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ARTICLE

Sanitary quality and diversity of culturable bacteria and yeasts in processed and *in natura* yerba mate (*Ilex paraguariensis* A. St.-Hil.)

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ABSTRACT: (Sanitary quality and diversity of culturable bacteria and yeasts in processed and *in natura* yerba mate (*Ilex paraguariensis* A. St.-Hil.). Yerba mate (*Ilex paraguariensis*) is a native plant of southern Brazil, whose leaves, after processing, are used as a beverage by the population of Brazil, Argentina, Paraguay and Uruguay. This study assessed the sanitary quality of the processed yerba mate (presence of thermophilic and mesophilic bacteria, molds and yeasts, *Salmonella* sp. and coliforms), and evaluated the microbial diversity (bacteria and yeasts) in leaves and in the processed yerba mate. Samples were collected in a yerba mate plantation field (leaves) and from local shops (processed yerba mate) of Vargeão city, Santa Catarina state, Brazil. Standard methods were used to determine the bacteria and fungi counts, as well as to isolate bacteria and yeasts from leaves and processed yerba mate. Bacteria and yeasts were identified through biochemical and physiological assays, and by rDNA gene sequencing. The main thermophilic bacterium found in both processed yerba mate and leaves was *Bacillus licheniformis*. The predominant mesophilic bacteria in leaves were *Pantoea ananatis*, *Staphylococcus sciuri* and *S. epidermidis,* while in processed yerba mate the predominant species were *Bacillus megaterium*, *B. amyloliquefaciens* and *Klebsiella pneumoniae*. The yeasts most frequently identified in leaves were *Aureobasidium pullulans* and *Sympodiomycopsis* sp., and those identified in the processed yerba mate were *Rhodosporidium kratochvilovae*, *Rhodotorula mucilaginosa* and *Sporobolomyces nylandii*. All microbiological parameters for processed yerba mate were in line with the current Brazilian legislation, as well as with the parameters established by the World Health Organization.
Keywords: food safety, microbiota, tea.

RESUMO: (Qualidade sanitária e diversidade de bactérias e leveduras cultiváveis na erva-mate (*Ilex paraguariensis* A. St.-Hil.) processada e *in natura*). A erva-mate (*Ilex paraguariensis*) é uma espécie nativa da região meridional do Brasil, cujas folhas, após o processamento, são utilizadas como bebida pelas populações do Brasil, Argentina, Paraguai e Uruguai. O objetivo deste trabalho foi verificar a qualidade sanitária da erva-mate processada (presença de bactérias mesófilas, termófilas, bolores, leveduras, *Salmonella* sp. e coliformes) e avaliar a diversidade microbiana (bactérias e leveduras) nas amostras *in natura* e após processamento. As coletas das amostras foram realizadas no município de Vargeão, Santa Catarina, Brasil. Foram utilizados métodos oficiais para as contagens de bactérias e fungos, assim como para o isolamento de bactérias e leveduras das folhas e da erva-mate processada. A identificação das bactérias e leveduras foi realizada por meio de testes bioquímicos, fisiológicos e pelo sequenciamento do gene do rDNA de cada amostra. O termófilo mais encontrado tanto na erva-mate processada quanto nas folhas foi o *Bacillus licheniformis*. Os mesófilos que predominaram nas folhas foram: *Pantoea ananatis*, *Staphylococcus sciuri* e *S. epidermidis*. Já na erva-mate processada os mesófilos mais frequentes foram: *Bacillus megaterium*, *B. amyloliquefaciens* e *Klebsiella pneumoniae.* As principais leveduras identificadas nas folhas foram: *Aureobasidium pullulans* e *Sympodiomycopsis* sp. Na erva-mate processada as leveduras identificadas foram: *Rhodosporidium kratochvilovae*, *Rhodotorula mucilaginosa*, *Sporobolomyces nylandii*. Verificou-se que todos os parâmetros microbiológicos para a erva-mate processada atenderam tanto à legislação brasileira vigente, como também aos parâmetros estabelecidos pela Organização Mundial da Saúde.
Palavras-chave: alimento seguro, microbiota, chimarrão.

INTRODUCTION

Yerba mate (*Ilex paraguariensis* A. St.-Hil.; Aquifoliaceae) is a native plant in southern Brazil, whose leaves, after processing, are used as a beverage by the population of Brazil, Argentina, Paraguay, Uruguay and other countries in South America. The beverage is usually prepared as an infusion with hot or cold water, called mate or tererê, respectively (Valduga 1994).

Microrganism counts must be determined in order to maintain the quality of the product, and to protect consumers' health. The WHO recommends that, for teas consumed in the form of an infusion or decoction (i.e. yerba mate), the counts of mesophilic bacteria should not exceed 10^7 CFU/g, and counts of molds and yeasts should not exceed 10^4 CFU/g (WHO 1998). The National Health Surveillance Agency (ANVISA) (Brazil 2001) mandates that for teas and similar products obtained by thermal processing, consumed in the form of an infusion or decoction, *Salmonella* sp. must be absent and the coliform count must not exceed10^3 CFU/g.

The microbiota inherent to foods is also very important, as they might affect its quality, causing deterioration, and even be a source of infections and intoxications, if the product is not effectively treated. This study determined the types of bacteria and yeasts present in the leaves of *Ilex paraguariensis* Saint Hilaire and in processed yerba

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mate, and evaluated the sanitary quality of processed yerba mate obtained in the city of Vargeão, Santa Catarina, Brazil.

MATERIALS AND METHODS

Sampling

The samples of yerba mate, both *in natura* and processed, were obtained monthly, during the first six months of 2013, in the city of Vargeão, Santa Catarina, Brazil.

A pool of approximately 50 g of leaves of *I. paraguariensis* was collected aseptically. Each pool comprised leaves from the apical portion (young leaves) and from the basal portions (mature leaves) of five plants, harvested randomly and with a healthy appearance. Each pool was packed in sterile plastic bags, kept at room temperature and transported to the laboratory. Microbiological determinations were made within 24 h after sampling. Concomitantly, five packages of processed yerba mate produced on the same plantation were acquired from local shops. A total of 30 packages of processed yerba mate were analyzed. They were transported to the laboratory in their original, intact and closed sale packages.

Microbiological tests

All samples were analyzed in duplicate. Coliforms and *Salmonella* sp. were analyzed as recommended by the National Regulatory standards number 62 of 08/26/2003 (Brazil 2003). Mesophilic bacteria, molds and yeasts were analyzed as recommended by the United States Food and Drug Administration (USFDA 2002). Thermophilic bacteria were quantified and isolated after decimal dilution of the samples in a 0.1% saline peptone solution and plating on Plate Count Agar, at 55 °C, for 24-48 h. All cultures were incubated in aerobiosis conditions.

Colony-forming units per gram (CFU/g) were calculated for *E. coli*, mesophiles, thermophiles, molds and yeasts; for *Salmonella* sp. the presence or absence of bacteria in 25 g of the sample was recorded. Additionally for mesophilic and thermophilic bacteria, the proportion of morphologically different colonies was also recorded, and representative isolates of each colony morphotype were isolated and examined by Gram and spore staining, catalase, oxidase, glucose oxidation and fermentation tests. For each of these morphologically and biochemically different isolates, DNA extraction and amplification of 16S rDNA were performed with 8 F and 926 R primers as described elsewhere (Misbah *et al*. 2005, Liu *et al*. 1997). The reagent concentrations for each reaction were: 2.4 mM $MgCl_2$, 80 nmol / uL of primer, 0.2 mM dNTPs, 0.5 U of Platinum® Taq DNA Polymerase (Invitrogen), 1 ng/μL DNA, and 1X buffer. The amplifications were carried out in a TC 5000 thermal cycler (Techne) under the following conditions: an initial cycle at 94 °C, 2 min; 35 cycles at 94 °C for 60 s, 58 °C for 60 s, 70 °C for 60 s and a final extension cycle of 72 °C for 6 min.

After quantification of yeasts and molds, morphologically different yeasts were selected and isolated in 2% acidified potato glucose agar (pH 3.5), incubated at 25 °C for 5 to 7 days. DNA of each isolate was extracted according to Osorio-Cadavid *et al*. (2009), and the ITS1--5.8S-ITS2 region was amplified using the ITS4 and ITS5 primers (White *et al*. 1990). Reaction mixtures were composed as follows: 3 mM $MgCl_2$, 0.64 pmol/ uL primer, 10 μM dNTPs, 1 U of Platinum ® Taq DNA Polymerase (Invitrogen), 1 ng/μL DNA, 1X buffer. The following thermal cycling parameters were used: an initial cycle at 94 °C for 5 min; 35 cycles at 94 °C for 15 s, 55 °C for 45 s, 72 °C for 90 s and a final extension cycle at 72 °C for 6 min.

The amplified products of the yeasts and bacteria were purified with a PureLink ® Quick Gel Extraction kit (Invitrogen), according to the manufacturer's recommendation. Sequences were obtained from the Amersham Biosciences MegaBACE 1000 automated sequencer, using standardized protocols in the Brazilian Genome Network, and the ABI-PRISM 3100 Genetic Analyzer automated sequencer, using the protocols established by the company Ludwig Biotech Brazil. Data sequences were edited by Finch TV software version 1.4.0 (Geospiza, Inc.; Seattle, WA, USA 2004) and analyzed with Nucleotide-nucleotide BLAST (blastn) available in the http://www.ncbi.nlm.nih.gov/blast program. The cut-off value for identity was similarity ≥ 98%, compared to type strains of each species.

Statistical Analysis

Microorganism counts of the yerba mate lots and between pools of leaves of *I. paraguariensis* were submitted to ANOVA and Tukey tests, with the Statistica 8.0 software, adopting a significance level of 95% ($p < 0.05$).

RESULTS AND DISCUSSION

Microbiological counts in yerba mate leaves

All microbiological counts from the pools of yerba mate leaves are presented in Table 1. A difference in CFU between pools was observed for mesophilic bacteria as well as for fungi (yeasts and molds). The counts of fungi in leaves were higher than those of mesophilic bacteria in four of the six pools examined. The mean of the mesophilic bacteria counts in leaves was $5.9x10^3$ CFU/g and of fungi was $3.0x10^4$ CFU/g. Thermophilic bacteria were isolated in only one pool of leaves examined.

Microbiological quality of processed yerba mate

The mean microbiological counts in the processed yerba mate lots are shown in Table 2. There was no significant difference among lots in the counts of mesophiles, thermophiles, coliforms, yeasts and molds; unlike the results for the leaves. This is probably due to the fact that the industrial processing of yerba mate produces a stable product compared to the leaves, which are exposed to a greater variation in humidity, temperature, contact with insects, etc.

Table 1. Mean counts of bacteria and fungi for each pool of *Ilex paraguariensis* leaves, in CFU/g, collected between January and June 2013 in Vargeão, SC, Brazil. Different letters in the same row indicate significant differences by Tukey test ($p < 0.05$).

	Counts (CFU/g)					
	Pool 1	**Pool 2**	**Pool 3**	**Pool 4**	**Pool 5**	**Pool 6**
	January	**February**	**March**	**April**	**May**	**June**
Mesophilic	$1.1x10^4$b	$5.0x10^2$d	$1.8x10^4$a	$6.1x10^3$c	$2.0x10^2$d	$7.5x10^1$d
Thermophilic	$5x10^0$a	0a	0a	0a	0a	0a
Yeasts and molds	$1.3x10^5$a	$1.4x10^4$bc	$3.9x10^3$c	$4.3x10^3$c	$2.9x10^4$b	$2.7x10^3$c

Mesophilic bacteria and fungi counts in processed yerba mate lots were lower than in the leaves. Although the leaf samples and processed yerba mate are not directly related (i.e. the leaves were not the same as those used to prepare the processed yerba mate), the lower counts of mesophilic bacteria and fungi in the processed samples were expected because of the heat treatment used to prepare yerba mate. The leaves are first singed using a propane flame, with input and output temperatures of 400 °C and 65 °C, respectively, for approximately 8 minutes (Mazuchowki 2000). This heating removes surface moisture and inactivates the enzymes that cause browning. The leaves are then further dried for several hours in a dryer at 80-120 °C (Peralta & Schmalko 2007). Similarly, a higher number of thermophilic bacteria was expected in processed yerba mate than in leaves, because of the bacteria's inherent resistance to heat. The amount of thermophilic bacteria observed in the processed yerba mate was below the limits described by Burgess *et al.* (2010), denoting good microbiological quality of this tea.

The good microbiological quality of the processed yerba mate was also confirmed by the absence of *Salmonella* sp. and the counts of coliforms, mesophilic bacteria and fungi below the accepted limits established by the Brazilian regulatory standards and by the WHO. Similar results were obtained in the studies of Horianski *et al*. (2012) and Barboza *et al*. (2006), who evaluated yerba mate "cancheada" (coarsely ground, i.e., less processed than standard yerba mate).

Diversity of thermophilic bacteria in leaves of Ilex paraguariensis and in processed yerba mate

Thermophilic bacteria were isolated in only one pool of leaves, collected in January, and *Bacillus licheniformis* was the only species found, with 5 CFU/g. In processed yerba mate, a higher number and diversity of thermophiles were found. *Bacillus licheniformis* was the predominant species (comprising 86.61% of the total counts), followed by *Bacillus subtilis* (11.97%), *Bacillus ginsengihumi* (0.70%) and *Bacillus smithii* (0.70%).

Bacillus licheniformis, the most frequent thermophilic species found on leaves and in processed yerba mate, is a ubiquitous organism and inhabits leather, paper, milk, bird feathers, the inner tissues of plants, and soil, and is also found in clinical samples (Ludwig *et al*. 2009, Mikkola *et al*. 2000). Some toxin-producing strains of *B. licheniformis* were involved in incidents of food poisoning with raw milk and baby food produced industrially (Mikkola *et al*. 2000). Although this species was found in these instances of food poisoning, its presence in yerba mate does not necessarily pose a risk, because of its low numbers, and because of the small amount of water remaining in the processed leaves, which impedes the multiplication of bacteria.

The same occurs with *B. subtilis*, the second most frequent thermophilic bacteria in yerba mate, which is ubiquitous and has been reported, although rarely, from gastrointestinal infections of animals (Earl *et al*. 2008, Pandey & Palni 1997 cited in Ludwig *et al*. 2009, Kramer & Gilbert 1989).

Diversity of mesophilic bacteria and yeasts in the leaves of Ilex paraguariensis

The different species of mesophilic bacteria isolated from leaves of *I. paraguariensis* and its respective CFU/g are listed in Table 3. The species with the highest number of CFU/g were: *Pantoea ananatis* (comprising 41.52% of the total CFU), *Staphylococcus sciuri* (23.82%), *Staphylococcus epidermidis* (16.47%) and *Staphylococcus saprophyticus* (13.75%). Although the microorganism detected in the largest amounts is a Gram-negative bacterium, the majority of species found were Gram-positive.

Members of the genus *Pantoea* sp. are found in various environments associated with plants and soil, as well as causing infections in plants, humans and animals (Grimont & Grimont 2005, Coutinho & Venter 2009). *P. ananatis*

Table 2. Mean microbiological counts (CFU/g) in each lot of processed yerba mate, between January and June 2013. Different letters in the same row indicate significant differences by Tukey test ($p < 0.05$).

	Counts (CFU/g)					
	Lot 1	**Lot 2**	**Lot 3**	**Lot 4**	**Lot 5**	**Lot 6**
Mesophilic	$\mathbf{1.6x10^2}$±$5.5x10^1$a	$\mathbf{5.0x10^2}$±$1.8x10^2$a	$\mathbf{3.5x10^2}$±$2.0x10^2$a	$\mathbf{2.0x10^2}$±$2.5x10^2$a	$\mathbf{2.9x10^2}$±$1.9x10^2$a	$\mathbf{2.8x10^2}$±$1.5x10^2$a
Thermophilic	$\mathbf{4.0x10^1}$±$1.4x10^1$a	$\mathbf{2.9x10^1}$±$4.4x10^1$a	$\mathbf{<1.0x10^1}$a	$\mathbf{1.0x10^0}$±$2.2x10^0$a	$\mathbf{<1.0x10^1}$a	$\mathbf{7.2x10^1}$±$8.9x10^1$a
Coliforms 35°C	$\mathbf{2.0x10^0}$±$4.4x10^0$a	$\mathbf{<1.0x10^1}$a	$\mathbf{4.0x10^0}$±$6.5x10^0$a	$\mathbf{2.0x10^0}$±$2.7x10^0$a	$\mathbf{5.0x10^0}$±$7.0x10^0$a	$\mathbf{4.0x10^0}$±$4.1x10^0$a
Coliforms 45°C	$\mathbf{<1.0x10^1}$a	$\mathbf{<1.0x10^1}$a	$\mathbf{3.0x10^0}$±$4.4x10^0$a	$\mathbf{2.0x10^0}$±$2.7x10^0$a	$\mathbf{2.0x10^0}$±$4.4x10^0$a	$\mathbf{1.0x10^0}$±$2.3x10^0$a
Yeasts and molds	$\mathbf{1.0x10^2}$±$9.3x10^1$a	$\mathbf{1.7x10^2}$±$8.3x10^1$a	$\mathbf{8.0x10^1}$±$8.0x10^1$a	$\mathbf{1.6x10^2}$±$2.2x10^2$a	$\mathbf{1.7x10^2}$±$9.0x10^1$a	$\mathbf{2.0x10^2}$±$9.3x10^1$a
Salmonella sp./25g	absent	absent	absent	absent	absent	absent

Table 3. Mesophilic bacteria counts in samples of leaves of *Ilex paraguariensis* after biochemical and sequencing identification (16S rRNA gene - identity ≥ 98%).

Species	Colony forming units (CFU/g)					
	Pool 1	Pool 2	Pool 3	Pool 4	Pool 5	Pool 6
Arthrobacter creatinolyticus	-*	$1.1x10^2$	-	-	-	-
Bacillus cereus	-	-	-	$2.0x10^2$	$7.5x10^1$	-
Bacillus flexus	-	$1.9x10^2$	-	-	-	-
Bacillus megaterium	-	-	$5.0x10^1$	-	$6.0x10^1$	-
Bacillus methylotrophicus	-	-	-	-	$1.5x10^1$	-
Bacillus pumilus	-	-	-	-	$3.0x10^1$	-
Bacillus thuringiensis	-	-	-	-	-	$7.5x10^1$
Enterobacter aerogenes	-	$5.0x10^0$	-	-	-	-
Escherichia hermannii	-	$9.5x10^1$	-	-	-	-
Pantoea ananatis	-	-	$1.3x10^4$	$2.1x10^3$	-	-
Pseudomonas oryzihabitans	-	-	$5.0x10^1$	-	-	-
Staphylococcus epidermidis	$6.0x10^3$	-	-	-	-	-
Staphylococcus haemolyticus	-	-	-	-	$2.0x10^1$	-
Staphylococcus pasteuri	-	$1.0x10^2$	-	-	-	-
Staphylococcus saprophyticus	$5.0x10^3$	-	-	-	-	-
Staphylococcus sciuri	-	-	$4.9x10^3$	$3.8x10^3$	-	-
Staphylococcus xylosus	$5.5x10^2$	-	-	-	-	-

* Species not found in this pool sample.

has been found on the surface of plants, and this bacterium may show antibacterial and antifungal activity *in vivo* as well as in *vitro* (Coutinho & Venter 2009).

Members of the genus *Staphylococcus* are mainly associated with skin and mucous membranes of warm-blooded animals, but are also found in environmental samples (Schleifer & Bell 2009). Species of *Staphylococcus* have been isolated from tobacco leaves, and from leaves and tanks of bromeliads (Perry 1969, Reginatto 2008).

Among the species of yeasts identified in leaves of *I. paraguariensis,* the most predominant was *Aureobasidium pullulans* (comprising 52.87% of the total counts), followed by *Sympodiomycopsis* sp. (45.01%), *Sporobolomyces ruberrimus* (0.60%), *Candida sake* (0.45%), *Rhodotorula mucilaginosa* (0.45%), *Sporobolomyces yunnanensis* (0.45%) and *Rhodosporidium kratochvilovae* (0.15%). *Aureobasidium pullulans* is a cosmopolitan yeast, popularly known as black yeast due to the production of melanin (Hoog 1993). Strains of *A. pullulans* are ubiquitous and found mainly in soil, water, phylloplane, wood and many other plant materials, rocks, and on limestone monuments (Urz'l *et al*. 1999, Slavikova *et al.* 2009). Species of *Sporobolomyces*, *Rhodotorula* and *Rhodosporidium* are common inhabitants of the phylloplane of various plants. *C. sake* has been reported on plant-associated substrates such as soil, flowers and decaying fruits, and is used as an agent in biocontrol of postharvest diseases (Fonseca & Inacio 2006, Lachance *et al*. 2011). Of great interest was the isolation of a possible new species of *Sympodiomycopsis*, which will be described later.

Diversity of mesophilic bacteria and yeasts in processed yerba mate

The different species of mesophilic bacteria isolated in the lots of yerba mate, and their respective counts (mean and standard deviation) are shown in Table 4. *Bacillus megaterium* (comprising 25.17% of the total counts) was the predominant species , followed by *Bacillus amyloliquefaciens* (22.80%), *Klebsiella pneumoniae* (11.36%), *Paenibacillus alvei* (9.41%), *Bacillus pumilus* (7.67%), *Bacillus cereus* (5.02%) and 10 other species in smaller numbers.

Only three Gram-negative bacteria were isolated, representing 16.73% of the total counts; Gram-positive bacteria predominated. All Gram-positive bacteria formed spores, with a predominance of the genus *Bacillus*. This predominance of sporulated bacteria is expectable, due to their resistance to heat treatment. *Bacillus megaterium* (the most frequent microorganism in yerba mate) and *P. alvei* are found in soil, the rhizosphere of plants, cattle feces, and food, and also in clinical samples (Ludwig *et al*. 2009, Hornitzky & Smith 1998, Djordjevic *et al*. 2000, Reboli *et al*. 1989). The ubiquitous *B. amyloliquefaciens* and *B. megaterium* have the ability to stimulate plant growth and inhibit the growth of certain pathogens (Idriss *et al*. 2002, Chakraborty *et al*. 2006).

Bacillus cereus was present in three lots of processed yerba mate, corresponding to 5.02% of the estimated total counts. It is a saprophytic bacterium in soil and is present in various kinds of foods, especially those of plant origin (Kramer 1989). The mean count in these lots was $2.4x10^1$ UFC/g. The infective dose to cause disease ranges from 10^5 to 10^8 viable cells or spores, and food containing more than 10^3 *B. cereus* per gram is not considered safe (Granum & Lund 1997). Here, this bacterium was found in low numbers, insufficient to initiate an infectious process, considering the low probability of multiplication in yerba mate due to its composition, and assuming that the product is consumed immediately after addition of water.

Klebsiella pneumoniae was the third most common mesophilic bacteria found in processed yerba mate. This

Table 4. Counts of mesophilic bacteria isolated, per lot of processed yerba mate, after biochemical and sequencing identification (16S rRNA gene - identity ≥ 98%).

Species	Colony forming units (UFC/g)*					
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
Bacillus aerophilus	-**	-	-	-	-	$\mathbf{4.7x10^1}$±$9.4x10^1$
Bacillus amyloliquefaciens	-	$\mathbf{2.5x10^2}$±$2.4x10^2$	$\mathbf{6.7x10^1}$±$1.0x10^2$	-	$\mathbf{5.0x10^0}$±$6.1x10^0$	-
Bacillus cereus	$\mathbf{3.1x10^1}$±$8.2x10^0$	$\mathbf{3.9x10^1}$±$3.4x10^1$	-	-	-	$\mathbf{2.0x10^0}$±$2.7x10^0$
Bacillus flexus	-	-	-	-	$\mathbf{1.0x10^1}$±$6.1x10^0$	-
Bacillus licheniformis	$\mathbf{2.1x10^1}$±1,.$4x10^1$	$\mathbf{3.5x10^1}$±$5.9x10^1$	-	$\mathbf{2.0x10^0}$±$2.7x10^0$	-	$\mathbf{6.0x10^0}$±$6.5x10^0$
Bacillus megaterium	$\mathbf{1.0x10^1}$±$7.0x10^0$	$\mathbf{4.6x10^1}$±$4.4x10^1$	$\mathbf{5.8x10^1}$±$6.8x10^1$	$\mathbf{3.5x10^1}$±$3.7x10^1$	$\mathbf{7.2x10^1}$±$9.9x10^1$	$\mathbf{1.6x10^2}$±$1.7x10^2$
Bacillus oleronius	-	-	-	$\mathbf{1.0x10^0}$±$2.2x10^0$	-	-
Bacillus pseudomycoides	$\mathbf{2.0x10^0}$±$4.4x10^0$	-	-	-	-	-
Bacillus pumilus	$\mathbf{3.9x10^1}$±$1.7x10^1$	$\mathbf{1.4x10^1}$±$7.4x10^0$	$\mathbf{2.0x10^0}$±$2.7x10^0$	-	$\mathbf{5.2x10^1}$±$9.7x10^1$	$\mathbf{3.0x10^0}$±$6.7x10^0$
Bacillus subtilis	$\mathbf{6.4x10^1}$±$2.8x10^1$	$\mathbf{1.1x10^2}$±$8.8x10^1$	$\mathbf{1.4x10^2}$±$1.5x10^2$	$\mathbf{1.5x10^1}$±$7.9x10^0$	-	$\mathbf{2.1x10^1}$±$2.5x10^1$
Bacillus thuringiensis	-	-	$\mathbf{1.0x10^0}$±$2.2x10^0$	-	-	$\mathbf{4.3x10^1}$±$9.6x10^1$
Erwinia amylovora	-	-	$\mathbf{4.4x10^1}$±$5.5x10^1$	-	-	-
Escherichia vulneris	-	-	$\mathbf{3.2x10^1}$±$4.4x10^1$	-	-	-
Klebsiella pneumoniae	-	-	-	$\mathbf{1.2x10^1}$±$2.6x10^1$	$\mathbf{1.5x10^2}$±$1.1x10^2$	-
Paenibacillus alvei	-	-	-	$\mathbf{1.3x10^2}$±$2.4x10^2$	-	-

*CFU/g expressed as mean and standard deviation.
** Species not found in this sample.

species is also found in many environments and is an opportunistic pathogen (Bagley 1985, Jiwa *et al*. 1981, Danielsson *et al*. 1979).

In the lots of processed yerba mate, yeasts were found in lower counts and in less diversity than in the leaves. The species most frequently found was *Rhodosporidium kratochvilovae* (comprising 68.75% of the total counts), followed by *R. mucilaginosa* (25%) and *Sporobolomyces nylandii* (6.25%). The two most frequent species were also found in the leaves. There is a scarcity of data describing their habitats or determining the relationship with animals, plants or other environments. Martins *et al*. (2001) reported, in a survey of microbiological contamination of medicinal herbs, that *R. mucilaginosa* is often present in these products. Takashima & Nakase (2000) continually found *S. nylandii* inhabiting the phylloplane of species of plants in Thailand. *R. kratochvilovae* is a ubiquitous yeast found in association with plants and water, but there is no report about the clinical importance of this species (Sampaio 2011).

This study revealed the presence of a wide diversity of bacteria and yeasts in leaves of *I. paraguariensis* as well as in processed yerba mate, with a predominance of Gram-positive bacteria in both kinds of samples. In processed yerba mate, sporulated Gram-positive strains predominated, and the microorganisms related to sanitary conditions were present in low numbers, indicating that these lots were within the microbiological parameters established by Brazilian law, suggesting that the processing used good manufacturing and hygiene practices.

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REFERENCES

BAGLEY, S. T. 1985. Habitat association of *Klebsiella* species. *Infect. Control*, *6*: 52–58.

BARBOZA, L. M. V., WASZCZYNSKYJ, N. & FREITAS, R. J. S. 2006. Avaliação microbiológica de erva-mate (*Ilex paraguariensis* St. Hil.). *Rev. Inst. Adolfo Lutz*, *65*: 123-126.

BRASIL. 2001. Agência Nacional de Vigilância Sanitária. Resolução RDC n. 12, de 2 de janeiro de 2001. Dispõe sobre o regulamento técnico sobre padrões microbiológicos para alimentos. Diário Oficial [da] República Federativa do Brasil, Brasília, DF, p.1-6. Available at:<http://portal.anvisa.gov.br/wps/wcm/connect/a47bab8047458b909541d53fbc4c6735/RDC_12_2001.pdf?MOD=AJPERES>. Accessed 26 August 2014.

BRASIL. 2003. Ministério da Agricultura, Pecuária e Abastecimento, Secretaria de Defesa Agropecuária. Métodos Analíticos Oficiais para Análises Microbiológicas para Controle de Produtos de Origem Animal e Água. Instrução Normativa nº 62, de 26/08/2003. Diário Oficial da União, Brasília, seção I, p. 14-51. Available at: <http://www.a3q.com.br/dmdocuments/Instru_Normativa_62.pdf>. Accessed 28 August 2014.

BURGESS, S. A., LINDSAY, D. & FLINT, S. H. 2010. Thermophilic bacilli and their importance in dairy processing. *Int. J. Food Microbiol*., *144*: 215–225.

CHAKRABORTY, U., CHAKRABORTY, B. & BASNET, M. 2006. Plant growth promotion and induction of resistance in *Camellia sinensis* by *Bacillus megaterium*. *J. Basic Microbiol*., *46*: 186-195.

COUTINHO, T. A. & VENTER, S. N. 2009. *Pantoea ananatis*: an unconventional plant pathogen. *Mol. Plant Pathol*., *10*: 325-335.

DANIELSSON, M. L., MÖLLBY, R., BRAG, H., HANSSON, N., JONSSON, P., OLSSON, E. & WADSTRÖM, T. 1979. Enterotoxigenic enteric bacteria in foods and outbreaks of food-borne diseases in Sweden. *J. Hyg*., *83*: 33-40.

DJORDJEVIC, S. P., FORBES, W. A., SMITH, L. A. & HORNITZKY, M. A. 2000. Genetic and Biochemical Diversity among Isolates of *Paenibacillus alvei* cultured from Australian Honeybee (*Apis mellifera*) Colonies. *Appl. Environ. Microbiol., 66*: 1098-1106.

EARL, A. M., LOSICK, R. & KOLTER, R. 2008. Ecology and genomics of *Bacillus subtilis. Trends Microbiol*., *16*: 269-275.

FONSECA, A. & INACIO, J. 2006. Phylloplane Yeasts. In: ROSA, C.

& PETER, G. (Eds.). *Biodiversity and ecophysiology of yeasts*. Berlin: Springer. p. 263-301.

GRANUM, P. E. & LUND, T. 1997. *Bacillus cereus* and its food poisoning toxins. *FEMS Microbiol. Lett., 157*: 223-228.

GRIMONT, P. A. D. & GRIMONT, F. 2005. *"Pantoea".* In: BRENNER, D. J., KRIEG, N. R. & STALEY, J. T. (Eds.). *Bergey's manual of systematic bacteriology: the Proteobacteria.* New York: Springer. p. 713-720.

HOOG, G. S. 1993. Evolution of black yeasts: possible adaption to the human host. *A. Van Leeuw., 63*: 105-109.

HORIANSKI, M. A., CASTRILLO, M. L., TAYAGUI, A. B. & JERKE, G. 2012. Calidad microbiológica de yerba mate canchada. *Rev. Cienc. Tecnol*., *14*: 30-33.

HORNITZKY, M. A. Z. & SMITH, L. 1998. Procedures for the culture of *Melissococcus pluton* from diseased brood and bulk honey samples. *J. Apic. Res., 37*: 292-294.

DRISS, E. E. S., MAKAREWICZ, O., FAROUK, A., ROSNER, K., GREINER, R., BOCHOW, H., RICHTER, T. & BORRISS, R. 2002. Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB45 contributes to its plant-growth promoting effect. *Microbiology*, *148*: 2097-2109.

JIWA, S. F., KROVACEK, H. & WADSTRÖM, T. 1981. Enterotoxigenic bacteria in food and water from an Ethiopian community. *Appl. Environ. Microbiol*., *41*: 1010-1019.

KRAMER, J. M. & GILBERT, R. J. 1989. *Bacillus cereus* and other *Bacillus* species. In: DYLE, M. P. (Ed.). *Foodborne bacterial pathogens.* New York: Marcel Dekker. p. 21-70.

LACHANCE, M. A., BOEKHOUT, T., SCORZETTI, G., FELL, J. W. & KURTZMAN, C. P. 2011. *Candida* Berkhout. In: KURTZMAN, C. P., FELL, J. W. & BOEKHOUT, T. (Eds.). *The yeasts: a taxonomic study*. Amsterdam: Elsevier. p. 1209-1211.

LIU, W. T., MARSH, T. L., CHENG, H. & FORNEY, L. J. 1997. Characterization of Microbial Diversity by Determining Terminal Restriction Fragment Length Polymorphisms of Genes Encoding 16S rRNA. *Appl. Environ. Microbiol*., *63*: 4516-4522.

LUDWIG, W., SCHLEIFER, K-H. & WHITMAN, W. B. 2009. "*Bacillaceae*". In: VOS, P., GARRITY, G. M., JONES, D., KRIEG, N. R., LUDWIG, W., RAINEY, F. A., SCHLEIFER, K-H. & WHITMAN, W. B. (Eds.). *Bergey's manual of systematic bacteriology: the Firmicutes.* New York: Springer. p. 20-193.

MARTINS, H. M., MARTINS, M. L., DIAS, M. I. & BERNARDO, F. 2001. Evaluation of microbiological quality of medicinal plants used in natural infusions. *Int. J. Food Microbiol*. *68*: 149-153.

MAZUCHOWSKI, J. Z. 2000. *Produtos alternativos e desenvolvimento da tecnologia industrial na cadeia produtiva da erva-mate.* Curitiba: Câmara Setorial da Cadeia Produtiva da Erva-Mate. Série PADCT III, n. 1.

MIKKOLA, R., KOLARI, M., ANDERSSON, M. A., HELIN, J. & SALKINOJA- SALONEN, M. 2000. Toxic lactonic lipopeptide from food poisoning isolates of *Bacillus licheniformis*. *Eur. J. Biochem*., *267*: 4068-4074.

MISBAH, S., HASSAN, H., YUSOF, M. Y., HANIFAH, Y. A. & ABUBAKAR, S. 2005. Genomic species identification of *Acinetobacter* of clinical isolates by 16S rDNA sequencing. *Singapore Med. J*., *46*: 461-464.

NCBI tool – National Center for Biotechnology Information; Available at: <http://www.ncbi.nlm.nih.gov>. Accessed 28 August 2014.

OSORIO-CADAVID, E., RAMÍREZ, M., LÓPEZ, W. A. & MAMBUSCAY, L. A. 2009. Estandarización de un protocolo sencillo para la extracción de ADN genómico de levaduras. *Rev. Colomb. Biotecnol*., *11*: 125-131.

PERALTA, J. M. & SCHMALKO, M. E. 2007. Modeling heat and mass transfer in the heat treatment step of yerba maté processing. *Braz. J. Chem. Eng. 24*: 73-82.

PERRY, J. J. 1969. Isolation of *Staphylococcus epidermidis* from Tobacco. *Appl. Microbiol., 17*: 647.

REBOLI, A. C., BRYAN, C. S. & FARRAR, W. E. 1989. Bacteremia and infection of a hip prosthesis caused by *Bacillus alvei*. *J. Clin. Microbiol*., *27*: 1395-1396.

REGINATTO, T. S. C. 2008. *Diversidade de bactérias associadas a bromélias do parque estadual de Itapuã-RS.* 83 p. Dissertation (Mestrado em Microbiologia Agrícola e do Ambiente UFRGS) – Universidade Federal do Rio Grande do Sul, Porto Alegre, 2008.

SAMPAIO, J. P. 2011. *Rhodosporidium* Banno. In: KURTZMAN, C. P., FELL, J. W. & BOEKHOUT, T. (Eds.). *The yeasts: a taxonomic study*. Amsterdam: Elsevier. p. 1531-1532.

SCHLEIFER, K-H. & BELL, J. A. 2009. "*Staphylococcaceae*". In: VOS, P., GARRITY, G. M., JONES, D., KRIEG, N. R., LUDWIG, W., RAINEY, F. A., SCHLEIFER, K-H. & WHITMAN, W. B. (Eds.). *Bergey's manual of systematic bacteriology: the Firmicutes.* New York: Springer. p. 392-426.

SLAVIKOVA, E., VADKERTIOVA, R. & VRANOVA, D. 2009. Yeasts colonizing the leaves of fruit trees. *Ann. Microbiol*., *59*: 419-424.

TAKASHIMA, M. & NAKASE, T. 2000. Four new species of the genus *Sporobolomyces* isolated from leaves in Thailand. *Mycoscience*, *41*: 357-369.

URZ'I, C., LEO, F., PASSO, C. & CRISEO, G. 1999. Intra-specific diversity of *Aureobasidium pullulans* strains isolated from rocks and other habitats assessed by physiological methods and by random amplified polymorphic DNA (RAPD). *J. Microbiol. Methods*, *36*: 95-105.

U.S. Food and Drug Administration. Bacteriological Analytical Manual Online. Available at: <http://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm2006949.htm>. Accessed 14 February 2014.

VALDUGA, E. 1994. *Caracterização química e anatômica da folha de Ilex paraguariensis St. Hil. e de algumas espécies utilizadas na adulteração do mate.* 119 p. Dissertation (Mestrado em Tecnologia de Alimentos UFPR) – Universidade Federal do Paraná, Curitiba, 1994.

WHITE, T., BRUNS, T., LEE, S. & TAYLOR, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: INNIS, M. A., GELFAND, D. H. J. J., SCHLOTTERER, C., HAUSER, M. T., HUESLER, A. & TAUTZ, D. (Eds.). *PCR protocols: a guide to methods and applications*. New York: Academic Press. p. 315-322.

WHO. 1998. WORLD HEALTH ORGANIZATON. *Quality control methods for medicinal plant materials.* Geneva: WHO.