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Research

Yeasts Bioproducts Prospection from Different Brazilian Biomes

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Summary

This research paper is a result of extensive work from a group of researchers participating in the BIOTA-FAPESP Program (FAPESP Research Program on Biodiversity Characterization, Conservation, Restoration and Sustainable Use), aimed at discovering, mapping and analyzing the diversity of the flora and fauna of the State of São Paulo, and also evaluating the possibilities of sustainable exploitation of this diversity, assisting in the formulation of conservation policies on forest remnant.

Several projects supported have resulted in information records on more than 12 thousand species and databases with the contents of 35 biological collections. The authors of this research paper have contributed with the isolation and selection of more than 1000 fungal strains evaluating their potential to produce enzymes, vitamins, and antimicrobial compounds. Thus strains which presented potential to industrial application were identified and registered in the Environmental Information System, SinBiota (sinbiota.cria.org.br), this database is open to the scientific community of Brazil and abroad.

This particularly study reports the bioprospection of yeasts from singular Brazilian biomes - Atlantic Rain Forest, Savannah and the transition area between both biomes– and their potential to produce extracellular lipase, biotin and riboflavin was also evaluated. The results obtained in our work showed that 120 yeasts were able to produce the target bioproducts, revealing that the Brazilian Atlantic Rain Forest is as an interesting bioprospection spot. Moreover two strains presented a dual potential production for the bioproducts analyzed, been identified as *Pichia caribbica* and *Candida oleophila.*

Abstract

This study reports the bioprospection of yeasts from different biomes in the State of São Paulo aiming the biotechnological production of enzymes and vitamins. A total of 427 yeasts were isolated from the Atlantic Rain Forest, Savannah and the transition area between both biomes. These strains were evaluated for their potential to produce extracellular lipase, biotin and riboflavin by selection on solid selective differential and liquid culture media. The results revealed 120 yeasts able to produce the target bioproducts, 31 as lipase producing, 38 biotin producing and 51 riboflavin producing strains. The Brazilian Atlantic Rain Forest biome presented itself as an interesting bioprospection spot since 28% of the isolates from this area presented potential for lipase production, 22% for riboflavin production and 6% for biotin production. The strain RP.C153 presented a dual potential production of lipase and riboflavin, while strain RP.J1308 produced lipase and biotin. Both were identified by molecular techniques as *Pichia caribbica* and *Candida oleophila,* respectively. The present work has a significant contribution to future studies regarding the bioprospection in Brazilian biomes and production, optimization, characterization and application of the target bioproducts.

Keywords: Bioprospection; Brazilian Biome; Yeast; Enzyme; Vitamin.

Introduction

Environmental microorganisms are an inexhaustible reservoir of genetic and functional diversity accumulated over millions of years of evolution and adaptation to numerous selective pressures. As a consequence, the isolation of microorganisms has become an important source of organic products with applications in all major industries of food, pharmaceutical, agricultural, fuels and others [1].

In this study, yeasts were isolated from Atlantic Rain Forest and Savannah biomes in the State of São Paulo, present in the cities of Ilha Bela, Campinas and RibeirãoPreto. They were bioprospected for lipase, biotin and riboflavin production. The Atlantic Rain Forest of Ilha Bela includes wet, cold and dark environments [2]. The Savannah region in RibeirãoPreto comprises weathered soils, with acidic pH ranging from 4.3 to 6.2 [3]. Campinas region is characterized by the transition between Atlantic Rain Forest and Savannah biomes. Since these regions have differences in soil, plant covering and climates, they were considered convenient to isolate a higher diversity of yeasts.

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The microbial prospection in natural sources has been recommended for obtaining new biocatalysts. Until 1980, only about 2% of the world's microorganisms were tested as sources of enzymes. More recently, thousands of microbial strains isolated from soil were tested for lipase production, revealing that 20% were lipases producers, including filamentous fungi and yeasts [4]. Microbial lipases are preferred for commercial applications due to their multiple properties, easy extraction procedures and manipulation, broad substrate tolerance, high temperature stability and unlimited supply. They have a remarkable ability to perform a variety of chemical transformations [5].

According to Treichel et al. [6] and Bussamara et al. [4] lipases from *Candida rugosa* and *Candida antarctica* have been extensively studied and applied in different fields, however there are several recent publications reporting the production of lipases by other yeasts, reinforcing some emerging lipase producing yeasts, which represent a hope for the biotechnological innovation in this area.

B vitamins are essential for the growth and development of organisms, and have an important role in the activity of enzymes that regulate metabolic reactions [7]. According to Félix et al. [8] riboflavin and biotin deficiency in livestock is one of the most frequent, since diets based on corn, soybean, cereals and protein supplements do not have the minimum values recommended by the NRC (National Research Council). Deficiency of riboflavin and biotin may result in the decrease of weight gain, diarrhea, congenital malformations, high mortality, impaired growth, reduced efficiency of nutrient absorption, oral dermatitis and paralysis of curved fingers in broilers [9, 10].The chemical synthesis for B vitamins production has disadvantages like high energy requirement, use of toxic compounds and side-products, resulting in a costly and high environmental impactful process [11]. Therefore, the development of a biotechnological process aiming the low cost application in animal feed expands the possibilities of getting a product with different characteristics. These are the main reason why this study was carried out.

Material and Methods

Yeasts Isolation

A total of 120 samples of fruits, flowers, seeds (20 each) and 60 soil samples were collected from the litter. Soil samples were collected up to a maximum depth of 5cm by using sterile spatulas and the soil was placed into sterile polyethylene bags. Locations selected are considered biodiversity hotspots, without having much change by mankind increasing the possibility to isolate novel strains. During collection process the weather in the Atlantic forest and transition region was rainy hindering the bioprospection. However the weather in RibeirãoPreto (savannah biome) was sunny which facilitated the samples collection.

One gram of each sample was diluted in 10 mL of sterile water. Aliquots of each dilution were overlaid on Yeast malt agar (YMA), supplemented with chloramphenicol (50 ppm) to prevent bacterial contamination. Inoculated Petri dishes were kept at 30^{0}C for 24 to 72 h. Every 24 h growing colonies were observed and transferred to YMA slants incubated at 30^{0}C until a favorable growth of the strain was obtained. The strains were incorporated into the Wild Microorganisms Collection of Food Biochemistry Laboratory of UNICAMP.

Screening for Lipase Production

Verification of lipase production was evaluated in a medium containing (g L^{-1}): Agar, 15.0; peptone, 10.0; NaCl, 5.0; $CaCl_2.2H_2O$, 0.1 and 10 mL of Tween 80, adapted from Sierra [12]. These components were mixed in a blender until they formed an emulsion. After incubation for 48 h at 30^{0}C, the plates were kept at 4^{0}C. A visible halo formed due to the deposition of calcium crystals was considered a positive reaction. The enzymatic activity was semi quantitatively estimated by the ratio between the halo diameter and the colony diameter [13].

Quantitative analysis of lipase production was performed with the liquid medium composed by 5% of corn steep water, 1% of soybean oil, 1% olive oil and 0.5% ammonium nitrate. Thus yeast strains were inoculated in Erlenmeyer's containing 75 mL of the medium described above in orbital shaker at 30^{0}C and 150 rpm for 48 h in duplicates. After yeast growth was observed, the flasks were centrifuged at 10000×gat 10^{0}C for 15 min. Lipase activity was measured in the supernatant with a system consisting of 5 mL of an emulsion composed of extra virgin olive oil and 7% arabicgum solution (25% olive oil: 75% gum solution), 2 mL of phosphate buffer pH 7.0, 0.1 M and 1 mL of supernatant. The system was incubated at 37^{0}C for 30 min in a thermostatic bath with agitation of 130 oscillations per min. The reaction was stopped by addition of 15 mL of acetone: ethanol (ratio 1:1), and the released fatty acids titrated against NaOH 0.05 M. One unit of lipase activity was defined as the amount of lipase required to liberate 1 µmol of fatty acid per minute per 1 mL of the supernatant in the described conditions [14].

Screening of Biotin and Riboflavin Producer Strains

Yeast isolates were grown in liquid medium containing (g L^{-1}): glucose, 20.0; maltose, 5.0; $(NH_4)_2C_4H_40_6$, 0.5; KH_2PO_4, 1.0; $MgSO_4.7H_2O$, 0.5; $C_3H_4(OH)(COO)_3Fe.H_20$ (1% solution), 0.5 mL; $ZnSO_4$ (0.2% solution), 0.5 mL; thiamin 50 µg; pH 7.0 [15]. Then, they were inoculated in Erlenmeyer's containing 15 mL of the described medium in orbital shaker at 30^{0}C and 150 rpm for 72 h. The biomass was separated by centrifugation at 10000×g for 15 min. The supernatant was used for riboflavin and biotin assays. HABA/Avidin H2153 (Sigma-Aldrich® - St. Louis, MO, United States) was used as the reagent to measure the biotin concentration, and the absorbance analysis was performed in a spectrophotometer at 500 nm. The concentration of riboflavin in the supernatant was also determined by fluorimetric method adapted from Knorr [16] in black 96-well microtiter, with the Fluostar Optima fluorimeter - BMG Labtech microtiter reader. The concentration of riboflavin in the supernatant was directly related to fluorescence values obtained at wavelength excitation (485 nm) and emission (520 nm).

Three dilutions were performed by varying the concentration of supernatant in 20% v/v, 30% v/v and 50% v/v. The linearity of the

absorbance of each supernatant was verified, assessing whether any compound influenced the absorbance values used to determine the concentration of the vitamins.

Molecular Identification of Strains RP.C153 and RP.J1308

The RP.C153 and RP.J1308 strains were identified by amplification and sequencing of the D1/D2 region of the 26S rDNA gene, using primers NL1 5'GCATA TCAATAAGCGGAGGAAAAG3' and NL4 5'GGTCCGTGTTTCAAGACGG3' in Gene Amp PCR System 9700 thermocycler. The sequencing was performed with the BigDye (Applied Biosystems) kit according to the manufacturer's recommendations in XL sequencer Genetic Analyzer 3500 (Applied Biosystems) [17].

The DNA sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) available in the National Center for Biotechnology Information (NCBI - www.ncbi.nlm.nih.gov/BLAST) website. Species identification was based on the maximum score, identity and cover. The phylogenetic tree was elaborated with MEGA software (Molecular Evolutionary Genetics Analisys) [18].

Results and Discussion

Screening of Lipase Producing Strains

From a total of 427 strains isolated (70% from savannah region, 14% from transition and 16% from Atlantic forest) 80 were able to form a calcium crystal halo around the colony (Figure 1), indicating a potential production of extracellular lipase.

Enzymatic activity (in triplicates) was determined in the supernatant of 33 strains which presented the ratio between the halo diameter and the colony diameter higher than 2.00. These results are listed in Table 1.

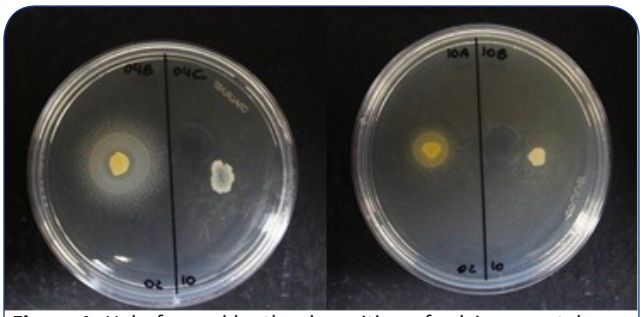


Figure 1: Halo formed by the deposition of calcium crystals surrounding the colonies, demonstrating the possible lipase activity (left). Strain which demonstrated no halo formation around the colony (right).

The results of enzymatic activity presented are promising, since 13 isolated strains presented a mean of enzyme activity higher than 20 UmL^{-1}. Burkert et al. [14] reported an enzyme activity f 20 U mL^{-1} after optimization of the same medium used in this study, to produce lipase by *Geotrichum* sp., thus showing the great potential of isolated yeasts for future studies regarding the production, optimization and its characterization.

Screening of Biotin Producing Strains

The results of biotin concentration in the medium, considering the different dilutions, are presented in Table 2. Therefore, 38 isolates presented biotin in the supernatant, which 34 were isolated from the RibeirãoPreto region (Savannah) and 4 from Ilha Bela (Atlantic Rain Forest). The strains from Campinas region did not demonstrated production of extracellular biotin.

Most studies developed in recent decades, involving the biotechnological production of biotin, regard the use of

Table 1: Enzymatic activities in the quantitative screening stage for lipase producing yeasts.

Strain	Lipase activity (U mL^{-1})	Strain	Lipase activity (U mL^{-1})	Strain	Lipase activity (U mL^{-1})
IB.03B	$7.55 \pm 0.49^{d,e,f}$	IB.35A	20.74 ± 1.47^{a}	RP.J1308	22.13 ± 0.49^{a}
IB.04B	$10.67 \pm 0.98^{d,e}$	IB.39A	21.44 ± 2.46^{a}	RP.K1.12	$20.05 \pm 0.49^{a,b}$
IB.05A	$8.24 \pm 2.46^{d,e,f}$	IB.40B	21.09 ± 1.96^{a}	RP.M201	20.4 ± 0.98^{a}
IB.07B	$9.29 \pm 0.98^{d,e,f}$	IB.46A	22.13 ± 1.47^{a}	C.15C	$11.37 \pm 0.98^{c,d,e}$
IB.10A	$6.51 \pm 0.98^{e,f}$	IB.48A	21.79 ± 0.98^{a}	C.01C	$17.97 \pm 0.49^{a,b}$
IB.17B	$6.51 \pm 0.00^{f,g}$	IB.41A	$17.62 \pm 0.98^{a,b,c}$	C.02A	$18.66 \pm 0.49^{a,b}$
IB.17C	$7.55 \pm 0.00^{d,e,f}$	IB.23C	20.74 ± 0.98^{a}	C.03B	$18.31 \pm 0.98^{a,b}$
IB.21A	$4.08 \pm 2.46^{f,g}$	IB.31B	20.40 ± 0.98^{a}	C.02B	Undetected
IB.23A	$7.90 \pm 3.44^{d,e,f}$	RP.C151	$12.76 \pm 0.00^{b,c,d,e}$	C.05C	20.49 ± 1.47^{a}
IB.33A	$17.62 \pm 0.98^{a,b,c}$	RP.C153	$13.45 \pm 0.00^{b,c,d}$	C.04B	21.091 ± 0.98^{a}
IB.33C	$17.97 \pm 1.47^{a,b}$	RP.K1.19	Undetected	C.14C	20.74 ± 0.49^{a}

[a, b, c] Results are presented as the mean of enzymatic activity (n = 3) ± standard deviation. Means with different letters show significant difference at $p > 0.05$.

Table 2: Biotin concentrations measured in the supernatant of yeasts cultures.

Strain	Biotin (µg mL^{-1})	Strain	Biotin (µg mL^{-1})	Strain	Biotin (µg mL^{-1})
IB.03B	0.28 ± 0.01^{g}	RP.C154	0.28 ± 0.12^{g}	RP.H21	3.95 ± 0.50^{f}
IB.16A	0.28 ± 0.01^{g}	RP.C156	18.61 ± 0.15^{a}	RP.J1.228	0.28 ± 0.13^{g}
IB.27B	0.28 ± 0.01^{g}	RP.D65	0.28 ± 0.02^{g}	RP.J1308	11.28 ± 0.07^{c}
IB.46B	7.61 ± 0.08^{e}	RP.D74	7.61 ± 0.40^{e}	RP.L1.177	0.28 ± 0.09^{g}
RP.A1.41	0.28 ± 0.01^{g}	RP.D77	11.28 ± 0.20^{c}	RP.L1.207	0.28 ± 0.24^{g}
RP.A1.60	18.61 ± 0.15^{a}	RP.E1.229	3.95 ± 0.12^{f}	RP.U08	0.28 ± 0.56^{g}
RP.A238	0.28 ± 0.01^{g}	RP.E10	18.61 ± 0.01^{a}	RP.X136	0.28 ± 0.01^{g}
RP.A242	7.61 ± 0.02^{e}	RP.E13	7.61 ± 0.01^{e}	RP.Y161	0.28 ± 0.01^{g}
RP.A250	11.28 ± 0.42^{c}	RP.E17	11.28 ± 0.12^{c}	RP.Y164	0.28 ± 0.05^{g}
RP.A252	14.95 ± 0.18^{b}	RP.E22	7.61 ± 0.01^{e}	RP.Z111	3.95 ± 0.12^{f}
RP.A254	0.28 ± 0.12^{g}	RP.E226	7.61 ± 0.01^{e}	RP.Z113	18.61 ± 0.01^{a}
RP.A256	0.28 ± 0.01^{g}	RP.F208	0.28 ± 0.03^{g}	RP.Z118	3.95 ± 0.03^{f}
RP.B131	3.95 ± 0.01^{f}	RP.F265	14.95 ± 0.12^{b}		

$^{a, b, c}$ Results are presented as the mean concentration of biotin present in the yeast supernatant culture (n = 3) ± standard deviation. Means with different letters show significant difference at p > 0.05.

recombinant DNA techniques. The best production results from this vitamin have been reported in the patent developed by Bower et al. [19] who used recombinant strains of *B. subtilis* resistant to 5 - (3-thienyl) pentanoic acid, with the *bio* gene over expressed (the gene responsible for synthesis of biotin), reaching a biotin production of more than 1 g L^{-1}.

According to Streit and Entcheva [11] although the yield achieved in the study above is very close to the profitable production, none of the strains mentioned in literature produced biotin enough to make a feasible biotechnological production. It should be considered that in many of these studies, there was addition of biotin precursors during cultivation, and the fermentation was performed in a complex medium making the process expensive.

Other factors may interfere in the microbial production of biotin like the instability of plasmids in the recombinant strains, the possibility of toxic effects of some metabolites and the cost of purification processes [20]. These factors reinforce the importance of bioprospection for novel producing biotin strains, as well as studies on the optimization of production, analysis of toxic metabolites, recombinant DNA technique and purification of this vitamin.

Screening of Riboflavin Producing Strains

The presence of riboflavin was detected in the culture supernatant of 51 isolates. The results listed in Table 3 show that 33 were isolated from Savannah region, 15 from Atlantic Rain Forest and 3 from transition region.

The industrial production of riboflavin using biotechnological processes, currently apply selected mutant microorganisms as *Candida famata* dep 8, *Bacillus subtilis*, and the filamentous fungi *Ahbyagossypi*. The yeast *C.famata* (also known as *C.flareri*, *Torolopsis candida*), has its ability to produce riboflavin widely studied, being considered the yeast with the greatest potential to produce this vitamin under iron limitation. Wild species of *C.famata* can accumulate about 600 mg mL^{-1}in the culture medium while other yeasts accumulate a maximum of 2 mg mL^{-1}[21].

It is evidenced in this study the importance of novel strains isolation for the production of this vitamin, since the literature reports few species capable of producing riboflavin profitably.

Identification of Lipase, Biotin and Riboflavin Producing Yeast Strains

The strain RP.J1308 produced lipase (22.13 U mL^{-1}) and biotin (0.11 mg mL^{-1}), and RP.C153 lipase (13.45 U mL^{-1}) and riboflavin (0.36 µg mL^{-1}). Both strains were selected to be identified by molecular techniques. RP.C153 strain showed 100% identity and higher scores when compared to the type strain *Pichia caribbica* NRRLY27274 (Figure 2). According to Vaughan-Martini et al. [22], *Pichia caribbica* is described as the ascosporic state of *Candida fermentati* and *Pichia guilliermondii*, belonging to *Pichia guilliermondii* clade. Strain RP.J1308 was 100% of identical to type strain of *Candida oleophila* ATCC28137, so the present study classified it as *C.oleophila* (Figure 3).

Novotný et al. [23] studied the lipase production by *C.guilliermondii* evaluating the influence of olive oil and glucose present in the culture medium. Concluding that the combination of both constituents significantly increased the enzyme production. Therefore the results presented in relation to RP.C153 strain (*Pichia caribbica*) and RP.J1308 strain (*Candida oleophila*) demonstrated considerable

Table 3: Riboflavin concentrations measured in the supernatant of yeasts cultures.

Strain	Riboflavin µg mL^{-1}	Strain	Riboflavin µg mL^{-1}	Strain	Riboflavin µg mL^{-1}
IB.03B	0.18 ± 0.05^{d,e,f,g,h,i}	RP.A251	0.07 ± 0.05^{g,h,i,j}	RP.N17	0.08 ± 0.02^{g,h,i,j}
IB.04B	0.17 ± 0.04^{e,f,g,h,i}	RP.B212	0.06± 0.04^{i,j}	RP.N19	0.06 ± 0.04^{i,j}
IB.08B	0.10 ± 0.05^{f,g,h,i,j}	RP.C153	0.36 ± 0.05^{b,c}	RP.O141	0.08 ± 0.03^{g,h,i,j}
IB.10A	0.13 ± 0.03^{f,g,h,i,j}	RP.C156	0.06 ± 0.04^{h,i,j}	RP.O144	0.07 ± 0.04^{h,i,j}
IB.10B	0.77 ± 0.07^{a}	RP.C162	0.14 ± 0.04^{f,g,h,i,j}	RP.O64	0.20 ± 0.02^{d,e,f,g}
IB.12A	0.45 ± 0.06^{b}	RP.C170	0.10 ± 0.05^{f,g,h,i,j}	RP.O73	0.15 ± 0.05^{e,f,g,h,i,j}
IB.12C	0.18 ± 0.03^{d,e,f,g,h,i}	RP.E17	0.12 ± 0.04^{g,h,i,j}	RP.D58	0.09 ± 0.03^{g,h,i,j}
IB.14A	0.11 ± 0.04^{f,g,h,i,j}	RP.E226	0.11 ± 0.03^{f,g,h,i,j}	RP.D64	0.15 ± 0.04^{f,g,h,i,j}
IB.21A	0.18 ± 0.05^{d,e,f,g,h,i}	RP.E235	0.07 ± 0.03^{g,h,i,j}	RP.D65	0.18 ± 0.05^{d,e,f,g,h,i}
IB.23B	0.15 ± 0.05^{e,f,g,h,i,j}	RP.F269	0.07 ± 0.03^{g,h,i,j}	RP.D83	0.29 ± 0.08^{c,d}
IB.26B	0.25 ± 0.05^{d,e}	RP.J1.SC	0.11 ± 0.03^{f,g,h,i,j}	RP.E1	0.36 ± 0.05^{b,c}
IB.33A	0.13± 0.02^{f,g,h,i,j}	RP.K1.12	0.05 ± 0.03^{j}	RP.E10	0.04 ± 0.03^{j}
IB.34A	0.07 ± 0.04^{h,i,j}	RP.LSc	0.11 ± 0.03^{f,g,h,i,j}	RP.E12	0.11 ± 0.04^{g,h,i,j}
IB.41C	0.21 ± 0.06^{d,e,f}	RP.M17	0.08 ± 0.03^{g,h,i,j}	RP.E16	0.08 ± 0.04^{g,h,i,j}
IB.47D	0.16 ± 0.06^{d,e,f,g,h,i,j}	RP.M21	0.05 ± 0.02^{j}	C.14A	0.08 ± 0.04^{g,h,i,j}
RP.A1.41	0.07 ± 0.04^{h,i,j}	RP.M22	0.05 ± 0.02^{j}	C.12B	0.04 ± 0.04^{g,h,i,j}
RP.A1.60	0.07 ± 0.05^{g,h,i,j}	RP.M25	0.08 ± 0.04^{g,h,i,j}	C.12C	0.03 ± 0.03^{f,g,h,i,j}

[a, b, c] Results are presented as the concentration of riboflavin present in the yeast supernatant (n = 3) ± standard deviation. Means with different letters show significant difference at p > 0.05.

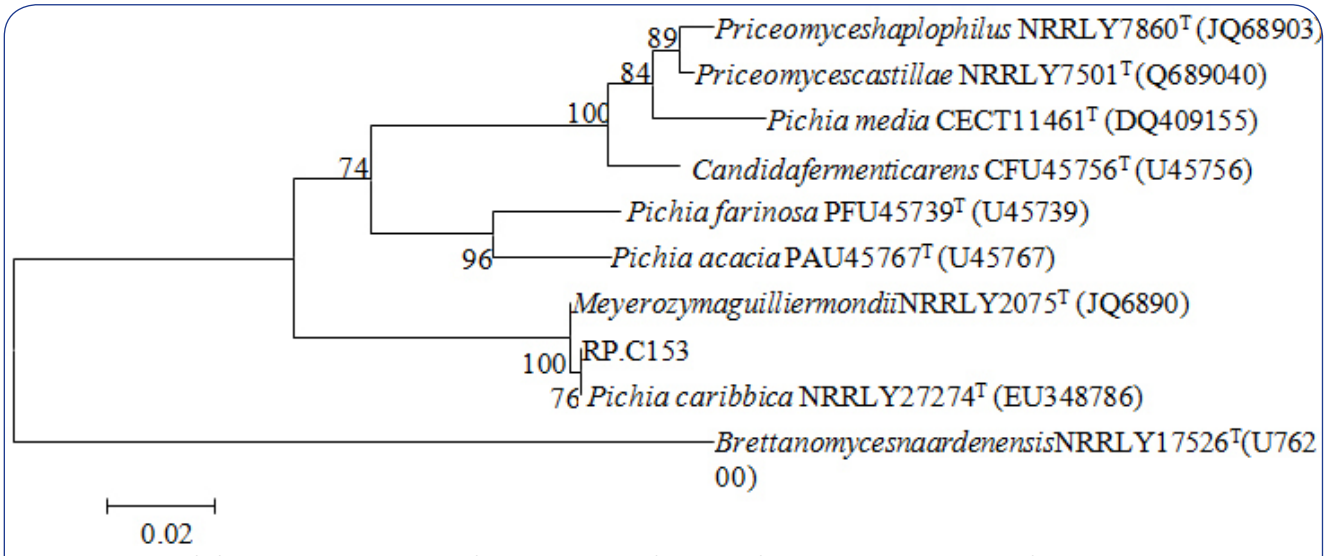


Figure 2: Phylogenetic tree comparing the RP.C153 strain distance, adopting *Brettanomycesnaardenensis* as outgroup.

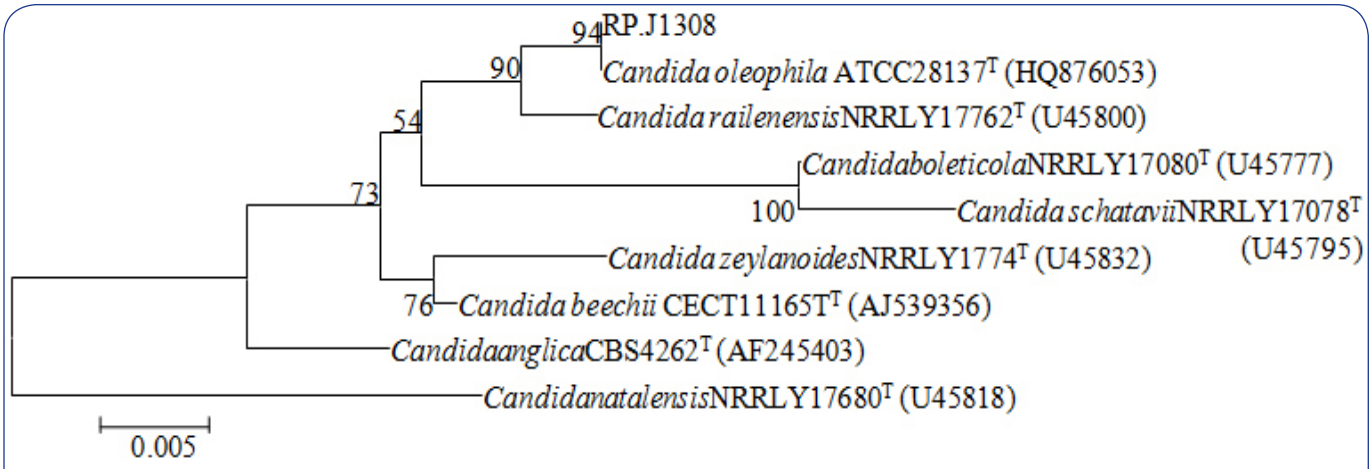


Figure 3: Phylogenetic tree comparing the RP.J1308 strain distance, adopting *Candida natalensis* as outgroup.

similarity to literature data, since the culture medium proposed in this study, used a combination of carbon source with olive oil and soybean, and both strains reached lipase activities values of (13.45 U mL^{-1} and 22.12 U mL^{-1}, respectively) demonstrating their potential for production of this enzyme. It is noteworthy that this study did not realize the optimization of lipase production by identified yeasts, been this step a suggestion for future studies.

Another aspect that describe the potential of *Candida* yeasts for lipase production was demonstrated in a study conducted by He and Tan [24], which used the response surface methodology and optimization of culture medium for enzyme production by the yeast *Candida* sp. This work reported a lipase activity of 6230 U mL^{-1} in cultivation in flasks and 9600 U mL^{-1} in cultivation in a 5 L bioreactor. Up to now, this strain of *Candida* sp. is the one with the greatest potential for lipase production reported in the literature.

According to Papon et al. [25], strains of *C. guilliermondii* have the ability to produce riboflavin in high concentrations under iron deficiency. The reason for this increase in production is not yet fully understood, however Abbas and Sibirny [26] emphasize that the excretion of riboflavin under iron deficiency plays an important role in the reduction of insoluble ion Fe^{3+} to the ion Fe^{2+}, which is more accessible by providing to the cells an additional source of iron.

For the selection of potential riboflavin producing yeast, the present study used the culture medium proposed by Strzelczyk et al. [15], which has Citrate of Iron III in its formulation. Therefore, a suggestion for future studies is the reduction of this component, in order to increase the production of extracellular riboflavin, using *Pichia caribbica*.

Regarding the production of Biotin, the genus *Candida* was studied by Hong et al. [20] and Suzuki et al. [27], and a production of extracellular Biotin of 1.8 mg $L^{-1}$and 8.3µg mL^{-1} was achieved respectively. The strain isolated in the present study RP.J1308 classified as *Candida oleophila*, was able to produce 11.28µg mL^{-1}demonstrating its potential for the production of this vitamin.

It is important to remark that a standard assay is essential for comparing the results of different studies. In several studies, research groups used different methodologies to determine the lipase activities and biotin and riboflavin concentration, which may result in differences in the substrates, incubation times, pH and temperatures of the reaction mixtures. The experimental data variability limits the comparisons among different studies. However it is evident that the present work has a significant contribution to future studies regarding production optimization, characterization and application of the prospected bioproducts.

Brazilian Biomes and Bioproducts Prospection

According to the results obtained during the selection of lipase, biotin and riboflavin producing yeast, it was possible to isolate from the biomes of Atlantic Rain Forest, Savannah and the transition region of these biomes, a significant amount of yeasts with potential for production of biocompounds analyzed (Figure 4). The present study also demonstrated the bioprospecting potential of the biomes present in the state of São Paulo, justifying its classification as a hotspot. Moreover the Atlantic Rain Forest biome can be considered as an interesting bioprospection spot since 28% of the isolates from this area presented potential for lipase production, 22% for riboflavin production and 6% for biotin production. This biome showed higher percentage values related to the bioproducts prospection when compared to the other regions (Figure 4).

Buzzini and Martini [28] also evaluated the biotechnological potential of microorganisms isolated from samples of water, soil, insects and plants collected from three different Brazilian rainforests, presenting that some strains had potential for production of lipases, esterases and proteases. Machado et al. [29] studied strains from Atlantic Forest region in Santos, São Paulo, isolating strains with potential for production of ligninolytic enzymes. Tauk-Torniselo et al. [30] evaluated microorganisms isolated from Atlantic Forest in Jureia-Itatins ecological station - São Paulo, isolating 1211 strains, which 67 had potential for production of cellulolytic enzymes.

This large capacity of the Brazilian Atlantic Rain Forest for

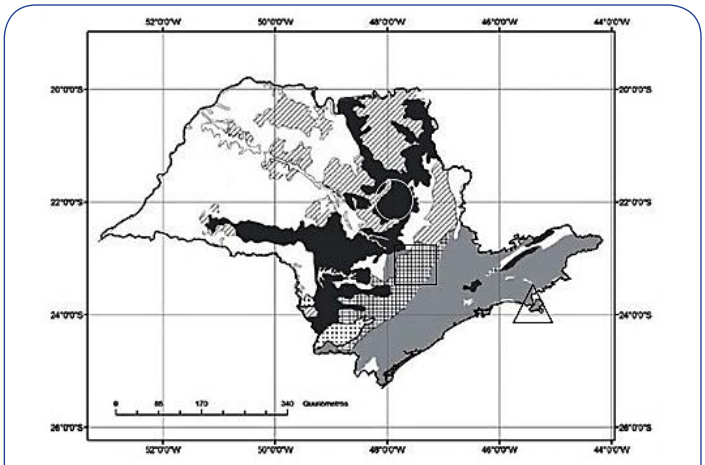
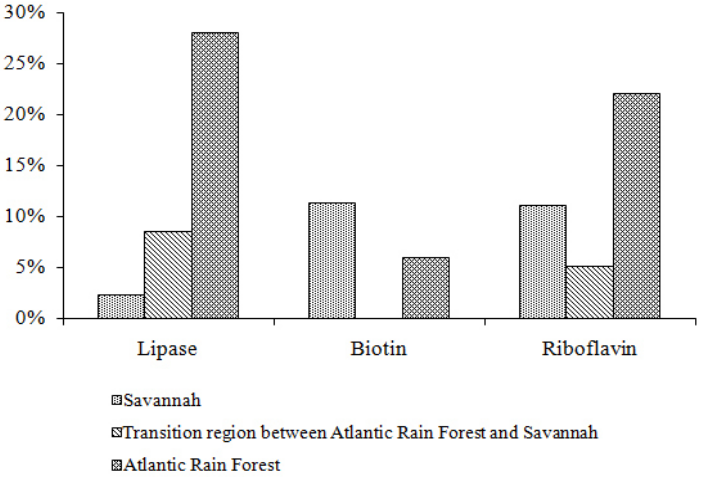


Figure 4: Yeasts isolated from regions of Savannah, Atlantic Rain Forest and transition region between both biomes which presented lipase, biotin and riboflavin potential production and percentages relating the isolates amount with the bioproducts producing yeast from each area.

○Savannah strains - Total: 300 (70%); Lipase producing: 7; Biotin producing: 34; Riboflavin producing: 33

□Transition region between Atlantic Rain Forest and Savannah strains - Total: 59 (14%); Lipase producing: 5; Biotin producing: 0; Riboflavin producing: 3

ΔAtlantic Rain Forest strains - Total: 68 (16%); Lipase producing: 19; Biotin producing: 4; Riboflavin producing: 15



isolation of microorganisms capable to produce several types of enzymes and other bioproducts can be related to the presence of a singular soil rich in organic material (as plant material and animal wastes decomposed) and humid environments creating an ideal condition for microorganisms growth, production and secretion of several bioproducts [31].

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