Taxonomy, morphology, physiology and DNA content of a new *Blastobotrys* yeast species with potential applications in Biotechnology

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Abstract

Lignocellulosic material residues, rich in xylose, are interesting feedstocks for biotechnology, due to their abundance and low-cost. *Saccharomyces cerevisiae* – the conventional yeast cell factory, cannot use xylose and other pentoses as substrate. So, there is an increased interest in the use of non-conventional yeasts, more suitable for specific applications. In this study, the isolation and identification of different non-conventional yeast strains was performed. Fifty isolates were obtained and molecularly identified, of which thirteen were grown in xylose media. Nine strains previously isolated by the group were also molecularly identified. During this step, strain IST508 belonging to a new yeast species of the genus *Blastobotrys* was isolated from olive tree soil in Alentejo. This new species, preliminarily designated *Blastobotrys solioliva* IST508 due to its ecological niche, presents low ITS identity (95%) to its closest match, *Blastobotrys proliferans*. Moreover, it exhibits a different morphology compared with the other described species of the genus and can assimilate and ferment a large range of carbon sources. This strain is also able to produce organic acids, like formic acid, in small concentrations, and the vitamin riboflavin. The complete genome of the haploid *Blastobotrys solioliva* IST508 was sequenced and analyzed. Sixty-five genes were chosen to be studied in detail, since they were previously associated with overproducing strains of riboflavin. All the genes involved in the main biosynthesis pathway of riboflavin were identified and gene duplications of *SEF1* and *MET2*, involved in the positive regulation of riboflavin main pathway and amino acid biosynthesis, respectively, were found.

Keywords: Non-conventional yeasts, *Blastobotrys sp.*, Lignocellulosic residues bioconversion, Added-value bioproducts, Riboflavin

Introduction

The yeast domain contains a wide variety of species, of which several present interesting traits for applications in biotechnology [1]. The study of non-conventional yeasts may uncover new strains with traits that can improve bioprocesses: they can be able to efficiently catabolize several sugars and other carbon sources and resist to different stresses, for example, improving the production of added-value products, like pharmaceuticals, chemical intermediates, biofuels, enzymes, among others [2,3].

One of the lines of biotechnology research nowadays is to find microbial strains with the capacity to utilize renewable feedstocks and transform them into biofuels or value-added products through the biorefinery concept, due to the increasing concerns on sustainability issues worldwide [2]. The largest low-cost source of renewable organic material includes lignocellulosic residues, generated through agricultural and forestry practices. Lignocellulosic residues are composed of cellulose, hemicellulose, and lignin [3]. Cellulose and hemicellulose can be processed to release sugar monomers, like glucose and xylose that can be used as substrates in bioprocesses. Xylose is the most abundant sugar of the biosphere after glucose [3]. The most widely used yeast in fermentation processes, *Saccharomyces*

cerevisiae, cannot metabolize xylose and other pentoses. In contrast, several non-*Saccharomyces* (non-conventional yeasts) can use this and other unusual sources as substrate [3].

Yeasts from the *Blastobotrys* genus are promising yeasts to be used in bioprocesses, due to their unusual characteristics: most of these strains are able to utilize a wide range of carbon sources, through assimilation or fermentation, including uncommon sources of carbon, like adenine, glycine, uric acid, n-hexadecane, putrescine and branched-chain aliphatic compounds, such as isobutanol, leucine and isoleucine [4–7]. Also, some yeast strains of *Blastobotrys* present unusual stress tolerance to high temperature (some strains can grow at up to 48ºC), dry environments, high salinity conditions and high osmotic pressures [4,8,9]. Currently, there are 24 species reported as belonging to this genus isolated from a wide variety of ecological (soil, water, air, plants and the gut of animals) and geographical sources [4,6]. Some, like *Blastobotrys raffinosifermentans*, are already being used in bioprocesses, for example, for the synthesis of enzymes naturally encoded in its genome, like tannases [8].

In this work, during the isolation and characterization of fifty different strains from twenty-six different species of non-conventional yeast, and also nine strains previously isolated by the group, a yeast strain isolated from the soil surrounding an olive tree, in *Ferreira do Alentejo, Portugal* (38°02'43.6"N 8°06'34.3"W), showed an identity percentage of approximately 95% with the *Blastobotrys* genus at the level of the ITS sequences was found. Due to this low identity, this species was proposed as a new anamorphic member of *Blastobotrys* and preliminarily designated as *Blastobotrys solioliva* IST508 and this work consists on its study and characterization.

Materials and Methods

Assimilation Tests

The assimilation tests were performed as described by Kurtzman et al. (2011) with minor modifications [4]. A pre-culture of *Blastobotrys solioliva* IST508 was prepared in 50 mL YNB (6.7 g/L Yeast Nitrogen Base, Difco), with 20 g/L of glucose and incubated for 24h. Afterwards, sterile glass tubes with 3 mL of YNB with 20 g/L of each compound (stock concentration of 100g/L) were inoculated with the pre-culture at an initial OD_{600nm} = 0.1. Compounds tested were fructose (Sigma-Aldrich), glucose (Merck), galactose (Sigma-Aldrich), ribose (Sigma-Aldrich), melezitose (Sigma-Aldrich), trehalose (Merck), sucrose (Sigma-Aldrich), xylose (Sigma-Aldrich), arabinose (Sigma-Aldrich), maltose (Sigma-Aldrich), galacturonic acid (Sigma-Aldrich), raffinose (Alfa-aesar), rhamnose (Merck), mannose (Sigma-Aldrich), myo-inositol (Merck), sorbitol (Sigma-aldrich), succinic acid (Fisher-scientific), mannitol (CalbioChem), xylitol (Sigma-aldrich), soluble starch (BDH chemicals), melibiose (Sigma-Aldrich), lactose (Sigma-Aldrich), inulin (Sigma-Aldrich), galactitol (Sigma-Aldrich, sorbose (Sigma-Aldrich, rybitol (Sigma-Aldrich, ethanol 5% (v/v) (Carlo Erba), methanol 5% (v/v) (VWR chemicals), glycerol 5% (v/v) (Sigma-Aldrich), ethanol 1% (v/v), methanol 1% (v/v), glycerol 1% (v/v). Vitamin requirements of the yeast were tested by inoculating a *Blastobotrys solioliva* IST508 pre-culture prepared as described before into a sterile glass flask with 3 mL of Vitamin-Free Yeast Base (5g/l ammonium sulfate (Panreac), 10g/L glucose, 10 mg/L L-Histidine monohydrochloride (Acros), 20 mg/L DL-Methionine (Sigma), 20mg/L Tryptophan (Merck), 0.85g/L Potassium phosphate monobasic, 0.15g/L potassium phosphate dibasic, 0.5g/L magnesium sulfate (Riedel-de-Haen), 0.1g/L sodium chloride, 0.1g/L calcium chloride, 500 µg/L boric acid (Merck), 40 µg/L copper sulfate (Merck), 100 µg/L potassium

iodide (Sigma-Aldrich), 200 μg/L ferric chloride, 400 μg/L manganese sulfate (Sigma-Aldrich), 200 μg/L sodium molybdate (Sigma-Aldrich), 400 μg/L zinc sulfate (Panreac) [4]. Osmotic tolerance was assessed by growing a *Blastobotrys solioliva* IST508 pre-culture prepared as described before in 3mL of 10% Sodium Chloride + 5% glucose (10% sodium chloride + 5% glucose media (100 g/L NaCl, 6.7 g/L Yeast Nitrogen Base, 50 g/L glucose) [4]. After 7 days of incubation at 30ºC, 250 rpm, optical densities at 600 nm were measured. A total of 3 replicates were performed for each assay, with exception to inulin, since the solution formed crystals after the first replicate, and methanol 5%, ethanol 5% and glycerol 5% assimilation, where only one replicate was done. The mean and standard deviation of the three replicates performed was calculated by the software GraphPad Prism.

Fermentation Tests

Fermentation sterile flasks with 16 mL of Fermentation Basal Media (4.5g/L yeast extract, 7.5g/L peptone, 0.0267 g/L bromothymol blue) and 4 mL of sugar (glucose, fructose, trehalose, galactose, maltose, xylose, raffinose, arabinose and sucrose) stock solution (100 g/L) were inoculated at an initial OD_{600nm} = 0.1 with a pre-culture prepared with approximately 7 hours of incubation at 30 ºC and 250 rpm, in 50 mL of YM liquid medium (6 g/L of malt extract, 1.8 g/L maltose, 6 g/L dextrose, 1.2 g/L yeast extract (ThermoFisher)). All flasks were capped with a perforated rubber cork and a needle was inserted in the middle of the cap to allow evaporation of gases, before incubation at 30 ºC without agitation for ~17 days. Fermentations were monitored daily by weighting the fermentation flasks and plotting weight loss over time. Three replicates were performed for each assay. The mean and standard deviation of the three replicates performed was calculated by the software GraphPad Prism.

Growth Analysis, Sugar Consumption and Determination of Extracellular Metabolites Produced by HPLC

Blastobotrys adeninivorans PYCC 4638, *Blastobotrys proliferans* PYCC 5116 and *Blastobotrys solioliva* IST508 were initially grown in 50 mL of YNB with 100 g/L of xylose or glucose. These cultures were grown overnight, at 30ºC and 250 rpm, and used to inoculate at an initial OD_{600nm}=0.1. Erlenmeyer flasks with 50 mL YNB containing 20g/L of carbon source (glucose or xylose). These flasks were incubated at 30ºC, 250 rpm, for 145 h. The growth of the yeast cultures was assessed by taking daily measures of the OD_{600nm} of the culture media. In addition, 500 μL of culture media at each point was taken, in order to analyze the metabolites produced and sugar consumption by High-Performance Liquid Chromatography (HPLC). These samples were centrifuged (8000 rpm, 3 min) and the supernatant was collected and frozen at -80ºC. These supernatants were diluted 10 times in 0,005 M H_2SO_4 in HPLC vials. Samples were analyzed by the HPLC system (Merck Hitachi, Darmstadt, Germany) with a refractive index detector (Hitachi LaChrom Elite L-7490) and a UV-Vis detector (Hitachi LaChrom Elite L-2420), and an Aminex HPX-87H column (300 mm × 7.8 mm, Bio-Rad) eluted at 65°C with 0.005M H_2SO_4 at a flow rate of 0.6 mL/min. The sugar concentrations (glucose and xylose) were determined, as well as the concentrations of glycerol and ethanol. Also, the concentrations of oxalic acid, citric acid, malic acid, succinic acid, formic acid and acetic acid were determined for each sample. Concentrations were estimated based on calibration curves previously prepared and three replicates were performed for each strain tested.

Riboflavin Detection Tests

Blastobotrys solioliva IST508 cells were grown on Erlenmeyer flasks with 50 mL of two different media:

YPD and YNB, and incubated for 3 days at 30ºC, 250 rpm. Afterwards, samples of both the culture media and also from the sterilized non-inoculated media were taken to be analyzed by HPLC. 500 µL of media from each flask was taken, in order to analyze the presence of riboflavin. These samples were centrifuged (8000 rpm, 3min) and the supernatant was collected. Also, a positive control with 1 g/L of riboflavin was prepared. These supernatants were diluted 10 times in water in HPLC vials. Samples were analyzed by the HPLC system (Merck Hitachi, Darmstadt, Germany) with a UV-Vis detector (Hitachi LaChrom Elite L-2420, at 271 nm) and a Chromalith Purospher® RP-18 endcapped column (Merck) eluted at room temperature with an isocratic mobile phase (48% H2O+ 52% acetonitrile), at a flow rate of 1mL/mim. Additionally, the samples were scanned in a UV-VIS spectrophotometer (Shimadzu) to obtain the absorbance spectrum at a wavelength ranging from 210 to 800 nm.

Genome Sequencing and Annotation

The genomic DNA of the strain *Blastobotrys solioliva* IST508 was sequenced and analyzed using the CLC Genomics Workbench 20.0 (https://digitalinsights.qiagen.com). The genes of the main pathway of riboflavin production were analyzed through BLAST against IST508 genome, as well as others associated with its overproduction [10–13]. For the genes with possible copies, protein motifs were also studied using the online bioinformatic tools Pfam, InterProScan and ScanProsite [14–16].

Results

The process of isolation and identification of fifty non-S*accharomyces* yeast strains, from twenty-six different species, plus nine strains previously isolated by the Biological Sciences Research Group (results not shown), led to the isolation of the strain IST508, isolated from olive tree soil and that presented an ITS identity of 95% and a D1/D2 identity of 98% with *Blastobotrys proliferans*. No ambiguities were found in the sequences obtained, indicating that it could be a new species. For this reason, from this point on, the new species will be referred as *Blastobotrys solioliva* IST508. Also, this strain showed a different morphology from its closest relative (results not shown).

To characterize *Blastobotrys solioliva* IST508 a set of tests was performed. In terms of its ability to ferment and assimilate, different carbohydrates were tested. Vitamins requirements, growth at different temperatures, and osmotolerance are other physiological tests that were performed [4].

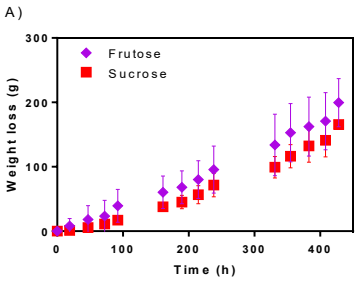
Assimilation Assays

To assess the assimilation ability of *Blastobotrys solioliva* IST508, yeast cells were cultured in YNB containing 20 g/L of each of the tested compounds. After 7 days of incubation, at 30ºC, the optical density at 600 nm was measured. It was observed that the strain of the novel species grew well, in general, in all the compounds tested, with exception of galacturonic acid, myo-inositol, lactose, soluble starch, methanol 1 and 5%, ethanol 5%, where growth was absent (OD smaller than 0.5). In rhamnose and 10% NaCl+5% Glucose growth was weak, reaching optical densities of 3. On the other hand, this strain grew considerably well when cultured with the sugars fructose, galactose, glucose, arabinose, xylose, sucrose, maltose, mannose, melezitose, raffinose, trehalose, melibiose, sorbose and other carbon sources, like glycerol 1% and 5%, sorbitol, mannitol, ethanol 1%, inulin, and in vitamin free-media (Vitamin requirements) reaching optical densities higher than 5. In rhamnose, ribose, succinic acid, xylitol, galactitol growth reached optical densities between 3 and 5.

Fermentation Assays

Fermentation is important in biotechnological applications and when describing a new species, the ability to ferment several carbon sources is usually tested. In this study, fermentation tests were carried out in 25 mL fermentation flasks capped with a rubber cork perforated with a needle to allow carbon dioxide release during fermentation. Fermentation basal media was supplemented with different carbohydrates (final concentration of 20 g/L) and inoculated with *Blastobotrys solioliva* IST508 yeast cells at an initial optical density (600nm) of 0.1. The fermentation flasks' weight was monitored daily, in order to follow carbon dioxide losses.

Figure 1 represents the weight loss, in grams, per time, in hours, of fermentation. The weight loss was calculated by the difference between the weight of the flask at a given time-point and the initial weight (at time-point 0). It is considered that the rate of weight loss is proportional to the carbon dioxide released hence it is a measure of the fermentation rate. When compared to *S. cerevisiae* strains, *B. solioliva* IST508 fermentation is very slow [17]. The fermentation profiles observed were higher when fructose and sucrose were added as carbon source, followed by glucose, raffinose, galactose and trehalose, in descending order (Figure 1). In the case of arabinose, xylose and maltose, the weight losses were minimal (results not shown).



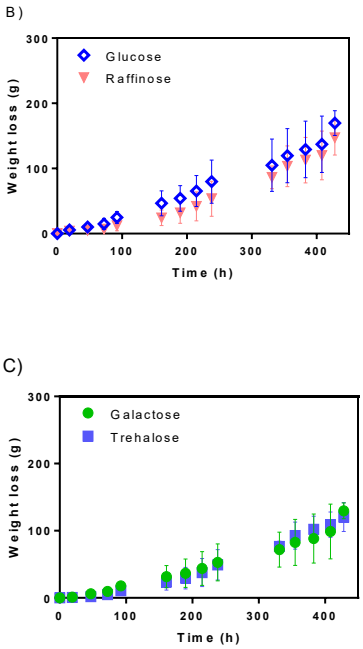


Figure 1 – Fermentation Assays. Weight lost by the flask, in grams, inoculated with the strain of the novel species Blastobotrys solioliva IST508, per fermentation time, in hours, with (A) Fructose and Sucrose, (B) Glucose and Raffinose and (C) Galactose and Trehalose as sole carbon source in each case.

Growth curve, Sugar Consumption and Extracellular Metabolites Produced by Blastobotrys solioliva IST508

The growth curve of *Blastobotrys solioliva* IST508 was monitored for 145h of incubation with either glucose or xylose as carbon sources. The type strains *B. adeninivorans* PYCC4638, *B. proliferans* PYCC5116 were used for comparison. In general, cells enter the exponential phase after 10 hours of incubation and after 50 hours leave it to enter into the stationary phase. Despite having similar curves, *Blastobotrys solioliva* IST508 reaches smaller OD_{600nm} in comparison to the other species tested.

HPLC analysis of the culture media composition during cultivation was also performed, allowing the description of the sugar consumption profiles for each species. In Figure 2, it is observed that both *B. adeninivorans* PYCC 4638 and *B. proliferans* PYCC 5116 are able to consume almost entirely the sugar sources present, in opposition to *Blastobotrys solioliva* IST508, where almost half of the initial

sugar (glucose or xylose) concentration are left in the growth medium due to growth stoppage.

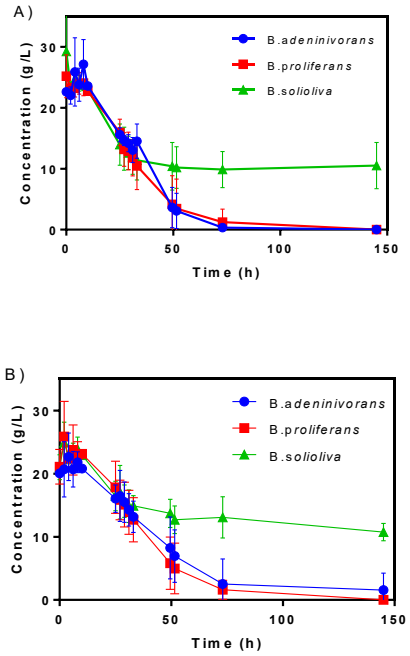


Figure 2 – Consumption of Glucose (A) and Xylose (B) by B. adeninivorans PYCC 4638, B.proliferans PYCC5116 and Blastobotrys solioliva IST508 during 145 hours of culture, cells were grown in YNB at 30ºC, 250 rpm for 145 hours.

Besides the analysis of glucose and xylose consumption, the production of glycerol, ethanol and organic acids (oxalic acid, citric acid, formic acid, acetic acid, malic acid and succinic acid) were also assessed for each species tested, since the pH of the media was around pH 2.0, after a few hours of incubation. Only the results concerning *B. solioliva* are shown. Glycerol or ethanol were not detected in any of the strains.

Blastobotrys solioliva IST508 produces similar compounds when growing on glucose and xylose, however, at different concentrations (Figure 3). When glucose is the carbon source, formic acid is the most produced acid, reaching max concentrations of approximately 2 mM, followed by malic and acetic acids, with concentrations around 1 mM. There is also a residual presence of succinic acid. When xylose is the carbon source, formic acid is also the most produced acid (3 mM), followed by succinic acid, malic acid and acetic acid (concentrations around 1 mM), and residual concentrations of oxalic acid are detected. Also, these concentrations started to increase only after 50h of incubation, reaching its maximum in the last point taken, in opposition to what occurs with glucose, where concentrations are almost constant after 20 h.

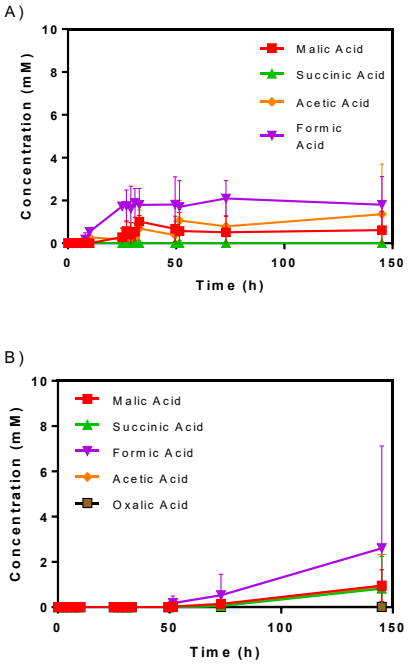


Figure 3 - Extracellular metabolites production by Blastobotrys solioliva IST508 during 145 h of culture, determined by HPLC analysis, with 20 g/L of either Glucose (A) or Xylose (B) as carbon sources, cells were grown in YNB at 30ºC, 250 rpm for 145 hours

In all three species tested formic acid is the most produced acid. In opposition, oxalic acid production is extremely rare.

Riboflavin Tests

When performing the growth curve analysis, it was observed that the flasks of *Blastobotrys solioliva* IST508 presented a bright yellow color after a few hours of incubation. The yellow color was observed either when glucose or xylose was used as carbon source, although the color was more intense in glucose medium, but the final ODs and the growth

curves were similar in both. The compound responsible for the alteration of the media color was secreted into the extracellular compartment by the cells. After some research on the literature, riboflavin was pointed out as a good candidate compound based on its color and water solubility.

In order to confirm this, samples of *Blastobotrys solioliva* IST508 in YPD and YNB culture media were taken and compared with samples from non-inoculated sterile media in order to compare the differences. A positive control of riboflavin (1 g/L) was used. The spectra of the different samples were analyzed. No differences between the spectra of YPD with or without *Blastobotrys solioliva* IST508 growth were observed. However, in the case of YNB, significant differences were seen between the inoculated and the sterile media. There is a clear resemblance between the positive control spectra, with riboflavin, and the spectrum from the culture media of *Blastobotrys solioliva* IST508 in YNB spectra, indicating that riboflavin is being produced by this species in this specific media.

The complete genome of the novel strain was sequenced. Sixty-five genes, previously associated directly or indirectly with riboflavin overproduction were chosen to be studied in this genome (results not shown) [10–13]. The strain was found to be haploid, with 11-14 Mb. All the genes involved in riboflavin biosynthesis were found and two genes, *SEF1*, a putative transcription factor related to riboflavin production pathway, and *MET2*, an enzyme involved in the methionine biosynthetic pathway [10,12], were found to have duplications in *Blastobotrys solioliva* IST508 genome (results not shown).

Discussion

The isolation and characterization of new non-conventional yeast strains could help to improve many bioprocesses used today [2,3]. During the search for these strains, the isolate *Blastobotrys solioliva* IST508 was found. It presented a small ITS identity with its closest match and since no ambiguities were observed in its sequence, it was believed that it could be a new yeast species. So, this work consisted on its study and characterization.

The anaerobic fermentation tests for *Blastobotrys solioliva* IST508, revealed that it can ferment different sugars, including fructose and sucrose. However, the fermentation profiles are slow when compared to *S. cerevisiae* strains, the most commonly used yeasts in fermentation processes [17,18], which indicates that this new yeast is not industrially competitive for fermentation. In terms of assimilation, which is the aerobic growth on different substrates [4], *Blastobotrys solioliva* IST508 showed the ability to assimilate several compounds with interest for industry such as fructose, xylose, and arabinose [19]. This strain is also able to grow on vitamin-free media, indicating that it does not need expensive supplements added to the media.

The analysis of the culture media content, during aerobic growth, was also monitored. The HPLC analysis showed that *Blastobotrys solioliva* IST508 did not consume all the sugars available (xylose and glucose), whereas in the cases of *B. proliferans* PYCC5116 and *B. adeninivorans* PYCC4638, this was not observed. The growth curves of these three species are similar, but *Blastobotrys solioliva* IST508 reaches slightly smaller OD_{600nm}. This could indicate that cells loose viability before consuming all the sugar, which could be caused by a lack in a vital nutrient or by the accumulation of toxic compounds, like the organic acids [20,21]. Probably this strain is more susceptible to the presence of these compounds in comparison to the others tested. In the future, cell viability assays should be performed, since measurements of the optical

density of the media only give insights on media turbidity and give no information on cell viability [22].

Ethanol and glycerol production, which is a by-product of the fermentation of sugar to ethanol in a redox-neutral process [23], was never observed. Indicating that this strain is Crabtree negative, in contrast to *S. cerevisiae,* since it appears to be favoring respiration over fermentation. Yeasts that display a Crabtree effect, ferment in the presence of oxygen and in high concentrations of glucose [24], which was not observed. The presence of organic acids in the culture media was also studied, due to the elevated acidity of the media. *Blastobotrys solioliva* IST508 is capable to produce different organic acids, like formic acid, succinic acid, and acetic acid, in small concentrations and both in the presence of xylose or glucose. However the concentrations obtained are considerable smaller when compared to other strains already used in industry, for example *Corynebacterium glutamicum* can produce up to 146 g/L (1.23 M) of succinic acid or *Aspergillus niger* that produces 240 g/L (1.25 M) of citric acid [25,26].

During culture of the novel species in YNB medium, it was observed that the liquid media turned bright yellow after a few hours of incubation and this color became more intense throughout growth, indicating that this yeast was producing and releasing a compound to the extracellular media. This unknown compound had to be water soluble and emit the yellow color and riboflavin was a candidate that reunited these characteristics.

After running the UV spectrum of *Blastobotrys solioliva* IST508 culture media (YNB) against the UV spectrum of riboflavin, it was observed that riboflavin had absorption peaks nearly at the same wavelengths of the compound present in the *Blastobotrys solioliva* IST508 growth medium. Production of this vitamin was only observed in a specific media, YNB, whereas no absorption peaks were detected in the YPD UV spectrum. This indicates that different media composition affects its production. Stahmann *et a*l. (2000) demonstrated that the presence of iron impaired riboflavin production [27]. In fact, YPD medium contains 25 μM of iron, while YNB medium has 1.2 μM, which accounts for a difference of over 20 times more iron in the first media [4,28]. Also, YNB medium contains pyridoxine hydrochloride, which is converted into the active form pyridoxal 5'-phosphate that is an essential cofactor for the enzymatic reaction that transforms serine into glycine, which was also proved to be a positive influencer in riboflavin's production [29,30]. In addition, riboflavin's overproduction has been related to oxidative stress and aging of cells. This vitamin acts as a cell reducing agent [10,12]. It is likely that riboflavin overproduction in *B. solioliva* IST508 is related with the oxidative stress generated by the dissociation of the weak acids, like formic acid, in the cytosol of the cells that influence the free radical production and inhibits cell metabolic activity, elucidating also why the cells stop consuming the sugar after some hours of incubation.

After sequencing the complete genome of the strain IST508, several genes directly or indirectly associated with riboflavin overproduction[10–13] were chosen to be studied through BLAST. All the genes from the pathway were found and the genes with possible copies were further studied regarding its protein motifs. For two genes, *SEF1* and *MET2*, there was a similarity in the protein family and in the motifs found. *MET2* is a homoserine acetyltransferase that catalyzes the first step unique to methionine biosynthesis, converting L-homoserine to O-acetyl-L-homoserine using acetyl-CoA as an acetyl group donor. This enzyme is also involved in the regulation of homoserine in other

pathways, being vital to cell growth and viability [31]. If this duplication results in the increased concentration of O-acetyl-L-homoserine, this molecule could be channeled into glycine production, thus increasing its concentration, which is known to increase riboflavin production [27,32]. However, more tests should be done to confirm this hypothesis. *SEF1*, in *Candida famata*, is responsible for riboflavin synthesis regulation. It is believed that this gene is involved in the positive regulation of this pathway, since its overexpression is connected to an increase in this vitamin's production [12]. The product of *SEF1* gene is a transcriptional regulator that belongs to Gal4 family of transcriptional activators and possesses zinc finger domain at its N-terminus. This regulator activates *RIB1* and *RIB7* promoters. Besides this, *SEF1* is also involved in iron homeostasis in *C. famata.* Levels of *SEF1* depend on iron concentration, where an iron deficiency leads to its increase, and consequently of riboflavin production. In this case, the vitamin could reduce practically insoluble Fe^{3+} ions to more soluble Fe^{2+} ions providing iron to the cells [12].

Conclusion

The isolation and study of new non-conventional yeasts can help the change towards more sustainable bioprocesses. For these reasons, there has been an increase in the interest in non-*Saccharomyces* yeasts. This study allowed to isolate and confirm the great diversity of non-conventional yeasts in the environment. Besides the isolation work of different strains, the novel species found, *Blastobotrys solioliva* IST508 represents and interesting candidate to be further explored. The most striking feature of *Blastobotrys solioliva* IST508 is its ability to produce riboflavin. Future work will involve the study of this yeast's capacity of producing riboflavin and other added-value products.

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