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ORIGINAL PAPER

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SIMULTANEOUS HIGH NUTRITIONAL SINGLE CELL OIL AND LIPASE PRODUCTION BY *CANDIDA VISWANATHII*

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ABSTRACT

Background. Omega fatty acids are a family of polyunsaturated fats associated with several health benefits. Lipases are enzymes with potential application in several food processes such as flavor and aroma, surfactants and formulations for the dairy and bakery industries. In this study, single cell oil and lipase production by *Candida viswanathii* CCR8137 were evaluated simultaneously from renewable carbon sources under nitrogen limitation.

Materials and methods. Enzyme and single cell oil were obtained in submerged cultivations supplemented with triolein, tributyrin, corn oil, sunflower oil, canola oil and olive oil. The effects of glucose on lipid accumulation, fatty acid profile, enzyme production and cell morphology were also evaluated.

Results. The highest lipid accumulation (44.5%, w/w) was obtained from triolein, whereas olive oil was the best inducer of lipase synthesis (26.8 U/mL). Nitrogen limiting cultivations were a key parameter for an organic source which showed higher lipid accumulation and enzyme production than the tested inorganic nitrogen source. Glucose was a poor inducer of lipase synthesis, though increased values of lipid accumulation were observed from this carbon source with a maximum of 63.1% (w/w). The fatty acid profile of lipids produced by *C. viswanathii* CCR8137 showed a high content of omega-9 fatty acid (C18:1 n-9). The addition of glucose to the culture media resulted in the synthesis of essential fatty acids: vaccenic, linolenic and eicosadienoic acids.

Conclusion. Therefore, *C. viswanathii* CCR8137 strain can be considered as an oleaginous yeast able to accumulate high concentrations of intracellular lipids, which are potential additives for food industry applications as well as being able to simultaneously synthesize high yields of lipase.

Keywords: single cell oil, essential fatty acids, unsaturated fatty acids, *Candida viswanathii*, lipase

INTRODUCTION

Oleaginous yeasts are industrially important organisms that assimilate carbon sources into lipids under nitrogen limiting conditions (Chi et al., 2011; Chang et al., 2015; Johnravindar et al., 2018). Oleaginous microorganisms

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are able to accumulate lipids to as much as 20% of the cell dry weight (the so-called single cell oils), which are rich in mono and polyunsaturated fatty acids from omega-3 and omega-6 families with promising applications associated with the food industry, commonly used as a supplement for child nutrition and additional dietetic purposes in the replacement of cocoa butter (Chang et al., 2015; Papanikolaou and Aggelis, 2011; Thevenieau and Nicaud, 2013; Zhang et al., 2014). Several oleaginous microorganisms have been reported in the literature, such as yeasts, filamentous fungi, bacteria, and microalgae, which are able to store intracellular triacylglycerols similar to those obtained from vegetable oils (Finco et al., 2016; Takaku et al., 2020). The main aspects of the biotechnology of microbial oils and fats are: a) the screening of new oleaginous microorganisms capable of producing high amounts of lipids, b) the optimization of culture conditions and development of innovative fermentation strategies, c) the simultaneous production of microbial compounds through non-competitive pathways (i.e. enzymes, biocontrols), d) the combination of various waste and by-products as carbon sources in order to overcome the issue regarding reduced/seasonal substrate availability, e) the production of single cell oil in pilot-scale operations that allows an integrated techno-economic evaluation of the process (Bellou et al., 2016; Diwan et al., 2018; Papanikolaou and Aggelis, 2019).

Lipases (triacylglycerol acyl hydrolases, E.C. 3.1.13) are naturally occurring enzymes in plants, animals, fungi and bacteria. Their basic function is to catalyze the hydrolysis of lipids into free fatty acids and glycerol at the interface of aqueous and organic solvents, which broadens their applications in various industries. Lipases catalyze a wide range of industrially important reactions such as transesterification, esterification, interesterification and show enantio-selectivity. For this reason, they are considered to be indispensable tools in the food, pharmaceutical, biofuel, diagnostic, chiral chemistry, drug, detergent, oleochemical, cosmetic, leather, and biosensor industries (Negi, 2019). Yeasts are industrial lipase producers due to their broad substrate specificity, selectivity and stability, and constitute the most important group of biocatalysts for biotechnological applications. Their enzymes are usually extracellular enzymes that facilitate the separation from fermentation media, from which the remaining biomass can be used for other industrial purposes (Salgado et al., 2020).

Candida viswanathii strains have previously been isolated from the soil and wastewater of oil refineries and their potential for enzyme production and bioremediation have been investigated (Fatima et al., 2007; Soares et al., 2008). The lipolytic activity of *C. viswanathii* was first studied by Almeida et al. (2013). In this cited study, the effects of soluble and hydrophobic carbon sources were linked to the induction and repression of lipase secretion. The optimum conditions for lipase production and the biochemical properties of the crude enzyme were characterized by the work of Almeida et al. (2013). Almeida et al. (2016) also presented the potential of *C. viswanathii* to produce lipase in a solid-state cultivation system using poultry fat as a carbon source. Purification and immobilization of *C. viswanathii* lipase produced in submerged conditions showed a potential application of this catalyst for the hydrolysis of triacylglycerols and phospholipids (Almeida et al., 2018). The accumulation of microbial oil by *C. viswanathii* was first studied by Ayadi et al. (2016) as a potential single cell oil using agro-industrial waste. *C. viswanathii* Y-E4 showed a high potential for oil accumulation using a wide range of substrates, especially from C5 and C6 sugars as well as glycerol and hydrophobic carbon sources. The fatty acid profile analysis revealed oleic acid to be the main component produced from different substrates. Guerfali et al. (2020) related the potential of *C. viswanathii* Y-E4 to biodiesel production using crude glycerol as an alternative feedstock for single cell oil production. The authors showed that the fatty acid profile was different from each tested culture condition with a predominance of long-chain fatty acids, such as linolenic acid.

In this work, *C. viswanathii* CCR8137 was cultivated using different carbon sources (tributyrin, triolein, olive oil, corn oil, sunflower oil, canola oil) under nitrogen limitation in submerged cultivations to evaluate lipid accumulation and lipase production. The effects of glucose on lipid accumulation and lipase production were also evaluated, as was the unsaturated fatty acid profile.

MATERIALS AND METHODS

Microorganism and cultivations

All cultivation experiments were carried out at the Laboratory of Biotechnology, Food Analysis and Product Purification (LABAP), HABITE – Biotechnology-Based Incubator, Federal University of Tocantins, Gurupi, Tocantins, Brazil. Stock cultures of *C. viswanathii* CCR8137 were streaked in PDA slant agar and incubated at 28°C for 72 h. Volumes of one milliliter from standardized inocula (10^7 cell/mL) were transferred to the culture media. Cultivations were carried out in Erlenmeyer flasks (125 mL) containing 20 mL of modified Vogel's medium at 28°C and 180 rpm for 72 h (Vogel, 1956). This culture medium was supplemented with the carbon sources triolein, tributyrin, corn oil, sunflower oil, canola oil and olive oil at a concentration of 1.0% (w/v). Additionally, 0.2% (w/v) yeast extract or NH_4NO_3 were added to the culture medium and tested as nitrogen sources. The culture media were sterilized at 121°C for 20 min. These cultivations were carried out at 28°C and 180 rpm, for 96 h. The culture broth was submitted to centrifugation at 7500 g, at 4°C for 15 min. Supernatant was used for enzyme and protein assays. The pellet was washed twice with distilled water and dried at 60°C for lipid analysis. All cultures were carried out in triplicate.

Effects of glucose on single cell oil and lipase production

The effects of glucose on lipase production and lipid accumulation were evaluated in an initial medium containing olive oil (1.0%, w/v). After 6 h of cultivation, glucose (1.0%, w/v) was added to the culture media. Glucose (1.0%, w/v) was also evaluated as sole the carbon source in the culture media. Samples were taken periodically to determine microbial growth, lipid accumulation and lipase production. The cultivations were carried out at 28°C and 180 rpm, for 96 h. The culture broth was submitted to centrifugation at 7500 *g*, at 4°C for 15 min. Supernatant was used for enzyme and protein assays. The pellet was washed twice with distilled water and dried at 60°C for lipid analysis. All cultures were carried out in triplicate.

Analytical methods

Enzyme activity assay. Lipase activity was assayed with p-nitrophenyl palmitate (pNPP) as a substrate (Almeida et al., 2018). pNPP was initially dissolved in 0.5 mL of dimethyl sulfoxide (DMSO), then diluted to 0.05 M with McIlvaine buffer pH 3.5, containing 0.5% Triton X-100 (w/v). The hydrolysis of pNPP was determined discontinuously at 40ºC by measuring the p-nitrophenol (pNP) released. After 5 min pre-incubation of 0.9 mL substrate solution in a water bath at 40°C, the reaction started with the addition of 0.1 mL diluted enzyme. The reaction was stopped after 1 and 2 min by heat shock (90ºC, 1 min), followed by the addition of 1 mL saturated sodium tetraborate solution. The absorbance was measured at 405 nm and the activity was determined according to a p-nitrophenol standard curve of $1.8 \times 10^4\ M^{-1}\ cm^{-1}$ molar absorptivity. Control assays were performed free of enzymes. One unit of enzyme activity was defined as the amount of enzyme that releases 1 μmol of pNP per mL per min.

Lipid extraction and fatty acid methyl ester preparation. Lipid extraction was carried out according to the Folch method (Folch et al., 1957). The reaction was performed at 25°C for 24 h using an extraction solution containing chloroform, methanol and water (2.0:1.0:0.8, v/v/v). The total lipid content was determined gravimetrically.

The *in-situ* extraction and transesterification of fatty acids were carried out following an adapted methodology from NREL (The National Renewable Energy Laboratory) (Wychen et al., 2015). 2 mL chloroform/methanol (2:1, v/v) and 3 mL HCl/methanol (5:95, v/v) solutions were added to test tubes for the transesterification reaction at 55°C for 60 min. 3 mL hexane was added to test tubes for the extraction of fatty acid methyl esters which were further analyzed by gas chromatography.

Gas chromatography analyses. The methylated fatty acids were analyzed in a gas chromatograph GC--2010 Plus (Shimadzu, Japan), equipped with a capillary column HP-88 (60 m × 0.250 mm × 0.20 μm) using helium as a carrier gas at a flow rate of 1 mL/min. One microliter of organic phase was analyzed after split injection (1:50). The temperatures of

the injector and the flame ionization detector were 250ºC and 200ºC, respectively. A temperature program was applied to the column oven which started at 140ºC for 5 min, with the temperature increasing at 4°C/min up to 240°C, which was kept for 10 min. The fatty acids were identified using a Supelco 37 component FAME mix standard (Supelco, USA).

Fermentation parameters. The fermentation parameters used to evaluate cultivation performance were lipase and lipid yields by substrate ($Y_{P/S}$) and biomass ($Y_{P/X}$); biomass values (Y_X); productivity of lipase (P_{lipase}), lipid (P_{lipid}) and biomass (P_X); as well as the specific rate of lipase production (q_L).

RESULTS AND DISCUSSION

Single cell oil and enzyme production from pure and renewable carbon sources

The *C. viswanathii* CCR8137 strain was analyzed for lipid accumulation and lipase production in submerged cultivations using different triacylglycerol carbon sources (Table 1). The results showed that tributyrin was deleterious for microbial growth, lipid accumulation and lipase production. On the other hand, the utilization of vegetable oils or triolein as the sole carbon sources induced better enzyme and biomass yields than those obtained from tributyrin. The highest value of lipid accumulation was observed using triolein, which was 44.5% of the total biomass (w/w), followed by olive oil (39.0%, w/w). Furthermore, olive oil induced the best lipase production value of 26,800.0 U/L, followed by triolein, linseed oil and sunflower oil. Tributyrin was also considered to be a repressor of lipase production. The utilization of vegetable oils as carbon sources is the major influencing factor in inducing the expression of lipase activity (Geoffry and Achur, 2018). Almeida et al. (2013) showed that the *C. viswanathii* strain is more adapted to hydrophobic substrates (e.g. triolein and olive oil) for producing high levels of lipase and also in support of expressive cell growth. Gomes et al. (2018) reported that Pequi oil is an efficient inducer of lipase synthesis by *C. viswanathii*. Oleic acid has also been investigated as an important inducer of lipase production by several filamentous fungi and yeasts. On the other hand, the use of olive oil as a suitable carbon source could be related to the intrinsic composition of vegetable oils, and the presence of several tocopherols and additional liposoluble vitamins, which are important for microbial growth (Almeida et al., 2013).

Lipid accumulation in oleaginous microorganisms from hydrophobic carbon sources (fats and oils) is possible due to a primary anabolic process called the *ex novo* pathway, occurring simultaneously with the production of lipid-free material, which is independent of nitrogen exhaustion in the medium (Papanikolaou and Aggelis, 2010). Tauk-Tornisielo et al. (2009) observed

Table 1. Lipid accumulation and lipase from pure and complex triacylglycerols by *C. viswanathii*

Triacylglycerol	Predominant fatty acids	Lipid accumulation						Lipase production				
		g/L	$Y_{P/S}$	$Y_{P/X}$	P_{lipid}	q	lipid %	U/L	$Y_{P/S}$	$Y_{P/X}$	P_{lipase}	q
Tributyrin	4:0 (100%)	0.55	0.55	0.352	0.008	0.0049	35.2	1,900.0	190.0	1,217.9	26.4	16.9
Triolein	18:1 (100%)	3.45	3.45	0.445	0.048	0.0062	44.5	26,400.0	2,440.0	3,406.4	366.7	47.3
Olive oil	18:1 (79%)	2.75	2.75	0.395	0.038	0.0054	39.6	26,800.0	2,680.0	3,856.1	372.2	53.5
Sunflower oil	18:2 (66%)	2.40	2.40	0.330	0.033	0.0046	37.2	21,900.0	2,190.0	3,016.5	304.2	41.9
Linseed oil	18:3 (53%)	2.10	2.10	0.277	0.029	0.0038	27.7	18,800.0	1,880.0	2,483.5	261.1	34.5

Cultures were carried out in Vogel medium with 1% (w/v) vegetable oils or animal fats and 0.2% (w/v) of nitrogen source, pH 6.0, 72 h, 200 rpm at 28°C.

$Y_{P/S}$ – product for substrate consumed. P – productivity, P/h, q – specific rate of product formation, P/biomass/h, lipid – formation of lipid in cells, %.

the best values of lipid accumulation by oleaginous filamentous fungi when vegetable oils were used as carbon sources in the culture medium. Papanikolaou and Aggelis (2019) reported that oleaginous microorganisms capable of accumulating lipids should possess an active lipase system in their enzymatic arsenal. The free fatty acids would be incorporated by active transport inside cells. In this sense, the *C. viswanathii* strain has been demonstrated to be a versatile alternative to biotechnological processes. Guerfali et al. (2020) achieved 51.9% lipid (w/w) after 166 h using crude glycerol as the sole carbon source by *C. viswanathii* Y-E4. This bioprocess can efficiently be employed as an integrated production set with additional benefits to manufacturers by reducing the costs ascribed to by product transportation, disposal, and refining.

The fermentation parameters analyzed in this study also demonstrated the direct influence of fatty acid composition in vegetable oils on enzyme production and lipid accumulation. The carbon sources composed of long-chain fatty acids and monounsaturated bonds provided the best yield and productivity values for biomass production (olive oil: $Y_{X/S}$ = 0.68 g/g; triolein: P_X = 0.108 g/h; respectively). Sunflower oil (18:2, 66%, w/w) and linseed oil (18:3, 53%, w/w) showed lower values of lipid accumulation and lipase production of 31.1% (w/w) and 21.9 U/mL for sunflower oil; and 28.9% (w/w) and 18,800.0 U/L for linseed oil, respectively. Therefore, the highest enzyme productivity and lipid accumulation were also observed in carbon sources composed of monounsaturated fatty acids. The lowest values of all the fermentation parameters evaluated in this study were obtained from tributyrin as the sole carbon source.

Effects of nitrogen sources on single cell oil and catalyst formation

The effects of nitrogen on lipid accumulation and lipase production are shown in Table 2. An inorganic nitrogen source (NH_4NO_3) resulted in the highest biomass production using triolein or olive oil. However, the lipase production increased with an organic nitrogen source (yeast extract) plus olive oil or triolein (26,800.0 U/L and 23,600.0 U/L, respectively), whereas ammonium nitrate was a poor inducer of lipase production using both carbon sources (olive oil: 16,400.0 U/L; triolein: 12,900.0 U/L). Nitrogen sources play an important role in the synthesis of enzymes. Since inorganic nitrogen sources can be metabolized quickly, organic nitrogen sources generally supply amino acids and additional growth factors which are necessary for cell metabolism and enzyme synthesis. Therefore, both organic and inorganic sources are used for lipase production from microbial cultivation (Mendes et al., 2019). Gomes et al. (2018) observed that peptone and yeast extract sources promoted high levels of lipase

Table 2. Lipid accumulation and lipase production from yeast extract and ammonium sulfate by *C. viswanathii*

Source of nitrogen	Lipid accumulation						Lipase production				
	g/L	$Y_{P/S}$	$Y_{P/X}$	P_{lipid}	q	lipid %	U/mL	$Y_{P/S}$	$Y_{P/X}$	P_{lipase}	q
Yeast extract											
Triolein	3.45	0.345	0.445	0.048	0.0062	44.5	26,400.0	2,640.0	3,406.4	366.7	47.3
Olive oil	2.75	0.275	0.395	0.038	0.0054	39.6	26,800.0	2,680.0	3,856.1	372.2	53.5
Ammonium nitrate											
Triolein	1.60	0.160	0.181	0.022	0.0025	18.2	12,900.0	1,290.0	1,461.0	179.2	20.3
Olive oil	3.40	0.340	0.407	0.047	0.0056	40.7	16,400.0	1,640.0	1,964.1	227.8	27.3

Cultures were carried out in Vogel medium with 1% (w/v) vegetable oils or animal fats and 0.2% (w/v) of nitrogen source, pH 6.0, 72 h, 200 rpm at 28°C.
$Y_{P/S}$ – product for substrate consumed. P – productivity, P/h, q – specific rate of product formation, P/biomass/h, lipid – formation of lipid in cells, %.

production by the *C. viswanathii* strain. Cultivation of *C. viswanathii* on solid-state substrates supplemented with yeast extract caused a 3.8-fold increase in lipase production (Almeida et al., 2016).

Oleaginous yeast growth in nitrogen limiting media along with excess carbon sources promotes the synthesis and accumulation of lipids in the form of triacylglycerols followed by significant modifications of the microbial growth rate (Takaku et al., 2020). On the other hand, the accumulation of lipids by the *ex novo* pathway from hydrophobic carbon sources may be independent from nitrogen exhaustion in the cultivation medium (Papanikolaou and Aggelis, 2010). In this work, cultivations of *C. viswanathii* CCR8137 using yeast extract resulted in a lipid production of 3.45 g/L (44.5%, w/w) with triolein as the carbon source. The addition of ammonium nitrate as a nitrogen source resulted in a lipid production of 3.40 g/L (40.7%, w/w) with olive oil and 1.6 g/L (18.2%, w/w) with triolein. Guerfali et al. (2020) reported the accumulation of lipids by *C. viswanathii* Y-E4 cultivated under nitrogen limiting conditions using yeast extract as the nitrogen source. Co-production of cellulase and lipid accumulation were evaluated under nitrogen limiting conditions by *Candida orthopsilosis* Y09GS34, which was able to simultaneously produce an extracellular cellulase and a single cell oil, which reached up to 63.74% of dry biomass (Kanti and Sudiana, 2015).

The fermentation parameters analyzed in this study showed the highest biomass yield ($Y_{X/S}$ = 0.92 g/g) from the association of triolein and NH_4NO_3. The biomass productivity under these experimental conditions was P_X = 0.123 g/h. The lipid accumulation yield per gram of consumed carbon source showed the highest mean value from cultivations containing triolein and yeast extract ($Y_{P/S}$ = 0.345 g/g), followed by olive oil plus NH_4NO_3 ($Y_{P/S}$ = 0.340 g/g). The value of lipid accumulation in cells obtained from cultivations containing triolein plus yeast extract was 44.5% of the total biomass, whereas the lipid accumulation specific rate showed a mean value of q = 0.0062 g/g/h. Similar values were observed in olive oil and NH_4NO_3 (q = 0.0056 g/g/h and 40.4% of the total biomass). On the other hand, the highest values of lipase yield by biomass, lipase productivity and lipase specific production rate were observed with olive oil plus yeast extract ($Y_{P/X}$ = 3,856.1 U/g, P = 372.2 U/h, q = 53.5 U/g/h).

Effects of glucose on lipid accumulation and lipase production

In these experiments, glucose was used as the sole carbon source and also added to the culture medium containing olive oil in the initial lag phase (Fig. 1). The cell morphology of *C. viswanathii* CCR8137 observed in cultivations containing olive oil and olive oil plus glucose showed typical oval shaped cells. Although the lipid accumulation in oleaginous microorganisms is related to carbohydrate sources (Athenaki et al., 2018; Gardeli et al., 2017), *C. viswanathii* CCR8137 presented cell morphological stress in the presence of glucose resulting in elongated cells with a pseudo hyphae formation.

When glucose was added to the culture medium as the sole carbon source, both lipid accumulation and enzyme production suffered a sharp decrease compared to cultivations containing hydrophobic substrates. Under these conditions, the lipid accumulation was 16.11% (w/w), whereas lipase production was 154.80 U/L with a biomass value of 3.6 g/L (Fig. 1). The highest growth and lipase production were observed with olive oil as the sole carbon source. The addition of glucose to the culture medium with olive oil resulted in decreased values of enzyme production and cell growth, although lipid accumulation increased 25% after 96 h cultivation (63.10%, w/w), compared with the highest values obtained from oleaginous carbon sources (Table 3). Lipolytic activity in oleaginous microorganisms is a tool for the assimilation of free fatty acids into the cells, which are utilized for growth or energy storage in the form of lipid droplets. These fatty acids can be modified in order to synthesize new fatty acids by *ex novo* lipid accumulation (Aggelis and Sourdis, 1997; Athenaki et al., 2018). Lipid accumulation from a glucose source is attributed to a fundamental physiological requirement, which is an excess of carbon source and nitrogen limitation leading to *de novo* lipid accumulation. Ayadi et al. (2016) observed an association of high lipase activity with lipid accumulation by *C. viswanathii* Y-E4, whereas *Saccharomyces cerevisiae* CTM-30015 showed low lipase activity and lipid accumulation.

The fermentation parameters were obviously influenced by the culture conditions, presenting the best results for lipase conversion ($Y_{P/S}$ = 2,367.91 U/g; q_L = 1.644 U/g/h, P = 328.90 U/h) from olive oil (Table 3).

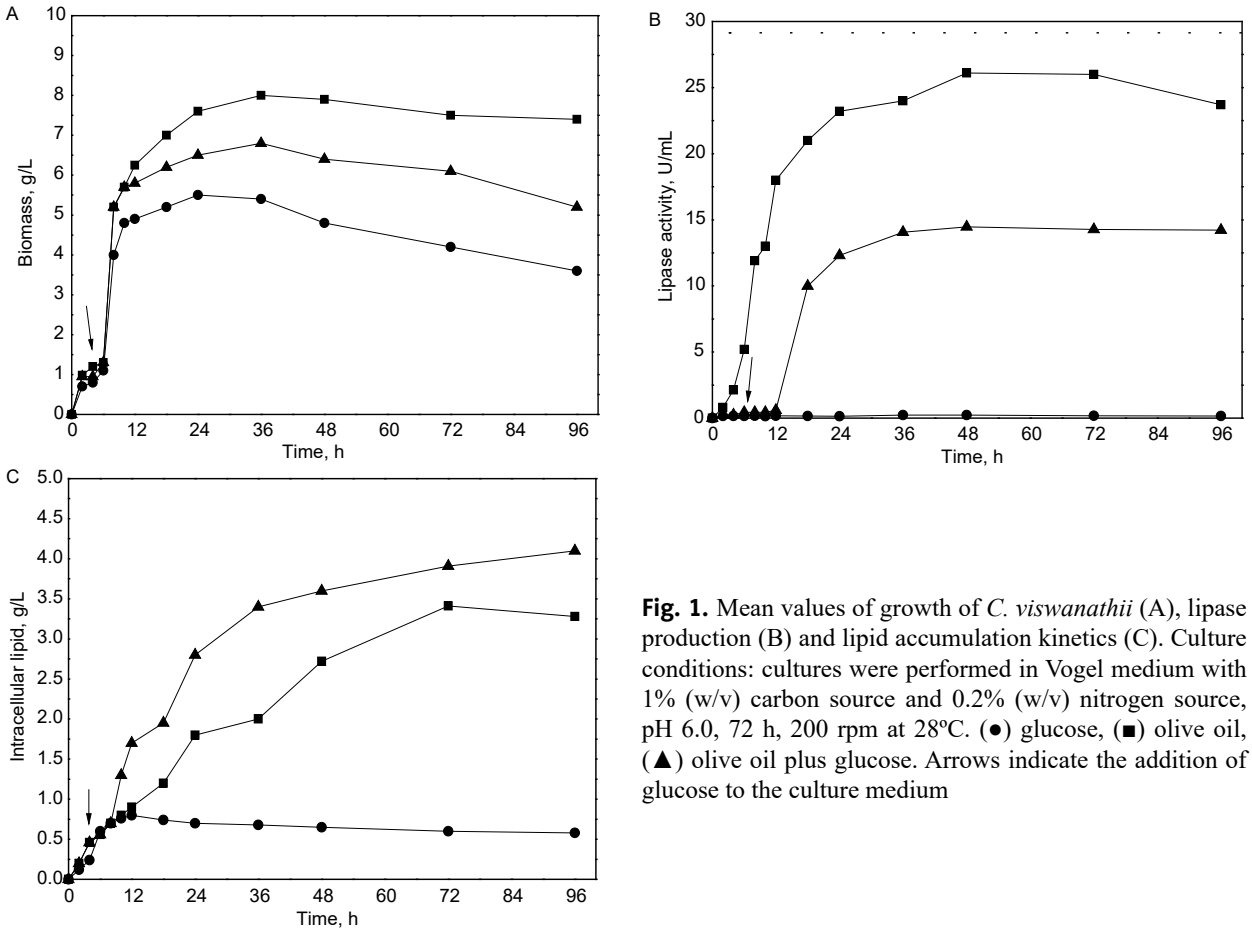

Fig. 1. Mean values of growth of *C. viswanathii* (A), lipase production (B) and lipid accumulation kinetics (C). Culture conditions: cultures were performed in Vogel medium with 1% (w/v) carbon source and 0.2% (w/v) nitrogen source, pH 6.0, 72 h, 200 rpm at 28ºC. (●) glucose, (■) olive oil, (▲) olive oil plus glucose. Arrows indicate the addition of glucose to the culture medium

Table 3. Lipid accumulation, lipase and biomass production by *C. viswanathi* after 96 h cultivation

Parameters	Glucose	Olive oil	Olive oil + glucose
Biomass, g/L	3.6	7.4	5.2
Lipase, U/L	154.80	23,679.16	14,222.10
Lipid, g/L	0.58	3.28	4.10
Conversion of substrate in lipase ($Y_{lipase/S}$)	15.48	2,367.91	1,422.21
Conversion of substrate in lipid ($Y_{lipid/S}$)	0.058	0.328	0.41
Conversion of substrate in biomass ($Y_{X/S}$)	0.36	0.74	0.52
Lipase conversion rate (q_L)	0.011	1.644	0.99
Lipid conversion rate (q_L)	0.0022	0.0061	0.0088
Productivity lipase (P)	2.15	328.90	197.52
Productivity lipid (P)	0.008	0.045	0.042

The maximum lipid accumulation was obtained from the medium containing olive oil plus glucose after 96 h cultivation (4.1 g/L, $Y_{P/X}$ = 0.41 g/g, q = 0.0088 g/g/h). Cultivations containing glucose as the sole carbon source resulted in lower lipid accumulations (0.58 g/L).

Fatty acid profile of single cell oil from *C. viswanathii* CCR 8137

The content of unsaturated fatty acids increased when glucose was used as the sole carbon source or added to olive oil (86.06% and 85.62%, w/w, respectively); whereas the saturated fatty acid content decreased (Table 4). The main fatty acid in the lipid composition was oleic acid (60.69–69.31%, w/w), followed by linoleic acid (12.55–12.84%, w/w). Linoleic acid was not detected in cultivations containing olive oil as the sole carbon source. Guerfali et al. (2020) showed a similar

Table 4. Fatty acid profile produced by *C. viswanathii* from different carbon sources

Parameters, %	Nomenclature	Glucose	Olive oil	Olive oil + glucose
Total lipid		16.11 ±0.89	55.40 ±0.13	63.10 ±2.87
Saturated fatty acid		13.94 ±0.40	33.36 ±0.51	14.38 ±0.76
Unsaturated fatty acid		86.06 ±2.87	66.64 ±0.86	85.62 ±4.46
Myristic acid	C 14:0	n.d.	n.d.	n.d.
Palmitic acid	C 16:0	10.80 ±0.28	17.56 ±0.13	10.80 ±0.59
Palmitoleic acid	C 16:1 Δ^{cis-9}	1.19 ±0.05	5.95 ±0.13	1.31 ±0.08
Stearic acid	C 18:0	3.14 ±0.12	15.79 ±0.38	3.59 ±0.18
Oleic acid	C 18:1Δ^{cis-9}	69.31 ±2.33	60.69 ±0.72	68.84 ±3.55
Vaccenic acid	C 18:1 $\Delta^{trans-11}$	1.80 ±0.05	n.d.	1.79 ±0.09
Linoleic acid	C 18:2 $\Delta^{cis-9,12}$	12.55 ±0.40	n.d.	12.84 ±0.69
Linolenic acid	C 18:3 $\Delta^{cis-6,9,12}$	0.79 ±0.03	n.d.	0.85 ±0.05
Eicosadienoic acid	C 20:2 $\Delta^{cis-11,14}$	0.42 ±0.01	n.d.	n.d.

fatty acid composition of lipids produced from glucose and pure glycerol by *C. viswanathii* Y-E4. The major component of the fatty acid profile was oleic acid (62.9–65.5%, w/w) followed by palmitic acid (9.8–14.8%, w/w) and stearic acid (8.5–9.3%, w/w). The accumulated lipid from glycerol showed a fatty acid profile mainly composed of linoleic acid (45%, w/w) and oleic acid (28.6%, w/w).

Interestingly, the presence of glucose in the culture medium promoted the production of vaccenic acid, linolenic acid and eicosadienoic acid, whereas these fatty acids were not detected in the lipids obtained from cultivations containing solely olive oil. The utilization of olive oil as the sole carbon source for single cell oil production could be a key factor in promoting the selective uptake of fatty acids into the cells, which were incorporated into microbial lipids synthesized by the *ex novo* pathway. In contrast, the use of glucose as the sole carbon source or added to olive oil could induce the expression of the *de novo* synthesis pathway, and consequently, elongases and desaturases could be involved in the synthesis of polyunsaturated long-chain fatty acids under nitrogen limiting conditions (Finco et al., 2016; Garay et al., 2014; Papanikolaou and Aggelis, 2003; Papanikolaou and Aggelis, 2010). The main fatty acids present in olive oil are oleic (55.0–83.0%, w/w), palmitic (7.5–20%, w/w), linoleic (3.5–21.0%, w/w) and stearic (0.5–5.0%, w/w) (Boskou et al., 2006). Vaccenic acid observed in the lipid accumulated by *C. viswanathii* CCR8137 has been associated with cytotoxic effects against tumoral cells due to its conversion to c9,t11-conjugated linoleic acid (Song et al., 2019). Vaccenic acid is naturally present in plant and milk lipids and can be obtained industrially by the incomplete biohydrogenation of polyunsaturated fatty acids. Linolenic and eicosadienoic acids are omega-6 series polyunsaturated fatty acids, widely recognized as nutraceutical molecules with attributed health benefits such as being prophylactic agents which reduce the risk of cardiovascular disease and fatal ischemic heart disease, as well as being preventive molecules of inflammatory responses and colorectal cancer (Cordova and Alper, 2018; Taha et al., 2019).

Among saturated fatty acids, myristic acid was not detected in the analyzed samples. Palmitic acid and stearic acid were detected in the lipid accumulated by *C. viswanathii* CCR8137 with fractions of about 10% and 3% (w/w) in the presence of glucose, and 17.56% and 15.80% (w/w), respectively, from olive oil as the sole carbon source.

CONCLUSION

Co-production of lipase and microbial lipid by *C. viswanathii* CCR8137 can be part of a fundamental industrial bioprocess which may offer prominent cost-effective food products of a high nutritional grade. The high levels of lipase production and lipid accumulation observed in this study highlight that *C. viswanathii* CCR8137 has several promising industrial applications. The fatty acid profile obtained from yeast cultivations exhibited a single cell oil rich in omega-9 family fatty acids, and the addition of glucose to the culture medium resulted in omega-3 and omega-6 family constituents, which are attractive molecules for nutraceuticals and dietary supplements.

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