

Research Article

## Lipid yields from oleaginous yeasts isolated from the north Peruvian Andes by culture media non-limiting nitrogen

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### Article Info

<https://doi.org/10.31018/jans.v13i2.2670>

Received: April 15, 2021

Revised: May 24, 2021

Accepted: May 29, 2021

### How to Cite

Alexander, M.P.C. *et al.* (2021). Lipid yields from oleaginous yeasts isolated from the north Peruvian Andes by culture media non-limiting nitrogen. *Journal of Applied and Natural Science*, 13(2), 607 - 615. <https://doi.org/10.31018/jans.v13i2.2670>

### Abstract

Oleochemicals can be obtained from oily yeasts due to their ability to produce a high lipid content. This research aimed to isolate them from the North Peruvian Andes with a lipid content greater than 20%. They were identified by sequencing internal transcribed spacer regions ITS of conserved ribosomal DNA (rDNA), evaluate their growth kinetics, biomass and lipid yields, using culture media with C/N 100:1+xylose (MS-1-7) and 2:1+glucose (MS-2-7). Growth kinetics up to the maximum stationary phase was evaluated using the parameterized Gompertz type II model. *Rhodotorula glutinis*, *R. mucilaginosa*, and *R. kratochvilovae* were selected. The C/N ratio in the culture medium influenced growth kinetics, biomass and lipids yields. With MS-1-7, a high specific growth rate ( $\mu_{max}$ ) was obtained, reaching the stationary phase between 6 to 9 h and the highest lipid accumulation between 23.1% and 31.5%. With the MS-2-7 medium, maximum biomass value obtained in the stationary phase between 37 and 51 h, which generated the highest biomass yields at the end of the entire process and lipid yield of 4.65, 5.59, and 8.80 g L<sup>-1</sup> in the strains mentioned. There is potential to obtain high lipid yields using a culture media non-limiting nitrogen, examining not only the C/N ratio. But also, the quantities, nature of the components, and type of oleaginous yeasts taking care to avoid a high carbon concentration to prevent the Crabtree effect.

**Keywords:** Lipid yield, Limiting and Non-limiting nitrogen, *Rhodotorula glutinis*, *Rhodotorula mucilaginosa*, *Rhodotorula kratochvilovae*

### INTRODUCTION

Oleochemicals are products derived from green chemistry. They are obtained from renewable raw materials, with a low level of potential hazards, which generate common toxicity substances. Their long chains and double bonds produce transesterification, epoxidation, amidation, and amination reactions (Zarli, 2020). Their growing economic potential is found in the markets for

food, personal care, detergents, cosmetics, pharmaceuticals, medicals, paints, resins, emulsifiers, plasticizers, biolubricants, biodiesel, and jet fuel (Spagnuolo *et al.*, 2019; Maina *et al.*, 2016). This demand is related to population growth and the interest in using products based on renewable and biocompatible resources (Zarli, 2020). There are also limitations on agricultural land expansion and concerns and environmental policy changes (OECD-FAO, Agricultural Out-

look 2015–2024) (Adrio, 2017). It is possible to obtain lipids or chemical oils sustainably by microbial synthesis, whose traditional production is based on raw materials of vegetable oils and fats. Oleaginous yeasts can synthesize them using glucose, xylose, L-arabinose, CO<sub>2</sub>, methane, or methanol (Zhang *et al.*, 2021). These are the most promising microorganisms in the production of the high content of cellular lipids. Compared to microalgae, molds, and bacteria, they can accumulate more than 70% of their biomass due to their high growth rate, productivity, and low areas required for production since they can be grown in bioreactors under controlled conditions (Vasconcelos *et al.*, 2019). Also, it can be used as raw material to produce biodiesel and adapted to the production of nutraceuticals using metabolic engineering (Dey and Maiti, 2013; Patel *et al.*, 2020). Also, can provide sustainable production of oleochemicals. They synthesize and accumulate lipids, primarily as triacylglycerides (Adrio, 2017). Besides using pure sugars as a substrate, it also can use waste rich in sugars (molasses and cheese whey), lignocellulosic materials, glycerol derived from biodiesel, starch, etc., residues from food processing (Maina *et al.*, 2016). Hydrolysis of lignocellulosic materials as pentoses and hexoses can be obtained (Maina *et al.*, 2016).

Different species of yeasts with oleaginous backgrounds of the genus *Candida*, *Cryptococcus*, *Rhodospiridium*, *Rhodotorula* have been found in the Arctic and Antarctica, at temperatures below -20 °C. Also, in coniferous forests in European Alps, Mont Blanc (4 810 m.a.s.l.) (Buzzini *et al.*, 2012; Zalar and Gunde-Cimerman, 2014; Yurkov *et al.*, 2012).

The main barrier for the industrial production of lipids is the cost at the fermentation stage, therefore adjusting it to an oleaginous system is imperative to obtain a higher yield (Karamerou and Webb, 2019). In this context, it has been reported that with a high C/N ratio of 84.9, 211.0, 76.9 using crude glycerol and glucose as a carbon source, high lipid contents in the cellular biomass of 54.3%, 65.1%, 45.51% respectively has been obtained with *Lipomyces starkeyi*, *Rhodospiridium turoloides*, and *Yarrowia lipolytica*. It is crucial to avoid a high C/N ratio that implies a high substrate concentration, producing growth inhibition and a fall in cell and lipid yield (Christophe *et al.*, 2012).

Also, it was reported that, due to the nitrogen limitation caused by an increase in carbon in the culture medium, high content of cellular lipids is produced in yeasts. Still, on the other hand, lower production of cellular biomass occurs, converting the carbon source by biosynthesis into storage lipids (Ratledge, 2002). Thus, some species can accumulate lipids up to 70% of their biomass dry weight (Papanikolaou and Aggelis, 2011). Studies carried out with the oleaginous yeasts *Cryptococcus curvatus* have shown that when is cultivated in lactose

and sucrose with limited nitrogen, there is an accumulation of a large amount of total intracellular sugars of up to 68% in the initial stage of fermentation, which decreases to 20% at the end (Tchakouteu *et al.*, 2014). The high content of lipids with deficiency or nitrogen-free has been found in 35 species of oleaginous yeasts. An increase in the C/N molar ratio from 150 to 350 increases the conversion efficiency of glucose to lipid in *Rhodotorula glutinis* from 0.25 to 0.40, but it has detrimental effects on cell viability. An exception to this is *Cryptococcus terricola*, which accumulates lipids during logarithmic growth instead of doing it later, and in the presence of excess nitrogen (Sitepu *et al.*, 2013; Sitepu *et al.*, 2014). Regardless of the carbon source, higher C/N ratios increase lipid yields in *R. toruloides* but decrease the specific growth rate (Lopes *et al.*, 2020). A C/N ratio greater than 20 stimulates lipid biosynthesis in oleaginous yeasts without producing a significant increase in biomass (Kot *et al.*, 2019). This research aimed to isolate oleaginous yeasts from the North Peruvian Andes with a lipid content greater than 20%. Identify them, evaluate their growth kinetics, biomass and lipid yields using culture media with C/N 100: 1 with xylose as carbon source, limiting nitrogen; and 2:1 with glucose as carbon source, non-limiting nitrogen.

## MATERIALS AND METHODS

This study was carried out at the Biomolecules Laboratory, Agroindustrial Science Department, National University of Trujillo, Peru.

### Equipment

Vertical autoclave, drying stove 1 400 W; analytical balance 205 g with precision 0.0001 g; centrifuge 6 000 rpm; optical microscope 40x; orbital shaker incubator four at 70 °C, 40-400 rpm; sonicator/ultrasonic bath 45 kHz with heater and temperature control  $\pm 3$  °C; vortex mixer 2 850 rpm; UV-C camera; multifunction gas extractor; Neubauer chamber.

### Reagents

Xylose, anhydrous glucose, peptone, yeast extract, agar, magnesium sulfate heptahydrate MgSO<sub>4</sub>·7H<sub>2</sub>O, sodium chloride NaCl, calcium chloride CaCl<sub>2</sub>, ammonium sulfate (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, monopotassium phosphate KH<sub>2</sub>PO<sub>4</sub>, chloramphenicol, citric acid C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>, phosphate sodium Na<sub>2</sub>HPO<sub>4</sub>, glycerol C<sub>3</sub>H<sub>8</sub>O, formaldehyde CH<sub>2</sub>O, absolute ethanol C<sub>2</sub>H<sub>5</sub>OH, petroleum ether, methylene blue.

Fig. 1 shows the sequence for isolation, treatment with ultrasound, and heat to determine the lipid content, molecular identification, and cultures obtained to evaluate growth kinetics in shake flasks, dry weight biomass yield, and lipids in oleaginous strains.

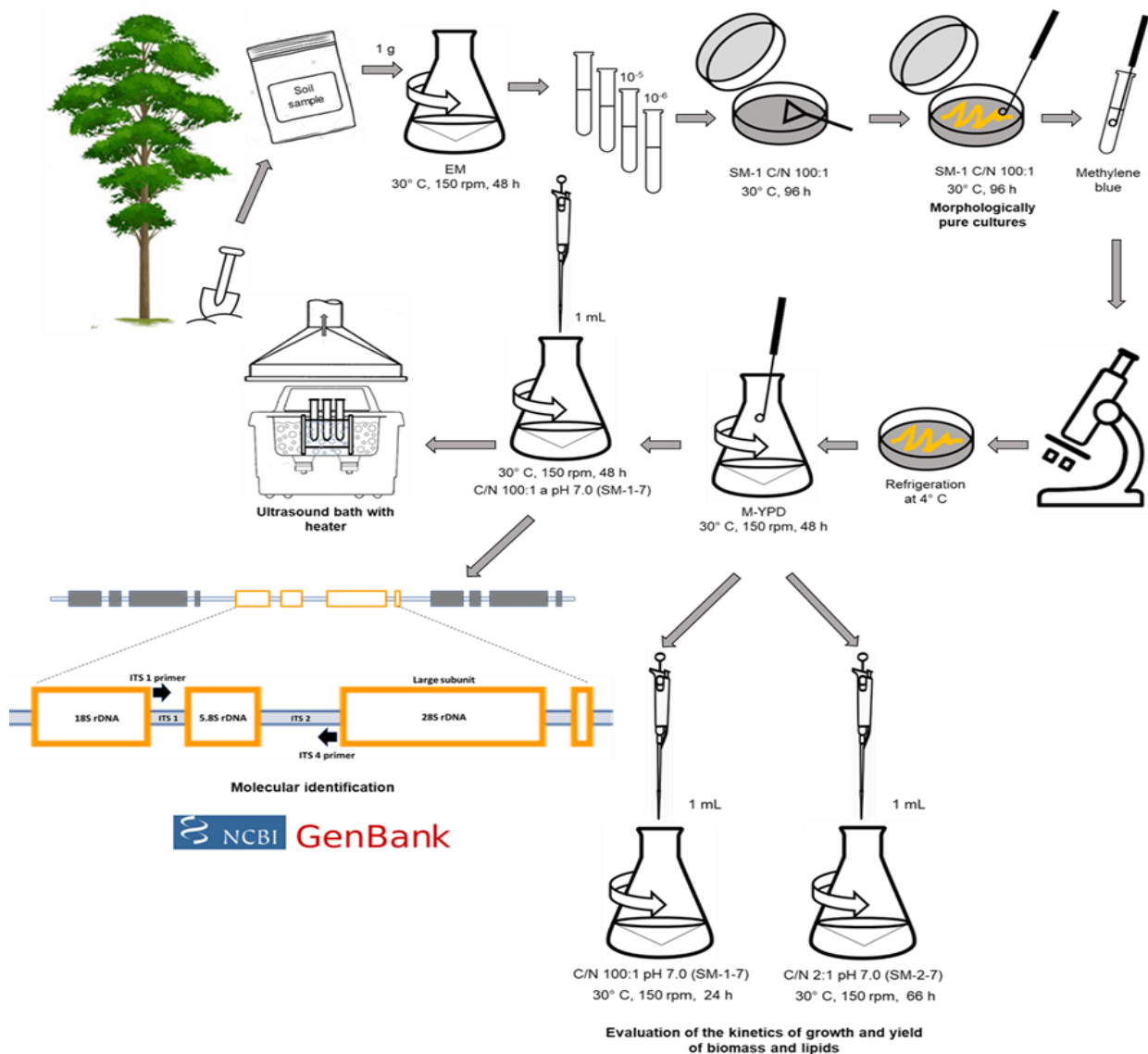
## Yeast isolation

Yeasts were isolated from nearby forests of the Porcon farm - Cajamarca, Peru (7° 02' 10.69" S 78° 38' 04.39" W, 3 148.5 m.a.s.l.; 7° 02' 32.66" S 78° 38' 28.87" W, 3 303.4 m.a.s.l.) and the El Paraiso forests - La Libertad, Peru (8° 00' 07.25" S 78° 27' 51.27" W, 3 398 m.a.s.l.). Approximately 20 g were taken per soil sample from random points forming a triangle at the sampling site, placed in ziplock bags. The samples were stored in refrigerators at 4 °C. From each homogenized sample, 1 g was taken, which was supplemented with an enrichment medium of composition (g L<sup>-1</sup>): glycerol 100, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1, KH<sub>2</sub>PO<sub>4</sub> 1, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.5, yeast extract 0.5. They were incubated at 30 °C and shaking at 150 rpm for 48 h. Serial dilutions of the enrichment medium were made up to 10<sup>-5</sup> and 10<sup>-6</sup>. These dilutions were seeded onto a surface in sterile Petri dishes

(previously dried at 20 °C for 12 h) with selective medium (SM-1) solid C/N 100: 1 and incubated at 30 °C for 96 h. The SM-1 composition (g L<sup>-1</sup>) was: xylose 20, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 5, KH<sub>2</sub>PO<sub>4</sub> 1, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.5, yeast extract 0.5, agar 20, distilled water 1 000 mL, chloramphenicol 0.05. The yeasts obtained were streaked again in SM-1 to get morphologically pure cultures incubated at 30 °C for 96 h. A yeast colony was selected at random, and simple staining with methylene blue was performed and observed under a microscope to verify its morphology. The cultures obtained were kept refrigerated at 4 °C.

## Form of calculation of the carbon-nitrogen ratio (C/N).

The carbon-nitrogen 2:1 ratio (Equation 1) was calculated taking glucose as a carbon source (20 g L<sup>-1</sup>), the nitrogen content of peptone (20 g L<sup>-1</sup>), and yeast ex-



**Fig. 1.** Schematic flow diagram for the isolation of oleaginous yeasts, treatment to determine lipid content, molecular identification and obtaining cultures to evaluate growth kinetics and biomass and lipid yield.

tract ( $10 \text{ g L}^{-1}$ ), where 40% is the percentage of carbon contained in glucose, 14.7%, and 10.5% is the percentage of nitrogen contained in peptone and yeast extract respectively. The carbon-nitrogen 100:1 ratio (Equation 2) was calculated by taking xylose as a carbon source ( $20 \text{ g L}^{-1}$ ). The nitrogen content was taken from ammonium sulfate ( $0.762 \text{ g L}^{-1}$ ), 40% of the carbon in xylose, and 21% of the nitrogen in ammonium sulfate.

$$\text{C/N} = \text{Glucose (g L}^{-1}\text{)} * 20 * 40 \% / \text{Peptone (g L}^{-1}\text{)} * 20 * 14.7\% + \text{yeast extract (g L}^{-1}\text{)} * 10 * 10.5\% = 2:1 \quad (\text{Eq.1})$$

$$\text{C/N} = \text{Xylose (g L}^{-1}\text{)} * 20 * 40\% / \text{Ammonium sulphate (g L}^{-1}\text{)} * 0.762 * 21 \% = 100:1 \quad (\text{Eq.2})$$

### Selection

The material was taken with a Kolle handle loop from each isolated sample and seeded by manual shaking in flasks with medium (M-YPD), incubating at  $30 \text{ }^\circ\text{C}$  with a shaking of 150 rpm for 48 h. The M-YPD (yeast extract peptone dextrose) had the composition ( $\text{g L}^{-1}$ ): yeast extract 10, peptone 20, glucose 20, 1 000 mL distilled water. Next, 1 mL of the samples enriched in M-YPD were added to the flasks with medium C/N 100:1 at pH 7.0 (SM-1-7) and incubated at  $30 \text{ }^\circ\text{C}$  with a shaking of 150 rpm for 48 h. The composition ( $\text{g L}^{-1}$ ) of the SM-1-7 medium was: xylose 40% 100 mL (400 g of xylose in 1 000 mL of water),  $(\text{NH}_4)_2\text{SO}_4$  0.762, NaCl 0.1,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.5,  $\text{CaCl}_2$  0.1, yeast extract 0.1, citrate phosphate buffer 900 mL (citric acid solution 741.15 mL and bibasic sodium phosphate solution 158.85 mL).

### Determination of lipid content

In MS-1-7 medium, 12 mL of the yeast culture was taken and added to previously weighed capped tubes. They were centrifuged at 3 000 rpm for 10 min, discarding the supernatant. The tubes, without caps, with yeast biomass, were dried at  $50 \text{ }^\circ\text{C}$  for 48 hours, then the tubes were capped and weighed, and determined the initial dry biomass, expressed as  $\text{g mL}^{-1}$ . Next, 5 mL of 5%  $(\text{NH}_4)_2\text{SO}_4$  solution was added, placing it on ice for 2 minutes, then placed in an ultrasound bath for 2 minutes at  $25 \text{ }^\circ\text{C}$  and 45 kHz. It was centrifuged at 3 000 rpm for 5 minutes; discarded the supernatant. Added 3 mL of absolute ethanol, shaken vigorously on agitator vortex for 5 seconds, then 1.25 mL of chloroform and 1.25 mL of petroleum ether were added, and then shaken in the vortex for 10 seconds. The tubes were then centrifuged at 3 000 rpm for 10 min. Then placed in a water bath equipped with a multifunction gas extractor for 2 hours at  $50 \text{ }^\circ\text{C}$ . Subsequently, they were placed in an oven at  $50 \text{ }^\circ\text{C}$  for 96 h to determine the final dry biomass. Equation 3 determined the percentage of the lipid content:

$$(\text{Initial dry biomass} - \text{final dry biomass}) / (\text{Initial dry biomass}) * 100 \quad (\text{Eq. 3})$$

Samples with a lipid content greater than 20% were seeded in solid M-YPD medium and kept refrigerated at  $4 \text{ }^\circ\text{C}$ .

### Molecular identification

Amplification of the genetic material of the sequences of internal transcribed spacer regions ITS of conserved ribosomal DNA (rDNA) was carried out using the Sanger technique. The analysis was limited to identifying the sequence similarities of the ITS regions (Gientka *et al.*, 2017).

### Form of evaluation of the kinetics of growth of oleaginous yeasts in MS-1-7 and MS-2-7 media

Material with a Kolle handle loop was taken from each isolated strain and seeded by manual shaking in flasks with M-YPD medium and incubated with shaking at 150 rpm at  $30 \text{ }^\circ\text{C}$  for 48 h. Enriched samples (1 mL) was taken and added to 50 mL flasks with MS-1-7 and MS-2-7 media, both at pH 7.0. The media were then incubated at  $30 \text{ }^\circ\text{C}$  with shaking at 150 rpm for 24 and 66 h, respectively. Yeast growth was quantified at 1 h intervals using the Neubauer chamber and 40X light microscopy. The Gompertz type II parameterized model contrasted the growth (Tjørve and Tjørve, 2017) (Equation 4):

$$Y = A \exp[-\exp(b - K_G t)] \quad (\text{Eq. 4})$$

Where: Y:  $\log(N/N_0)$ , N is the number of yeasts (cells  $\text{mL}^{-1}$ ) as a function of time t (h) and  $N_0$  is the number of initial yeasts (cells  $\text{mL}^{-1}$ ) at time t = 0 h. A is the upper asymptote (absolute maximum growth value), b is a constant,  $K_G$  is the coefficient of the growth rate. With the use of Software R v.3.6.1., the growth curves were plotted and the parameters were determined as follows: specific growth speed  $\text{h}^{-1}$  ( $\mu_{\max} = A * b$ ), latency phase h ( $\lambda = (b-1)/K_G$ ) and generation time h ( $G = \ln 2 / \mu_{\max}$ ).

### Oil yeast lipid yield in MS-1-7 and MS-2-7 media.

After 24 hours of growth in MS-1-7 medium, 66 h in MS-2-7 medium, evaluated the lipid content following the procedures described above and their yield.

## RESULTS

### Isolation and selection of yeasts for lipid content

Three strains were selected from 18 cultures obtained from samples taken in the geographical areas of the North Peruvian Andes. The strains were isolated from nearby forests of the Porcon farm – Cajamarca: CON-5 and POR-3 and El Paraiso forests - La Libertad: EP-5, with lipid content of 31.5%, 29.7%, and 23.1%, respectively.

### Molecular identification

The ITS regions of the rDNA of the yeast strains encod-

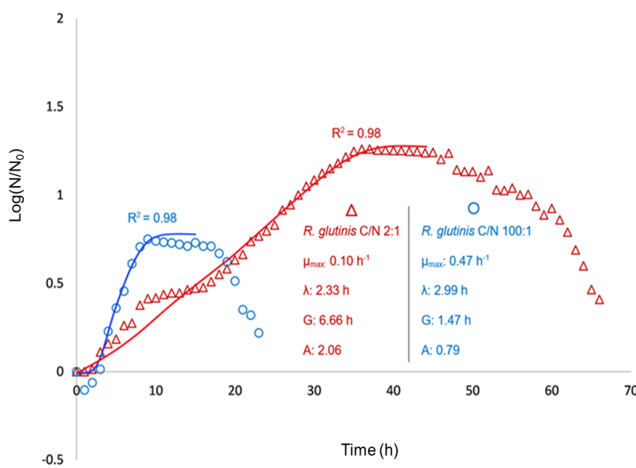
ed as CON-5, EP-5, and POR-3, were identified: *Rhodotorula glutinis*, *Rhodotorula mucilaginosa*, and *Rhodotorula kratochvilovae*, respectively. The primers 5'-3' ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) were used to identify which were incorporated into Genbank as LC413754.1; MK215798.1, and LC390313.1, respectively.

**Evaluation of the growth kinetics of oleaginous yeasts in MS-1-7 (C/N 100: 1) and MS-2-7 (C/N 2: 1) media**

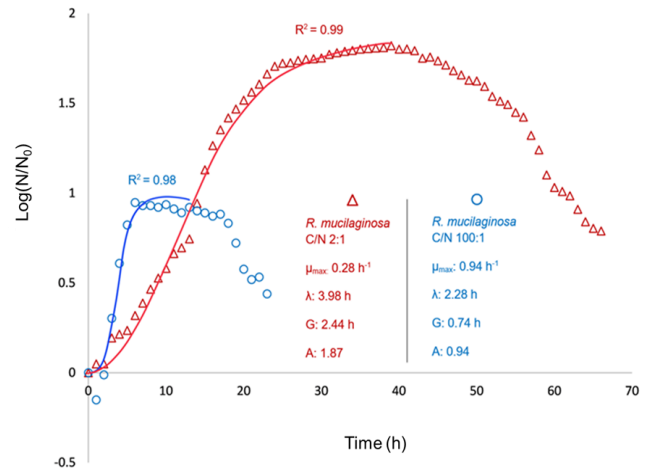
In Fig. 2, 3, and 4, the growth of the three strains of *Rhodotorula* (*glutinis*, *mucilaginosa*, and *kratochvilovae*) observed in MS-2-7 medium (C/N 2:1) took a long time to reach a higher value of cellular biomass A (log N/No), compared to that obtained with the MS-1-7 medium (C/N 100:1). The three strains of *Rhodotorula* in MS-2-7 were determined to reach

their maximum value of A at 37, 39, and 51 h, values higher than 9, 6 and 9 h obtained in MS-1-7.

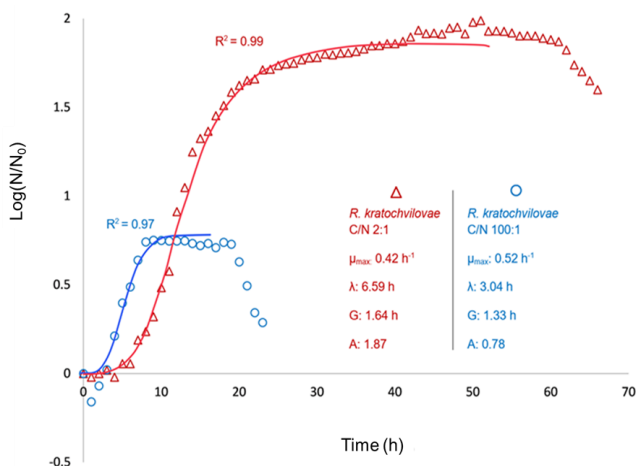
Similarly, Fig. 2, 3, and 4 show the curves modeled with the parameterized Gompertz type II model for the growth of the three *Rhodotorula* strains up to a maximum stationary phase, in the media with C/N 2:1 and C/N 100:1, with their respective kinetic parameters indicating statistical consistency of the model with R<sup>2</sup> values between 97% and 99%. The three strains of *Rhodotorula* showed a lower value of μ<sub>max</sub>, higher values of A and G using the medium MS-2-7 (C/N 2:1) compared to MS-1-7 medium (C/N 100:1) was used. Regarding the duration of λ, *R. glutinis* in medium with a C/N ratio of 2:1 was 2.33 h, less than the λ of 2.99 h obtained in medium with C/N ratio of 100:1. For *R. mucilaginosa* and *R. kratochvilovae*, the values were the opposite, since *R. mucilaginosa* and *R. kratochvilovae* in medium with C/N 2:1 ratio presented λ of 3.98 and 6.59 h respectively, while in medium with C/N



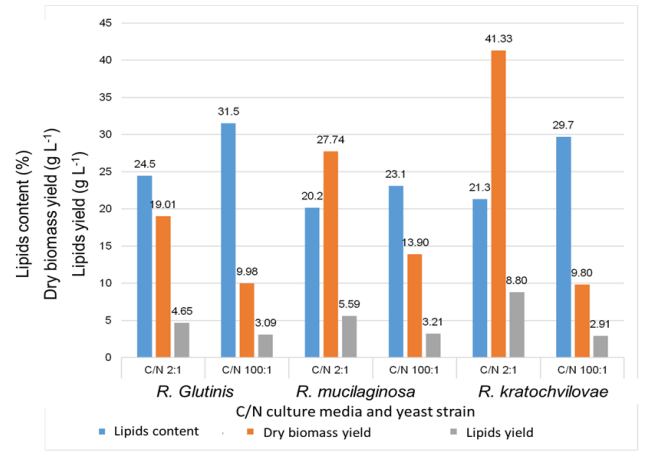
**Fig. 2.** Growth curve of *R. glutinis* in the culture media MS-2-7 and MS-1-7 with C/N 2:1 and C/N 100:1 ratios, respectively.



**Fig. 3.** Growth curve of *R. mucilaginosa* in the culture media MS-2-7 and MS-1-7 with C/N 2:1 and C/N 100:1 ratios, respectively.



**Fig. 4.** Growth curve of *R. kratochvilovae* in the culture media MS-2-7 and MS-1-7 with C/N 2:1 and C/N 100:1 ratios, respectively.



**Fig. 5.** Lipid content, dry biomass and lipid yields in the three *Rhodotorula* strains using culture media with C/N 2:1 and 100:1.

ratio 100:1 presented a lower value of  $\lambda$ , which was 2.28 and 3.04 h respectively.

**Lipid yield with MS-1-7 (C/N 100:1) and MS-2-7 (C/N 2: 1) media.** Fig. 5 shows the behavior of the three *Rhodotorula* strains in the culture media with C/N ratios 2:1 and 100:1. Strains in medium with a C/N ratio of 100:1 obtained a higher percentage of lipids, of 31.5%, 23.1%, and 29.7%; compared to values lower than 24.5%, 20.2%, and 21.3% obtained with a medium with a C/N ratio 2:1, for *R. glutinis* (CON-5), *R. mucilaginoso* (EP-5) and *R. kratochvilovae* (POR-3) respectively.

On the other hand, contrary to the medium with a C/N ratio of 2: 1, they presented higher values of biomass and lipid yields of the order of 19.01 g L<sup>-1</sup>, 27.74 g L<sup>-1</sup>, and 41.33 g L<sup>-1</sup>; as well as 4.65 g L<sup>-1</sup>, 5.59 g L<sup>-1</sup>, and 8.80 g L<sup>-1</sup>. This result compared to the medium with a C/N ratio 100:1 with lower values of biomass and lipid yields 9.98 g L<sup>-1</sup>, 13.90 g L<sup>-1</sup> and 9.80 g L<sup>-1</sup>; as well as 3.09 g L<sup>-1</sup>, 3.21 g L<sup>-1</sup>, and 2.91 g L<sup>-1</sup> for *R. glutinis* (CON-5), *R. mucilaginoso* (EP-5) and *R. kratochvilovae* (POR-3) respectively.

## DISCUSSION

The three selected cultures showed a greater than 20% lipid content, which is the minimum content required to be considered oleaginous yeast (Ageitos *et al.*, 2011). The sampled areas were chosen based on the evidence that *Rhodospiridium* and *Rhodotorula*, generally known as potential lipid producers, have an important role as saprophytes or parasites in coniferous and deciduous forests (Sláviková and Vadkertiová, 2000; Buzzini *et al.*, 2012; Yurkov *et al.*, 2012) and alluvial soils (Wuczowski and Prillinger, 2004). Also, based on the evidence of resisting temperatures below -20 °C (Zalar and Gunde-Cimerman, 2014) and altitudes close to 4 000 m.a.s.l. (Buzzini *et al.*, 2012). The areas where the samples were taken fall within the characteristics mentioned as the Porcon pine forests. On the other hand, some studies reveal the presence of yeasts of the genus *Rhodotorula* in habitats with low pH levels and with the presence of heavy metal or fossil fuel residues (Muñoz-Silva *et al.*, 2019; Gupta *et al.*, 2016); which explains its presence in the sampled area of El Paraíso forests, located near a river contaminated with mining tailings.

For molecular identification, the universal primers ITS1 and ITS4 were used, which can be directed to the conserved 18S, 5.8S, and 28S rDNA sequences, to be able to amplify the ITS1 and ITS2 regions, which vary according to the species depending on the length and sequence of the amplicon (Fujita *et al.*, 2001). The ITS encoded regions deposited in public databases such as GenBank are the most widely used (Toju *et al.*, 2012).

Regarding the maximum values of A reached in the three strains of *Rhodotorula* using the MS-2-7 (C/N 2:1), which yielded higher values than with the MS-1-7 medium (C/N 100: 1), there is information that a C/N ratio greater than 20 does not produce a significant increase in biomass, but does stimulate yeast lipid biosynthesis. Lower C/N ratios influence cell growth because the available forms of carbon and nitrogen are used mainly to satisfy cells' multiplication and development needs. Therefore, when nitrogen availability is reduced in the medium (MS-1-7), the growth rate decreases and the excess carbon is used to synthesize lipids as a storage substance (Ratledge, 2002; Kot *et al.*, 2019).

The Gompertz model has been used to evaluate the growth kinetics of oleaginous yeasts strains, obtaining R<sup>2</sup> values between 0.96 and 0.99 for *Lipomyces starkeri* and *Pichia pastoris*, respectively (Sierra, 2013; Chang *et al.*, 2006). In the present study, three *Rhodotorula* strains showed an R<sup>2</sup> of 0.98 to 0.99, which indicates that the model fits with the experimental values up to a maximum stationary phase, equally in media with C/N 2:1 and 100:1.

A longer time to reach high A values as observed in the three strains of *Rhodotorula* using the C/N 2:1 medium has resulted in lower  $\mu_{max}$  values concerning the C/N 100:1 medium. This aspect, as mentioned, can be influenced by a lower value of C/N (Kot *et al.*, 2019).

Research has shown that oleaginous yeasts do not have an overactive fatty acid biosynthesis system but can produce significant amounts of acetyl-CoA, the basic unit for their biosynthesis (Papanikolaou and Aggelis, 2011), which is reflected in the processing time. It has been observed that the C/N ratio influences the value of  $\lambda$ . Thus a 100:1 C/N ratio allows a rapid adaptation with a lower value of  $\lambda$  and a higher value of  $\mu_{max}$  with an immediate and more extraordinary transformation to lipids. (Fig. 2, 3, 4, and 5). But the carbon source is very important; in *R. glutinis*, it has been reported that high production of lipids can reach 66.0% using glucose as a carbon source (Kot *et al.*, 2016). An aspect considered is xylose used carbon source in the medium with a C/N ratio of 100:1, while glucose was used as a carbon source in the medium with a C/N ratio 2:1. In this regard, it has been reported that the *R. glutinis* strain T216 produced 36.6% of lipids, whose optimal conditions occurred when glucose was used as a carbon source, accumulating a higher concentration of lipids of 49.25% concerning the cellular biomass (Dai *et al.*, 2007). Glucose and xylose can be used by oily yeasts isolated from soil or other natural sources to produce lipids. Still, glucose is the preferred sugar for any oily yeasts, and high C/N ratios favor higher production. For xylose metabolism, yeast must be able to absorb pentose into the cell via sugar transporters. The overexpression of three regulatory enzymes (xylose

reductase, xylitol dehydrogenase, and xylulokinase) can aid yeast growth (Sreeharsha and Mohan, 2020), and this is an aspect that may have favored *R. mucilaginoso* and *R. kratochvilovae* at a lower value of  $\lambda$ .

Studies show a more significant accumulation of lipids by yeast *R. kratochvilovae* and *R. glutinis* in a limiting nitrogen medium (Jiru *et al.*, 2017; Yen *et al.*, 2019), in line with findings shown in Fig. 5 for the *Rhodotorula* strains with C/N 100:1. The accumulation of lipids in a culture with a deficiency of nitrogen is used for protein synthesis and cell growth. Still, when rapidly depleted, cells enter a state of survival in which they must increase their reserve materials, transforming the substrate into fats (Meesters *et al.*, 1996). Similar results to the present investigation have also been obtained with *R. kratochvilovae* using a culture medium with a 40:1 C/N ratio, resulting in 20.2% lipid content. In comparison, in a medium with a C/N ratio of 120:1, they obtained a lipid content of 42.5% (Jiru *et al.*, 2017). Likewise, for *R. glutinis* there is a consensus that lipid production is stimulated and increases at high C/N ratios, which are formed in the secondary metabolism of yeast using acetyl CoA as a precursor. In this regard, a C/N 20:1 ratio has been equally used with amounts of glucose 16.2 g L<sup>-1</sup>/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.0 g L<sup>-1</sup>, obtaining a low biomass production and total lipid content. When using glucose 57.0 g L<sup>-1</sup>/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 4.885 g L<sup>-1</sup>, high biomass production stimulated by the amount of nitrogen has been obtained. With high C/N ratios 70:1 and 120:1 (glucose 57 g L<sup>-1</sup>/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.0 g L<sup>-1</sup>, and glucose 97.4 g L<sup>-1</sup>/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.0 g L<sup>-1</sup>, high values of total lipids and biomass at the maximum stationary phase in cultures with *R. glutinis* have been achieved.

It should be noted that an increase in the C/N ratio 70:1 to an intermediate ratio of 100:1 results in a curb in the increase in total lipids, producing even a slight decrease (Braunwald *et al.*, 2013). On the other hand, it has been shown that *R. toruloides* can present an efficient biomass production and storage lipids accumulation in nitrogen-rich media, provided that phosphorus or sulfate is the limiting factor of cell growth. According to what was stated above, it is shown that high values of biomass and lipid content can be obtained. One aspect to be highlighted is observed in Fig. 5, noting that *Rhodotorula* cultures in media with C/N 2: 1 ratio can provide lower lipid content, between 13% to 28%, concerning C/N 100:1 medium, but when achieving a more significant amount of biomass, the lipids yield was higher (4.65, 5.59 and 8.80 g L<sup>-1</sup>) in *R. glutinis*, *R. mucilaginoso* and *R. kratochvilovae* respectively, using glucose as a carbon source, noticeably with *R. kratochvilovae*. The present investigation has been carried beyond the maximum stationary phase to the logarithmic death phase, but it could not exceed the maximum growth process escalation. Another aspect to consider is the appropriate C/N ratio and the amounts

of the components that contribute carbon and nitrogen, coming from different substrate sources and the type of oil yeast (Karamerou and Webb, 2019). It is recommended to avoid the Crabtree effect caused by a high carbon concentration (Christophe *et al.*, 2012; Braunwald *et al.*, 2013).

## Conclusion

Oleaginous yeasts were isolated and identified from the North Peruvian Andes with a greater than 20% lipid content: *Rhodotorula glutinis*, *R. mucilaginoso*, and *R. kratochvilovae*. The C/N ratio in the culture medium influenced the oleaginous yeasts kinetics, modeled with the parameterized Gompertz type II equation. Also, C/N affects biomass and lipid yields. With MS-1-7 (C/N 100:1) with xylose as carbon source, limiting nitrogen, a high  $\mu_{max}$  was obtained, reaching the stationary phase between 6 to 9 h, as well as lipid accumulation between 23.1% and 31.5%. With the MS-2-7 medium (C/N 2:1) with glucose as a carbon source, non-limiting nitrogen obtained the maximum biomass value in the stationary phase between 37 and 51 h, which generated the highest biomass yield at the end of the whole process with a lipid yield of 4.65; 5.59 and 8.80 g L<sup>-1</sup>. There is potential to obtain high lipid yields using a culture media non-limiting nitrogen, examining not only the C/N ratio. But also, the quantities, nature of the components, and type of oleaginous yeasts taking care to avoid a high carbon concentration to prevent the Crabtree effect.

## ACKNOWLEDGEMENTS

To the National Council of Science, Technology and Technological Innovation (CONCYTEC) and Active Science of Peru, for providing the resources that allowed the realization of this research (Agreement N° 165-2017-FONDECYT).

## Conflict of interest

The authors declare that they have no conflict of interest.

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