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MYCOCINOGENIC YEASTS ISOLATED FROM AMAZON SOILS OF THE MARACÁ ECOLOGICAL STATION, RORAIMA-BRAZIL

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ABSTRACT

The 240 yeasts isolated from soils of the Maracá Ecological Station in the Brazilian Amazon were identified and screened for mycocin production. These strains included 82% of ascomycetous and 18% basidiomicetous affinities and the prevalent species were *Candida etchellsii, Candida famata, Candida robusta, Candida rugosa, Candida valida, Debaryomyces hansenii, Cryptococcus albidus, Cryptococcus laurentii, Rhodotorula glutinis, Rhodotorula minuta* and *Rhodotorula mucilaginosa*. Mycocins able to kill some yeasts were produced by 6 strains identified as *Issatchenkia* sp., *Saccharomyces exiguus*?, *Williopsis saturnus*, var. *subsufficiens*, and 3 *W. saturnus* according to 26S rDNA D1/D2 region sequence and phenotypic data.

Key words: Mycocins, killer yeasts, tropical soil, *Issatchenkia, Saccharomyces, Williopsis.*

INTRODUCTION

Some yeasts strains produce and excrete extracellular toxins, mycocins, that are lethal to other sensitive yeast strains. Production of proteins with specific toxicity to related organisms, associated to specific immunity, is known to occur in the Ustilaginales, myxomycetes, paramecia and bacteria. The bacterial proteins with such capacity are denominated bacteriocins and similar compounds produced by yeasts have been called "killer" toxins but now are called mycocins and the strains that produce them as mycocinogenic (9). The *S. cerevisiae* mycocin system was first discovered in 1963 and has been the most studied. It is coded by a double stranded RNA gene that has been found only in *Saccharomyces* and the related genus *Zygosaccharomyces* (9,32). In other genera studied, the mycocins are coded in plasmidial DNA or chromosomal DNA, being activated at low pH and characterized as proteins or glicoproteins, with two or three subunits (9,24). This natural phenomenon is a potential mechanism of competition by interference or amensalism, with the production of toxic compounds preventing access of sensitive yeasts to the resources and resulting in decreased population size of the less competitive species. Competition by interference generally occurs to obtain more resources by exclusion of other individuals or populations (1,8,23). Mycocinogenic activity has been detected in 80 species distributed in 20 ascomycetous and basidiomycetous yeast genera. Most of the mycocinogenic yeasts are in the genera *Candida, Cystofilobasidium, Cryptococcus, Debaryomyces, Filobasidium, Hansenula, Hanseniaspora, Kloeckera, Kluyveromyces, Metschnikowia, Pichia, Rhodotorula, Saccharomyces, Sporodiobolus, Williopsis* and *Zygosacharomyces* (9). Mycocinogenic activity has been detected mainly in yeasts isolated from fruits, plant exudates, and plant structures, but rarely from soils (3,23,25).

One of the most important conditions for detection of the mycocinogenic activity is the pH of the culture medium used for screening tests. This activity is generally expressed in acidic conditions between pH 3 to 6, with optimums ranging from 4 to 5 (33). Exceptions can be observed in mycocins that are inactivated by high temperature and tested at lower temperature

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(13). Mycocins are more stable in solid media, because agitation can cause their inactivation (30). Glucose-peptone-yeast extract-agar medium (GYP) or malt agar with phosphate-sodium citrate buffer is most often used for these tests. The relation between the inoculum sizes of the sensitive and mycocinogenic strains influences the test sensitivity. In some cases the addition of 10-15% glycerol increases the size of the inhibition zone, and facilitates interpretation (9,13,17).

Mycocins are of industrial importance for microbiological control of fermentation processes, and as a model for the study of secretion mechanisms involving extracellular proteins and glicoproteins (17). The application of mycocinogenic yeasts in the fermentation and beverage industry has grown with the objective of eliminating wild strains responsible for product deterioration and production of undesirable compounds (18,32). The mycocins have only antifungal activity and are inactive against bacteria and protozoans (9). Some mycogenic strains act against pathogenic yeasts, such as *Candida albicans* and *Candida glabrata*, and filamentous fungi, suggesting potential application against fungal infections (14,20,28). Our objective was to detect 'in vitro' mycocin producing yeasts from Amazon soils, and identify these strains by conventional and molecular methods.

MATERIALS AND METHODS

Yeasts were isolated from soil samples collected from the Maracá Ecological Station, located on Maracá Island 130 km from Boa Vista, in the Amazon state of Roraima, Brazil. The Ultisoils are the predominant soils on the Island, especially oxissoil, with the occurrence of red-yellow ultisoils, in euthrophic and distrophic forms. Yeast isolations were done by the spread plate method on acidified Y-M Agar medium (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose and 2% agar) with 200mg/L of chloramphenicol, 0.15% sodium propionate, and pH adjusted to 4.5 with HCl and incubated 3 to 5 days at 25 ± 3ºC (2,22). Selected colonies were streaked on Y-M agar to obtain pure cultures and maintained at 8 ± 4ºC on 2% glucose- 0.5% yeast extract- 2% malt extract- 0.1% monosodium phosphate 2% agar slants covered with sterile mineral oil.

Mycocinogenic activity was tested in triplicate, on YM agar medium supplemented with 0.003% of methylene blue and 15.0% of glycerol and buffered with 0.01 M citrate buffer to pH 4.2. The strains *C. glabrata* Y-55 (NCYC 388, IMUFRJ 50.083) and *Pichia heedii* 83.504-2, sensitive to the majority of the known mycocins, were grown for 24 h at 21ºC on YM agar, then were suspended in distilled water at 4 x 10^5 cells/ml and spread on the medium surface with sterile swabs. The yeasts that were tested for mycocin production were grown in the same way and inoculated in streaks on medium after inoculation with the sensitive strain. The plates were incubated at 23ºC and were observed daily for 3 days. Isolates were considered as mycocinogenic if they produced an inhibition zone with no growth and adjacent blue zone indicating cellular death of the sensitive strain. Strains of *Pichia kluyveri* (IMUFRJ 51.498) and *P. ohmeri*-like (IMUFRJ 51.535) were employed as positive controls with mycocinogenic activity. The confirmation of activity was obtained through the replica-plating technique in which the sensitive test strains were incorporated individually into the agar medium and 25 strains to be tested for mycocin production were then inoculated onto each plate. The incubation conditions, positive controls and evaluation were the same as noted above. To test the ability of mycocinogenic yeasts to eliminate yeasts from soil communities, 121 selected strains were used as lawns to determine the sensitivity to the 6 toxin-producing yeasts.

The phenotypic characterization and identification of yeast cultures was done according to Kurtzman and Fell (11) and Barnett *et al.* (5). Representative isolates of different species were characterized by the size of the ITS region and the isolates producing mycocins also by sequencing the D1/D2 region of the rDNA large subunit (7,10,12,26,29). DNA was extracted from pure cultures and amplified by PCR using the universal primers ITS 1 and ITS 4 or NL-1 and NL-4. Extracted DNA was purified for sequencing using the Concert Rapid PCR purification System (Gibco BRL), sequencing done using the Big Dye Termination Cycle Sequencing kit (ABI PRISM) and GeneAmp 9700 program according to the manufacturer's recommendations. Electrophoresis of the sequence reaction mixtures was done with an automatic DNA sequencer (ABI PRISM model 310 Genetic Analyzer). The sequence was edited using the DNASIS for Windows (version 2.1) software and aligned by the ClustalW program. Yeasts were identified using BLAST analysis and the GeneBank database. The designation of species followed by the suffix "-like" indicates that the organism is similar to this species but sufficiently different to be a new species, whereas the suffix "?" indicates the yeast appears to be of the named species but the identification is uncertain.

RESULTS

The 240 yeast isolates were grouped into 16 genera and 66 species, with 82% of the strains having ascomycetous affinity (Table 1). Only 6 strains, all of ascomycetic affinity had micocinogenic activity with the same profiles against both the *C. glabrata* and *P. heedii* test strains. These included *Issatchenkia* sp. (probable teleomorph of *Candida pseudolambica*), *Saccharomyces exiguus* ?, *W. saturnus* var. *subsufficiens*, and 3 *W. saturnus*. The activity of the 6 mycocinogenic strains against yeasts from the soil community is presented in Table 1. *Issatchenkia* sp., and *W. saturnus* were isolated from samples collected during the dry season, whereas *S. exiguus*? and *W. saturnus* var. *subsufficiens* were collected during the rainy season.

Table 1. Amazon forest soil yeasts and the number of them that were sensitive to mycocins produced by six of the isolates.

Yeasts	Number of isolates	*W. saturnus* RR41	*W. saturnus* RR14	*W. saturnus* RR19	*W. saturnus* RR177	*Issatchenkia* sp.	*S. exiguus* ?
ASCOMYCETOUS		a(b)					
Candida spp. (9 species)	10	0(8)	0(8)	0(8)	0(8)	0(8)	0(8)
Candida boidini	1	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)
Candida bombicola	2	0(2)	0(2)	0(2)	0(2)	0(1)	0(1)
Candida colliculosa	5	0(3)	0(3)	0(3)	0(3)	0(1)	0(1)
Candida edax?	2	0(2)	0(2)	0(2)	0(2)	0(2)	0(2)
Candida etchellsii	14	2(4)	2(4)	2(4)	2(4)	2(4)	2(4)
Candida famata	12	2(4)	2(4)	2(4)	2(4)	2(4)	2(4)
Candida famata-like	2	0(2)	0(2)	0(2)	0(2)	0(2)	0(2)
Candida gropengiesseri?	1	1(1)	1(1)	1(1)	1(1)	0(1)	0(1)
Candida lusitaniae	1	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)
Candida holmiii	1	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)
Candida holmiii-like	1	1(1)	1(1)	1(1)	1(1)	0(1)	0(1)
Candida oleophila	4	1(2)	1(2)	1(2)	1(2)	0(2)	0(2)
Candida oregonensis-like	1	1(1)	1(1)	1(1)	1(1)	0(1)	1(1)
Candida reukaufii	2	0(2)	0(2)	0(2)	0(2)	0(2)	0(2)
Candida robusta	13	0(4)	0(4)	0(4)	0(4)	0(4)	0(4)
Candida rugosa	7	2(4)	2(4)	2(4)	2(4)	2(4)	2(4)
Candida sake	3	1(2)	1(2)	1(2)	1(2)	1(2)	1(2)
Candida schatavii?	1	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)
Candida sorbophila	1	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)
Candida tannotolerans?	1	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)
Candida valida	7	1(3)	1(3)	1(3)	1(3)	1(3)	1(3)
Candida versatilis -like	1	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)
Debaryomyces hansenii	15	4(4)	4(4)	4(4)	4(4)	0(4)	0(4)
Debaryomyces occidentalis	4	1(2)	1(2)	1(2)	1(2)	1(2)	1(2)
Debaryomyces vanrijiae	5	0(2)	0(2)	0(2)	0(2)	0(2)	0(2)
Geotrichum candidum	2	1(1)	1(1)	1(1)	1(1)	0(1)	0(1)
Issatchenkia occidentalis	3	0(2)	0(2)	0(2)	0(2)	0(2)	0(2)
Issatchenkia orientalis	1	1(1)	1(1)	1(1)	1(1)	0(1)	0(1)
Issatchenkia sp.	1	1(1)	1(1)	1(1)	1(1)	0(1)	0(1)
Lipomyces tetraporus	3	0(1)	0(1)	0(1)	0(1)	0(2)	0(2)
Pichia bispora-like	1	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)
Pichia farinosa?	1	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)
Pichia galeiformis?	1	1(1)	1(1)	1(1)	1(1)	0(1)	1(1)
Pichia inositovora -like	5	0(2)	0(2)	0(2)	0(2)	0(2)	0(2)
Pichia membranifaciens	3	2(2)	2(2)	2(2)	2(2)	0(2)	0(2)
Pichia spp. (3 species)	3	0(3)	0(3)	0(3)	0(3)	0(3)	0(3)
Saccharomyces cerevisiae	4	2(3)	2(3)	2(3)	2(3)	0(3)	0(3)
Saccharomyces exiguus ?	1	1(1)	1(1)	1(1)	1(1)	1(1)	0(1)
Schizoblastosporion s. -henrici	1	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)
Torulaspora delbruecki	3	1(2)	1(2)	1(2)	1(2)	0(1)	0(1)
Williopsis saturnus RR41	1	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)
Williopsis saturnus	3	0(3)	0(3)	0(3)	0(3)	0(3)	0(3)
Zygosaccharomyces sp.	2	0(2)	0(2)	0(2)	0(2)	0(2)	0(2)
BASIDIOMYCETOUS							
Cryptococcus albidus	8	1(4)	1(4)	1(4)	1(4)	0(4)	1(4)
Cryptococcus curvatus	2	0(2)	0(2)	0(2)	0(2)	0(2)	0(2)
Cryptococcus hungaricus	3	0(2)	0(2)	0(2)	0(2)	0(2)	0(2)
Cryptococcus laurentii	11	0(4)	0(4)	0(4)	0(4)	0(4)	0(4)
Fellomyces fuzhouensis	1	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)
Rhodotorula aurantiaca	1	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)
Rhodotorula glutinis	14	0(4)	0(4)	0(4)	0(4)	0(4)	0(4)
Rhodotorula minuta	7	0(2)	0(2)	0(2)	0(2)	0(2)	0(2)
Rhodotorula mucilaginosa	27	0(5)	0(5)	0(5)	0(5)	0(5)	0(5)
Sporobolomyces roseus	2	0(2)	0(2)	0(2)	0(2)	0(2)	0(2)
Sporobolomyces shibatanus	2	1(2)	1(2)	1(2)	1(2)	1(2)	1(2)
Trichosporon asteroides	1	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)
Trichosporon ovoides	4	0(1)	0(1)	0(1)	0(1)	0(1)	0(1)

a= number of sensitive strains; b= number of tested strains.

The *Williopsis* strains showed mycocinogenic activity against many yeasts from their soil habitat (48% of all strains, 36% of ascomycetous species, 14.5% of basidiomycetous species) compared with *S. exiguus* ? (19% of all strains, 16.4% of ascomycetous species and 15% of basidomycetous species) and *Issatchenkia* sp. (9.5% of all strains, 14.5% of ascomycetous species and 8% of basidomycetous species). The 4 *Williopsis* strains presented equal activity profiles in relation to the species of sensitive strains, producing growth inhibition and cellular death zones of about 12 mm diameter. The activity of *Issatchenkia* sp. and *S. exiguus*?, produced inhibition and death in a 10-mm diameter zone. All of these mycocinogenic strains fermented glucose, 2 had low carbon source assimilation profiles with less than 9 positive results and 4 had intermediate assimilation profiles of 11 to 19 out of the 37 carbon sources tested. Strain RR115 failed to show the latent growth on glucosamine characteristic of *C. pseudolambica*, but otherwise was consistent with the description of this species and formed ascospores typical of the genus *Issatchenkia*. Strain RR169 fit the description of *S. exiguus*, the teleomorph of *Candida holmii*.

The ITS region length of *Issatchenkia* sp., of about 430 bp, and *S. exiguus* ?, 700 bp, were consistent with those of similar species, while *W. saturnus* and like strains, with fragments of 600 bp, were compatible with type strain (IMPPG 51.700). The D1/D2 region nucleotide sequence from *Issatchenkia* sp. was 99% similar (527/530bp) to that of *C. pseudolambica* (GenBank U71063), indicating that it should represent the teleomorph of this species. That of strain RR169 had 90% similarity (253/279bp) with *S. exiguus* (GenBank U68553) meaning that it should be closely related. The pairing of D1/D2 region of 26 rDNA of *W. saturnus* var. *subsufficiens* RR41 with Gene Bank data for the type strain (U75960), resulted in 98.2% (487/496bp) similarity; while the sequences of *W. saturnus* RR14 showed 85.4% (287/336), *W. saturnus* RR19 showed 92.4% (340/368bp), and *W. saturnus* RR177, presented 91.1% (224/246bp) similarity with the sequence of the type strain.

DISCUSSION

The frequency of mycocinogenic strains isolated from natural environments varies for different microhabitats but is greater than that found among isolates from culture collections (23,24). Most of the reports of mycocinogenic yeasts in natural environments, including tropical ones, refer to ephemeral habitats like fruits with high concentrations of sugar, and associated insects (3). The percentage [and isolated strains] of mycocin producing yeast species in communities found in tropical fruits was 24% of the species [13% of strains] for Amapa fruit, 27% of the species [10% of strains] and for guava from a forest area, and for Cactus stem necrosis 12% of the species [6.4% of strains] (1,3). In association with animals mycocinogenic yeasts from the marsupial *Didelphis marsupialis* fecal pellets 6.9% of the species [10% of strains], *Drosophila* 18% of the species [9% of strains] and mangrove invertebrates 15% of the species [27% of strains], but for yeasts from the marsupial *Philander frenata* and rodent fecal pellets mycocins were not detected (1,2,4, and unpublished data) in their yeast communities. Mycogenic yeasts in water from bromeliad tanks had 5% of the species [2% of strains] and from a swamp water 8% of the species [8% of strains] (unpublished data). In most of the habitats mycocinogenic yeasts were not among the prevalent species. However, in some cases such as those of *K. aestuarii* in mangrove invertebrates, *P. kluyveri* in cactus necrosis and guava fruits and *W. saturnus* in swamp and bromeliad waters they were among the prevalent yeasts. In Amazon soils we found the percentage of mycocinogenic species to be most similar to that found in bromeliad tank and swamp waters in previous studies using similar methodology of detection. Soils are generally more stable and lower nutrient level habitats than fruits, and should favor less fermentative and broader assimilation profile species. However, they are not at all homogenous in nature, but a mosaic of microhabitats some of which can be nutrient rich such as dead plant or animal matter. In the lower nutrient level habitats, fast growth and elimination of nutrient competitors may not be as much of an advantage in soils when there is not much to compete for and mycocinogenic species would use some energy making toxins. Other factors allowing persistence with lower levels of more diverse nutrient resources could have more importance in habitats like soil.

In the present work we used two phylogenetically distant strains, which were sensitive to the majority of the described toxins, and we found a similar diversity of mycocinogenic yeasts to that obtained in other studies using only 1 or 2 sensitive strains (1,3,24). The utilization of more than one sensitive strain to detect mycocin production has been indicated in order to detect a greater number of mycocinogenic isolates (9,24,32). The use of a larger number of more diverse test strains applied to isolates of more diverse origin should reveal more cultures with this activity. This was the case for a collection of isolates from diverse substrates of the Brazilian Atlantic Forest tested for mycocin production against a set of yeasts of industrial or medical importance (6). It is also probable that the basidiomycetous yeasts are more important in the tropical soil habitat than indicated by our data because the culture medium and incubation conditions used in this study were more favorable for the isolation of ascomycetous yeasts.

Sequence analysis of the D1/D2 region of the 26SrDNA gene with about 550 base pairs has been shown by Kurtzman and Robnett (12) to allow identification of most ascomycetous yeast species and their anamorphs. Strains that show more than 1% of nucleotides substitution in this region are probably different species, while strains with substitutions in up to 3 nucleotides (about 0.5%) belong to a same species or a closely related one. Comparison of sequences of this region of type

and authentic cultures deposited in the GenBank (12) allow identification in most cases of described yeasts to the species level and can show the closest species affinity of undescribed species. Spore production and the alignment of D1/D2 sequence from strain RR115 indicated it is the teleomorph of *C. pseudolambica* so it has been designated as *Issatchenkia* sp. and its phenotypic characteristics are in agreement with this identification (21). Our partial D1/D2 sequence data suggest that strain RR169 may be a new species similar to *S. exiguus* of the "*sensu lato*" group so it has been designated here as *S. exiguus*? although its phenotypic characteristics are typical of this species. A new species with high phenotypic similarity to existing *Saccharomyces* species has been isolated previously from Brazilian forest microhabitats (15). *W. saturnus* includes several varieties reflecting a well documented high level of variation within this genetically related group, but without delineation among them at the species level. Strain RR41 had a high sequence similarity, when compared with the type culture of *W. saturnus* var. *subsufficiens* and was also consistent in phenotypic characteristics with this organism. However strains RR14, RR19, and RR177 had an intermediate level of partial D1/D2 sequence similarity but based on incomplete sequences of the region. They are closely related to *W. saturnus* and have a broad mycocin activity profile which is typical of this species, but do not fit any of the existing five varieties. All 4 of the *Willopsis* isolates were in the same clade, but 3 may be distinct from existing varieties of *W. saturnus*.

W. saturnus is known to have a relatively broad spectrum of mycocinogenic activity and is a cosmopolitan species, frequently isolated from soil and related habitats (4,11,16,31). The presence in soil at relatively high frequency of isolation and mycoinogenic properties we found for this group are consistent with previous reports. *Candida pseudolambica*, the probable anamorph of *Issatchenkia* sp RR 115, has been found to occur in stools, forages and temperate climate soils and a similar yeast has been isolated from mangrove invertebrates (4), but has not been found previously in tropical soils. *S. exiguus*, and its anamorph *C. holmii*, has a record of occurrence in soils, fruits, juices of fruits and products of fermentation, preserved vegetables, in temperate environments (5,11) and has been reported from a tropical mangrove (4) Brazilian Atlantic Forest marsupial fecal pellets (2) and polluted estuarine waters (22). There is no record in the literature of mycocinogenic activity for *S. exiguus* nor for this *Issatchenkia* sp, but the genus *Issatchenkia* is part of the *P. membranifaciens* clade, which includes many mycocin producers as does the *Saccharomyces* clade, so the presence of this characteristic could be expected in these species. The 4 *Williopsis* isolates had identical results of activity against other strains from the soil community but *S. exiguus*? and *Issatchenkia* sp. were different from these and from each other in this activity suggesting they may produce different toxins.

The presence of non-mycocynogenic strains of species reported as including producers such as *C. famata*, *R. mucilaginosa*, *Cr. albidus* and *Cr. laurentii*, demonstrates the variability of this characteristic. This could result from poor taxonomic delineation of these species in studies relying mostly on a few differences in biochemical tests for identification, or genetic variation among strains. For example, our *C. famata* isolates varied in sensitivity to mycocins. This species is poorly delineated by conventional taxonomic methods so much of the data attributed to this species may be for other misidentified or undescribed species that can fall within its broad phenotypic description. Mechanisms of control and expression of mycocin production for the majority of the known strains, and sensitivity and resistance relationships among strains needs further study (9). Different levels of sensitivity to mycocynogenic activity have been used as a factor for intraspecific distinction in *C. albicans* and *S. cerevisae* (19,27).

Mycocinogenic yeasts have a broad geographic distribution in diverse microhabitats, although they generally make up a relatively small portion of their yeast communities. Our preliminary data indicate the yeast community in Amazon soils to be diverse. We have shown the presence of mycocinogenic yeasts in a limited collection of yeasts from soils in a small Amazon region. Further studies of yeasts in tropical soils should allow discovery of new species and novel mycocinogenic yeasts.

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RESUMO

Leveduras micocinogênicas de solos da Estação Ecologica de Maracá, Roraima-Brasil

Duzentos e quarenta linhagens de leveduras foram isoladas de amostras de solos da Estação Ecológica de Maracá, na Amazônia Brasileira, as quais representam 82% de leveduras de afinidade ascomicética e 18% basidiomicética. As especies dominantes foram *Candida etchellsii, Candida famata, Candida robusta, Candida rugosa, Candida valida, Debaryomyces hansenii, Cryptococcus albidus, Cryptococcus laurentii, Rhodotorula glutinis, Rhodotorula minuta* and *Rhodotorula mucilaginosa*. A capacidade das leveduras produzirem e excretarem toxinas letais a cepas sensíveis de leveduras, atividade micocinogênica, foi investigada. Seis

linhagens foram capazes de produzir micocinas: *Issatchenkia* sp., *Saccharomyces exiguus*?, *Williopsis saturnus* var. *subsufficiens*, e 3 *W. saturnus* identificadas conforme os dados de taxonomia molecular baseados nas seqüências da região D1/D2 do 26S rDNA.

Palavras-chave: micocinas, leveduras "killer", solos tropicais, *Issatchenkia*, *Saccharomyces*, *Williopsis*.

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