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Yeasts isolated from bovine rumen selected to degrade lignocellulosic roughage

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Abstract. *Duarte ER, Lima SM, Nere JC, Freitas CES, Maia HAR, Júlio ADL, Abrão FO, Alves JMS, Santos VLD, Geraseev LC, Cota J. 2024. Yeasts isolated from bovine rumen selected to degrade lignocellulosic roughage. Biodiversitas 25: 1159-1166.* Commercial yeast strains are viable alternatives to chemical additives in ruminants; however, they are not self to the rumen ecosystem. The present study was conducted to compare the degradation of *Urochloa decumbens* (Stapf) R.D.Webster (UD) hay and sugarcane bagasse (SB) by eight selected yeast strains, naturally present in the rumen cows and control without microorganisms. Eight yeast isolates that showed >6 log CFU per mL of ruminal fluid were selected and identified as four *Pichia kudriavzevii* isolates, three *Rhodotorula mucilaginosa* isolates and one *Candida tropicalis* isolate, according to their morphological and physiological characteristics and by analyses of the D1 - D2 variable domain sequences of the 28S rRNA gene. The strain V5 (*R. mucilaginosa*) showed higher cell biomass production than other strains of this species in fermentations with UD ($P<0.05$). Two isolates of *Pichia kudriavzevii* (V10 and V62) and one of *R. mucilaginosa* (V5) promoted a higher reduction of dry matter (DM) of UD than other yeast. The strain V5 was also the most efficient to reduce DM of SB ($P <0.05$). These selected yeasts could represent a potential alternative for use in animal diets and for industrial applications.

Keywords: Biomass production, ruminal microbiota, sugarcane bagasse, tropical forage

INTRODUCTION

The complex ruminal microbiota may supply proteins, energy, and vitamins to the ruminants, which contribute to their growth and production. This ecosystem comprises known species of bacteria, anaerobic filamentous fungi, and protozoa (Silva et al. 2019). Nevertheless, yeast species can be naturally detected in ruminal fluid, constituting up to 6.76 log of colony forming unit (CFU) per mL of ruminal fluid in healthy cattle of different ages (Abrão et al. 2014; Fernandes et al. 2019). However, the role of the biotechnological potential of these eukaryotes needs to be better elucidated.

Research evaluating the incorporation of yeast from exogenous sources in the ruminant diet has shown a reduction in the overall ruminal oxygen content and increased ruminal bacterial populations and microbial protein production of the rumen (Marrero et al. 2015; Huebner et al. 2019). The use of yeast and yeast product supplementation during specific periods of creation or with a specific diet can maximize the performance of the ruminants (Baker et al. 2022). *Saccharomyces cerevisiae*, the most important exogenous yeast used in ruminant supplementation, can reduce lactic acid levels, control the ruminal pH, and favor cellulose digestion (Vyas et al. 2014; Jiang et al. 2017).

This yeast also prevented diseases and reduced fecal excretion of pathogenic bacteria (Garcia et al. 2019) and this could have been attributed to bioactive peptides from yeast that have provided antimicrobial, antioxidative and immunomodulatory effects (Sánchez and Vázquez 2017). The supplementation with live yeast can favor feed intake and growth rate and improve milk production and milk fat value. These effects could be caused by yeast's ability to stimulate fiber digestion and stabilize the rumen pH. These small eukaryotes could counteract the acidotic effect in high-concentrate diets in the rumen by competing with lactate-producing (Amin and Mao 2021).

Diets with yeast supplementation could favor the protozoa modulation in the rumen, which could exhibit positive effects on fiber digestibility through modulation of ruminal pH (Bayat et al. 2015). The yeast can stimulate the growth of ciliate protozoa, which integrate cis-9, trans-11 CLA, and trans-11, 18:1 into their cells. The fatty acids within the cells of these protozoa eventually become available for absorption

in the small intestine after the microbes get degraded during acidic digestion in the abomasum (Francisco et al. 2019).

However, some of the commercial *S. cerevisiae* strains have exhibited limited growth in the rumen (Garcia et al. 2019; Huebner et al. 2019). The natural occurrence of yeast in the ruminal fluid has been reported for cattle fed with different sources of tropical roughage (Abrão et al. 2014; Fernandes et al. 2019). Surprisingly, in our previous study, a higher population of ruminal yeast in adult Zebu cows (>6.7 log10 CFU per ml of rumen fluid) than young in cattle raised on pastures of *Uroclhoa* spp. was detected (Abrão et al. 2014). Yeast biochemical profiles were evaluated considering the growth of 44 nutrient sources. Four profiles were detected among the five yeast isolates obtained from steers. The yeast from the cows showed five distinct profiles, whereas isolates from calves showed two. The D1/D2 variable domains of the large subunit ribosomal RNA sequences were evaluated. Among 16 yeast isolates from the steers, four different biochemical profiles, belonging to *Torulaspora* cf. *globosa* were observed, indicating intra-specie variability of the strains. For cows, were identified *Wickerhamomyces* cf. *anomalus*, *Cryptococcus (Bulleromyces) laurentii* and *Pichia kudriavzevii*. In calves, the biochemical profile seven, similar to the cows, belongs to the same species (*P. kudriavzevii*). The yeast of profile 10 was also identified as this species, showing intra-specie variation of biochemical characteristics (Abrão et al. 2014). In another study from Brazil, the yeast population from rumen ranged from 3.84 to 6.76 log10 CFU per mL and 77 yeast isolates from rumen fluid were identified. The most frequent species yeast detected were *P. kudriavzevii*, *Candida rugosa*, *Candida pararugosa*, *Candida ethanolica* and *Magnusiomyces capitatus* (Fernandes et al. 2019).

The characterization of these autosomes yeasts from adult cows that grazed on tropical pastures would support the selection of isolates that may facilitate the degradation of cell walls in roughages with high lignocellulose concentrations during dry seasons. In this study, the aims were to select, identify and evaluate the growth and biomass production, of yeast strains isolated from bovine rumen in submerged fermentations containing roughages with high concentrations of lignocellulose.

MATERIALS AND METHODS

Isolates of yeast from rumen

The ruminal fluids were collected from 75 Nellore cows raised in northern Minas Gerais State, Brazil, located at approximately 16º 51' S and 44º 55' W. This region presented a dry season from May to September and a rainy season from December to February and, according to the Köppen classification, has a humid tropical climate with a dry summer (As).

These cows were supplemented only with a mineral mixture for beef cattle and were raised in an extensive system on pasture containing *Urochloa* spp. The ruminal fluid was collected by puncture of the ventral rumen sac and the yeasts were cultured at 39ºC as reported by Abrão et al. (2014). Eight yeast isolates with different morphologic aspects were selected and evaluated for degradation of *Urochloa decumbens* (Stapf) R.D.Webster (UD) hay and sugarcane bagasse (SB). These isolates were selected because the yeast showed high populations in the rumen environment of eight adult Nellore cows (>6 log CFU per mL of ruminal fluid). The yeast isolates were grown in Sabouraud broth (ACUMEDIA®, Michigan, USA) with glycerol at 5% and stored in an ultra-freezer at -80ºC and deposited at public

Yeast Culture Collection of the Universidade Federal of Minas Gerais, Brazil. The yeast isolates were collected experiments submitted and approved by the Ethics Committee on Animal Experiments of the UFMG (protocol nº 156/05 and 128/2013), regulated by the National Council for Control of Animal Experimentation of Brazil regulated by the National Council for Control of Animal Experimentation of Brazil. The authorization for the study of the yeast and plant species collected was approved by National Management System of Brazil Genetic Heritage and Associated Traditional Knowledge (cadastres AC14923, ABAA22D and A3B530C).

Characterization and identification

Preliminarily, we grouped the yeast strains according to colony morphology, and physiological tests were performed according to the procedures described by Kurtzman et al. (2011). For molecular analyses, the yeast isolates of rumen were grown on Sabouraud agar (ACUMEDIA®, Michigan, USA) for two days, and DNA was extracted using glucanase (Glucanex; Novo Nordisk, Ferment Ltd., Dittingen, Switzerland) in one phenol extraction followed by two phenol/chloroform extractions (Abrão et al. 2014).

The D1-D2 variable domain sequences of the 28S rRNA gene were amplified by polymerase chain reaction (PCR) using primers NL1 (5'-GCA TAT CAA AAG GAA GAG TAA GCC-3 ') and NL4 (5'-GGT AAG CTT CGC TGT CCG G - 3'). The DNA concentration was adjusted to 100 ng μ/L using a NanoDrop 1000ND (NanoDrop Technologies for use in sequencing reactions. A DYEnamic (Amersham Biosciences, USA) was used for sequencing in a Mega-BACE 1000 automated sequencing system. The rDNA sequences were analyzed using BLASTn (v.2.215) of BLAST 2.0 at the National Center for Biotechnology Information (NCBI) website. Conspecific strains differed by no more than three among the 500-600 nucleotides of the D1/D2 domains and the isolates with 99% sequence similarity to deposited sequences were considered as the same species (Kurtzman et al. 2011).

Phylogenetic analyses

The D1-D2 sequences of the RNA gene from the yeast isolates were used to reconstruct their phylogenies using the MEGA X version 10.1 (BETA) program (Kumar et al. 2018). Analyses were performed individually with the sequences belonging to the Basidiomycota and Ascomycota, which were aligned using the Clustal W. For each genus, the alignments were performed by including sequences of other yeast strains deposited in GenBank. Akaike's information criterion was used to identify the most

appropriate evolution model for the dataset of each phylum.

These datasets were estimated by the Maximum Likelihood Method, based on the Tamura - Nei model and discrete Gamma distribution was used to model the evolutionary rate differences between sites [5 categories (+ G, parameter=0.4430)] for Basidiomycota and [5 categories (+ G, parameter=0.3043)] for Ascomycota. Tree robustness was estimated by bootstrap analysis with 1000 replicates and all nucleotide sequences were submitted to GenBank, assigned MN380262 to MN380269 accession numbers.

Lignocellulosic materials

The material of *U. decumbens* (UD) hay was collected during the dry season (March to June) on a farm located in the Montes Claros region, Northern Minas Gerais, Brazil and the sugarcane bagasse (SB) was provided by alcohol and sugar company São Judas (SADAbio), located in the Jaíba city at the same region. The species of these forages were identified according to their morphological characteristics expressed in the flowering periods (Panda et al. 2014).

These fibrous materials were dried at 40ºC for 72 hours and grounded in a Wiley knife mill producing 1-3 mm particle sizes. Subsequently, subsamples were subjected to chemical composition analysis as described in Table 1. The samples were analysed as reported in Duarte et al. (2021) according to the Association of Official Analytical Chemists (AOAC) for DM (method 934.01), ash (method 942.05), crude protein (CP, method 954.01), and ethereal extract (EE, method 920.39). Neutral detergent fiber (NDF, and acid detergent fiber (ADF) analyses (method 973.18 of AOAC) were performed using an ANKOM200 Fiber Analyzer unit (ANKOM Technology Corporation, Fairport, New York, USA). Lignin content was obtained by acid hydrolysis in Van Soest method.

Fermentations

In the first experiment, submerged fermentation occurred in tubes with 30 mL of liquid medium (medium C) containing 5 g of ammonium sulfate, 1 g of potassium monobasic phosphate, 0.5 g of heptahydrate magnesium sulfate per liter and The roughage samples was weighed on an analytical balance and packed 0.33 g of carbon source (SB or UD as sole carbon sources, Table 1) in non-woven textile (NWT made of polypropylene polymer with 100 µm of pores) bags (3×3 cm) and added to the culture medium for further autoclaving (Duarte et al. 2021).

Table 1. Chemical composition of *U. decumbens* and sugarcane bagasse

Parameters	*U. decumbens*	Sugarcane bagasse
DM (% in NM)	95.38	97.16
NDF (% in DM)	82.26	86.89
ADF (% in DM)	53.04	57.89
Lignin (% in DM)	7.50	6.15
CP (% in DM)	3.06	2.25
EE (% na DM)	1.02	0.08
Mineral (% in DM)	5.89	3.36

Notes: DM: dry matter; NM: natural matter; NDF: neutral detergent fiber; ADF: acid detergent fiber; CP: crude protein; EE: ethereal extract

The inocula of each yeast strain were prepared after incubation in Sabouraud agar medium plus chloramphenicol (150 mg/L) for 48 hours. Subsequently, yeast colony masses were added and incubated in Sabouraud broth at 37ºC for 48 hours. After this period, 3 mL of inoculums containing approximately 3.2×10^4 CFU / mL was added to tubes containing 30 mL of C medium that contained the lignocellulosic substrates SB or UD. The same volume of the culture medium with no inoculums was added to the control tubes.

The tubes were incubated in a shaker (NT 715, Novatécnica, São Paulo, Brazil) at 37ºC and 150 RPM for seven days. The pH of the medium was measured before and after incubation using a digital potentiometer (pH 1800, Waterproof Pen pH Tester, Instrutherm, São Paulo, Brazil).

After this period, the tubes were centrifuged at 4000g to separate the yeast mass, which, together with the non - woven textile (NWT -100 g/m^2) bags, were kept in a circulating oven (TE - 394/3, Tecnal, São Paulo, Brazil) for four days at 40ºC until they presented constant weight. Subsequently, the dry yeast mass and dry matters of SB or UD were obtained using a moisture determinant (MOC63u Shimadzu, Kyoto, Japan).

Data analysis

The experiment was conducted in a 2×9 factorial design with four replications, comparing the eight yeast isolates and the control (without microorganisms), and the degradation of the two substrates (UD or SB). The variables observed were pH, dry yeast mass, dry mater (DM) of lignocellulosic material residues and their degradation rate.

Reduction of DM = 1 - (final DM / initial DM)

Degradation percentage= (DM reduction with yeast - DM reduction of control)/initial DM × 100

After normality and homogeneity test, the data were subjected to analysis of variance and were compared to a 5% of significance the by Sccott-Kont test.

RESULTS AND DISCUSSION

Identification and molecular characterization of yeasts from bovine rumen

Yeast was detected for 34 of 75 cows evaluated, showing a population of 1.4×10^4 colony forming units (CFU)/ mL of ruminal fluid. Among the eight selected isolates with higher populations (>10^6 CFU/mL), three yeast species were identified, considering morphology and physiological tests and molecular analyses: *P. kudriavzevii* (4 isolates) *Rhodotorula mucilaginosa* (3 isolates) and one *Candida tropicalis* (Table 2). Phylogenetic analyses of D1-D2 domain sequences of the 28S rRNA confirmed the biochemical classification, showing these isolates clustered with reference strains of their respective species and intraspecific variations were not detected (Figures 1 and 2, and supplementary material). These sequences were registered in the World Federation for Culture Collections (Table 2) and shown in the supplementary material.

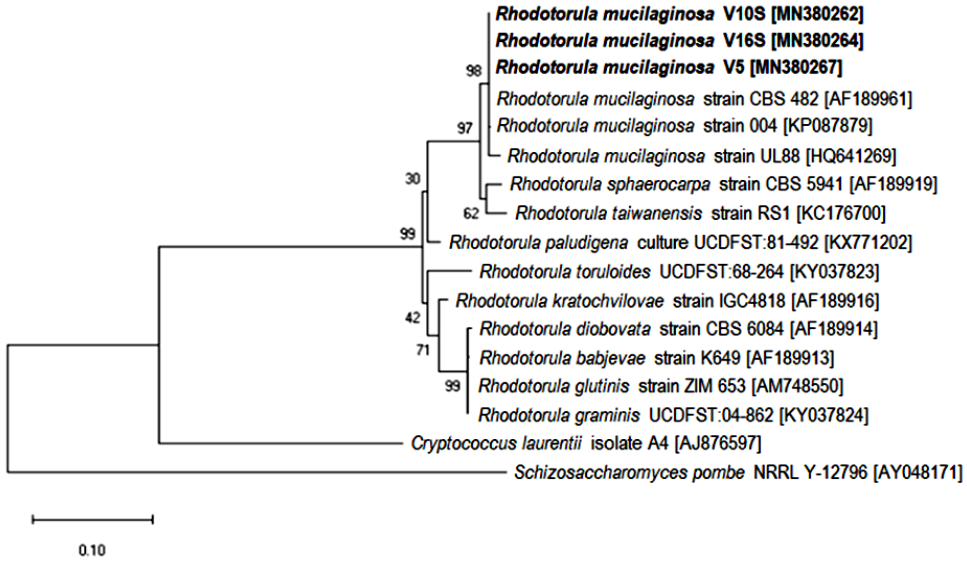

Figure 1. Consensus trees representing phylogenetic analyses of the sequences of the D1-D2 variable domain (28S rRNA gene) of yeast isolated from the rumen of Zebu cows from phylum Basidiomycota compared to sequences of their species and strains more closet and relevant deposited in GenBank. The sequences were rooted using the *Schizosaccharomyces pombe* NRRL Y - 12796 [AY048171] isolate as an external group. Notes: The isolates names and access codes of this study are in bold

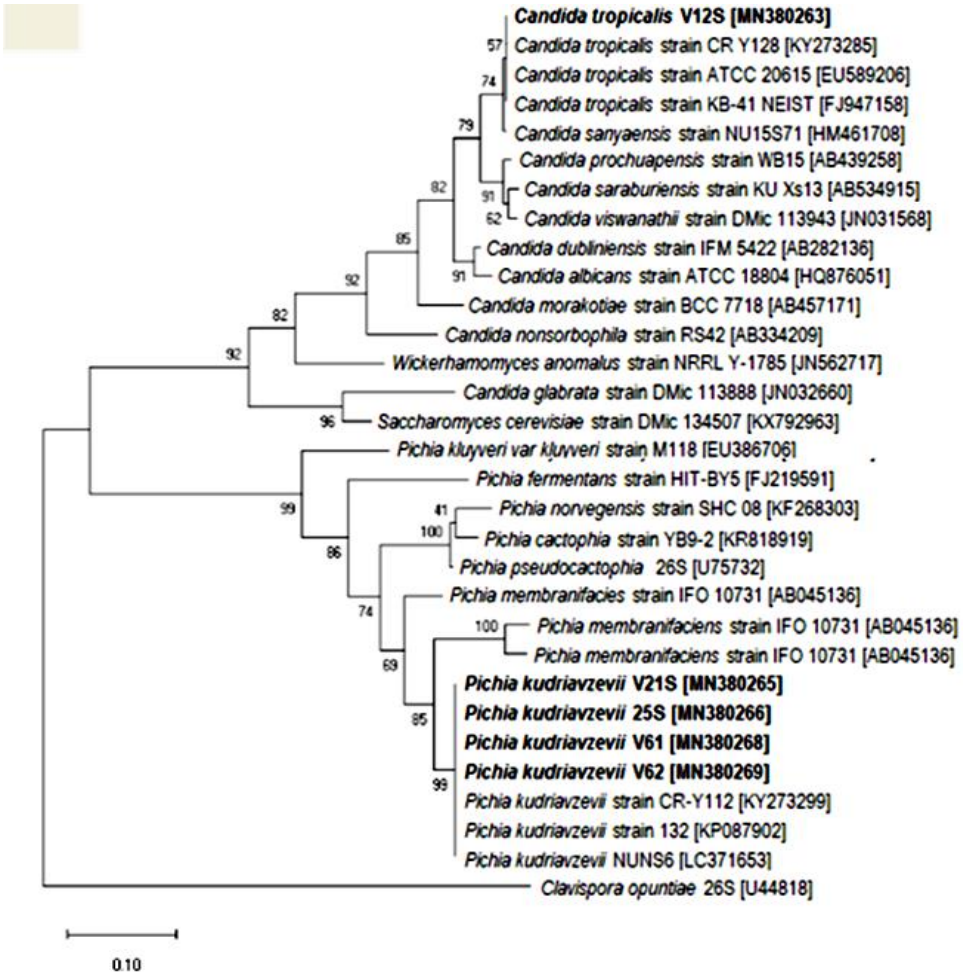

Figure 2. Consensus trees representing phylogenetic analyses of the sequences of the D1-D2 variable domain (28S rRNA gene) of yeast isolated from the rumen of Zebu cows from phylum Ascomycota comparing to sequences of their species and strains more closet and relevant deposited in GenBank. The sequences were rooted using the *Clavispora opuntiae* 28S isolate [U44818] isolate as an external group. Notes: The isolates names and access codes of this study are in bold

Table 2. Molecular identification by sequence analyses of D1/D2 variable domains of the large rDNA subunit of yeast from the rumen of Zebu cows raised in tropical pastures

Yeast strains	Accession number*	Pro.posed identification	Nº of bp analyzed	Identity (%)	Query coverage (%)	BLAST results [nº acc. GenBank] and nº strain from culture collection
V5	[MN380264]	*Rhodotorula mucilaginosa*	582	100	100	*R. mucilaginosa* DAMB1[MK968443.1]
V10S	[MN380262]	*Rhodotorula mucilaginosa*	587	99	100	*R. mucilaginosa* SM03UFAM [MN268779.1]
V16S	[MN380264]	*Rhodotorula mucilaginosa*	584	99	100	*R. mucilaginosaenv1* [MN075224.1]
V21S	[MN3802265]	*Pichia kudriavzevii*	580	100	100	*P. kudriavzevii* Z2 [MK310151.1]
V25S	[MN380266]	*Pichia kudriavzevii*	579	100	100	*P. kudriavzevii* OE9 [LC487598.1]
V61	[MN380268	*Pichia kudriavzevii*	579	100	100	*P. kudriavzevii* KKP 3005 [MK881743.1]
V62	[MN380269]	*Pichia kudriavzevii*	583	100	100	*P. kudriavzevii* CR - Y112 [KY273299.1]
V12S	[MN380263]	*Candida tropicalis*	587	100	100	*C. tropicalis* A1 [MK409681.1]

Notes: Culture Collections registered in World Federation for Culture Collections. [a]D1/D2 sequences of 28 rRNA gene

Degradation of lignocellulosic substrates

In this study, there was a significant interaction ($p<0.01$) between the factors analysed (forage type and yeast isolates). Yeast mass productions were significantly higher in fermentations using UD than SB ($p<0.01$, Table 3). In fermentations containing SB, no significant differences were detected in cell mass production between the evaluated yeast strains (Table 3). However, for fermentations with UD, the strains of *R. mucilaginosa* (V16S and V10S) presented lower mass production than other strains ($p <0.05$).

The strain V5 and V62, in UD medium, and V62 together with V61, in SB medium, promoted more reduction of the final pH of fermentation medium in comparison to other strains ($p<0.05$, Table 3). The strain V12S (*C. tropicalis*) increased the final pH of the medium containing SB.

The V5, V10S, and V62 (*P. kudriavzevii*) showed a higher percentage of dry matter reduction of UD than other strains ($p <0.05$); furthermore, the strain V5 was the most efficient in dry matter reduction of SB (Table 4). Dry matter reduction of UD was higher than SB by the yeast strains ($p <0.05$).

Discussion

The three yeast species identified in this study (*P. kudriavzevii*, *R. mucilaginosa*, and *C. tropicalis*) have also been reported in the ruminal microbiota analysis of cattle fed with tropical forages (Abrão et al. 2014, Fernandes et al. 2019). The species detected most frequently in the present study was *P. kudriavzevii*, which is an ambiguous species present in natural environments and occurs in traditionally fermented foods and drinks on different continents, contributing to the production of flavor compounds and exhibits probiotic properties (Chu et al. 2023; Ganapathiwar et al. 2023). This yeast has a high tolerance to ethanol, pH, high temperature, hyperosmotic stress and lignocellulosic inhibitors, which are notable characteristics for industrial applications (Mukherjee et al. 2017).

In another study, *P. kudriavzevii* was the most frequent yeast in the rumen of cattle from South of Minas Gerais, Brazil, which may be due to its ability to better adapt to ruminal conditions. All strains showed growth in anaerobic conditions and in temperatures that predominate in the rumen (Fernandes et al. 2019). The supplementation of the strain *P. kudriavzevii* YSY2 to Holsteins cows promoted the detoxification of aflatoxin B. Additionally, the yeast improved fed feed intake and milk component yield, showing potential as a dietary supplementation (Intanoo et al. 2020). This species was also the dominant yeast in the bovine rumen that can survive efficiently in the rumen fluid and produce cellulase and large amounts of biomass (Suntara et al. 2021). These characteristics, coupled with the noteworthy potential for the production of various enzymes (Kurtzman et al. 2011), likely played a role in the establishment and colonization of this species within the ruminal environment of the cows assessed in the current study.

Additionally, research evaluated yeast strains isolated from fermented fish as candidate ruminant probiotics based on *in vitro* rumen fermentation characteristics. The strain *P. kudriavzevii* (B-5P) was selected to be utilized as probiotics for ruminants based on their potential to improve rumen fermentation in vitro, considering dry matter digestibility and organic matter digestibility and also the total volatile fatty acid) production (Ardani et al. 2023).

In Pune, India, the yeasts *P. kudriavzevii* and *Candida glabrata* were isolated from buffalo rumen e exhibited significant tolerance towards furfural, 5-HMF, acetic acid, and ethanol. These two strains can produce ethanol at 45ºC with a fermentation efficiency of 86.7% and 86%, respectively, showing ethanologenic potential (Avchar et al. 2021).

Strains of *C. tropicalis* were also identified in a study of fistulated cows fed with *Trijolium pratense* L. (Clarke and Menna 1961). In an analysis conducted in northern Minas Gerais, Brazil, evaluating the rumen microbiota of goats fed with tropical pasture, 90% of the total yeast isolated corresponded to the species *P. membranifaciens* and 10% to *C. tropicalis* (Abrão et al. 2011). A strain of *C. tropicalis* (BPU1) was also isolated from the rumen of the Malabari goat. This yeast showed the production of biosurfactant and polyhydroxybutyrate in a simple mineral salt medium, using vegetable oil as the sole carbon source (Prakasan et al. 2013). Strains of *P. kudriavzevii* and *C. tropicalis* were isolated from the rumen of dairy cattle in Thailand. In cows, these strains increased the apparent digestibility of dry matter when compared with one *S. cerevisiae* strain and the milk protein was highest when *C. tropicalis* was fed. Thus, feeding ensiled rice straw (RS) with novel Crabtree negative yeast could improve RS digestion, rumen fermentation, and milk protein content in dairy cows (Suntara et al. 2021).

Table 3. Mean and SEM of initial and final pH, yeast biomass (mg), dry matter (DM) reduction and degradation rate (%) of sugarcane bagasse (SB) and *Urochloa decumbens* (UD) in submerged fermentation containing yeast from rumen fluid of Nelore cows

Yeast isolates	pH (sugarcane bagasse)				pH (*Urochloa decumbens*)				Yeast biomass (mg)			
	Initial	SEM	final	SEM	Initial	SEM	final	SEM	SB	SEM	UD	SEM
R. mucilaginosa												
V5	7.29	0.14	6.56^{Ab}	0.17	6.73	0.038	5.82 ^{Bc}	0.083	16.1 ^{Ba}	0.59	27.3 ^{Aa}	1.68
V16S	7.10	0.17	6.47^{Ab}	0.12	6.61	0.026	6.69 ^{Aa}	0.079	14.1 ^{Ba}	0.93	16.4 ^{Ab}	0.48
V10S	7.07	0.15	6.32Ab	0.22	6.81	0.063	6.69Aa	0.045	10.5 ^{Ba}	0.62	14.0 ^{Ab}	0.78
P. kudriavzevii												
V21S	6.93	0.10	6.55^{Ab}	0.13	6.76	0.055	6.62 ^{Aa}	0.034	16.8^{Ba}	0.29	20.6 Aa	0.34
V62	6.79	0.06	5.83^{Ac}	0.04	6.75	0.056	6.30 ^{Ab}	0.042	17.5 ^{Ba}	0.67	23.0 Aa	0.47
V25S	7.03	0.11	6.39^{Ab}	0.06	6.63	0.030	6.67 ^{Aa}	0.110	16.7 ^{Ba}	0.68	23.4 ^{Aa}	1.65
V61	7.12	0.17	5.87^{Bc}	0.06	6.77	0.051	6.57 ^{Aa}	0.177	15.0 ^{Ba}	0.89	22.2 ^{Aa}	0.78
C. tropicalis												
V12S	6.85	0.11	7.22^{Aa}	0.05	6.97	0.057	7.16 ^{Aa}	0.017	19.3 ^{Ba}	0.50	26.4 ^{Aa}	0.94
Control	7.35	0.17	6.76^{Ab}	0.04	7.06	0.045	6.78 ^{Aa}	0.079	00.0 ^{Ab}	0.00	00.0 ^{Ac}	0.00
p-value (interaction)	0.47		0.002		0.56		0.002		0.0001		0.017	

Notes: Average with different lowercase letters in the column, comparing the different inoculums, and uppercase letters in lines (comparing forages) are different by Sccott-Kont test considering $p<0.05$

Table 4. Mean and SEM of dry matter (DM) reduction and degradation rate (%) of sugarcane bagasse (SB) and *U. decumbens* (UD) in submerged fermentation containing yeast from rumen fluid of Nelore cows

Yeast isolates	Dry matter reduction (g)*				Degradation rate (%)**			
	SB	SEM	UD	SEM	SB	SEM	UD	SEM
R. mucilaginosa								
V5	0.1271 ^{Ba}	0.011	0.1733^{Aa}	0.004	3.96 ^{Aa}	0.396	3.99 ^{Aa}	0.671
V16S	0.1093 ^{Bb}	0.004	0.1604 ^{Ab}	0.009	0.0^{Bb}	0.14	0.08^{Ac}	0.406
V10S	0.1140 ^{Bb}	0.004	0.1741 ^{Aa}	0.003	0.0^{Bb}	0.08	4.20^{Aa}	0.242
P.kudriavzevii								
V21S	0.0976 ^{Bb}	0.001	0.1709 ^{Ab}	0.005	0.00 ^{Bc}	0.00	3.2 ^{Ab}	0.408
V62	0.0992 ^{Bb}	0.006	0.1728 ^{Aa}	0.006	0.00 ^{Bc}	0.00	3.83 ^{Ba}	0.329
V25S	0.0859 ^{Bb}	0.005	0.1677 ^{Ab}	0.004	0.00 ^{Bb}	0.039	2.29 ^{Ac}	0.117
V61	0.0789 ^{Bb}	0.002	0.1653 ^{Ab}	0.006	0.00 ^{Bb}	0.00	1.56 ^{Ac}	0.117
C. tropicalis								
V12S	0.0921 ^{Bb}	0.002	0.1612 ^{Ab}	0.003	0.00 ^{Bb}	0.00	0.32^{Ab}	0.0001
Control	0.1140 ^{Bb}	0.005	0.1601 ^{Ab}	0.003	-		-	
p-value (interaction)	0.015		0.047		0.0143		0.0487	

Notes: Average with different lowercase letters in the column, comparing the different inoculums, and uppercase letters in lines (comparing forages) are different by Sccott-Kont test considering $p<0.05$. Reduction of DM (g) = (final DM / initial DM); **Degradation percentage=(DM reduction with yeast -DM reduction of control)/Initial DM×100

In this study, three isolates were identified as *R. mucilaginosa*, which was additionally detected in ruminal fluid samples obtained from fistulated cows and in the hay utilized for feeding these animals (Clarke and Menna 1961). In another study involving three fistulated Holstein cows, the researchers isolated yeast colonies and identified the Levica 18 strain, which was 98% similar to *R. mucilaginosa* (Marrero et al. 2015). The genus *Rhodotorula* is common in the environment and is frequent in soil, water, milk, fruit juices and air samples (Kurtzman et al. 2011).

The occurrence of yeast in the rumen environment of cattle fed on pastures is still poorly supported in the scientific literature despite their high population, especially in adult cows raised on low-quality pastures (Abrão et al. 2014). Studies associated with the rumen microbiota have frequently ignored the presence of these yeast fungi in the rumen ecosystem (Fernandes et al. 2019). Although the three yeast species identified in this study have also been reported in other studies of this ruminant site, the role of these microorganisms should be better elucidated in the ruminal ecosystem of animals of different categories fed with different diets.

The highest yeast mass productions and percentage of DM degradation of UD could be associated to lower leave of protein and mineral salt present in SB evaluated. Additionally, these yeast strains were isolated from cows fed with UD, which could have selected samples more adapted to the degradation of this pasture in the rumen than SB. The strain V12 (*C. tropicalis*) increased the final pH of medium for both substrates, possibly through its photolytic action, as reported by Ramos et al. (2015).

Studies have showed that this species has potential benefits by improving fibrous material digestion and antioxidant function, and enhancing the microbial activities in the rumen. Supplementation with *C. tropicalis* or flavonoids improved rumen fermentation. However, the combination of *C. tropicalis* and flavonoids did not promote a synergistic effect on health or rumen fermentation in pre- and post-weaning calves (Kong et al. 2019).

In this present study, the strains *R. mucilaginosa* V5 and V10S and V62 (*P. kudriavzevii*) promoted higher reduction of dry matter reduction of UD representing potential yeast for improving the production of cattle raised in pastures of this forage. The strain V5 was the yeast most efficient for in vitro degradation of SB what could contribute to ethanol production processes from this industrial waste.

The yeast *R. mucilaginosa* and *P. kudriavzevii* are potential producers of enzymes involved in the oxidative degradation of lignocellulosic biomass, such as superoxide dismutase based on the information available from the UniProt p database (Cragg et al. 2015; Deligios et al. 2015). Genes coding for enzymes that act on the degradation of hemicellulose and lignin as 1,3-β-glycosidases, mannosidases, trehalases, esterases and deacetylases were reported in *P. kudriavzevii* (Cragg et al. 2015). Sequencing of the complete genome of this species revealed that yeast has the potential for fermentation of xylose, xylitol dehydrogenase and xylulokinase, enzymes that are considered important in the production of second-generation ethanol (Cragg et al. 2015; Deligios et al. 2015). There are no reports in the scientific literature about lesions or diseases in ruminants associated with *P. kudriavzevii* or *R. mucilaginosa*. Additionally, in this study, the yeast isolates were detected in the ruminal fluid in high concentrations, and the cows were healthy, showing no visible clinical alteration. In the present study *Candida tropicalis* was not selected because it has promoted a heightened prevalence of candidiasis in the respiratory tract, urinary tract, and gastrointestinal tract of the human in countries of Asia and South America (Wang et al. 2021). This *Candida* species is a zoonotic pathogen, and in recent years, there has been a growing incidence of fungal infections in dairy cows, leading to mastitis (Asfour et al. 2022).

The strain V5 showed a higher percentage of dry matter reduction for both lignocellulosic substrates, even in a very poor culture medium used in the submerged fermentation. This strain of *R. mucilaginosa*, a typical non-pathogenic species, might be an ideal strain to be selected and evaluated with further tests to improve the digestibility of UD and to represent a natural yeast probiotic for cattle raised in tropical pastures.

In this study, among isolates with larger populations in the ruminal fluid of cows raised in tropical pastures, *P kudriavzevii*, *R. mucilaginosa* and *C. tropicalis* were identified. These yeasts show higher biomass production and higher rates of dry matter degradation in submerged fermentations containing UD than SB. The strains V5 (*R. mucilaginosa*), V10S, and V62 (*P. kudriavzevii*) promote higher dry matter reduction of UD and V5 is the most effective for the matter degradation of SB.

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