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Research Paper

Oleaginous yeasts from Antarctica: Screening and preliminary approach on lipid accumulation

Silvana C. Viñarta[1,2], M. Virginia Angelicola[1], J. Maximiliano Barros[1], Pablo M. Fernández[1], Walter Mac Cormak[3], Manuel J. Aybar[2] and Lucía I.C. de Figueroa[1]

[1] PROIMI-CONICET, Planta Piloto de Procesos Industriales Microbiológicos (PROIMI), Tucumán, Argentina
[2] INSIBIO, CONICET-UNT, Instituto Superior de Investigaciones Biológicas (INSIBIO), Tucumán, Argentina
[3] Instituto Antártico Argentino (IAA), Buenos Aires, Argentina

The capability of 17 *Rhodotorula* spp. isolated from Antarctica to accumulate intracellular lipids in nitrogen-limited medium was investigated. As results, 10 isolates were selected by Nile red staining, while 12 isolates were selected as oleaginous by analysis of total lipid content (20.4–73%, w/w of dry biomass). The higher lipid production and accumulation was exhibited for six strains belonging to three species of *Rhodotorula* (*Rhodotorula glutinis*, *Rhodotorula glacialis*, and *Rhodotorula laryngis*). This is the first report where *R. laryngis* have been identified within oleaginous specie. Lipid accumulation was evaluated comparatively in two nitrogen-limited glucose-based media (MI and MII). MI (low C/N ratio) was more suitable for biomass and lipid production while in MII (high C/N ratio) total lipid content was improved. *R. glutinis* R4, *R. glacialis* R15, and *R. glutinis* R48 showed high lipid concentrations (4.65–6.93 g L^{-1}) and they were able to accumulate large amounts of lipids per gram of biomass (47–77%, w/w). A similar profile in fatty acids composition and content of neutral lipids to vegetable oils was observed, indicating that lipids produced by oleaginous Antarctic yeasts can be considered an alternative feedstock for biodiesel production. Antarctica represents an important source of oleaginous yeasts with adaptive capabilities to accumulate considerable amounts of lipids with biotechnological interest at 15 °C and 25 °C.

Additional supporting information may be found in the online version of this article at the publisher's web-site.

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Introduction

Oleaginous microorganisms, microbes that can accumulate more than 20% (w/w) lipids by dry weight biomass, are receiving increasing attention for their potential use in commercial production of oil for food, chemical, and energy applications [1]. Oleaginous microorganisms can accumulate high amounts of neutral storage lipids under appropriate cultivation conditions and they are considered as source of triacylglycerols (TAG) for biodiesel production [2–5].

Microbial lipids from oleaginous microorganisms represent a valuable alternative feedstock for biodiesel manufacturing because of their similarity in fatty acid composition and energy with vegetable oils. Moreover, microbial lipids have also many advantages (short life cycle, low affection by venue, season and climate, easy to scale-up) that promise to overcome many limitations of plant oils in biodiesel manufacturing [4–6].

Yeasts have been of high industrial interest as sources of renewable oleochemicals, including fuels and platform for lubricants, adhesives, solvents, and polymers. Oleaginous yeasts are capable to synthesize and accumulate high amounts of neutral lipids (up to the 70% of biomass weight), mostly consisting of TAG [2]. Besides, they have advantages over bacteria, moulds, and algae due to its unicellular relatively high growth rate and rapid lipids accumulating ability in specialized intracellular compartments known as lipids bodies. Nitrogen limitation in presence of an excess of carbon source is the most efficient condition to induce lipogenesis. Under these conditions oleaginous yeasts produce high

Correspondence: Silvana C. Viñarta, Laboratory of Fungal Biotechnology, PROIMI-CONICET, Av. Belgrano y Caseros, T4001MVB, Tucumán, Argentina
E-mail: scvinarta@hotmail.com

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amounts of storage lipids [2, 3, 7]. Yeasts can also utilize low-cost fermentation media such as nutritional residues from agriculture and industry [2, 8].

A few species of oleaginous yeasts have been identified and deeply investigated in the genera *Yarrowia*, *Candida*, *Rhodotorula*, *Rhodosporodium*, *Cryptococcus*, and *Lypomyces* belonging to Ascomycota and Basidiomycota phyla [6, 9, 10]. The exploration of the natural biodiversity is a promising strategy to identify novel oleaginous species. Psychrophilic yeasts are understudied sources of biodiversity, which can play a novel role in biotechnology, thus offering an alternative to conventional microorganisms. They are attracting academic and industrial attention for their huge biotechnological potential due to their distinctive ability to grow and metabolize at low temperatures. To thrive successfully in low temperature environments, psychrophiles have evolved a complex range of structural and functional adaptations [6, 10] and they can accumulate lipids in high amounts and they produce a wide range of fatty acids and considerable quantities of polyunsaturated fatty acids. A few studies have considered psychrophilic yeasts for lipid production. Antarctic yeasts represent a group of cold-adapted microorganisms relatively unexplored.

Between oleaginous genera, *Rhodotorula* is widely known and various strains of *Rhodotorula* isolated from different environments were reported as good producers of large amounts of lipids in the triglycerides form. However, few studies have addressed psychrophilic and psychrotolerant *Rhodotorula* species [10].

To our best knowledge, the occurrence of oleaginous Antarctic yeasts and their lipids accumulation were scarcely investigated. In addition, the capability to accumulate lipids of *Rhodotorula* species isolated from Antarctica has not been studied comparatively.

The aim of this work was to select oleaginous Antarctic yeasts with high potential for lipid production as novel candidates for alternative feedstock for biotechnological purposes as biodiesel industry.

Materials and methods

Microorganisms

Yeasts were obtained from the Microbiological Resources Center Culture Collection (MIRCEN) of PROIMI-CONICET Institute, San Miguel de Tucumán, Argentina. Antarctic yeast were previously isolated from soil samples collected, during the 2011/12 austral summer [11], near the Argentinean scientific research station (Dr. Carlini), located on the Potter Caleta, 25 de Mayo Island (62°14′18″S, 58°40′00″W), Antarctica. Seventeen yeast strains belonging to seven species of *Rhodotorula*, previously identified by Rovati et al. [11] were studied. *Rhodotorula creatinivora* (R2, R34, R45), *Rhodotorula glutinis* (R4, R48), *Rhodotorula laryngis* (R8, R12, R21, R32, R35, R49, R54), *Rhodotorula pallida* (R13), *Rhodotorula glacialis* (R15), *Rhodotorula arctica* (R22, R23), and *Rhodotorula mucilaginosa* (R29) were evaluated for lipid production. *Saccharomyces cerevisiae* ATCC 32051 (Sc) and *Cyberlindnera jadinii* M9 (M9) were included as negative control.

Culture conditions

Yeasts were grown and maintained on YM-agar ($g\,L^{-1}$: yeast extract 3, malt extract 3, peptone 5, dextrose 10) and incubated at 15 °C for 3–7 days. For screening of oleaginous yeasts and for observations of lipid accumulation assays, yeasts were aerobically cultured in two nitrogen-limited glucose-based media, MI ($g\,L^{-1}$: glucose 30, yeast extract 1.5, NH_4Cl 0.5, $MgSO_4 \cdot 7H_2O$ 1.5, KH_2PO_4 7, Na_2HPO_4 5, $CaCl_2 \cdot 2H_2O$ 0.1, $ZnSO_4$ 0.01; pH = 5.5) and MII ($g\,L^{-1}$: glucose 30, yeast extract 0.75, $(NH_4)_2SO_4$ 0.1, $MgSO_4 \cdot 7H_2O$ 1.5, KH_2PO_4 0.4, $CaCl_2 \cdot 2H_2O$ 0.22; pH = 6.0). One hundred milliliters media were inoculated with a 10% (v/v) of inoculum and incubated at 250 rpm during 120 h. Inocula were prepared by transferring a 1-day-old colony grown on MI-agar to MI or MII media and incubated at 250 rpm during 24 h. Growth temperature was 25 °C for all yeast strains psychrotolerant (R4, R8, R12, R13, R21, R22, R29, R32, R35, R48, R49, R54) and 15 °C for the psychrophilic R15 and R23 isolates. Growth of yeasts at 25 °C was observed previously in MI-agar plates in order to design a bioprocess of lipid accumulation at this temperature. MI (C/N = 40) and MII (C/N = 110) were selected from literature for similar studies with oleaginous yeasts [12–14].

For analytical determinations, samples were taken under sterility conditions at different times of culture as below described.

Selection of oleaginous yeasts

Screening of oleaginous yeasts was performed using nitrogen-limited media, MI and MII, as above described and by qualitative and quantitative analysis.

Qualitative selection by Nile red staining

Conventional Nile red fluorescence staining technique was performed according to Kimura et al. [12], after 48, 72, and 120 h of culture in MI. Yeast cells were observed with magnifications of 40, 60, and 100× using a fluorescence microscope (Nikon Eclipse 80i; Tokio, Japón) under which the Nile red-stained lipid bodies

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show yellow-gold emission. The best micrographs of different yeasts were recorded.

Quantitative analysis of lipid content

Total lipids content were determined after 120 h of culture in MI broth. Yeasts were incubated in aerobic conditions on a rotatory shaker at 250 rpm and 15 °C or 25 °C. Dry biomass weight and total lipid content were determined gravimetrically at 105 °C. *S. cerevisiae* ATCC 32051 and *C. jadinii* M9 were included as negative control. Yeasts with values of total lipid content above or equal to 20% (w/w) of dry biomass were selected as oleaginous.

Lipids analysis: extraction and quantification

Lipid extraction of yeasts was carried out with solvents (chloroform:methanol, 2:1, v:v) according to standard methodology [15]. Samples of 50 ml of yeasts culture to the final time of fermentation were processed. The biomass was removed by centrifugation (7400×*g*), 10 min, 25 °C) and washed twice with distilled water. The pellet was frozen at −80 °C and then lyophilized and pulverized. Lipids were extracted from 0.02 g of lyophilized biomass with chloroform:methanol (2:1, v/v) and constant stirred with glass beads during 3 h at room temperature. After that, the samples were centrifuged (7400×*g*, 10 min, 5 °C) and the organic phase of supernatants containing lipids was recovered and completely evaporated in the vacuum (Savant speedvac® Plus SC110A; UVS400A universal vacuum system plus). Lipids were determined gravimetrically at 105 °C (over-night) until constant weight.

Comparative study of lipids production

A comparative study of lipid accumulation in MI and MII media was performed in *Rhodotorula* spp. selected as oleaginous. Determinations of biomass, lipid production, and total lipids content were performed after 120 h of culture.

Biomass determination by dry cell weight

The biomass of culture broth was removed by centrifugation and washed twice with the same volume of distilled water. Biomass was dried at 105 °C to constant weight.

Determination of TAG by thin layer chromatography (TLC)

A qualitative analysis of microbial lipids was performed by TLC according to Alvarez et al. [16]. Lipids extraction from yeasts cultures was performed as previously described. The extracts were then concentrated to 50% of its original volume and subjected to TLC using plates silica gel 60 F_{254} Aluminium sheets 20 × 20 cm (Merck Millipore) and hexane:diethyl ether:acetic acid (90:10:1, by vol) as system solvent for TAG analysis. Triolein (Sigma–Aldrich, St. Louis) was used as reference substance. Olive and soybean oils were included as control of vegetable TAG. TLC plates were revealed with iodine vapor [16].

Faty acids analysis

To determine the relative composition of fatty acids (FA), the microbial lipids were subjected to methanolysis [10] and the fatty acyl methyl esters (FAME) were analyzed by GC using an Agilent Technologies (Model 6890) equipped with a flame ionization detector (FID) and automatic injector, and a HP-5 capillary column (30 m × 0.32 mm i.d., 0.25 μm). Carrier gas was N_2 with a flow rate of 15.0 ml min^{-1}. The injection temperature was 270 °C; initial temperature was 40 °C, increasing to 190 °C at a rate of 23 °C min^{-1} and holding for 4 min, then increasing to 290 °C at a rate of by increasing 8 °C min^{-1} (5 min). Temperature of detector was 300 °C. The fatty acids were identified by comparison with retention times to reference standards.

Statistical analysis

Statistical analyses were performed using Minitab Statistical Software Release 15 for Windows (Minitab Inc., State College, PA). All results are expressed as mean values of at least triplicate determinations. Means were compared and analyzed using one-way analysis of variance (ANOVA) with Tukey–Kramer multiple comparisons tests. Differences were considered statistically significant for $p < 0.05$ with a 95% confidence interval.

Results

Screening of oleaginous yeasts

Oleaginous yeasts were selected according to their capacity to accumulate lipids in limited-nitrogen medium by a combination of qualitative and quantitative techniques. Firstly, a qualitative selection was performed using the Nile red staining and fluorescence microscopy. Subsequently, the lipid content of the yeasts was quantified. Thus, the most efficient yeasts for storing intracellular lipids were selected.

Qualitative selection of oleaginous yeasts by Nile red staining

The Nile red staining was performed in 17 yeast strains belonging to seven species of *Rhodotorula* from Antarctica. Yellow-gold emission was presented in all Antarctic yeasts and lipid bodies were observed by fluorescence microscopy (Fig. 1). The number, size, and shape of lipid bodies as well

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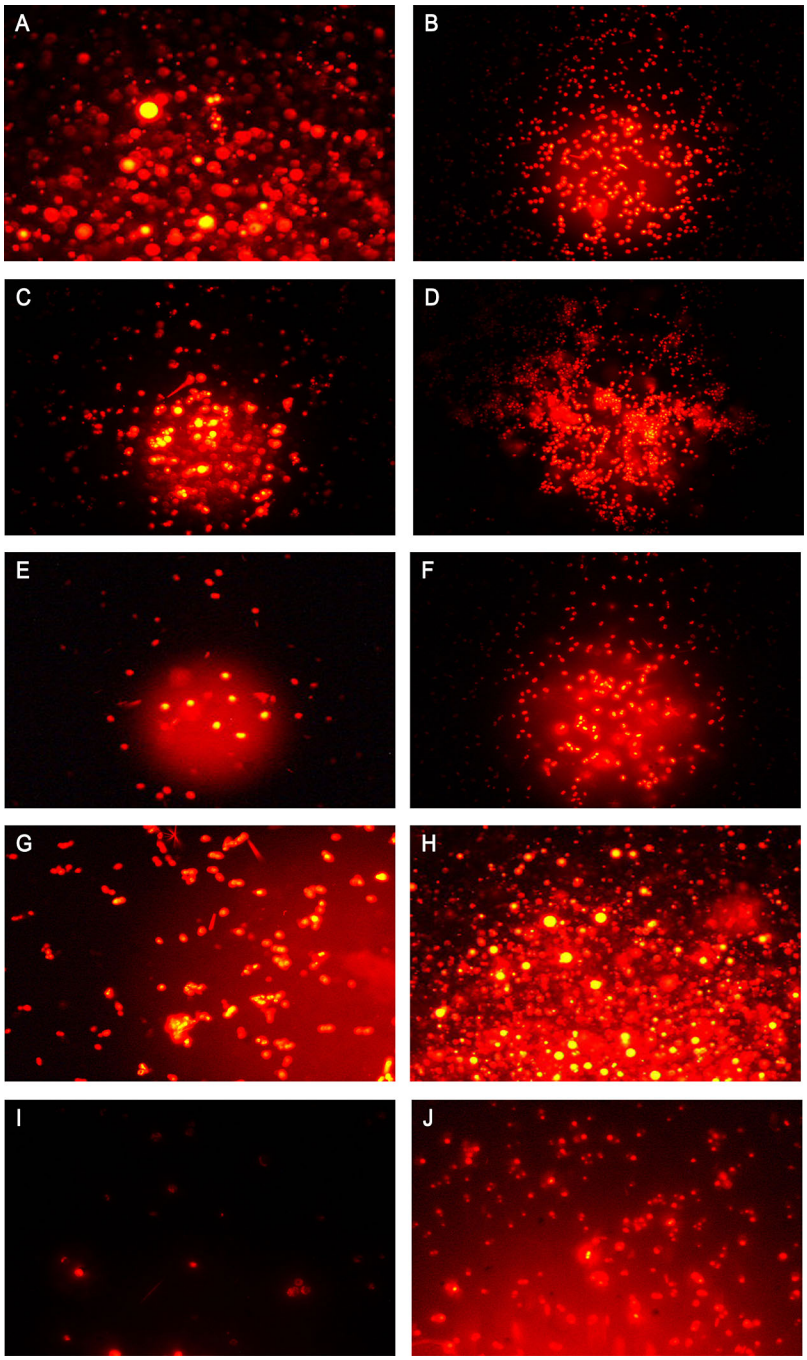

Figure 1. Fluorescence micrographs of lipids bodies from Antarctic *Rhodotorula* spp. (A: *R. glutinis* R4; B: *R. pallida* R13; C: *R. glacialis* R15; D: *R. arctica* R22; E: *R. laryngis* R32; F: *R. laryngis* R35; G: *R. creatinovora* R45; H: *R. glutinis* R48) and negative controls (I: *Saccharomyces cerevisiae* ATCC 32051 and J: *Cyberlindnera jadinii* M9) after 120 h of culture in MI medium after staining with Nile red. Magnification used for micrographs was 40×, except for R4, which was 60×.

as, intensity and persistence of fluorescence were different for each strain (Fig. 1). Ten isolates were selected as oleaginous yeasts on the basis of their emitted fluorescence intensity and the higher persistence of fluorescence emission. Six isolates (R4, R15, R32, R35, R45, and R48) were considered as the best lipid-producers according to the higher fluorescence intensity (Fig. 1).

In contrast, for *S. cerevisiae* ATCC 32051 and *C. jadinii* M9, included as negative controls, these characteristics were not observed in the same conditions and time of

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culture. In these yeasts, low intensity and persistence of fluorescence emission at the beginning of the microscopic observation was observed but disappear a few seconds later (Fig. 1I and J).

Yeasts cultivated in MI medium during 48, 72, and 120 h did not show any differences in the fluorescence emitted (Supporting Information Fig. S1), suggesting that it is possible to observe lipid accumulation in yeasts, starting at 48 h of culture. However, this technique is not suitable for monitoring the accumulation of lipids in function of time.

Quantitative selection of oleaginous yeasts

Lipid quantification was performed after 120 h of culture in MI broth. As results, 12 of 17 isolates of Antarctic yeasts were identified as oleaginous and potential lipid producers with total lipid accumulation between 20.4 and 73% w/w of dry biomass (Fig. 2A). The higher lipid content (36–73%, w/w) was observed in decreasing order for: R15 > R4 > R48 > R45 > R32 > R35. As expected, lipid contents of selected yeasts were significantly higher than negative controls (14.27 and 8.87%, w/w lipid contents for *C. jadinii* M9 and *S. cerevisiae* ATCC 32051, respectively). Moreover, lipid production was determined in $g\,L^{-1}$ as shown in Fig. 2B. *R. glacialis* R15 presented higher capacity for lipid production ($6.92\,g\,L^{-1}$) and higher percentages of lipid accumulation (Fig. 2). As results of intracellular lipid quantification *R. glacialis* R15, *R. glutinis* R4, *R. glutinis* R48, *R. laryngis* R32, and *R. laryngis* R35 were selected as potential candidates for lipids production. Results of the quantitative selection coincide with the qualitative selection results. The isolates considered as the best lipid producers according to the higher fluorescence intensity, indeed had the higher lipid content and production.

To our best acknowledge, this work constitutes the first report of *R. laryngis* as oleaginous specie.

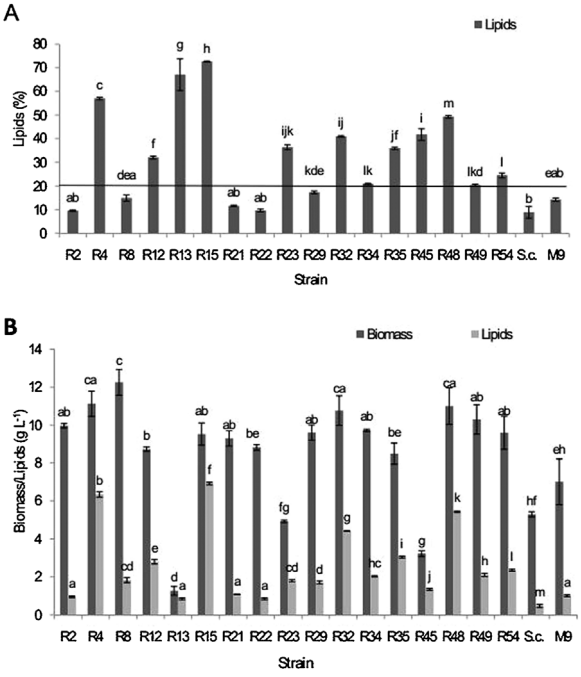

Figure 2. A: Percentage of total lipid content (% according total cell dry weight) of Antarctic yeasts strains and negative control yeasts, after 120 h of culture in MI. The cutting line indicates 20% w/w of accumulated lipids. B: Production of biomass and lipids in $g\,L^{-1}$ of Antarctic yeasts and negative controls yeasts after 120 h of culture in MI. Data are mean ± standard deviation (error bars) of three independent experiment. Values followed by the same letter are not statistically different ($p \leq 0.05$).

Comparative study of lipids production

A comparative study of lipid accumulation in two nitrogen-limited glucose-based media (MI and MII) was performed in five yeasts strains previously selected according to their higher lipid content and higher intensity of fluorescence emission. Values of biomass, lipid production, and percentage of accumulated lipids (according to dry biomass) of yeasts after 120 h of culture are shown in Fig. 3.

Biomass and lipid production were significantly superior in MI for all yeasts, while the percentage of lipid accumulation was better in MII (Fig. 3).

In both media, *R. glutinis* R4, *R. glacialis* R15, and *R. glutinis* R48 showed higher percentage of lipid content (47–75%, w/w using MI and 69–77%, w/w using MII) and lipid production ($4.65–6.93\,g\,L^{-1}$ in MI and $3.15–3.45\,g\,L^{-1}$ in MII). *R. glutinis* R4 and *R. glutinis* R48 showed significant increase in the percentage of accumulated lipids when they were cultured in MII (Fig. 3C), thus accumulation of lipids was dependent on the C/N ratio. Lipid accumulation of *R. glacialis* R15 was not significantly different in both media and this yeast exhibited the higher percentage of lipid accumulation (75%, w/w) in MI (Fig. 3C). Not significant differences were observed in lipid production of these three isolates in MII medium (Fig. 3C).

R. glutinis R4, *R. glacialis* R15, and *R. glutinis* R48 were selected for further analysis of microbial lipids (TAG and FA analysis), according to the high capacity to produce and accumulate large amounts of lipids.

Determination of TAG by thin layer chromatography (TLC)

The presence of TAG in microbial lipids was determined qualitatively by TLC for the three yeast strains selected as better candidates for lipids production.

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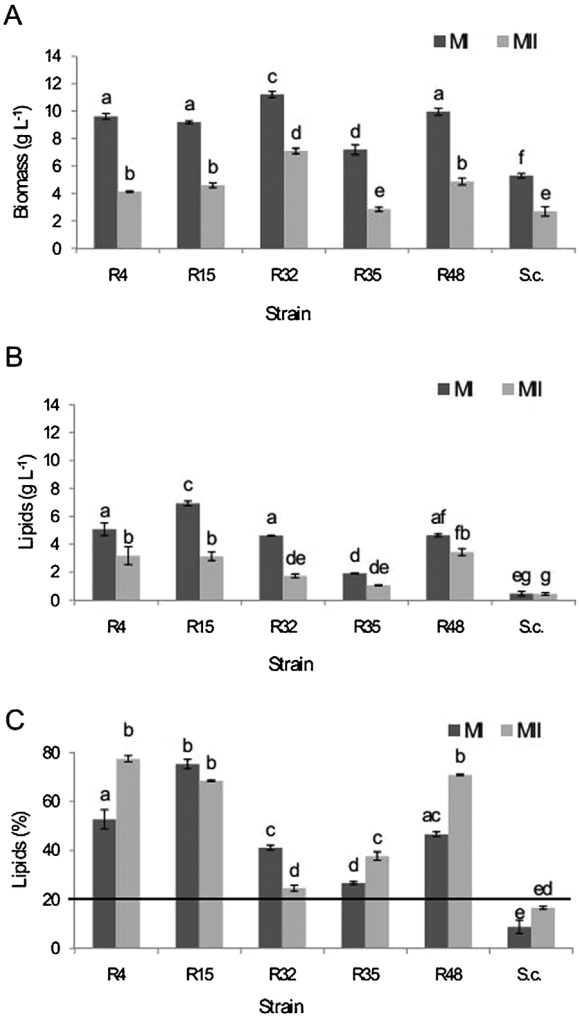

Figure 3. A–C: Biomass ($g L^{-1}$), Lipid production ($g L^{-1}$), and percentage of lipid accumulation (% according to total cell dry weight) of selected oleaginous yeast strains, and negative control after 120 h of culture in MI and MII media with glucose as carbon source. The cutting line indicates 20% w/w of accumulated lipids. Data are mean ± standard deviation (error bars) of three independent experiment. Values followed by the same letter are not statistically different ($p \leq 0.05$).

As was revealed by TLC, *R. glutinis* R4, *R. glacialis* R15, and *R. glutinis* R48 are able to synthesize TAG under assayed culture conditions (Fig. 4). Results demonstrate that oleaginous yeasts selected can be considered as an alternative source of triglycerides for biotechnological applications.

Fatty acids composition analysis

The fatty acids (FA) with chain length ranging from 14 to 18 carbons dominated the FA profile of Antarctic yeasts strains. Relative abundance of FA with chain length between C14 and C18 is shown in Table 1. The FA composition was similar in the three yeast strains (Table 1). Eighteen-carbons FA were the most abundant (85.59–91.07%). The dominants FA are linoleic acid (31.21–57.59%) and oleic acid (26.41–37.59%). These data are comparable to the results reported by other authors on the FA composition for oleaginous yeasts [6, 10, 13, 17]. The FA compositional profiles and the content of neutral lipids are quite similar to vegetable oils as soybean oil and rapeseed oil [11, 14, 17]. Interestingly, abundance relative of linolenic acid (C18:3n3) in *R. glacialis* R15 and *R. glutinis* R48 was 19.37 and 15.21%, respectively.

Discussion

This work explored the production of intracellular lipids of 17 cold-adapted *Rhodotorula* spp. from Antarctica.

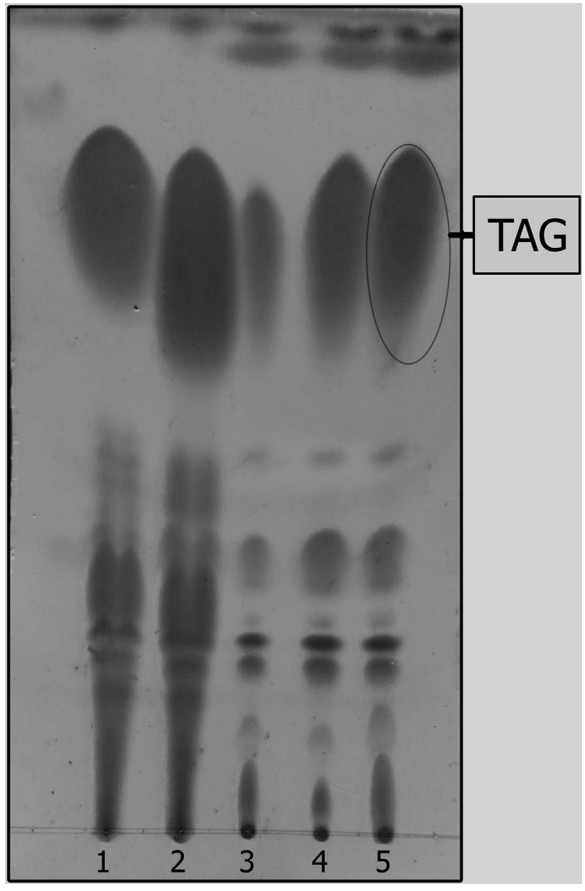

Figure 4. Lipid analysis by TLC of oils extracts of Antarctic yeasts isolates (R4, R15, and R48) and vegetable oils. Lanes: 1 – olive oil; 2 – soybean oil; 3 – R4; 4 – R15; 5 – R48. Yeasts were grown in MII medium after 120 h of culture time. Lipid extracts from the three yeast strains were resolved using a solvent system to separate TAG from other lipid species. TAG standards were used to identify the R_f value for TAG under the chromatographic conditions used. The TAG accumulated by the Antarctic yeasts exhibit a similar profile to vegetable oils according to the obtained spots.

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Table 1. Fatty Acid (FA) composition of *R. glutinis* R4, *R. glacialis* R15, and *R. glutinis* R48 growth in MI medium.

	Relative abundance of FA (w/w %)									
Strain	**C14:0**	**C15:0**	**C15:1**	**C16:0**	**C16:1**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**Total C18**
R. glutinis R4	0.60	1.12	0.52	6.93	1.28	1.90	26.41	57.59	3.64	89.54
R. glacialis R15	0.30	0.70	0.23	6.81	0.87	1.56	29.26	40.88	19.37	91.07
R. glutinis R48	0.55	0.63	0.27	12.38	0.58	1.58	37.59	31.21	15.21	85.59

Yeast strains accumulated high amounts of lipids within the biomass when they were cultured in limited-nitrogen glucose-based media.

Lipids bodies were observed by fluorescence microscopy. Results of lipid bodies staining were consistent with observations previously performed by other authors who have indicated that lipid bodies in oleaginous microorganisms have different shapes depending on species and culture conditions [12, 13]. In addition, fluorescence emission observed in Fig. 1 was similar to those described in several oleaginous yeasts [12, 13, 18].

Biomass, lipid production and percentage of lipid accumulated (20.4–77%) exhibited by Antarctic yeasts (Figs. 2 and 3) were comparable and even superior to other oleaginous yeasts as *Rhodosporidium toruloides* Y4, *R. glacialis* DBVPG 4785, *Cryptococcus curvatus* NRRLY-1511, *Candida Freyschussii* among others [10, 17, 19, 20]. Data were also similar and consistent to the results reported for *Rhodotorula* spp. by other authors [7, 10, 21–23]. Furthermore, 73–77% w/w of lipid accumulation obtained by *R. glacialis* R15 is one of the highest values reported and described at the moment for species of *Rhodotorula* [10, 17, 23, 24]. These results are relevant because *R. glacialis* R15, can accumulate 73% w/w of lipid after 120 h with $30\,g\,L^{-1}$ of glucose in nitrogen-limited medium MI. Amaretti et al. [10] reported for *R. glacialis* DBVPG 4785 lipids content of 68 and 50% w/w after 340 and 120 h, respectively, in a liquid nitrogen-limited medium with an excess of glucose ($120\,g\,L^{-1}$).

To the best of our acknowledgment, this work constitutes the first report of *R. laryngis* as oleaginous specie. These data are relevant because the oleaginous phenotype appears to be uncommon as it is observed in approximately 70 of the known 1500 yeast species [25] and results demonstrated that the exploration of the natural biodiversity is a promising strategy to identify novel oleaginous species.

Most of oleaginous yeasts accumulate storage lipids when a nutrient becomes exhausted but the carbon source is still available and continues to be assimilated by the cells which progressively becomes obese [2, 7]. When Antarctic yeasts strains were cultured in the limited-nitrogen glucose-based media, MI (C/N = 40) and MII (C/N = 110) effects of medium composition on biomass, lipid production and total lipid content were observed (Fig. 3). These results were not surprising because the C/N ratio is a very important factor for lipid accumulation [2, 7]. According to data of comparative analysis, MI (C/N = 40) was more suitable for biomass and lipid production while, total lipid content was improved in MII (C/N = 110). Thus, an increase of percentage of lipid content was obtained when the C/N ratio was increased (Fig. 3C) with MII though a decrease of biomass was also observed (Fig. 3A). Some authors have pointed that MII composition (nature and concentration of components) is more favorable than MI to induce the accumulation of a greater amount of lipid storage in oleaginous yeasts [12–14]. The assimilation of nitrogen source of MII increase the concentrations of a variety of metabolites implicated in diverse mechanisms that interact with the lipids biosynthetic pathways in yeasts [26].

Results of FA composition for oleaginous Antarctic yeasts were similar and comparable to those observed earlier in oleaginous yeasts [6, 10, 13, 17, 27]. In addition, the FA compositional profiles and the content of neutral lipids are quite similar to soybean oil, rapeseed oil, sunflower oil, and cotton seed oil [10, 13, 17].

The high oleic and linoleic acids content observed here, and common for yeasts, is considered favorable for biodiesel applications [10, 13, 17–19]. Furthermore, it has been concluded that yeast oils would be appropriate for use as biodiesel at a similar blending ratio as biodiesel originating from vegetable oils [10, 13, 17, 18].

A similar profile in FA composition and content of neutral lipid to vegetable oils was observed, indicating that lipids produced by oleaginous Antarctic yeasts can be considered as an alternative feedstock for biodiesel production [6, 10, 13, 17]. Abundance relative of linolenic acid (C18:3n3) in *R. glacialis* R15 and *R. glutinis* R48 was higher than those produced by *R. glacialis* DBVPG [10] and *R. toruloides* DMKU-TK16 [13]. Linolenic acid is an omega-3 fatty acid and it is very important in the food and pharmacological industries. Results indicate that the microbial lipids produced by these Antarctic yeasts would

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also be of interest for other applications including food and pharmacological industries.

The high capacity of *R. glutinis* R4, *R. glacialis* R15, and *R. glutinis* R48 to produce and accumulate large amounts of lipids was demonstrated. These yeasts represent interesting candidates for microbial lipids production for biotechnological applications.

Namely, several strains of *R. glutinis* and *R. glacialis* were characterized as oleaginous by other authors [10, 21, 23] and the applicability of their TAG in the biodiesel production showed good perspectives [24, 28, 29]. For this reason, *R. glutinis* R4, *R. glacialis* R15, and *R. glutinis* R48 represent a potential source of TAG for biodiesel generation. On the other hand, *R. glutinis* R4 and and *R. glutinis* R48 can produce significant amount of TAG at 25 °C, therefore they constitute a feasible alternative for the development of economically viable bioprocesses.

It was evidenced that the Antarctica represents an important source of oleaginous yeasts with adaptive capabilities to accumulate considerable amounts of lipids at 25 °C. These findings may also contribute to bioprospecting of Antarctic yeasts.

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Conflict of interest

No conflicts of interest declared.

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