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1 **Bacteria associated to human saliva are major microbial components of Ecuadorian**
2 **indigenous beers (*chicha*)**

3

4 **Ana L. Freire¹, Sonia Zapata¹, Juan Mosquera¹ Maria Lorena Mejia¹, and Gabriel**
5 **Trueba¹**

6 ¹ **Instituto de Microbiología, Universidad San Francisco de Quito, Quito, Ecuador.**

7

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10 microbiota, indigenous beer, *Streptococcus salivarius*, artisanal fermented beverages, *Streptococcus*
11 *mutans*, fermented cassava, lactic acid bacteria, saliva, chewed indigenous beer

12

13 Corresponding autor: Gabriel Trueba, gtrueba@usfq.edu.ec

14 **ABSTRACT**

15 Indigenous beers (*chicha*) are part of the indigenous culture in Ecuador. The
16 fermentation process of these beers rely probably on microorganisms from: fermenting
17 substrates, environment and human microbiota. We analyzed the microbiota of artisanal beers
18 (including a type of beer produced after chewing boiled cassava) using bacterial culture and
19 16S-based tag-encoded FLX amplicon pyrosequencing (bTEFAP). Surprisingly, we found
20 that *Streptococcus salivarius* and *Streptococcus mutans* (part of the human oral microbiota)
21 where among the most abundant bacteria in chewed cassava and in non-chewed cassava
22 beers. We also demonstrated that *S. salivarius* and *S. mutans* (isolated from these beers)
23 could proliferate in cassava mush. *Lactobacillus* sp. was predominantly present in most types
24 of Ecuadorian *chicha*.

25

26 **INTRODUCTION**

27 The domestication of fermenting bacteria and yeast predated the domestication of
28 animals and plants; ancestral hominids adapted to metabolize alcohol long time before the
29 Neolithic period (*Carrigan et al., 2015*). The organoleptic and psychotropic effects
30 associated with the consumption of accidentally fermented fruits or cereals may have
31 motivated early humans to replicate this process. Additionally, fermentation may have
32 provided unintended benefits as fermenting bacteria may have reduced the risks of foodborne
33 diseases in ancient societies (*Nakamura et al., 2012; Lewus et al., 1991; Fooks & Gibson,*
34 *2002; Tesfaye et al., 2011*); it is still unclear whether these microorganisms confer additional
35 health benefits (*McNulty NP, 2011*). The use of alcoholic beverages has played a crucial role
36 in the evolution of human societies (*Joffe, 1998*), nevertheless, very little is known about the
37 process of domestication and evolution of these fermenting microorganisms (*Libkinda, et al.,*
38 *2011*).

39 Many fermenting microorganisms have originated in the environment and food
40 substrates (*Martini, 1993*), others resemble microorganisms found in the human microbiome
41 suggesting human (skin or intestine) origins (*Agapakis & Tolaas, 2012*); in fact some
42 modern fermented dairy products contain intestinal bacteria (*Walter, 2008*).

43 Indigenous people from South America (such as Ecuador) prepare a type of beer
44 known as *chicha* which is made with either corn, boiled cassava or the fruit of the palm
45 *Bactris gasipaes* (chonta); some cassava beers include an additional chewing step before the
46 fermentation process. A recent report showed that bacteria present in chewed cassava beers
47 were mainly *Lactobacillus* sp (*Colehour, et al., 2014*). We analyzed the microbial diversity
48 (using culture dependent and culture independent techniques) in different types of Ecuadorian
49 *chicha*.

50 MATERIALS & METHODS

51 Sample collection

52 Four samples of *chicha* (indigenous beer) from two geographical regions of Ecuador
53 (Andean and Amazon regions) were collected. These samples included beer made with both
54 chewed cassava (CC), mashed cassava (MC), mashed chonta (CB) and ground corn (CoB)
55 (Table 1). The samples of CC and MC were purchased from the same household. All these
56 products were obtained from rural communities. None of these beers were pasteurized, nor
57 had they any commercial additives or preservatives. All samples were refrigerated (2 to 8° C)
58 after collection; a 2 mL aliquot of sample was stored at -20°C, for molecular phylotyping.

59 Plate count of lactic acid bacteria (LAB)

60 A 20 mL aliquot of each sample was homogenized in 180 mL of a sodium citrate
61 solution (10^{-1} dilution) and ten-fold dilutions were made in saline solution (NaCl 0.9%). One
62 mL of each dilution was inoculated in MRS (pH 5) and M17 (pH 7, 0.5% dextrose) by pour
63 plate method. Two incubation temperatures were used (37°C and 43°C) under aerobic and
64 anaerobic conditions, for 3 to 5 days. The incubation time varied because of the different
65 bacteria present on each product.

66 Phenotypic characterization

67 Ten to twenty colonies (showing different morphology) were randomly picked from
68 each sample. Six to ten colonies that had the characteristics of lactic acid bacteria (oxidase
69 negative, catalase negative, Gram positive rods and cocci) were isolated for further
70 characterization. Strains were stored at -20°C in MRS or M17 broth with 20% of glycerol.

71 Genotypic characterization of bacterial colonies

72 From each colony the 16S ribosomal gene was amplified and sequenced. DNA
73 extraction was performed with the DNAzol Reagent (Life Technologies, 2001). One pure

74 colony was needed, and all the steps were followed as recommended by the manufacturer.
75 DNA was stored at -20°C until used. The 16S ribosomal gene was amplified in 25ul
76 containing: 1X PCR buffer, 2.5mM MgCl₂, 0.25mM dNTP's, 0.2uM 27F primer (5'-
77 AGAGTTTGATCCTGGCTCAG-3'), 0.2uM 1492R primer (5'-
78 GGTTACCTTGTTACGACTT-3') (Martin, et al., 2001), 0.5U GoTaq Flexi DNA
79 polymerase (Promega, Madison), 5uL of sample DNA and Milli-Q water. The times and
80 temperatures used for the amplification were: melting (94°C, 1 minute), annealing (56°C, 30
81 seconds), elongation (72°C, 30 seconds), this routine was repeated for 30 cycles, and final
82 extension (72°C, 10 minutes);. Amplicons were subjected to gel electrophoresis (1% agarose
83 gel), sequenced at Functional Biosciences (Madison, WI) and DNA sequences analyzed using
84 Seqmatch (Ribosomal Database Project <http://rdp.cme.msu.edu/>) and submitted to GenBank;
85 the accession numbers are: KT722809 to KT722833).

86 **High throughput sequencing analysis**

87 In order to complement the culture-based protocols, we investigated the microbial
88 diversity using FLX amplicon pyrosequencing. DNA was extracted from all beer samples
89 using DNeasy Plant Mini kit (Qiagen) following manufacturer's protocols, but instead of
90 using AE buffer for elution, we used same volume of PCR Milli-Q water. DNA samples from
91 four types of beer were sent to CD Genomics (NY, USA), for 16S-based phylotyping. DNA
92 was subjected to bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) using
93 primers 939F-5'TTGACGGGGGCCCGCAC3' and 1492R-5'TACCTTGTTACGACTT3'.
94 For fungal sequences we used ITSf- 5'CTTGGTCATTTAGAGGAAGTAA3' . Resulting
95 sequences (minimum length = 250 nucleotides) were trimmed and quality scored using
96 USearch (<http://drive5.com/>); chimeras were detected using UCHIIME (<http://drive5.com/>) in de
97 novo mode and were compared using BLASTn to a ribosomal database. Identity values were

98 used to make assignments to the appropriate taxonomic levels: greater than 97% identity
99 were resolved at the species level and between 95% and 97% at the genus level. The number
100 of bacterial sequences we obtained were: 2,965 readings for CC, 3,320 for MC, 3,046 for CB
101 and 15,623 for CoB. For fungi we obtained 6,763 readings from CC, 6,925 from MC and
102 6,558 from CB. We did not carry out fungi analysis of CoB. All sequences were submitted to
103 Sequence Read Archive, accession number are SRP070493; SUB1342311 and SUB1351168.

104

105 ***Streptococcus salivarius* and *Streptococcus mutans* growth in cassava solution**

106 To rule out the possibility of *S. salivarius* or *S. mutans* contamination, one colony of a
107 pure culture of each bacteria (obtained from beers) was diluted in 25mL of sodium citrate
108 (2%) separately. Subsequently, 1 mL of this cell suspension was used to inoculate tubes
109 containing 9mL of sterile (autoclaved) chewed cassava solution (10%) and incubated at 37°C
110 under anaerobic conditions. A 100 µL aliquot from each incubated tube was extracted and
111 plated in M17 (this was done by triplicate) at 0, 24, 48 and 72 hours of inoculation. Results
112 from each day were compared to determine the ability of these bacteria to grow in chewed
113 cassava solution.

114 **Statistical analysis**

115 We used Mann-Whitney U test to test whether *S. salivarius* and *S. mutans* were able
116 to grow in cassava solution. Shannon indices were calculated using the formula $H =$
117 $-\sum p_i \log(p_i)$, p_i being the relative frequency of the abundance of each species found.
118 Principal component analysis (PCA) of the bacterial species and abundance of the four
119 beverages was performed using the software SPSS v21 (IBM Corp, Armonk, NY).

120 **RESULTS**121 **Characterization of bacterial isolates**

122 Twenty-five bacterial isolates (cultured from the 4 beer types) were characterized by
123 16s rDNA sequencing showing 99% to 100% identity when compared with GenBank
124 sequences (Table 2). The predominant bacterial species in all beers were *Lactobacillus*
125 *fermentum* (16%), *Lactococcus lactis* (16%), *Leuconostoc mesenteroides* (16%), and
126 *Streptococcus salivarius* (16%); followed by *Lactobacillus plantarum* (8%), *Weissella*
127 *confusa* (8%), *Lactobacillus casei* (4%), *Lactobacillus pantheris* (4%), *Lactobacillus*
128 *parabuchneri* (4%), *Lactobacillus paracasei* (4%) and *Streptococcus mutans* (4%). The most
129 diverse bacterial composition (using culture-dependent techniques) found in CoB (6 bacterial
130 species), followed by the CC (5 bacterial species), CB (3 bacterial species) and MC (2
131 bacterial species). Intriguingly cassava beers contained human salivary bacteria: both CC and
132 MC, had *Streptococcus salivarius* while CC had also *S. mutans* (Table 2).

133 **High throughput sequencing analysis**

134 The beer with greater diversity was CC (31 bacterial species), followed by CoB (26
135 bacterial species), CB (21 bacterial species), MC (20 bacterial species). The predominant
136 bacterial species in CC were *Lactobacillus* spp. (40.9%) followed by human microbiota
137 bacteria: *Streptococcus salivarius* (31.94%), *Streptococcus parasanguinis* (5.41 %),
138 *Streptococcus pneumoniae* (3.65%). The most prevalent bacteria in MC were *Streptococcus*
139 spp. (83%) followed by *Lactococcus* sp. (9.32%); the majority of streptococci have been
140 described as part of the human microbiota: *Streptococcus salivarius* (65%), *Streptococcus*
141 *pasteurianus* (7.74%), and *Streptococcus parasanguinis* (3.47%). The most prevalent bacteria
142 in CB were *Weissella confusa* (46%), *Weissella* sp. (20%), and *Lactococcus lactis* (9%). The
143 dominant bacteria in CoB were *Weissella* sp. (19%) and *Lactobacillus plantarum* (12.5%),

144 *Lactococcus garviae* (2.76%) *Lactobacillus brevis* (2.5 %) (Table 3). The dominant fungal
145 species present in different beers analyzed was very similar; *Saccharomyces cerevisiae* was the
146 most abundant comprising 92% of all the taxa detected (Table 4).

147 **Growth of *S. salivarius* and *S. mutans* in cassava solution**

148 *Streptococcus salivarius* (Figure 1) and *S. mutans* (Figure 2) grew in chewed
149 cassava solution. After 48 hours of culture (*S. salivarius*) and 72 hours (*S. mutans*), bacterial
150 counts went down.

151 **Diversity estimations**

152 CC was the beverage with the most species diversity (H=1.06, E= 0.71), followed by
153 CoB (H=0.94, E=0.66), CB (H=0.71, E=0.54), and MC (H=0.59, E=0.45). The evenness
154 values followed the same pattern and suggest that CC is also the most heterogeneous in terms
155 of species (Hayek, 2010; Pielou, 1966).

156 **Principal component analysis**

157 The type of beer (fermenting substrate) accounted for 90.4% of the bacterial species
158 variability and cassava beers had more similar bacterial composition and abundance than the
159 other types of beer; interestingly CB and CoB also showed similarity (Figure 3).

160 **DISCUSSION**

161 Our study found higher bacterial diversity in beer that contained human saliva (Tables
162 2 and 3); therefore, saliva may not only speed up the fermentation process (by providing
163 amylases as suggested by Henkel, 2005) but also may offer an additional bacterial inoculum
164 which may favor this process. This finding may provide additional explanation for the
165 adoption of such a peculiar process in the beer's manufacture.

166 Our study also demonstrate the presence of oral streptococci such as *S. salivarius*, *S.*
167 *mutans*, *S. parasanguinis* in cassava beers; these bacteria may thrive on carbohydrates
168 present in the oral cavity after starchy meals (Moye, et al., 2014; Burne, et al., 1998). Oral
169 bacteria *S. salivarius* and *S. mutans* were cultured from cassava chicha (with saliva and
170 without saliva) in large numbers and were shown to grow in mashed cassava under
171 laboratory conditions. Oral bacteria in beer without human saliva may indicate contamination
172 of fermenting containers (or utensils). Fermenting bacteria are known to produce biofilm in
173 containers (Kebede et al., 2007) and both types of cassava beers were obtained from the same
174 household, and probably they use the same pots for both type of beers. It is possible that
175 some strains of *S. salivarius* from these beers may be adapting to the fermentation process;
176 *Streptococcus thermophilus*, a bacteria used as starter in yogurt (Burton, et al., 2006) may
177 have evolved from *S. salivarius* (Hols, et al., 2005). Future studies should investigate the
178 prevalence of *S. salivarius* in larger number of cassava *chichas* from other locations and find
179 out whether the strains of *S. salivarius* isolated from beers are different from those isolated
180 from human saliva.

181 A recent study failed to detect *S. mutans* and *S. salivarius* in *chicha* prepared with
182 chewed cassava in Ecuador (Colehour, et al., 2014). The disagreement between both studies
183 may result from differences in samples in both studies; Colehour, et al., 2014 collected beers

184 that were fermenting for 4 days while we collected samples that were fermenting for 3 days.
185 Beer microbiota changes overtime (*Steinkraus, 2006*) and in the case of *S. mutans* and *S.*
186 *salivarius* we observed a sharp increase and decline in bacterial populations in 24 hours
187 (Figures 1 and 2). Unlike Colehour, et al., 2014, we also carried out bacterial cultures.

188 Reduction on streptococci populations may be due to the consumption of all the
189 nutrients, accumulation of toxic metabolites, autolysis. (*Dufour & Lévesque, 2013*). Also,
190 these bacteria are known to form biofilm (*Ajdic, et al., 2002; Li, et al., 2002*) which may
191 change bacterial location and reduction of planktonic cells. Additionally, unlike our study
192 Colehour, et al., 2014 found predominance of *L. reuteri* which is known to antagonize *S.*
193 *salivarius* (*Nikawa, et al., 2004; Corby, et al., 2005*). Similar to previous studies (*Colehour,*
194 *et al., 2014; Elizaquivel, et al., 2010; Puerari, et al., 2015*), *Lactobacillus* was a dominant
195 genus of lactic bacteria in chicha found in both culture dependent and independent
196 assessments.

197 Our study complements previous microbiological analysis carried out in *chicha* and
198 shows for the first time the potential adaptation of *S. salivarius*, *S. mutants* (and possibly
199 other streptococci from the human upper respiratory tract) to grow in cassava mush. The
200 study not only shows how bacteria from human microbiota may adapt to artisanal
201 fermentative processes but also shows that chewed chicha may potentially transmit human
202 pathogens such as *S. mutans*, one of the causative agents of dental plaque and cavities
203 (*Loesche, 1986*); *Streptococcus mutans* can be transmitted person to person probably
204 throughsaliva (*Baca et al., 2012*). This is especially relevant because these types of beers are
205 consumed as early as 2 or 3 days after preparation.

206 The main limitation of our study was the low number of samples analyzed of each
207 beer. However this limitation does not invalidate the main findings of this study.

10

208 Additionally, the culture medium (MRS) is not suitable to culture *Lactobacillus* from cereals
209 (*Minervini, et al., 2012*), therefore we may have underestimated the bacterial diversity in
210 these beers.

211

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214

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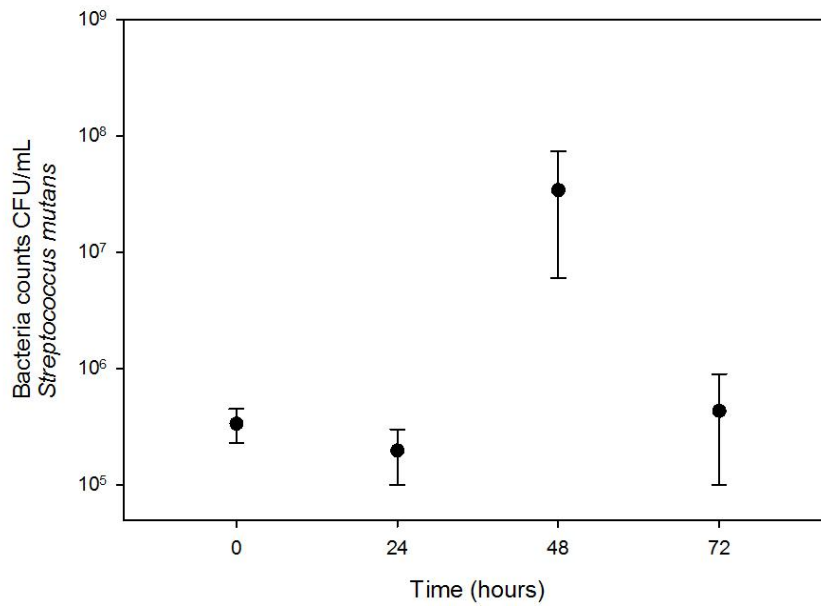
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315

FIGURES



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318 **Figure 1. Growth of *S. salivarius* in sterile chewed cassava solution.** There is a significant
319 increase in CFU (Mann-Whitney U test) at the 24 hours of incubation compared with those at
320 inoculation time (0 hours).

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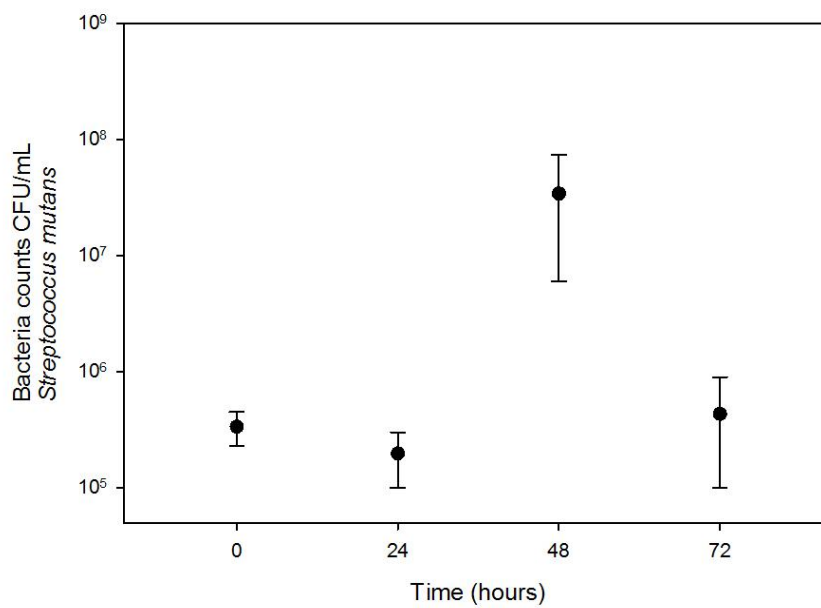
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333 **Figure 2. Growth of *S. mutans* in chewed cassava solution.** There is a significant increase
334 in CFU (Mann-Whitney U test) at the 48 hour of incubation compared with those at the
335 inoculation time (0 hours).

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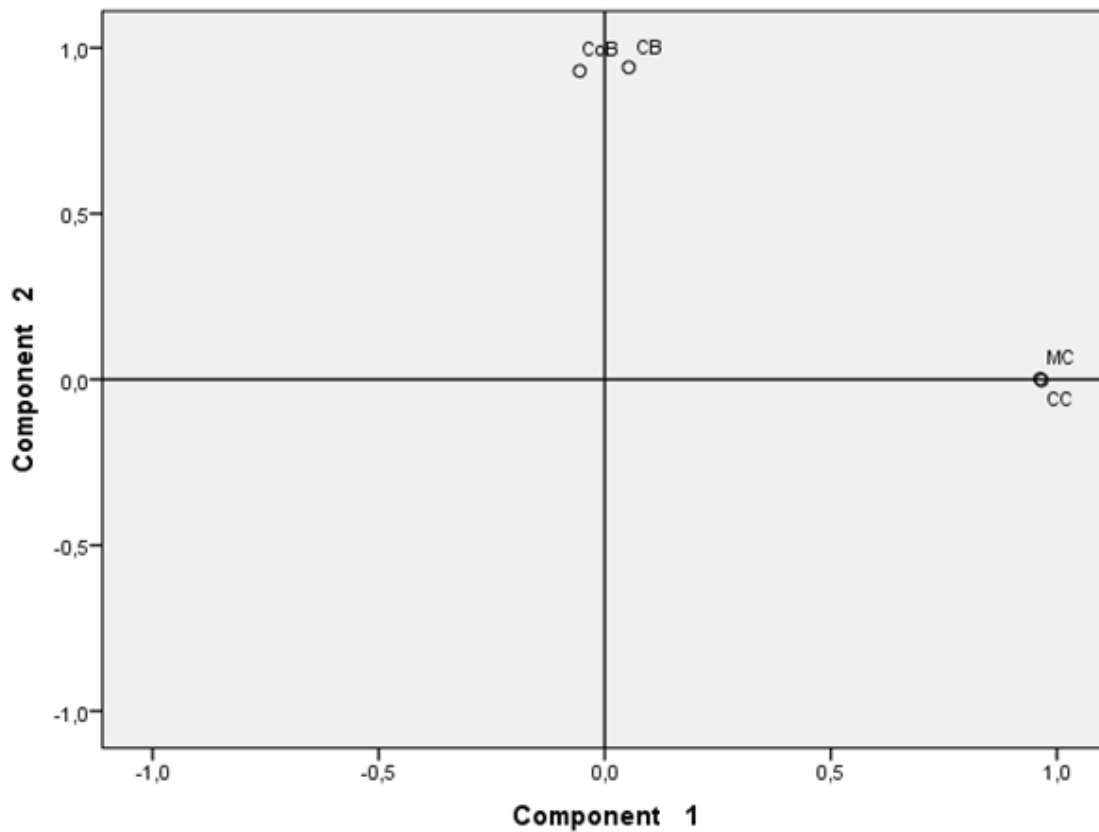
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344 **Figure 3. Principal component analysis of beers' microbiota.** Beers made with cassava
345 (MC and CC) formed a cluster different from the cluster formed by beers made with either
346 chonta (CB) or corn (CoB). Each pair of beverages that form a group share a similar bacterial
347 species profiles and abundance.

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352 **Table 1. Description and site of collection of the different types of indigenous beers**
353 **analyzed.**

Main ingredient	Substrate Scientific name	Geographic al region	Site of collection	Time of fermentation
Chewed cassava	<i>Manihot esculenta</i>	Amazon	Puyo	3 days
Mushed cassava	<i>Manihot esculenta</i>	Amazon	Puyo	3 days
Chonta	<i>Bactris gasipaes</i>	Amazon	Tena	2 days
Corn (jora)	<i>Zea mays</i>	Highlands	Pifo	2 days

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369 **Table 2. Bacteria isolated from the four beer samples.** All the 25 strains were obtained by
 370 bacterial cultures in MRS and M17 and 16s ribosomal gene from colonies was amplified and
 371 sequenced.

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Sample	Isolate ID	Culture Media	Growth condition	Identification (16S)
Chewed cassava beer	25 A2	MRS	Anaerobic	<i>Leuconostoc mesenteroides</i>
	25 C2	MRS	Aerobic	<i>Lactobacillus fermentum</i>
	25 E2	M17	Anaerobic	<i>Streptococcus mutans</i>
	25 F1	M17	Aerobic	<i>Lactococcus lactis</i>
	25H1	M17	Aerobic	<i>Streptococcus salivarius</i>
Mushed cassava beer	26 A1	MRS	Anaerobic	<i>Lactobacillus fermentum</i>
	26 B1	MRS	Anaerobic	<i>Lactobacillus fermentum</i>
	26 C2	MRS	Aerobic	<i>Lactobacillus fermentum</i>
	26 E2	M17	Anaerobic	<i>Streptococcus salivarius</i>
	26 F2	M17	Anaerobic	<i>Streptococcus salivarius</i>
	26 G1	M17	Aerobic	<i>Streptococcus salivarius</i>
Chonta beer	27 A1	MRS	Anaerobic	<i>Lactobacillus plantarum</i>
	27 B1	MRS	Anaerobic	<i>Weissella confusa</i>
	27 C1	MRS	Aerobic	<i>Weissella confusa</i>
	27 E1	M17	Aerobic	<i>Lactococcus lactis</i>
	27 F2	M17	Anaerobic	<i>Lactococcus lactis</i>
	27 G2	M17	Aerobic	<i>Lactococcus lactis</i>
Corn beer	61 B2	MRS	Anaerobic	<i>Lactobacillus casei</i>
	61 G1	M17	Anaerobic	<i>Leuconostoc mesenteroides</i>
	61 G2	M17	Anaerobic	<i>Lactobacillus plantarum</i>
	61 H1	MRS	Anaerobic	<i>Lactobacillus parabuchneri</i>
	61 I1	MRS	Anaerobic	<i>Lactobacillus paracasei</i>
	61 J1	MRS	Anaerobic	<i>Lactobacillus pantheris</i>
	61 K1	M17	Anaerobic	<i>Leuconostoc mesenteroides</i>
	61 L1	M17	Anaerobic	<i>Leuconostoc mesenteroides</i>

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379 **Table 3. Most predominant bacterial species (abundance of more than 0.1%) found by**
 380 **pyrosequencing analysis of samples from 4 types of chicha.** Chewed cassava (CC),
 381 **mashed cassava (MC), chonta (CB) and corn (CoB).** Numbers indicate percentages and “+”
 382 **indicates that bacterium recovered in culture.**

Bacterial species	CC	MC	CB	CoB	Cultured	Possible origins
<i>Bacillus amyloliquefaciens</i>	0.0	0.5	0.0	0.00	-	Environment
<i>Carnobacterium maltaromaticum</i>	0.0	0.0	1.0	0.1	-	Environment
<i>Enterobacter asburiae</i>	0.5	0.0	0.0	0.0	-	Environment
<i>Enterobacter cancerogenus</i>	0.5	0.0	0.0	0.0	-	Environment
<i>Enterobacter sp</i>	1.3	0.0	0.1	0.0	-	Environment
<i>Fructobacillus sp</i>	0.0	0.0	0.0	3.8	-	Vegetables
<i>Gluconacetobacter intermedius</i>	0.0	0.0	0.0	0.6	-	fermented food
<i>Kluyvera ascorbate</i>	0.4	0.0	0.0	0.0	-	Human gut, food
<i>Lactobacillus brevis</i>	8.4	0.1	2.5	0.6	-	Environment, gut
<i>Lactobacillus camelliae</i>	0.0	0.0	0.0	7.3	-	Environment, gut
<i>Lactobacillus casei</i>	0.0	0.0	0.0	3.1	+	Environment, gut
<i>Lactobacillus delbrueckii</i>	8.0	0.0	0.0	0.0	-	Environment, gut
<i>Lactobacillus fermentum</i>	6.5	3.8	0.0	0.0	+	Environment, gut
<i>Lactobacillus harbinensis</i>	0.0	0.0	0.0	2.1	-	Vegetables
<i>Lactobacillus manihotivorans</i>	1.8	0.0	0.0	0.0	-	Vegetables
<i>Lactobacillus parabuchneri</i>	0.0	0.0	0.0	1.4	+	Oral microbiota
<i>Lactobacillus paracasei</i>	0.0	0.0	0.0	8.6	+	Environment, gut
<i>Lactobacillus paracollinoides</i>	0.0	0.0	0.0	16.0	-	Environment, gut
<i>Lactobacillus plantarum</i>	10.8	0.0	12.4	0.1	+	Environment, gut
<i>Lactobacillus sp</i>	3.4	0.0	0.7	1.3	-	Environment, gut t
<i>Lactobacillus vaccinostrercus</i>	1.2	0.0	0.2	0.0	-	Environment, gut
<i>Lactococcus garviae</i>	0.0	0.0	2.8	0.0	-	Fermented food
<i>Lactococcus lactis</i>	2.1	0.0	8.9	0.0	+	Environment, gut
<i>Lactococcus sp</i>	0.2	9.3	1.0	0.2	-	Gut
<i>Leuconostoc citreum</i>	0.0	1.5	1.2	0.0	-	fermented food
<i>Leuconostoc lactis</i>	1.7	0.1	0.2	0.8	-	Environment
<i>Leuconostoc sp</i>	0.0	0.0	0.1	4.6	-	Vegetables
<i>Oenococcus kitaharae</i>	0.0	0.0	0.0	1.2	-	Vegetables
<i>Serratia sp</i>	1.0	0.0	0.0	0.0	-	Environment
<i>Streptococcus gallolyticus</i>	0.0	0.5	0.0	0.0	-	Oral microbiota
<i>Streptococcus oralis</i>	1.4	0.2	0.0	0.0	-	Oral microbiota
<i>Streptococcus parasanguinis</i>	5.4	3.5	0.0	0.0	-	Oral microbiota
<i>Streptococcus pasteurianus</i>	0.0	7.7	0.0	0.0	-	Human gut
<i>Streptococcus pneumoniae</i>	3.6	0.5	0.0	0.0	-	Human nasopharynx
<i>S. pseudopneumoniae</i>	0.5	0.0	0.0	0.0	-	Human nasopharynx
<i>Streptococcus salivarius</i>	32.0	65.0	0.0	0.0	+	Oral microbiota
<i>Streptococcus sp</i>	2.5	2.3	0.1	0.0	-	Human microbiota
<i>Streptococcus thermophilus</i>	1.2	2.59	0.0	0.0	-	Vegetables
<i>Streptococcus vestibularis</i>	0.4	0.8	0.0	0.0	-	Oral microbiota

<i>Weissella cibaria</i>	0.1	0.0	0.9	0.9	-	Vegetables
<i>Weissella confusa</i>	0.5	0.1	45.9	25.3	+	Vegetables
<i>Weissella paramesenteroides</i>	0.5	0.0	0.1	0.0	-	Environment
<i>Weissella sp</i>	0.2	0.3	19.8	19.4	-	Vegetables

Table 4. Most predominant fungal species found by pyrosequencing analysis of samples from 3 types of *chicha*. Chewed cassava (CC), mashed cassava (MC) and chonta (CB). The numbers indicate percentages.

Fungal species	CC	MC	CB	Possible origins
<i>Saccharomyces cerevisiae</i>	92.533	92.023	92.033	Vegetables
<i>Penicillium citrinum</i>	0.03	0.021	0.062	Soil
<i>Debaryomyces hansenii</i>	0.636	0.547	0.549	Sea water
<i>Hanseniaspora uvarum</i>	0.044	0.056	0.075	Vegetables
<i>Wallemia muriae</i>	0.118	0.115	0.137	Salty water
<i>Wallemia sp</i>	1.316	1.701	1.602	Salty water
<i>Aspergillus sp</i>	0.089	0.047	0.032	Soil
<i>Pichia kudriavzevii</i>	1.05	1.5	1.32	Vegetables
<i>Aspergillus versicolor</i>	0.104	0.138	0.135	Soil
<i>Pichia burtonii</i>	0.118	0.123	0.107	Vegetables
<i>Hyphopichia burtonii</i>	0.089	0.067	0.073	Starch substrates
<i>Cyberlindnera sp</i>	0.532	0.54	0.545	Waste deposits
<i>Pichia sp</i>	0.044	0.04	0.054	Soil
<i>Saccharomyces bayanus</i>	0.104	0.132	0.096	Vegetables
<i>Galactomyces sp</i>	3.149	2.908	3.133	Rumen, fermented food
<i>Pichia fermentans</i>	0.044	0.042	0.047	Vegetables

