



Yeast isolation and identification during on-farm cocoa natural fermentation in a highly producer region in northern Brazil

Isolamento e identificação de leveduras durante a fermentação natural de cacau em uma região altamente produtora no norte do Brasil

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The cocoa seeds from Brazilian Amazon are recognized for the high international market value in addition to the desirable aroma and taste. We aimed to identify yeast cultures in a natural cocoa fermentation process in one of the Amazonian regions of great importance in the cocoa beans market. Natural fermentation was carried out for seven days according to the methodologies of the producer and at 24 h intervals, seed samples were collected and physical-chemical and microbiological analyzes were performed. The contents of lipids, proteins, ash and moisture did not differ ($p \geq 0.05$) differently from temperature, pH and total titratable acidity ($p \leq 0.05$). We identified three species in the fermentation: *Pichia kudriavzevii*, *Torulaspora delbrueckii* and *Saccharomyces cerevisiae*, the most common being during the process. We can verify the importance of knowing the microbiota active in cocoa fermentation to propose improvements during this process of great economic importance for the Amazon region.

Keywords: Amazon region, chocolate, *Saccharomyces*

As sementes de cacau da Amazônia brasileira são reconhecidas pelo alto valor no mercado internacional, além do aroma e sabor desejáveis. Nosso objetivo foi identificar culturas de leveduras em um processo de fermentação natural do cacau em uma das regiões amazônicas de grande importância no mercado de grãos de cacau. A fermentação natural foi realizada por sete dias de acordo com as metodologias do produtor e em intervalos de 24 horas foram coletadas amostras de sementes e realizadas análises físico-químicas e microbiológicas. Os teores de lipídios, proteínas, cinzas e umidade não tiveram diferença estatística ($p \geq 0,05$) diferentemente da temperatura, pH e acidez total titulável ($p \leq 0.05$). Identificamos três espécies na fermentação: *Pichia kudriavzevii*, *Torulaspora delbrueckii* e *Saccharomyces cerevisiae*, sendo a mais comum durante o processo. Podemos verificar a importância de se conhecer a microbiota ativa na fermentação do cacau para propor melhorias durante este processo de grande importância econômica para a Região Amazônica.

Palavras-chave: Região Amazônica, chocolate, *Saccharomyces*

1. INTRODUCTION

Fermentation is one of the first steps in chocolate manufacturing from cocoa (*Theobroma cacao* L.) seeds. The combination of wooden boxes and the practice of turning for five to seven days is a traditional procedure still widely used [1]. The fermentation process occurs spontaneously in the presence of different microorganisms, with the predominance of yeasts, lactic bacteria and acetic bacteria [2].

The diversity of yeasts present in cocoa fermentation is usually associated with the locality and process conditions (available nutrients, pH, temperatures, oxygen, etc.) that directly influence the fermenting bacteria and yeasts' activity. Several studies reveal a wide diversity of yeasts in cocoa fermentation in different countries [3, 4].

The follow-up of some parameters is used as a reference for successful fermentation, among them: processing time, temperature of the environment and the mass, turning, pH and the acidity of the pulp, the fermentation system and existing microbiota [5].

Yeasts are the first microorganisms active in the fermentation process of cocoa beans, which convert the sugars present in the pulp into ethanol and produce enzymes that degrade the pectin present in the pulp, favouring the growth conditions of other microorganisms [6]. Therefore, isolating and identifying them is of great importance to assess their diversity and distribution during cocoa fermentation.

The diversity of fungal microorganisms in the Amazon region is wide and recent studies on cocoa fermentation prove this. The first researches about the identification and performance of yeasts and molds during cocoa fermentation in the Brazilian Amazon were in the cities of Medicilândia and Tucumã (both in Pará state), also reporting the role of enzymatic production in the fermentation process [7, 8]. On the other hand, Serra et al. (2019) [9] identified the cocobiota in Pará state through metagenomic analysis, a pioneering study in the region.

In 2019, the state of Pará returned to the leadership of cocoa production in Brazil, with around 135 thousand tons harvested from the fruit. Which is the result of climatic favours, control of the spread of witches' broom and greater competitiveness in the market [10].

This study aimed to identify the active yeasts during the fermentation of cocoa beans from Tomé-Açu, State of Pará, Brazil, one of the largest chocolate producing regions in the Brazilian Amazon.

2. MATERIAL AND METHODS

2.1 Material and Fermentation Assay

Cocoa fruits of the Forastero variety were harvested in a local property of Tomé-Açu, Pará State, Brazil (02°28'41.3"S and 48°16'50.7"W) and manually opened with stainless steel knives 48 h after the harvest. The fermentation process was carried out during seven days (168 h) and after this period, cocoa beans were submitted to a natural drying process. Approximately 90 kg of cocoa seeds were divided into three wooden boxes (n=3) with dimensions of 1.0 m length × 0.40 m width × 0.30 m height. The boxes were covered by banana leaves and burlap bags to keep the storage fermentation temperature. Both did not undergo any previous hygiene treatment in order to maintain the natural microbiological conditions in order to serve as natural inocula of microorganisms, providing the start the natural fermentation process in the boxes.

After 48 h from the beginning of the process, cocoa seeds were turned in the boxes, to enable oxygenation and to facilitate the aerobic microorganisms adaptation into the medium.

The temperature of fermentation was measured at five random points (considering the surface, the middle and the bottom) in each fermentation time. Around 100 g of seed samples were collected from each temperature measurement point and were stored in sterile polyethylene bags and kept under refrigeration (4 ± 2 °C). At the end of the fermentation process, the samples were chilled and sent to the Laboratory of Biotechnological Processes/UFPA (Belém, Pará, Brazil), where they were frozen (-18 ± 2 °C) for physical-chemical analysis and refrigerated (4 ± 2 °C) for microbiological analysis.

2.2 Methods

2.2.1 Obtaining yeasts from banana leaves

Sterile swabs were rubbed in random areas (25 cm²) on the banana leaves used in the fermentation process of the cocoa [11]. The swabs were placed in conical Falcon type tubes, containing 25 mL of peptone water (pH 7.20, Kasvi, São José dos Pinhais, PR, Brazil), added with 15% sterile glycerol and kept under freezing (-18 °C) until the moment of analysis. The tubes were vortexed for 2 minutes for cell detachment, being inoculated directly via spread plate technique in petri dishes with Potato Dextrose Agar (PDA, pH 5.6, Himedia, Mumbai, India) with chloramphenicol (Sigma-Aldrich Chemical Co., St. Louis, MO, USA, 100 mg/L) to inhibit the bacterial growth and were incubated inverted for 72 h at 30 °C.

2.2.2 Obtaining yeasts during fermentation

During the fermentation process, at intervals of 24 h, aliquots of cocoa beans were collected until the end of this stage, totalizing 168 h of natural fermentation (7 days). During the first 48 hours of fermentation, analyses were performed at four hours intervals because the predominant yeast activity in this period of time is elucidated in the literature [3, 6].

Twenty grams of cocoa beans samples were aseptically macerated and homogenized in 180 mL 0.1% peptone water (Kasvi) for 2 min obtaining the dilution of 10⁻¹. In sequence, decimal serial dilutions were taken until 10⁻⁸. Subsequently, 1 mL of each serial dilution was inoculated into sterile petri dishes via pour-plate technique and homogenised with PDA agar (Himedia) [8] supplemented with chloramphenicol (Sigma-Aldrich Chemical Co., 100 mg/L). The inoculated plates were incubated at 30 °C for 48 h. The result of the plate count was expressed in log CFU g⁻¹ of cocoa beans.

Ten colonies randomly chosen out of twenty to fifty colonies were collected after incubation (30 °C for 96 h) for each petri dish for all times of fermentation. The colonies were picked via the depletion technique twice in sterile petri dishes containing PDA agar and incubated for 72 h at 30 °C.

2.2.3 DNA extraction and Polymerase Chain Reaction (PCR)

The genomic DNA of the yeasts was extracted using the AxyPrep kit (Axygen, New York, USA) according to the manufacturer's instructions. The extracted and purified DNAs were stored under freezing (-18 °C) for further analysis.

Aliquots of the extracted DNA₇ were added to a solution containing ultrapure water (Ambion, Austin, TX, USA), 10× buffer solution (Invitrogen, Carlsbad, CA, USA), dNTP mix 10 mM (Invitrogen), MgCl₂ 50 mM (Invitrogen) and Taq DNA polymerase 5U/μL (Invitrogen). The 5.8S – Internally Transcribed Spacer (5.8S-ITS) region was amplified by the PCR analysis with the pair of primers ITS-1 (forward) (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (reverse) (5'-TCCTCCGCTTATTGATATGC-3') (Invitrogen) [12, 13] at a final concentration of 10 pmol each. The PCRs conditions, adapted based in Fujita et al. (2011) [12], Chang et al. (2001) [14] and Chen et al. (2001) [15] studies were: initial denaturation at 95 °C for 5', 35 cycles of 94 °C for 1', 55.5 °C for 2', 72 °C for 2' and a final extension at 72 °C for 10', performed in a Labtrace thermocycler (model K960, Hangzhou, Zhejiang, China).

The PCR products were purified sequentially with isopropanol 65% and ethanol 70% at 1.800 × g and 10 °C for 45 min and 10 min, respectively. Afterwards, the cycle sequencing reactions were performed with ~20 ng of amplified DNA using the Big Dye Terminator kit v. 3.1 (Thermo-Fisher Scientific, Waltham, MA, USA), as recommended by the manufacturer. The bi-directional reactions were sequenced and read in an ABI 3730 DNA Analyzer (Thermo-Fisher Scientific). Afterwards, bi-directional reads were assembled to generate FASTA sequences in Geneious R10 (Biomatters, Auckland, New Zealand) and then aligned in MAFFT v. 7.2 [16].

The phylogenetic analysis based on maximum likelihood was conducted using the PHYML package (using the GTR+G substitution model, with 1,000 bootstrap replicates) [17] implemented in Geneious R10. All the sequences were submitted for the GenBank database (<https://www.ncbi.nlm.nih.gov>).

2.2.4 Physico-chemical analysis of the cotyledons

The husks and the embryo of cocoa seeds were extracted and the cotyledons were grounded using an analytical mill (model A1 1b, Ika, Staufen, Germany). Titratable acidity (TTA – 31.06.06, AOAC), pH (970.21, AOAC), moisture (931.04, AOAC), ash (972.15, AOAC), total lipids (963.15 AOAC) and proteins (970.22 AOAC) were measured according to the Association of Official Analytical Chemists – AOAC [18]. Moisture, ash, total lipids and the proteins analyses, were carried out only at the beginning and at the end of the fermentation process, because the values of these analyses do not present a considerable variation during the process. All analyses were carried out in triplicates.

2.2.5 Statistical Analysis

Replicates analyses (temperature of fermentation, pH and titratable acidity) were submitted to analysis of one-way ANOVA followed by comparison by Tukey test ($p \leq 0.05$). The statistical analyses were undertaken using the Statistica 7.0 software (StatSoft, Inc., Tulsa, USA).

3. RESULTS AND DISCUSSION

3.1 Diversity of yeasts during the cocoa fermentation

Two yeasts species were isolated from the banana leaves used in the fermentation process of cocoa beans. Banana leaves showed, on average, a yeast count of 2.7 log CFU/cm². The isolates were *Pichia kudriavzevii* and *Torulasporea delbrueckii* species (Table 1). A total of 32 yeast isolates were obtained during the fermentation process. Four species were identified: *Pichia kudriavzevii* (5 strains; 15.6% of the strains), *Torulasporea delbrueckii* (2 strains, 6.3% of the strains) and *Saccharomyces cerevisiae* (24 strains, 75.0% of the strains). The presence of the *Trichosporon asahii* can probably be considered environmental contamination (one strain; 3.1% of the strains) (Table 1).

The composition of the yeast species during the fermentation process is directly responsible for the development of the chocolate flavour and aroma. Several species have been characterized during the fermentation process.

The count value found in this study is within the count range reported by Papalexandratou et al. (2011) [19], who studied spontaneous fermentations in cocoa troughs in Ecuador, found counts on the surface of plant materials (fruits and leaves). Fernández Maura et al. (2016) [20] studied the diversity of environmental and intrinsic yeasts from cocoa bean fermentations in Cuba, and found that environmental samples (equipment and vegetable surfaces) showed a greater diversity of yeasts compared to fermentations.

Banana leaves are used empirically to reduce heat losses during fermentation or to avoid contact of the seeds with the soil, in case of fermentation in heaps and are known to be an essential source of yeast initiating the fermentation process. Since most of the microorganisms that naturally contaminate the seeds come mainly from wooden boxes with residues from previous fermentations and banana leaves, placed on top of the troughs during fermentation [6, 21].

The yeast species composition during the fermentation process is directly responsible for developing flavour and aroma of chocolate. Several species have been characterized during the fermentation process and we identified four species during the fermentation process. Recently, Serra et al. (2019) [9] identified the microbial population of cocoa fermentation in Pará state by metagenomic analysis and found results similar to this study: the presence of *S. cerevisiae*, *P. kudriavzevii* and *T. asahii* (Table 1).

Table 1. Species identification of isolated yeasts from banana leaves and the fermentation process of cocoa seeds in the Brazilian Amazon using ITS sequences. BLAST n scores, e-values and pair wise identities are shown for the obtained ITS sequences matched against the ITS sequences of the type strains of *Pichia kudriavzevii* (CBS 5147, KY104577.1), *Saccharomyces cerevisiae* (CBS 1171, NR_111007.1), *Torulaspota delbrueckii* (CBS 1146, NR_111257.1) and *Trichosporon asahii* (CBS2479, NR_073341.1).

Isolate	GenBank accession	Score (%)	E-value	Pairwise identity (%)
Banana Leaves				
<i>Pichia kudriavzevii</i>	MT121010	100	0.0	100
<i>Pichia kudriavzevii</i>	MT121011	100	0.0	100
<i>Torulaspota delbrueckii</i>	MT121012	100	0.0	100
During fermentation				
<i>Pichia kudriavzevii</i> Sample12	KY794726	100	0	100
<i>Pichia kudriavzevii</i> Sample16	KY794727	100	0	100
<i>Pichia kudriavzevii</i> Sample22	KY794723	99.8	4.69e-171	99.7
<i>Pichia kudriavzevii</i> Sample44	KY794724	100	0	100
<i>Pichia kudriavzevii</i> Sample46	KY794725	99.8	2.64e-174	99.7
<i>Saccharomyces cerevisiae</i> Sample6	KY794728	99.7	0	99.5
<i>Saccharomyces cerevisiae</i> Sample7	KY794729	99.8	0	99.5
<i>Saccharomyces cerevisiae</i> Sample8	KY794730	99.8	0	99.7
<i>Saccharomyces cerevisiae</i> Sample10	KY794731	99.8	0	99.5
<i>Saccharomyces cerevisiae</i> Sample13	KY794732	99.8	0	99.7
<i>Saccharomyces cerevisiae</i> Sample14	KY794733	99.8	0	99.6
<i>Saccharomyces cerevisiae</i> Sample19	KY794734	99.8	0	99.7
<i>Saccharomyces cerevisiae</i> Sample20	KY794735	99.6	0	99.3
<i>Saccharomyces cerevisiae</i> Sample21	KY794736	99.8	0	99.6
<i>Saccharomyces cerevisiae</i> Sample23	KY794737	99.8	0	99.6
<i>Saccharomyces cerevisiae</i> Sample24	KY794738	99.2	0	99.6
<i>Saccharomyces cerevisiae</i> Sample25	KY794739	99.6	0	99.2
<i>Saccharomyces cerevisiae</i> Sample26	KY794740	99.7	0	99.6
<i>Saccharomyces cerevisiae</i> Sample29	KY794741	99.8	0	99.6
<i>Saccharomyces cerevisiae</i> Sample30	KY794742	99.3	0	99.6
<i>Saccharomyces cerevisiae</i> Sample31	KY794743	99.8	0	99.6
<i>Saccharomyces cerevisiae</i> Sample32	KY794744	99.8	0	99.6
<i>Saccharomyces cerevisiae</i> Sample33	KY794745	99.7	0	99.4
<i>Saccharomyces cerevisiae</i> Sample34	KY794746	99.8	0	99.6
<i>Saccharomyces cerevisiae</i> Sample37	KY794747	99.8	0	99.6
<i>Saccharomyces cerevisiae</i> Sample39	KY794748	99.8	0	99.6
<i>Saccharomyces cerevisiae</i> Sample40	KY794749	99.8	0	99.6
<i>Saccharomyces cerevisiae</i> Sample43	KY794750	99.8	0	99.5
<i>Saccharomyces cerevisiae</i> Sample48	KY794751	99.8	0	99.6
<i>Torulaspota delbrueckii</i> Sample36	KY794752	99.9	0	99.9
<i>Torulaspota delbrueckii</i> Sample38	KY794753	99.9	0	99.9
<i>Trichosporon asahii</i> Sample42	KY794754	100	0	100

Saccharomyces cerevisiae had the largest number of isolates in the process we investigated (75.0%), being present from start to finish. The presence of *S. cerevisiae* contributes to the improvement of the sensory characteristics of cocoa-based beverages [22]. Also, it reduces the concentration of lactic acid, hydrogen peroxide removal and favouring the production compounds to growth other bacteria [23, 24]. In Cuba, *S. cerevisiae* is prominent at the start of the fermentation process, mostly if initiated 48 h after harvesting [20]. Probably this occurs due to the release of sugars surrounding the fruit flesh, which is the substrate for yeast proliferation [6, 25].

A recent review [3], highlighted that in the last two years (2018-2020), *S. cerevisiae* proved to be an excellent option for obtaining cocoa beans with adequate fermentation as low levels of acidity, acceptable levels of phenolic compounds and protection against aflatoxins.

The species *Pichia kudriavzevii* was reported in many cocoa seed fermentation studies in different countries such as Ghana, Mexico, Ivory Coast [3, 4]. Meersman et al. (2013) [26] showed that *Pichia* species were present, but not to the same extent as *S. cerevisiae*. The authors indicate that both species support pH and temperature variations. *P. kudriavzevii* was reported in the Amazon by Serra et al. (2019) [9]. *Pichia kudriavzevii*, like *P. manshurica*, can produce many volatile compounds such as alcohols, aldehydes and esters, that are important for the final product quality [27].

The first report of the isolated yeasts in the Brazilian Amazon was carried out in Medicilândia and Tucumã [8]. The authors also founded *S. cerevisiae* and *P. kudriavzevii* during the natural cocoa fermentation, elucidating that these species are common in the Amazon region.

For the first time, inocula with *P. kudriavzevii* were used for the fermentation of cocoa beans in the Amazon [28]. In this study, the authors associated this species with *S. cerevisiae* (with a proportion 1:1) and obtained cocoa beans with low levels of putrefactive amines and acidity, higher levels of pH, a good amount of methylxanthines and phenolic compounds, making it a good option for the use of cocoa seed fermentation, unlike a fermentation without the inocula addition or only with *S. cerevisiae* or *P. kudriavzevii*.

Torulasporea delbrueckii was used as a culture starter by Visintin et al. (2017) [29]. This yeast species associated with *S. cerevisiae* produced fermented almonds with a different aromatic profile, becoming another alternative for use.

On the other hand, a specie is widely used as a starter crop in the last two years, precisely because it provides characteristics peculiar to fermented almonds. In the studies by Mota-Gutierrez et al. (2018) [30] and Santos et al. (2020) [31], this yeast species was also able to reduce fermentation times and acidity levels of the final product [3], which could be seen in the present study.

3.2 Physico-chemical analyses of the cocoa beans during fermentation

The results of the physical-chemical analysis and counting of yeasts present in fermentation are shown in Table 2.

An increase in temperature was observed, especially after 24 h (40.7 °C) and until 48 h. An increase in total acidity was verified after the first 48 h due to microorganisms' activity, although a reduction was detected after 72 h of the fermentation process. There were no significant differences in moisture, ash, fat and protein analyses ($p > 0.05$) between the first and the seventh days of the process. During the first 48 h of fermentation, yeasts have expressive participation while transforming pulp fermentable sugar (glucose) and other components into ethanol [32]. This intense activity elevates the temperature, which is essential for killing the embryo and directly influencing product quality [6]. The consumption of the citric acid in the pulp by the lactic acid bacteria together yeasts in the first times of fermentation and the pH values of the pulp provides a favourable environment for the subsequent proliferation of lactic and acetic bacteria [32, 33].

Acidity is consequential for the fermented seeds' market value because it influences protease activity, which is highest at pH 5.0 to 5.5. Protease activity is essential for promoting hydrolysis of proteins responsible for the desirable chocolate flavour [13]. The proposal of a Cocoa Index [34] suggested a combination of many different chemical parameters (fat, titratable acidity, total phenolic, catechin and epicatechin, organic acids and heavy metals) as an index of fermentation quality that directly influence the product's flavour and aroma. According this Cocoa Index, the appropriate pH and TTA values must be from 5.60 to 6.57 and from 10.53 to 19.37 mEq NaOH/100 g, respectively. The fermentation reported here had 11.21 mEq NaOH/100 g (Table 2), indicating its high quality.

Table 2. Means of physico-chemical analyses and yeast counting during natural cocoa fermentation in Tomé-Açu, PA, Brazil.

Hours of Fermentation (h)	Temperature of Fermentation (°C)	pH	Titratable acidity (meq. NaOH 0.1 N/100 g) ¹	Moisture* (%)	Ashes* (%)	Lipids* (%)	Proteins* (%)	Yeast Counting (log CFU g ⁻¹)
0	29.0±0.78 ^c	5.93±0.51 ^{ab}	11.54±1.76 ^d	39.42±0.88	2.43±0.07	48.31±0.99	12.92±0.33	7.20
4	30.1±0.63 ^c	5.99±0.07 ^{ab}	12.59±3.34 ^d	n.a.	n.a.	n.a.	n.a.	7.45
8	31.5±0.85 ^{bc}	5.97±0.12 ^{ab}	13.34±2.03 ^{cd}	n.a.	n.a.	n.a.	n.a.	7.08
24	40.7±1.96 ^a	4.68±0.15 ^{cd}	41.62±4.47 ^b	n.a.	n.a.	n.a.	n.a.	9.15
32	41.0±0.93 ^a	4.51±0.15 ^{cd}	46.60±5.74 ^{ab}	n.a.	n.a.	n.a.	n.a.	5.61
48	42.1±0.61 ^a	4.43±0.14 ^d	52.3±2.35 ^a	n.a.	n.a.	n.a.	n.a.	7.20
72	39.0±1.34 ^a	4.79±0.22 ^{cd}	38.60±1.65 ^b	n.a.	n.a.	n.a.	n.a.	9.74
96	34.3±1.52 ^b	5.45±0.36 ^{bc}	23.59±1.51 ^c	n.a.	n.a.	n.a.	n.a.	7.23
120	29.3±0.95 ^c	5.96±0.35 ^{ab}	15.89±1.87 ^{cd}	n.a.	n.a.	n.a.	n.a.	6.11
144	29.3±0.81 ^c	6.35±0.36 ^{ab}	13.84±1.21 ^{cd}	n.a.	n.a.	n.a.	n.a.	5.81
168	30.8±0.60 ^c	6.69±0.28 ^a	11.21±1.97 ^d	40.57±0.43	2.24±0.24	48.58±0.80	12.53±0.35	5.18

Means ± standard deviation with different letters in the same column are statistically different (Tukey test, $p \leq 0.05$).

¹meq. NaOH 0.1 N/100 g: milliequivalent sodium hydroxide solution 0.1N per 100 g sample.

n.a.: not analyzed because during the fermentation process, these variables does not vary statistically.

*: in dry base

Other publications have reported a lower pH of the fermented product. Some authors [35] found that initial pH values ranged between 4.5 and 4.0. The studies of Camu et al. (2007) [5], Samagaci et al. (2014) [36] and Arana-Sánchez et al. (2015) [2], also reported initial pH values lower than this study. The behaviour of pH is a strong evidence that the microbiota in Amazon cocoa fermentation conduce to a distinct fermentation process when compared with studies conducted in other countries.

The content of lipids was below that reported by Tuentler et al. (2020) [37] carried out on the National variety and all, well below that reported by Afoakwa et al. (2008) [38] for the Forastero variety. However, at the same time that the fermentation and drying process does not seem to influence the variation in fat content, the variety of the fruit should not be the only factor to be studied [39]. The values are within the Cocoa Index proposal, which recommends lipids levels above 30% to consider fermented cocoa beans of good quality [34].

High levels of total lipids associated with fermentation and prolonged storage times can trigger the high activity of lipase enzymes that act in the breakdown of triglycerides providing an increase in the amount of free fatty acids, thus promoting an increase in the production of sour almond notes [40].

Alcohols are formed by the metabolization of fermentable sugars present in cocoa pulp by yeasts and some lactic acid bacteria [4, 6]. However, some studies point to their formation due to thermal degradation of amino acids [41, 42] which can probably give desirable notes to chocolate as a final product in this study.

4. CONCLUSIONS

In this study, we identified the diversity of four yeast species that act during the cocoa fermentation process in the Brazilian Amazon region: *Saccharomyces cerevisiae*, *Torulaspota delbrueckii*, *Pichia kudriavzevii* and *Trichosporon asahii*. Knowing the current microbiota is important to propose several other studies in the region, as already happens with the production of starter cultures, thus being a possibility for obtaining fermented cocoa beans with characteristics of the region. For this, establish the role that each yeasts species plays on cocoa fermentation is the possibility for future studies. It is important to note that the bacterial diversity also plays an essential role in fermentation, but this was not evaluated in the present study.

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