p-NPP) as substrate (Almeida, Tauk-Tornisielo, & Carmona, 2013). p-NPP was first dissolved in 0.5 mL of dimethyl sulfoxide and then diluted to 0.5 mM with McIlvaine buffer (pH 4.0) containing 0.5% Triton X-100. The hydrolysis of p-NPP was determined discontinuously at 40°C by measuring the release of p-nitrophenolate (p-NP). After 5 min of preincubation, 0.9 mL of this substrate was placed in a water bath, and the reaction was started by adding 0.1 mL of an appropriately diluted sample. The reaction was stopped at different intervals by heat shock (90°C, 1 min), followed by the addition of 1 mL of saturated sodium tetraborate solution. The absorbance was measured at 410 nm, and the activity was determined according to the standard curve using p-nitrophenol (p-NP molar extinction coefficient: 1.72×10^4 M cm^{-1}). Controls were prepared without the enzyme. One unit of enzyme activity was defined as the amount of enzyme that releases 1 μmol of p-NP per mL per min.

Fermentation parameters

The results obtained from cultivations were also assayed for substrate, biomass yield on substrate, lipase yield on substrate, biomass productivity, lipase productivity and specific rate of lipase production (Darvishi, Nahvi, Zarkesh-Esfahani, & Momenbeik 2009):

Biomass and lipase yield (Y), according Equation 1, 2 and 3:

$$Y_{X/S} = \frac{X_1 - X_0}{S_0 - S_1} \quad (1)$$

$$Y_{P/S} = \frac{P_1 - P_0}{S_0 - S_1} \quad (2)$$

$$Y_{P/X} = \frac{P_1 - P_0}{X_1 - X_0} \quad (3)$$

X = biomass (g L^{-1});
P = lipase (U L^{-1});
S = substrate (g L^{-1});
$Y_{X/S}$ = biomass yield on substrate consumed (g g^{-1});
$Y_{P/S}$ = lipase yield on substrate consumed (U g^{-1});
$Y_{P/X}$ = lipase yield on biomass produced (U g^{-1}).

Productivity and specific rate, according Equation 4, 5 and 6:

$$P_X = \frac{X_1 - X_0}{t} \quad (4)$$

$$P_L = \frac{P_1 - P_0}{t} \quad (5)$$

$$q_L = \frac{P_{X/S}}{t} = \left(\frac{P_f - P_0}{X_f - X_0}\right) * \frac{1}{t} \quad (6)$$

P_X = biomass productivity (g hour^{-1});
P_L = lipase productivity (U hour^{-1});
q_L = specific rate of lipase production (U g^{-1} hour^{-1}).

Statistical analysis

The results are expressed as the mean ± standard error of the mean and were compared using variance analysis followed by Tukey's test.

Results and discussion

Selection of culture medium

Initially, various nutrient medium compositions supplemented with 2.0% olive oil were evaluated for *C. viswanathii* growth and lipase production (Figure 1).

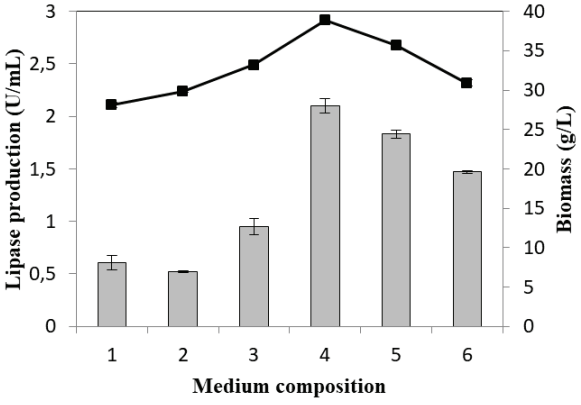

Figure 1. Effect of different medium compositions on the lipase production (—) and cell growth (■) of *C. viswanathii* in submerged cultivation. Cultivations were performed using 2% olive oil with constant shaking (200 rpm) at pH 6.0 and 28°C for 72 hours.

Lipase production and biomass growth were the highest with medium 4 (2.1 U mL^{-1} and 38.83 g L^{-1}, respectively). Medium 4 was composed of K_2HPO_4 (5.5 g L^{-1}), $KHPO_4$ (15.0 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.5 g L^{-1}), and yeast extract (10.0 g L^{-1}). This medium composition has been used for lipase production by *Rhizopus delemar* with sunflower oil and Tween 80 as carbon sources (Açikel et al., 2010). Intermediate values were observed with medium 5, which demonstrated the second highest lipase and biomass levels (1.83 U mL^{-1} and 35.7 g L^{-1}, respectively), followed by medium 6 with a lipase level of 1.47 U mL^{-1} and biomass of 30.9 g L^{-1}. Low levels of enzyme activity and growth were observed with medium 1, 2, and 3.

The parameters including biomass yield on substrate consumed, biomass productivity, lipase yield on substrate consumed, lipase productivity, and specific rate of lipase production were calculated for all medium compositions (Table 1). The results indicated that the nutrient composition of medium 4 was suitable for lipase production and biomass growth ($Y_{P/S}$ = 108.90 U g^{-1}, $Y_{X/S}$ = 2.01 g g^{-1}, $Y_{P/X}$ = 54.08 U g^{-1}, P_L = 29.16 U hour^{-1}, P_X = 0.54 g hour^{-1}, and q_L = 0.75 U g^{-1} hour^{-1}). However, the composition of medium 5 was also moderately suitable ($Y_{P/X}$ = 38.56 U g^{-1}, P_L = 19.11 U hour^{-1}, and q_L = 0.53 U g^{-1} hour^{-1}).

Table 1. Fermentation parameters of biomass and lipase production by *C. viswanathii* with different medium compositions.

Media	$Y_{X/S}$ (g g^{-1})	$Y_{P/S}$ (U g^{-1})	$Y_{P/X}$ (U g^{-1})	P_L (U hour^{-1})	P_X (g hour^{-1})	q_L (U g^{-1} hour^{-1})
1	1.47	31.77	21.66	8.47	0.39	0.30
2	1.56	27.22	17.43	7.22	0.41	0.24
3	1.66	50.00	28.60	13.19	0.46	0.39
4	2.01	108.90	54.08	29.16	0.54	0.75
5	1.81	92.90	51.27	25.41	0.49	0.71
6	1.60	84.97	47.62	20.41	0.43	0.66

Cultivations were performed using 2 % (w v-1) olive oil with constant shaking (200 rpm) at pH 6.0 and 28°C for 72 hours.

According to the results, *C. viswanathii* had a high level of biomass and lipase activity with minimal nutritional requirements [potassium (K^+), magnesium (Mg^{2+}), and yeast extract]. In medium 5, the presence of sodium (Na^+), calcium (Ca^{2+}), ammonium $(NH_4)^{2+}$, and micronutrient solution decreased lipase production and microbial growth. Mg^{2+} has been described in the literature as an important macronutrient for microbial growth and lipase stabilization (Tan, Zhang, Xu, & Zhang, 2004). The addition of Ca^{2+} and Fe^{2+} has been found to inhibit lipase production by *Penicillium camemberti*; these ions could form complexes with ionized fatty acids, changing their solubility and behavior at the oil-water interface (Tan et al., 2004). In another study, lipase production by *Pseudomonas gessardii* was slightly inhibited in the presence of Na^+, Fe^{2+}, and Mg^{2+}; however, Ca^{2+} had a stimulatory effect on lipase productivity (Ramani, Kennedy, Ramakrishnana, & Sekarana, 2010). In this sense, the use of minimal basal medium for lipase production by *C. viswanathii* is an advantageous approach for scaling up the process of submerged fermentation, which can reduce enzyme production costs and facilitate the downstream processes used to isolate and characterize the produced enzyme.

Selection of vegetable oils

The total fatty acid profile of pequi oil is composed of 65.5% oleic acid and 34.5% palmitic acid, whereas that of buriti oil is composed of 84.4%

oleic acid and 16.6% palmitic acid (Ferreira et al., 2011). Oleic acid is considered as the most important triacylglycerol fatty acid that can induce lipase production in several microorganisms including yeasts, bacteria, and filamentous fungi (Treichel, Oliveira, Mazutti, Luccio, & Oliveira, 2010). In comparison with buriti oil, pequi oil demonstrated the highest lipase production level (1.66 U mL^{-1}) owing to its fatty acid composition and nutritional value (Figure 2). Andiroba and tucum oils induced moderate levels of lipase production (0.4 U mL^{-1}), whereas castor oil induced the lowest lipase production (0.17 U mL^{-1}) and biomass growth (13.2 g L^{-1}). Andiroba oil is a rich source of essential fatty acids including oleic, palmitic, myristic and linoleic acids and contains non-fatty components such as triterpenes, tannins and isolated alkaloids such as andirobine and carapine (Gilbert et al., 1999). These components present anti-nutritional effects for organisms and can act as inhibitors for several enzymes (Barbosa Filho et al., 2006). Tucum oil has lauric acid as predominant fatty acid in total triacylglycerol content. This fatty acid was reported as weak inductor for lipase production by *C. viswanathii* under submerged conditions (Almeida et al., 2013).

The results of lipase production with castor oil may be attributed to the anti-nutritional effect of ricinoleic acid (88.3% of the total fatty acid profile), an unsaturated omega-9 fatty acid called 12-hydroxy-9-cis-octadecenoic acid. This result was also observed with *Lasiodiplodia theobromae* which the presence of castor oil in the culture medium resulted in the lowest lipase activity and fungal growth (Venkatesagowda, Ponugupaty, Barbosa, & Dekker, 2015). The inhibitory effect of the unusual hydroxy fatty acid on lipase production and microbial growth has also been observed for *Candida rugosa* in submerged cultivation using coconut oil, a hydroxy substituted C-14 fatty acid (Lakshmi, Kangueane, Abraham, & Pennathur, 1999). Fermentation parameters demonstrated that pequi oil is efficient for lipase production ($Y_{P/S}$ = 83.00 U g^{-1}, $Y_{P/X}$ = 106.41 U g^{-1}, P_L = 23.05 U $hour^{-1}$, and q_L = 1.48 U g^{-1} hour) (Table 2).

Biomass production using the different vegetable oils did not result in significant statistical differences ($p < 0.05$), indicating that these vegetable oils could be used to grow *C. viswanathii*. However, certain oils may have an inhibitory effect on lipase synthesis. This study highlighted for the first time the use of pequi oil for lipase production in submerged cultivation. Pequi oil and fruits are used for cooking as well as in traditional medicine for the treatment of colds, coughs, bronchitis, edema, and burns; on the other hand, buriti oil is applied in the cosmetic industry because of its emollient properties and ability to act as an adjuvant in sun protection (Ferreira et al., 2011). The use of pequi or buriti oils as carbon sources for lipase production and growth demonstrated the effectiveness of *C. viswanathii* lipase as a biocatalyst with these oils as substrates.

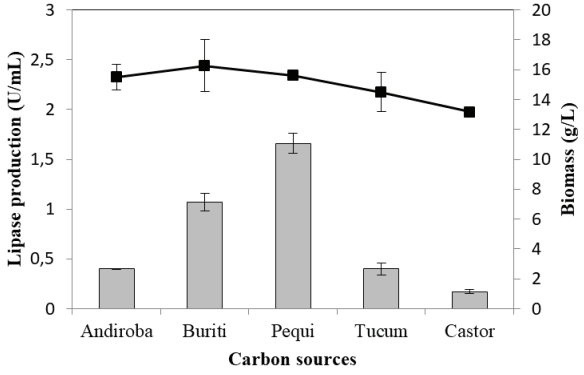

Figure 2. Effect of Amazonian oils on the lipase production (▬) and growth (■) of *C. viswanathii*. Cultivations were performed using 2% of each oil with constant shaking (200 rpm) at pH 6.0 and 28°C for 72 hours.

Table 2. Fermentation parameters of biomass and lipase production by *C. viswanathii* with different Amazonian oils.

Vegetable oils	Predominant fatty acids (%)*	$Y_{X/S}$ (g g^{-1})	$Y_{P/S}$ (U g^{-1})	$Y_{P/X}$ (U g^{-1})	P_L (U $hour^{-1}$)	P_X (g $hour^{-1}$)	q_L (U g^{-1} $hour^{-1}$)
Andiroba	18:1(49.8)	0.78	20.12	25.79	5.55	0.21	0.35
Buriti	18:1 (73.3)	0.83	54.56	65.72	14.86	0.23	0.91
Pequi	18:1 (56.0)	0.78	83.00	106.41	23.05	0.21	1.48
Tucum	12:0 (47.3)	0.73	20.31	27.58	5.55	0.20	0.38
Castor	18:1-ωOH (88.3)	0.72	9.29	12.90	2.36	0.18	0.18

Cultivations were performed in medium 4 using 2 % (w v^{-1}) vegetable oils with constant shaking (200 rpm) at pH 6.0 and 28°C for 72 hour. *Amazon oils industry.

Selection of nitrogen sources

Generally, microorganisms provide high yields of lipase when organic nitrogen sources are used. Nitrogen sources play an important role in the biosynthesis of lipase by microorganisms. Inorganic nitrogen sources can be used quickly, while organic nitrogen sources can supply cell growth factors and amino acids needed for cell metabolism and enzyme synthesis (Dheeman, Frias, & Henehan, 2010). Different nitrogen sources may be used in the lipase fermentation process. In this study, various organic and inorganic nitrogen sources were evaluated at a concentration of 1% (w v^{-1}): yeast extract, meat peptone, soy protein, casein, urea, ammonium chloride, and ammonium sulfate (Figure 3). The culture media were supplemented with 2% (w v^{-1}) pequi oil.

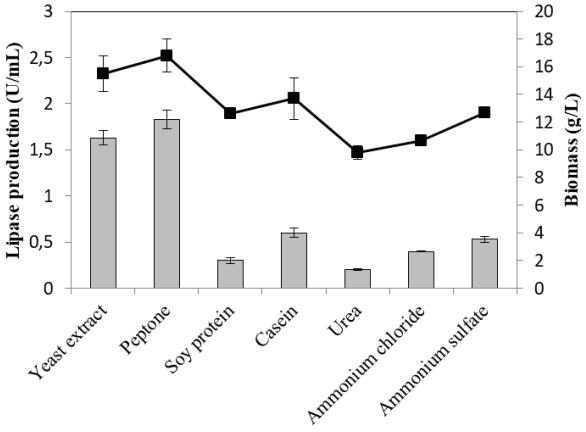

Figure 3. Effect of nitrogen sources on the lipase production (▬) and biomass growth (■) of *C. viswanathii*. Cultivations were performed using 2% pequi oil with constant shaking (200 rpm) at pH 6.0 and 28°C for 72 hours.

Among the organic nitrogen sources, peptone and yeast extract supported the highest cell growth (16.8 and 15.5 g L^{-1}, respectively) and the highest lipase production (1.83 and 1.63 U mL^{-1}, respectively). Moderate cell growths were observed with casein and soy protein (13.7 and 12.6 g L^{-1}); however, they had a negative effect on lipase production. Urea resulted in the lowest growth (9.8 g L^{-1}) and lipase production (0.2 U mL^{-1}). *C. viswanathii* cultured with peptone exhibited high biomass production ($Y_{X/S}$ = 1.31 g g^{-1}), lipase yield on substrate consumed ($Y_{P/S}$ = 142.74 U g^{-1}), lipase productivity (P_L = 25.41 U $hour^{-1}$), biomass productivity (P_X = 0.23 g $hour^{-1}$), and specific rate of lipase production (1.51 U g^{-1} $hour^{-1}$) (Table 3). Inorganic nitrogen sources, such as NH_4Cl and $(NH_4)_2SO_4$, could support *C. viswanathii* growth; however, lipase production may be decreased by these sources.

Table 3. Fermentation parameters of biomass and lipase production by *C. viswanathii* with different nitrogen sources.

Nitrogen sources	$Y_{X/S}$ (g g^{-1})	$Y_{P/S}$ (U g^{-1})	$Y_{P/X}$ (U g^{-1})	P_L (U $hour^{-1}$)	P_X (g $hour^{-1}$)	q_L (U g^{-1} $hour^{-1}$)
Yeast extract	0.78	82.03	105.16	22.63	0.21	1.46
Peptone	1.31	142.74	108.93	25.41	0.23	1.51
Soy protein	1.19	28.35	23.81	4.16	0.17	0.33
Casein	1.30	56.98	43.79	8.33	0.19	0.61
Urea	0.79	16.13	20.41	2.77	0.13	0.28
NH_4Cl	0.69	25.90	37.53	5.55	0.15	0.52
$(NH_4)_2SO_4$	0.77	32.12	41.71	3.397.36	0.18	0.58

Cultivations were performed in medium 4 using 2 % (w v^{-1}) pequi oil with constant shaking (200 rpm) at pH 6.0 and 28°C for 72 hours.

Organic nitrogen sources such as meat peptone, soy protein, casein, and yeast extract are regarded as suitable sources for lipase production by filamentous fungi and yeast (Tan et al., 2004). Conversely, inorganic nitrogen sources have been described in the literature as unsuitable for lipase production. Lipase production by the indigenously isolated marine fungi *Aspergillus sydowii* was found to reach maximum levels when using ammonium chloride at 3.5% (Bandiya & Ramana, 2012). Oliveira and Lima (2014) observed that the microbial growth of *Fusarium* sp. was favored by ammonium sulfate and low level of this inorganic nitrogen source increased the lipase production.

In a study on *Yarrowia lipolytica* LgX64.81, mineral nitrogen sources did not have a significant effect on both cell growth and lipase production; in contrast, a marked increase in lipase productivity was observed following the addition of certain organic nitrogen sources such as tryptone (Fickers, Nicaud, Gaillardin, Destain, & Thonart, 2004). Inorganic nitrogen was used in submerged cultivation of *Aspergillus sydowii* to produce lipases. Ammonium chloride promoted the maximum lipase production using 3.5% (w v^{-1}) (Bandiya & Ramana, 2012). Jia et al. (2015) evaluated the production of lipase by *Aspergillus niger* different when using peptone, beef extract, and soybean meal with or without $NaNO_3$, respectively. Soybean meal was selected as nitrogen source because of its low cost and the ease of accessibility. Herein, the lipase activity was not significantly different when supplemented with sodium nitrate, which suggested that sodium nitrate can be omitted.

Time-course of cultivation

The time-course of lipase production and microbial growth in medium 4 with 2.0% (w v^{-1}) pequi oil and 1.0 % (w v^{-1}) peptone was assessed for 72 hours. The enzyme produced was highest in the culture at 60 hours (2.35 U mL^{-1}) (Figure 4). The fermentation parameters under these conditions were $Y_{P/S}$ = 118.10 U g^{-1}, $Y_{P/X}$ = 93.96 U g^{-1}, P_L = 32.64 U $hour^{-1}$, $Y_{X/S}$ = 1.25 g g^{-1}, P_X = 0.34 g $hour^{-1}$, and q_L = 1.30 U g^{-1} $hour^{-1}$. Typical microbial growth and lipase production were also observed. Lipase production was associated with cell growth, and lipase production decreased in the stationary phase.

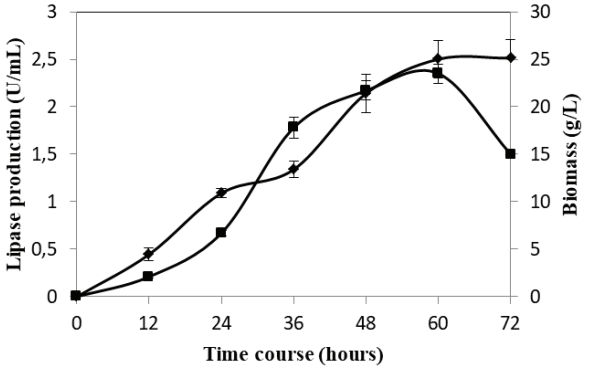

Figure 4. Time-course of lipase activity (■) and growth (●) of *C. viswanathii*. Cells were cultured in medium 4 using 2.0 % (w v^{-1}) pequi oil with constant shaking (200 rpm) at pH 6.0 and 28°C.

Conclusion

In this study, *C. viswanathii* cultivation revealed that medium composition mainly influenced lipase production more than microbial growth. Potassium and magnesium were found to be important nutrients for lipase production, whereas sodium, calcium, and ammonium may act as inhibitors. Pequi oil was used as a carbon source for growth and induced the highest levels of lipase production after 60 hours when cultured with meat peptone as the sole nitrogen source. These results suggest that *C. viswanathii* lipase can be used as a biocatalyst for oil modification under submerged conditions.

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