UNIVERSIDAD SAN FRANCISCO DE QUITO

Colegio de Postgrados

Characterization and technological properties of predominant lactic acid bacteria isolated from traditional fermented foods from Ecuador

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Tesis de grado presentada como requisito para la obtención del título de Magíster en Microbiología

Quito

Mayo de 2012

Universidad San Francisco de Quito
Colegio de Posgrado
HOJA DE APROBACIÓN DE TESIS

Characterization and technological properties of predominant lactic acid bacteria isolated from traditional fermented foods from Ecuador

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DEDICATORIA

Este trabajo está dedicado a mi esposo a quien amo profundamente, por ser mi compañero de aventuras en este viaje que se llama vida.

A mis padres y hermanos por tener fé en mis sueños y proyectos y apoyarme incondicionalmente en todo momento.

A mi sobrino Alejandro, por ser una constante fuente de inocencia y alegrar mis días con sus detalles.

Finalmente a Dios, el mejor de los maestros, a quien sencillamente le debo todo.

AGRADECIMIENTOS

Quiero agradecer a la Universidad San Francisco de Quito y al Instituto de Microbiología por la beca de estudios que me fue otorgada para realizar mis estudios de Maestría.

A mis compañeras y amigas Carito, Cristina, Thamara, Verónica, Daysita y Emilia por compartir su conocimiento y brindarme su amistad en cada momento.

Un agradecimiento especial a Rosita Bayas y mi padre Sr. Ángel Guamán por su apoyo durante la toma de muestras, y a Elizabeth Bolaños por su colaboración en el análisis microbiológico.

Finalmente al Dr. Gabriel Trueba, Dra. Mariela Serrano y Dra. Sonia Zapata un eterno agradecimiento por su asistencia y dirección a lo largo de la ejecución de esta tesis.

Abstract

Ninety-six samples of traditional fermented foods were collected from 20 Ecuadorian provinces. A total of 119 lactic acid bacteria (LAB) were isolated and characterized by phenotypic and 16S ribosomal DNA (rDNA).

47% of isolates were identified as facultative heterofermentative *Lactobacillus sp*. The next predominant groups of lactic acid bacteria (31%) were homofermentative cocci belonging to the genera *Pediococcus*, *Lactococcus* and *Enterococcus;* 11%. were obligate heterofermentative bacteria such *Lactobacillus* sp. and 11% heterofermentative cocci (*Weissella* and *Leuconostoc)*.

Biochemical properties such as production of bacteriocin, exopolysaccharide (EPS), proteinases and acidification, were evaluated in vitro with the purpose of identification of potential starter strains. The most of strains (82%) showed caseinolytic activity; contrastingly EPS and bacteriocin production were a rare trait.

This manuscript represents the first characterization of traditional Ecuadorian fermented foods-associated LAB, using phenotypic and genetic approaches.

Resumen

Noventa y seis muestras de alimentos fermentados tradicionales de Ecuador fueron colectados de 20 diferentes provincias de Ecuador. Un total de 119 bacterias ácido lácticas (LABs) fueron aisladas y caracterizadas por fenotipo y amplificación de 16S ribosomal DNA. (rDNA).

47% de los aislados fueron identificados como heterofermentativos facultativos Lactobacillus sp. El siguiente grupo predominante de LABs (31%) fueron cocos homofermentativos pertenecientes al género Pediococcus, Lactococcus y Enterococcus, 11% fueron bacterias heterofermentativas obligatorias tales como algunas especies de Lactobacillus sp. y el 11% de cocos heterofermentativos (*Weissella* y *Leuconostoc*). Propiedades bioquímicas tales como producción de bacteriocinas, exopolisacáridos, proteinasas y acidificación fueron evaluadas in vitro con el propósito de identificar cepas con potencial de cultivos iniciador. La mayoría de cepas (82%) mostraron actividad caseinolítica, en contraste producción de EPS y bacteriocinas fueron características poco frecuentes.

Este trabajo representa la primera caracterización de alimentos tradicionales fermentados asociados a LABs usando métodos fenotípicos y genotípicos.

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1. INTRODUCTION

The diet is part of heritage of any human culture. Foods are prepared in different ways in the distinct geographical regions and they reflect environmental factors such as availability of ingredients, and religious beliefs, laws, etc. (McWilliams 2007). World dietary culture is based on cereal diets: (1) rice based Eastern food culture, (2) wheat/barley-based Western-Australian food culture, and (3) sorghum/maize based in African and South American food cultures (Tamang 2010). Similarly, diverse fermentation techniques have been described around the world.

1.1 Lactic Acid Bacteria and Fermented Foods

Fermentation is probably one of the oldest food preservation methods, the historic use of lactic acid bacteria (LAB) in fermented foods dates back at least 8,000 years ago as suggested by written records coinciding with the development of dairy farming in India, Mesopotamia and Egypt (Edward 2008).

LAB's are bacteria that produce lactic acid from glucose (or lactose) fermentation, and share some metabolic similarities. They are gram-positive, non-sporulating,

non-motile, acid tolerant, cocci or rods (Hutkins, 2006). Historically the genera *Lactobacillus, Leuconostoc*, *Pediococcus*, and *Streptococcus* formed the core of this group. However from food technology point of view, the principal LAB genera are: *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus, and Weissella.* (Jin *et al*., 2009)

Artisanal food fermentation occurs spontaneously due to naturally occurring microorganisms in raw materials known as “non-starter lactic acid bacteria” (NSLAB) because they are not intentionally added to food to promote fermentation, Lactobacilli dominate this group (Williams and Banks, 1997; Fitzsimons *et al*., 1999; Swearingen *et al*., 2001), although pediococci, micrococci and leuconostocs have also been found in raw microflora of artisanal dairy products. (Manolopoulou *et al*., 2003; Callon *et al*., 2004)

NSLAB in artisanal dairy products determines organoleptic qualities such as flavors and textures in yoghurt, cheeses, sauces, fermented vegetables and bakery products. The distinct complexity of the microflora in different regions of the world makes every product unique. (Cogan *et al*., 1997; Beresford *et al*., 2001)

The success and prevalence of fermented foods in time, perhaps is in part due to the intrinsic advantages of the fermentation, such as the preservation of perishable foods from the production of lactic acid and other organic acids such as metabolites, H_2O_2, diacetyl and bacteriocins. (Olaoye et al., 2008) In tropical

countries where dairy products are difficult to store, starchy foods (cassava, maize, sorghum, etc.) are the basis of the daily diet.

1.2 Biological Importance of Fermented Foods

Several researchers claimed beneficial effects of fermented foods such as increased bioavailability of minerals, the production of antioxidants and omega-3 polyunsaturated fatty acids (Tamang 2007, Liong 2008). Fermentation is also known to enhance the digestibility of lactose, (Simango, 1997) reduction of pH below 4 which prevent proliferation of pathogenic bacteria, and may also reduce undesirable substances present in raw foods such as phytates and tannins, (Gadaga *et al*., 1999). Another example is degradation of cyanogenic glycoside linamarin, which can be detoxified by species of *Leuconostoc*, *Lactobacillus* and *Streptococcus* in fermented foods such as gari.(Westby and Twiddy, 1991) Finally, this process may reduce the energy required for refrigeration and subsequent cooking (Simango, 1997).

1.3 Probiotic Properties of Fermented Foods

The concept of probiotics was first proposed by Nobel Prize winner Elie Metchnikoff in 1908, who suggested that longevity of Bulgarian peasants resulted from their consumption of fermented milk products (Mercenier *et al*., 2002; Chuayana *et al*., 2003; Tannock, 2003). Metchnikoff suggested that when the fermented milk products were consumed, *Lactobacillus* could prevent "fouling" in

the large intestine and positively influenced the microflora of the gut, decreasing toxic microbial activities there. (Mercenier *et al*., 2002; Chuayana *et al*., 2003)

In 1998 Salminen *et al.,* defined probiotics as ‘‘foods containing live bacteria which are beneficial to health’’ this concept was replaced in 2001 by the Food and Agriculture Organization (FDA) and World Health Organization (WHO), which defined probiotics as ‘‘live micro-organisms which when administered in adequate amounts, confer a health benefit on the host.’’

LAB’s are the most common type of microorganisms used as probiotics. Strains belonging to the genera *Lactobacillus*, *Bifidobacterium* (Yateem *et al*., 2008) and *Enterococcus* (Ljungh and Wadström, 2006) are the most widely used and commonly studied probiotic bacteria.

To be considered as probiotics, these microorganisms must satisfy several criteria described below. (Salminen and von Wright, 1998)

- Survival of the environmental conditions on the location where it must be active
- Proliferation and/or intestinal colonization.
- No immune reaction against the probiotic strain.
- No pathogenic, toxic, allergic, mutagenic or carcinogenic reaction by the probiotic strain itself, its fermentation products or its cell components after decrease of the bacteria.
- Genetically stable, no plasmid transfer.
- Easy and reproducible production.

- Viable during processing and storage.

In 1994, the World Health Organization deemed probiotics as “the next-most important immune defense system when commonly prescribed antibiotics are rendered useless by antibiotic resistance” (Kailasapathy and Chin, 2000).

1.4 Health benefits of probiotics

The type of health benefits provided by the consumption of probiotics can classified in two: first through the "probiotic effect" resulting from the bacterial-host interaction and the second through the "biogenic effect" resulting from the ingestion microbial metabolites as bioactive peptides, vitamins, etc. that occur during fermentation process.(Mozzi et al., 2010)

The therapeutic use of probiotics has been considered in the cases of lactose intolerance (Suvarna and Boby, 2005), antibiotic-associated diarrhea (Plummer *et al.,* 2004) and *Helicobacter pylori* infection (Ouwehand *et al*., 2002). Other conditions have also reported to benefit from probiotics such as: gastroenteritis, irritable bowel syndrome, depressed immune function, cancer, and genitourinary tract infections (Stanton *et al.* 2005). Additionally, probiotics may improve intestinal mobility and relieve constipation, (Ouwehand *et al*., 2002). They also reduce the cholesterol levels in serum (Liong and Shah, 2005). Fig 1. Some reports claimed that probiotics have anti-cancer properties, which are of three types: (1) elimination of procarcinogens; (2) modulation of procarcinogenic enzymes; and (3) tumor suppression (Wollowski *et al*., 2001).

Pool-Zobel (1993) based on evidence from studies *in vitro* and in *in vivo* suggested that lactic acid bacteria decrease the incidence of DNA damage (Pool-Zobel *et al.,* 1993) and other changes associated with the carcinogenic process (Rowland, 1996) Furthermore, there is evidence that LAB promote reduction of intestinal bacterial enzyme activities (glucuronidase, nitroreductase, and azoreductase which may convert procarcinogens into carcinogens (Ling et al., 1994).

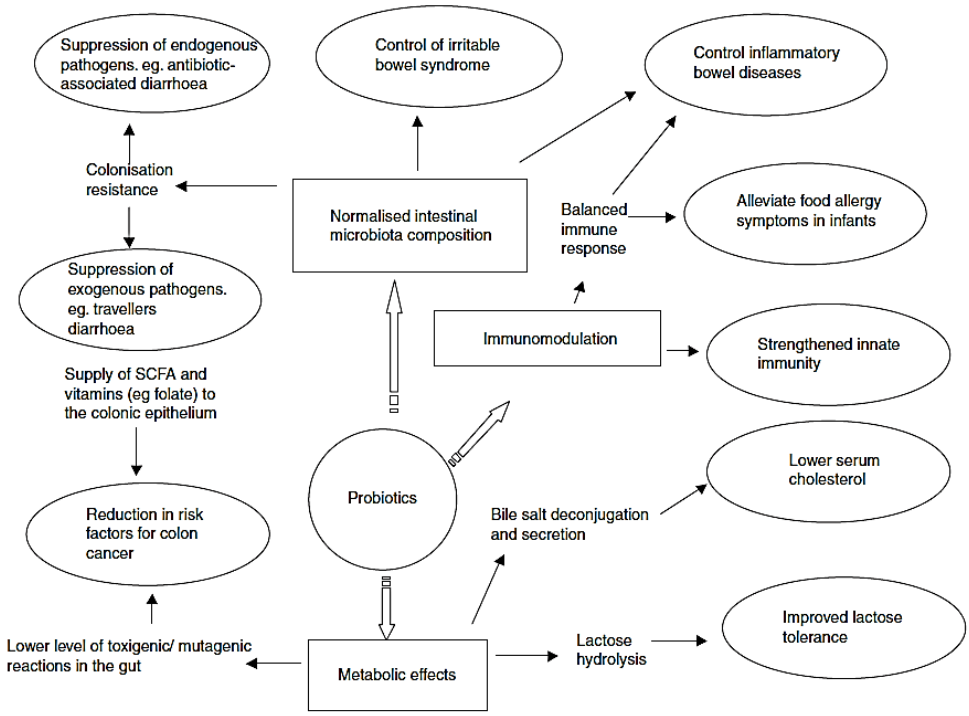


Figure 1. Various health benefits from probiotics consumption. (Parvez *et al.,* 2005)

Despite all these reports describing health benefits, recent evidence coming from metanalysis of several studies suggests that the evidence of health benefits is still missing (Osborn and Sinn, 2009) (Mallon *et al.,* 2008)

Additionally, a recent study indicated that probiotic bacteria are unable to colonize human intestines (Mah *et al.,* 2007). This colonization deficiency may be caused by adaptation to milk causing evolution lactic bacteria evidenced by genome decay (Van de Guchte et al., 2006)

1.5 Economic Importance

Consumer interest in healthy foods and beneficial microorganisms is growing. Food fermentations, derived from milk, meat and plants with probiotic products represent a total global market value of over 100 Billion Euro. Cheese, yogurt and other fresh dairy products constitute the most economically important products (FAO, 2011). According with Global Probiotics Market Magazine in 2010 “The market for probiotic bacteria in foods and supplements is the most rapidly growing segment in the fast moving consumer goods and expected to grow by 10 % each year” With an annual growth rate of 7.1%, probiotic sales will be at US$1.1 billion in 2010. In Japan probiotics are present in more than 50% of their dairy products, especially yoghurts, but also in some soups, fruit and vegetable juices, and cereals. (Market & Market., 2010)

1.6 Technological Importance LAB’s

The need for standardization of products and processes as well as the interest for maintaining food safety has promoted the use of LAB as starter cultures by the industry, especially dairy products. Starter cultures, are carefully selected, purified and propagated in fermentation tanks in order to produce the type of fermentation required (homolactic, heterolactic, etc.) The starter cultures either consist of one

pure strain of bacteria or yeasts or of a combination of strains of different microbial species (Ross *et al.,* 2005).

According to Salminem (1995) among the physiological functions of LAB's are several of great importance in food processing and maturation that influencing the final organoleptic qualities of the products:

- Fermentation of carbohydrates, leading to a pH decrease important in the clotting phenomenon and reduction or prevention of the growth of adventitious micro-flora.
- Protein hydrolysis which causes the texture and, partially, taste of cheese.
- Synthesis of flavor compounds (e.g. acetaldehyde in yoghurt and cheese)
- Synthesis of texturing agents (e.g. extracellular polysaccharides), which may influence the consistency of the product.
- Production of antimicrobial compounds.
- Production of proteolytic enzymes for degradation of complex proteins such as casein.

An overview of the starter bacteria used in dairy fermentations and some of their relevant physiologic properties is given in Table 1.

Table 1: Starter organisms for dairy products. (Heller, 2001)

Species[1]	Growth temperature			Lactic acid fermentation		Lactic acid	Final pH
	Minimum	Optimal	Maximum	Homofermentative	Heterofermatative		
		°C				%	
Lb. delbrueckii subsp. *bulgaricus*	22	45	52	+		1.5–1.8	3.8
Lb. delbrueckii subsp. *lactis*	18	40	50	+		1.5–1.8	3.8
Lb. helveticus	22	42	54	+		1.5–2.2	3.8

Lb. acidophilus	27	37	48	+		0.3–1.9	4.2
Lb. kefir	8	32	43		+	1.2–1.5	—
Lb. brevis	8	30	42		+	1.2–1.5	—
Lb. casei subsp. *casei*		30		+		1.2–1.5	—
S. thermophilus	22	40	52	+		0.6–0.8	4.5
Lc. lactis subsp. *lactis*	8	30	40	+		0.5–0.7	4.6
Lc. lactis subsp. *cremoris*	8	22	37	+		0.5–0.7	4.6
Lc. lactis subsp. *lactis* biovar. *diacetylactis*	8	22–28	40	+		0.5–0.7	4.6
Ln. mesenteroides subsp. *cremoris*	4	20–28	37		+	0.1–0.2	5.6
Ln. mesenteroides subsp. *dextranicum*	4	20–28	37		+	0.1–0.2	5.6
Bifidobacterium (*bifidum, infantis,* etc)	22	37	48			0.1–1.4	4.5

[1]*Lb., Lactobacillus; S., Streptococcus; Lc., Lactococcus; Ln., Leuconostoc.*

1.7 Future trends

An important direction followed by researchers in LAB, probiotics and starter cultures must be in genetics in order to implement new characteristics in technologically interesting strains. For example, besides acidification, EPS or bacteriocins producer strains are desirable in order to manufacture fermented products in which raw materials should not be heat treated, and where the contamination level could be reduced by the action of modified lactic acid bacteria.

Given the important role of microorganisms in the manufacture of dairy products, it is necessary to control the activities of microbial starter cultures. This means ensuring they grow in enough quantity and time, and that they produce the correct amount and type of metabolites, enzymes, flavors, etc. (Hutkins, 2006).

To make these techniques feasible, it is important achieve the following:

- Identify genetics determinants of certain physiological characteristics of LAB's
- Use methods that allow a stable transfer of genes.
- Dispose of vectors donor and recipient strains of the same species in order to obtain a

  Generally Recognized as Safe (GRAS) microorganism
- Solve regulatory problems and constraints related to genetically modified microorganisms. (Salminem, 1995)

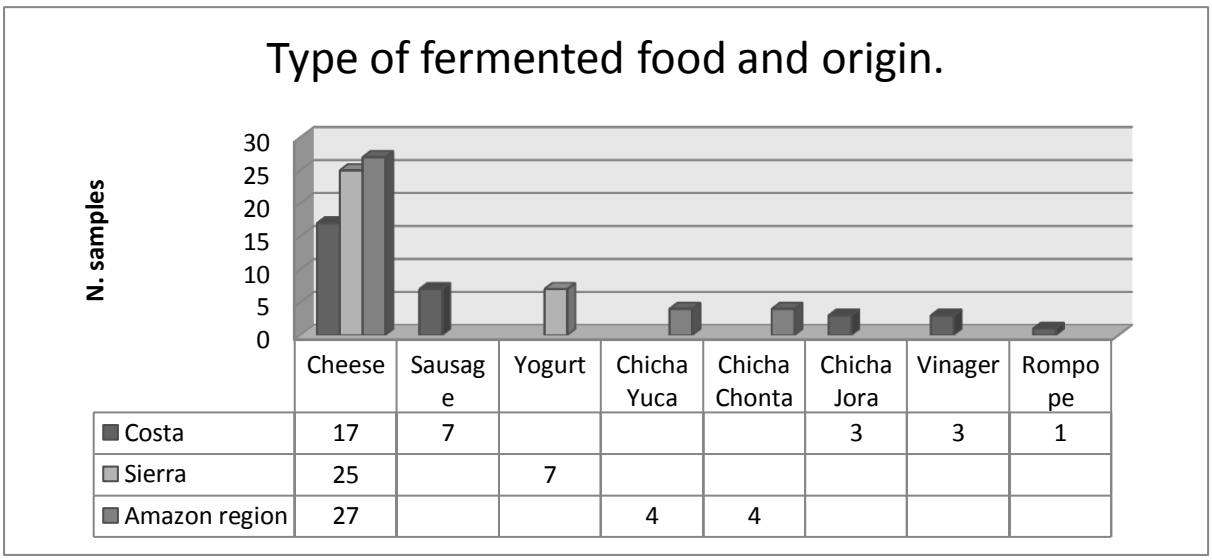
Ecuadorian fermented foods should contain organisms with unique characteristics that can be associated with probiotic and technological properties. Unfortunately at present, there is no adequate information on the spectrum of microorganisms associated with traditional fermented foods from Ecuador. For this reason, the aim of the present study was isolation and identification of a large number of lactic acid bacteria from traditional fermented food from Ecuador and their technological assessment as starter cultures.

2. MATERIALS AND METHODS

2.1 Sample collection

A total of 98 fermented food samples were collected from three geographical regions of 20 provinces of Ecuador, Coast region (31) , Andean region (32) , and Amazon region (35) (see Table 1)

Table 1: *Type of fermented food and origin.*



	Cheese	Sausage	Yogurt	Chicha Yuca	Chicha Chonta	Chicha Jora	Vinager	Rompope
Costa	17	7				3	3	1
Sierra	25		7					
Amazon region	27			4	4			

2.2 Cultivation and isolation of LAB

For microbiological analysis, 20g of each sample was homogenized with 180 ml sterile 2% Trisodium citrate solution ($Na_3C_6H_5O_7$). Serial dilutions (10^{-2} to 10^{-7}) of the homogenates were prepared in sterile 0.85% NaCl and 1ml of each dilution was plated on MRS agar pH 5.7 (Merck, Germany) and M17 agar supplemented with 0.5%, glucose pH 7.2 (Merck, Germany).

Dilutions 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} were incubated at 37^{o}C, and dilutions 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were incubated at 45^{o}C for 3-5 days under aerobic conditions and under anaerobic conditions.

1 ml of 10^{-1} dilution was inoculated in 9ml MRS and M17 supplemented with 0.5% glucose (GM17) broth and sterile skimmed milk, incubated anaerobically at 30^{o}C and 45^{o}C for 24 h and plated on MRS and GM17 agar and incubated anaerobically at 30^{o}C and 45^{o}C for 3 to 5 days.

Twenty to 100 colonies were randomly selected from plates and re-isolated. Gram positive and catalase negative isolates were transferred to MRS or GM17 broth containing 15% of sterile glycerol and stored at –80^{o}C.

2.3 Phenotypic characterization

A total of 119 isolated strains were examined for detecting 5 starter properties

2.3.1 Acidification activity

One millilitre of overnight culture was transferred to 9 ml of sterile milk and incubated at 37°C and 45°C for 18h. Before and after incubation pH was measured in sterile milk using pH strips (Macherey-Nagel-Düren).

2.3.2 Bacterial growth curve

5ml of culture media (MRS or GM17) were inoculated with 1ml of fresh overnight culture and optical density was monitored each hour for 10 hours at 25^{o}C, 37^{o}C and 45^{o}C.

2.3.3 Proteinase activity

Detection of caseinolytic activity was performed using a modified version of the procedure of Hill and Gasson (1986).Bacteria were collected from the Milk Citrate Agar (MCA) plates and resuspended in ammonium acetate buffer (100 mM, pH 6.8) up to obtain a density of about 3^{8} cells per ml. Cell suspension was mixed with the same volume of β-casein solution (5 mg/ml) in ammonium acetate buffer (100 mM, pH 6.8) (Sigma Chemie GmbH, Deisenhofen, Germany). The suspensions of cells and β-casein were incubated for 3 h at 37°C or 45°C and then centrifuged to remove the cells. Degradation of β-casein was analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli *et al*., 1970). Supernatants were taken and mixed with the sample loading buffer (125 mM TrisHCl, pH 6.8, 10 mM EDTA, 4% SDS, 25% glycerol, 5% 2-mercaptoethanol and 0.07% bromophenolblue) in a 1:1 volume ratio. Prior to loading, samples were heated at 100°C for 2 min and analyzed on 15% acrylamide gels (w/v). Gels were stained with Coomassie brilliant blue R250 (Serva, Heidelberg, Germany) and distained in a mix of methanol (20%) and acetic acid (7%).

2.3.4 EPS production

The protocol described by Mayeux *et al*., 1962 was used. Bacterial strains are cultivated in MSE (Mayeux Sandino Ellik) agar with 2% w/v glucose at 37°C for 18h.Production of extracellular polysaccharides EPS was inspected visually, strains positives showed elastic and slimy texture.

2.3.5 Bacteriocin production

For detecting bacteriocin activity, agar-well diffusion assay was used (Tagg and McGiven, 1971). Semisolid GM17 or MRS (0.7% agar w/v), containing lactococci or lactobacilli indicator strains, was overlaid on solid GM17 and MRS (1.5% agar w/v) plates, respectively. Wells were made in the lawn of hardened semisolid agars. Aliquots (50 µl) of supernatant of overnight cultures (16 h) were poured in the wells. The plates were incubated overnight at 30°C. A clear zone of inhibition around the well was taken as a positive signal for antimicrobial activity. *Lactococcus lactis* subsp. *lactis* BGMN1-596 and *Lactobacillus paracasei* subsp. *paracasei* BGBUK2-16/K4 were used as indicator strains.

2.4 Genotypic characterization

2.4.1 DNA extraction, PCR amplification and sequencing

Genomic DNA was released from cells using the heat shock/boiled-cell method (Pandey *et al.,* 2007), 5 isolated colonies from an axenic culture were transferred to 300µl of sterile water and heating at 100°C for 10 min. Suspension was centrifuged at 13,000 rpm for 3 min and stored immediately at - 20°C overnight**.**

Amplification of 16S rRNA gene were performed using universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3')

(Martin-Laurent *et al.*, 2001). PCR mixture consisted of 1X PCR Buffer (Promega), 0.25 mM of each dNTP, 0.2 uM of each primer, 0.6 U GoTaq DNA polymerase (Promega, Madison USA), 5 ul of DNA extracts, and Milli-Q water to a total volume of 25 ul. PCR conditions were as follows: After an initial denaturation step at 94°C for 1 min, 30 amplification cycles were performed (94°C for 30 s, 50°C for 30 s, 72°C for 30 s) and a final extension at 72°C for 10 min. Amplicons were analyzed by electrophoresis in 1.% (w/v) agarose gel. PCR products were directly sequenced in both directions by Functional Biosciences. USA.

2.4.2 Sequencing analysis

The sequences were treated using Pregap and Gap softwares included in the Staden Package (Bonfield and Staden, 1996) and were compared to entries of homologous sequences in the GenBank.

Sequence alignments were done utilizing ClustalW software (Thompson et al., 1994). Neighbour-Joining analysis was performed using MEGA version 4.0 software (Kumar et al., 2004) with the Kimura-2 parameter model and using uniform rates among sites.

3. RESULTS

A total of 119 isolates were obtained from Ecuadorian traditional fermented products (Table 1). Majority of the isolates (68) belonged to genus *Lactobacillus* followed by *Enterococcus (15)*, *Pediococcus* (13), *Lactococcus (10), Leuconostoc (7) and Weisella (6)* held in GenBank accession numbers: JQ446453-JQ446571 (Figure 2-6)

A small percentage (5%) of the strains, (3 *Lactobacillus* and 2 *Lactococcus* strains were identified as high acid producers (ΔpH>2), Qazan (*Lactococcus)* isolated from cheese was the strain with the highest acidifying activity (ΔpH>2.5).

All *Pediococcus* strains, 92 % of *Lactobacillus* spp. strains, 50% of *Weisella* strains, and 80% of *Lactococcus and Enterococcus* sp. were found to produce proteinases. In contrast, none *Leuconostoc* isolates was proteinase positive. Eight percent of isolates produced EPS in MSE agar (4 *Leuconostoc*, 1 *Enterococcus,* and 3 *Lactobacillus).*

Two *Lactobacillus* isolates (QX2) and one *Leuconostoc* (QX5an) isolate produced bacteriocins. The bacterial growth inhibition was not due to low pH, because the supernatants were neutralized to pH 6.5 before testing against the organisms.

On average, isolates incubated at 37°C and 45°C reached the stationary phase at hour 10 and 8 respectively to ≈ 0.7 OD at 600 nm. While in the case of isolates incubated at room temperature, on average at the end of follow-up (hour 10) they were still in exponential growth phase.

In the phylogenetic analysis it was evidenced that there is no co-relation between the geographic distribution of the isolates and the highest percentage of identity, for example in Figure 1 shows a value of 100% identity between corresponding isolates P. acidilactici like strains of this study and reference sequences taken from GenBank from France and Argentina.

4. DISCUSION

The present study describes some of the biodiversity of the microbiota associated to Ecuadorian traditional acidic fermented foods analyzed is represented by *Lactobacillus* 57%, *Enterococcus* 13%, *Pediococcus* 11%, *Lactococcus* 8%, *Leuconostoc* 6%, and *Weissella* 5%.

The most predominant LAB's strains from fermented foods in this study were facultative heterofermentative strains, 35 isolates, belonged to *Lactobacillus (*99% identity to *L. plantarum)* and 13 *Lactobacillus (*99% *homology to L. fermentum*). These results are in agreement with previous reports indicating that *L. plantarum* is dominant specie in vegetal and milk fermentation (Ngaba and Lee, 1979; Ben Omar *et al*., 2000; Amoa-Awua *et al*., 1996). (Watabe *et al.* 1998) The taxonomic status of some of this isolates was confirmed by phylogenetic analysis (Figure 2.)

L. plantarum strains occur in late phases of fermentation (Ben Omar *et al*., 2000) and terminate many of the spontaneous lactic fermentations such as silage and vegetable fermentations (Daeschel *et al*., 1987). In 2006, Kostinek *et al,* reported a presence of 32 LAB strains from fermented cassava with one or more desirable biochemical properties for selection as starter cultures. Of these 60% were identified as *L. plantarum*. Several other studies report *L. plantarum* as the most frequently specie found in different foods and silages, which showed that it play an important role in natural fermentation processes, which is confirmed in our study. (Sawitzki *et al*., 2008) (Rantsiou, *et al*., 2008) (Tzanetakis *et al*., 1992) (Cai et al 1999)

Other predominant strains founded were *Pediococcus*, *Lactococcus* and *Weissella* which belonged to homofermentative cocci group. *Pediococcus* was mainly isolated from cheese; (Table. 3) however this bacterium is not common in dairy products (Beukes *et al*. 2001). In our study *Pediococcus* was mainly isolated form sausage. *Pediococcus* has been used since 1950 as starter culture for the production of sausages, the ability of pediococci of to grow and metabolize under conditions of a_w reduced and moderate salt concentration favors the growth of pediococci in this kind of foods. (Everson, *et al* 1970) In this study Leuconostoc was isolated mainly from chicha, in 2001 Beukes et al reported Leuconostoc as the major bacterial population in Kimchi and sauerkraut, which are obtained from a spontaneous fermentation from plant material similarly to chicha.

While members of the genus *Weissella* were isolated from cheese (Table S1, Table 3) and these bacteria have been found in fresh vegetables, sugar cane, meat samples, and also from clinical samples from animals and humans (Björkroth *et al*. 2002). *Lactococcus* was isolated from cheese and sausage and it has been reported in plants, vegetables, and cereals products (De Vuyst, L *et al*., 1994) (Salama *et al*., 1995)

Enterococcus species were isolated from 6 products suggesting fecal contamination. (Franz *et al*. 1999)

Acidification to pH levels lower than 4.2 constitutes a major food safety factor (Holzapfel, 1997). In this study three Lactobacillus strains *Lb. curvatus* (1), *Lb.*

rhamnosus (2), and two *Lactococcus lactis* strains were identified as high acid producers (ΔpH>2), a *Lactococcus lactis* strain isolated form cheese was the isolate with the highest acidifying activity (ΔpH>2.5 in18h incubation).A rapid decrease in pH during the initial steps of fermentation ensures a prevention o reduction of the growth of adventitious microflora. The most acidifying strains are good candidate for dairy fermentation as primary atarter organisms, whereas the poor acidifiers can be used as secondary cultures.

Bacteriocin production was detected in two isolates *Lactobacillus plantarum* (QX2) and *Leuconostoc mesenteroides* (QX5an). Various bacteriocins produced by *L. plantarum* have been described, i.e. plantaricin A (Daeschel et al, 1990), plantaricin B, (West y Warner 1998) Several plantaricins have been reported to inhibit *Listeria* spp. and other gram positive bacteria (Atrih et al. 1993 ; Garriga et al. 1993)

Nine isolates produced EPS in MSE agar, 4 *Ln mesenteroides*, 2 *L. fermentum,* 2 *L. plantarum,* and 1 *Enterococcus lactis. Leuconostoc mesenteroides* has been previously reported to increase viscosity in pulque (Sánchez, 1967; Ulloa, 1976). Numerous studies report the formation of dextran from different strains of bacteria that were primarily *Leuconostoc* strains. Dextran in food industry is being used as thickener for yogurt and ice cream. (Kim and Robyt, 1995)

Proteolytic activity of dairy lactic acid bacteria is essential for the bacterial growth in milk and it is also responsible for the development of organoleptic properties of different fermented milk products (Axelsson, 1998; Christensen *et al*., 1999). In

this study 83% of strains were found to produce proteinases (100% of *Pediococcus* and *Lactobacillus* strains 50% of *Weissella* strains, and 80% of *Lactococcus* and *Enterococcus* sp.) indicating that most of these isolates can be used in fermented dairy foods. Proteinase activity is a characteristic widely found between lactic acid bacteria, particularly those isolated from milk products, (Bockelmann, 1995).

Nucleotide sequences from Ecuadorian isolates did not show any tendency to group with Andean or Latin-American isolates. This suggests that the origin of these bacteria may be broad, however this may not rule out local diversity due to adquisition of laterally transferred genes.

Our results demonstrate the diversity of LAB in dairy and non-dairy fermented foods in Ecuador and provide data and strain resource for further study involved in probiotic strain selection and starter culture design.

5. ACKNOWLEDGMENTS

We thank Rosita Bayas for her support in sample collection, and Elizabeth Bolaños for their technical assistance. This work was supported by CSK Food Netherlands.

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SUPPLEMENTARY INFORMATION

Table 3: Bacterial species and products from which were isolated

Identified species	N. isolated
Vinegar	
Lb. fermentum	1
Lb. plantarum	2
Yogurt	
Lb. plantarum	3
Lb. casei	1
Ln. mesenteroides	1
Chicha	
Lb. plantarum	10
Lb. fermentum	5
Lb. paracasei	1
E. lactis	1
Sausage	
Lb. sakei	1
Lc. garviae	2
P. pentosaceus	4
Lc. lactis	2
Cheese	
Lb. plantarum	21
Lb. rhamnosus	7
Lb. fermentum	7
Ln. mesenteroides	6
Lc. lactis	6

W. paramesenteroides	6
E. faecalis	5
P. pentosaceus	5
Lb. casei	4
E. italicus	3
P. acidilactici	3
E. durans	3
E. faecium	2
Lb. paraplantarum	2
P. stilesii	1
Lb. zeae	1
E. casseliflavus	1
Lb. pentosaceus	1
Lb. curvatus	1

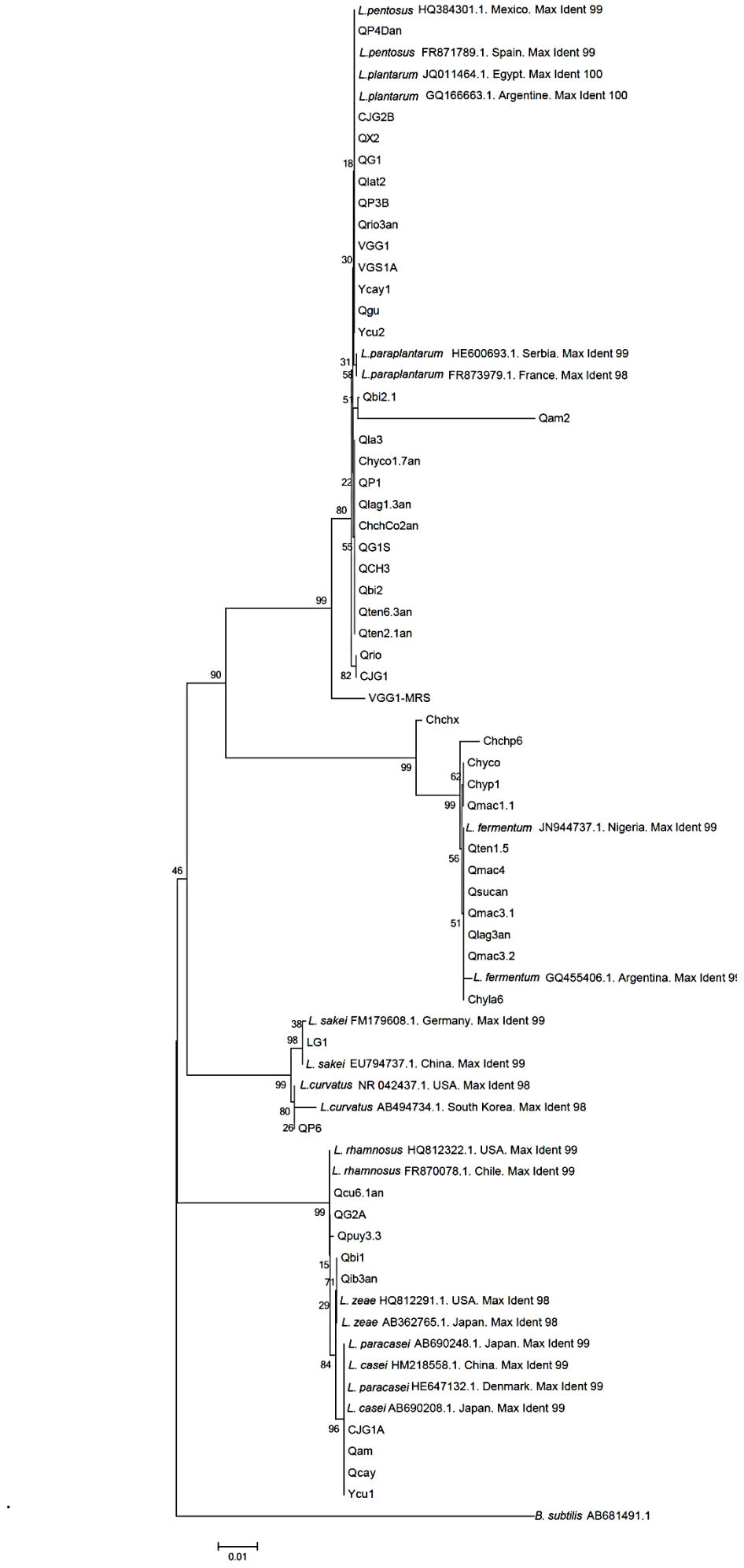
L.pentosus HQ384301.1. Mexico. Max Ident 99
QP4Dan
L.pentosus FR871789.1. Spain. Max Ident 99
L.plantarum JQ011464.1. Egypt. Max Ident 100
L.plantarum GQ166663.1. Argentine. Max Ident 100
CJG2B
QX2
18
QG1
Qlat2
QP3B
Qrio3an
VGG1
30
VGS1A
Ycay1
Qgu
Ycu2
L.paraplantarum HE600693.1. Serbia. Max Ident 99
31
58
L.paraplantarum FR873979.1. France. Max Ident 98
51
Qbi2.1
Qam2
Qla3
Chyco1.7an
22
QP1
Qlag1.3an
80
ChchCo2an
55
QG1S
QCH3
Qbi2
99
Qten6.3an
Qten2.1an
Qrio
90
82
CJG1
VGG1-MRS
Chchx
Chchp6
99
Chyco
62
Chyp1
99
Qmac1.1
L. fermentum JN944737.1. Nigeria. Max Ident 99
Qten1.5
56
Qmac4
Qsucan
Qmac3.1
51
Qlag3an
Qmac3.2
L. fermentum GQ455406.1. Argentina. Max Ident 9
Chyla6
46
38
L. sakei FM179608.1. Germany. Max Ident 99
98
LG1
L. sakei EU794737.1. China. Max Ident 99
99
L.curvatus NR 042437.1. USA. Max Ident 98
80
L.curvatus AB494734.1. South Korea. Max Ident 98
26
QP6
L. rhamnosus HQ812322.1. USA. Max Ident 99
L. rhamnosus FR870078.1. Chile. Max Ident 99
Qcu6.1an
99
QG2A
Qpuy3.3
15
Qbi1
71
Qib3an
29
L. zeae HQ812291.1. USA. Max Ident 98
L. zeae AB362765.1. Japan. Max Ident 98
L. paracasei AB690248.1. Japan. Max Ident 99
84
L. casei HM218558.1. China. Max Ident 99
L. paracasei HE647132.1. Denmark. Max Ident 99
L. casei AB690208.1. Japan. Max Ident 99
96
CJG1A
Qam
Qcay
Ycu1
B. subtilis AB681491.1
0.01

Fig. 2. Phylogenetic tree of 16S rDNA sequences *Lactobacillus* strains using Neighbor-joining method. QP4Dan, CJG2B, QG1, QX2, Qlat2, QP3B, VGG1, QRio3an, VGS1A, Ycay1, QGu, Ycu2, Qbi2.1, Qla3, Chyco1.7an, QP1, Qlag1.3an, ChchCo2an, QG1S, QCh3, QBi2, Qten6.3an, QTen2.1an, QRio, CJG1, VGG1, Chchx, Chchp6, Chyco, Chyp1, Qmac1.1, Qten1.5, Qmac4, QSuc an, Qmac3.1, Qlag3an, Qmac3.2, Chyla6, LG1, QP6, Qcu6.1an, QG2A, Qpuy3.3, Qbi1, Qib3an, CJG1A, QAm, Qcay, YCu1, are isolates described in this paper, other sequences were obtained from GenBank. The corresponding bootstrap values (100 replications) are shown on each branch.

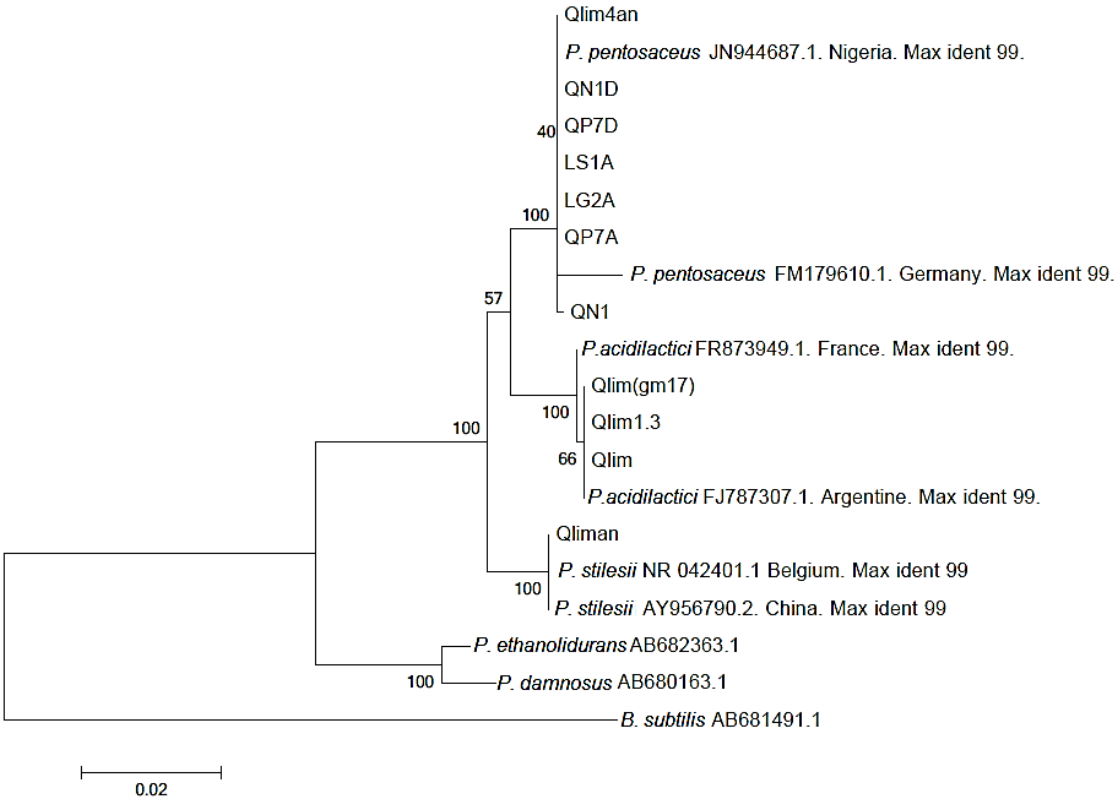

Fig. 3. Phylogenetic tree of 16S rDNA sequences *Pediococcus* strains using Neighbor-joining method. QN1D, Qlim4an, QP7D, LS1A, LG2A, QP7A, Qlim (gm17), Qlim 1.3, Qlim, Qliman are isolates described in this paper, other sequences were obtained from GenBank. The corresponding bootstrap values (100 replications) are shown on each branch.

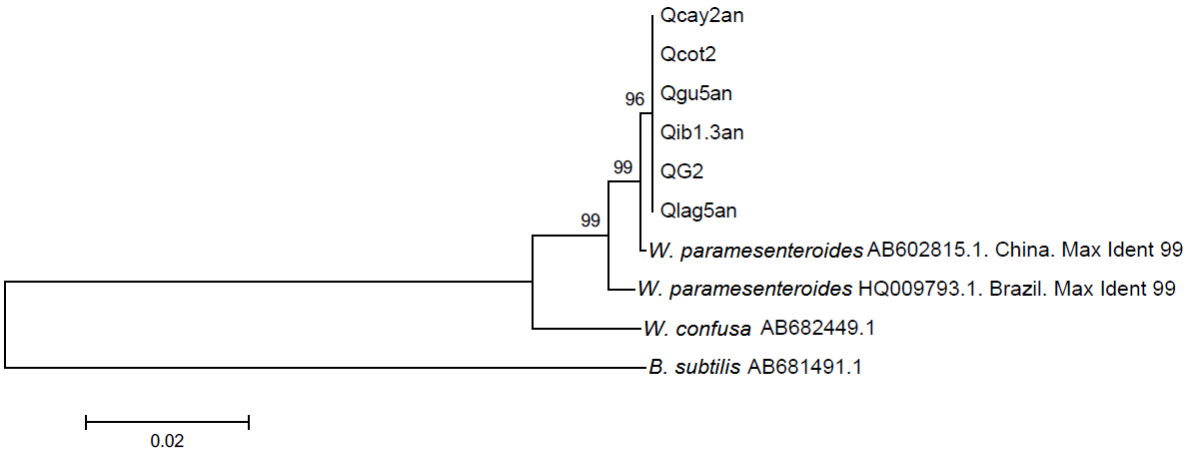

Fig 4. Phylogenetic tree of 16S rDNA sequences *Weissella* strains using Neighbor-joining method. Qcay2an, Qcot2, Qgu5an, Qin1.3an, QG2, Qlag5an are isolates described in this paper, other sequences were obtained from GenBank. The corresponding bootstrap values (100 replications) are shown on each branch.

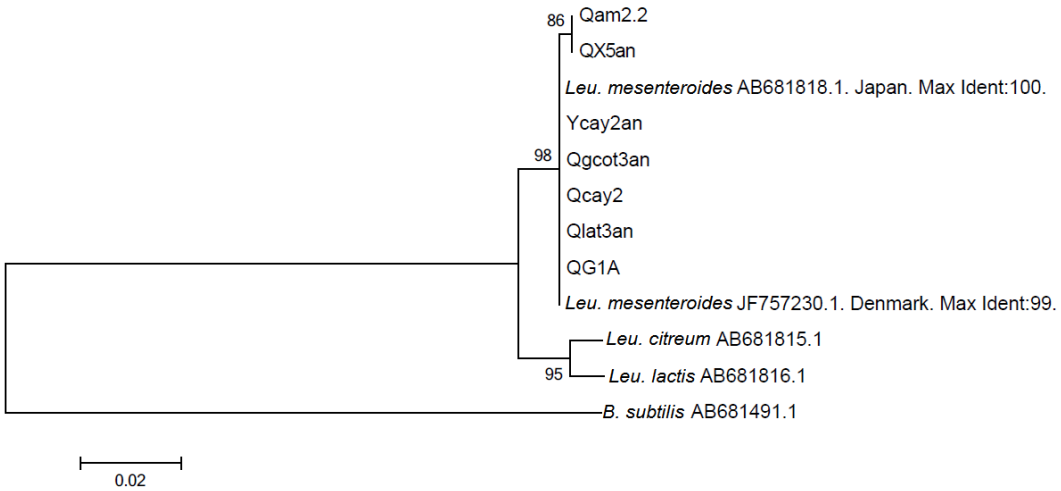

Fig 5. Phylogenetic tree of 16S rDNA sequences *Leuconostoc* strains using Neighbor-joining method. Qam2,2, QX5an, Ycay2an, Qgcot3an, Qcay2, Qlat3an, QG1A, are isolates described in this paper, other sequences were obtained from GenBank. The corresponding bootstrap values (100 replications) are shown on each branch.

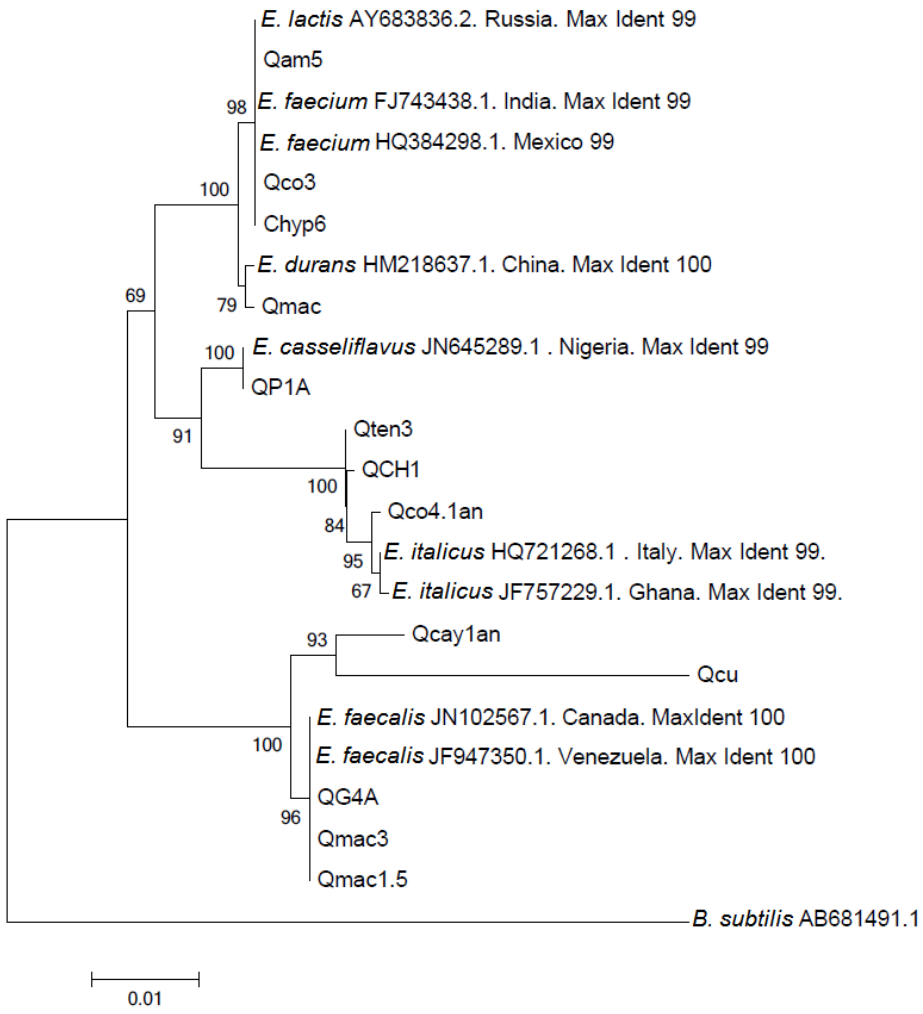

Fig 6. Phylogenetic tree of 16S rDNA sequences *Enterococcus* strains using Neighbor-joining method. Qam5, Qco3, Chyp6, Qmac, QP1A, Qten3, QCh1, QCo4.1an, QG4A, Qmac3, Qmac1.5 are isolates described in this paper, other sequences were obtained from GenBank. The corresponding bootstrap values (100 replications) are shown on each branch.

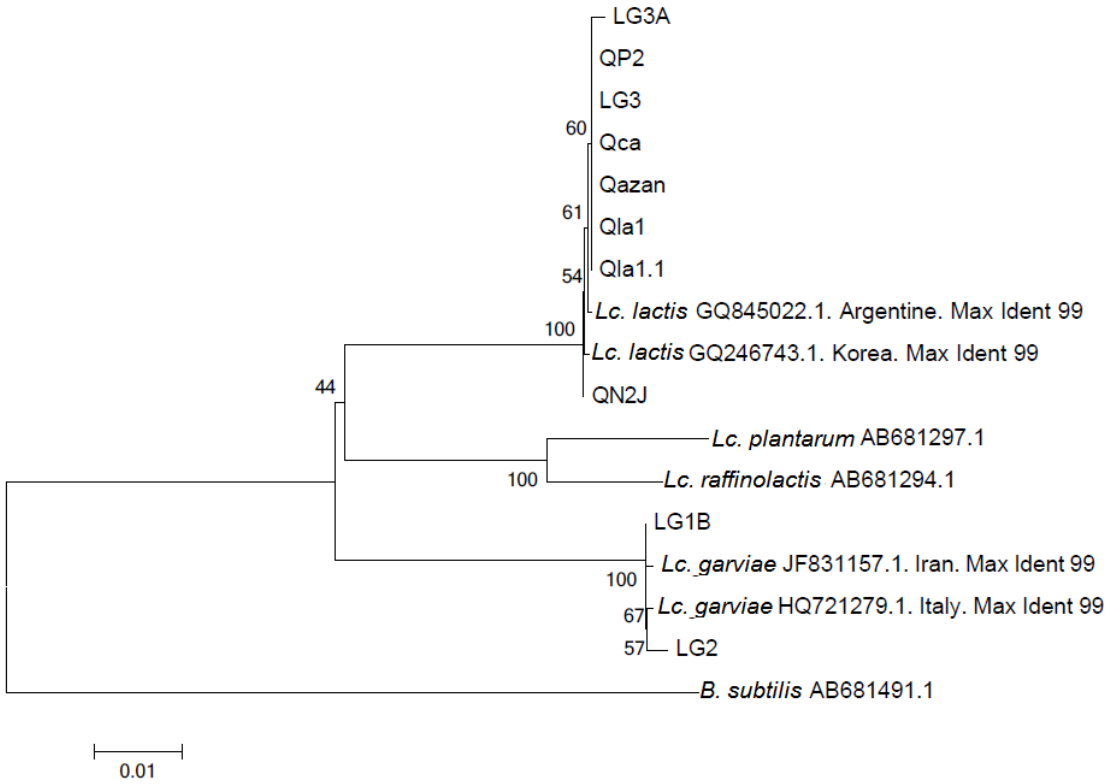

Fig 7. Phylogenetic tree of 16S rDNA sequences *Lactococcus*, strains using Neighbor-joining method. LG3A, QP2, LG3, QCa, Qazan, Qla1, Qla1.1, QN2J, LG1B, LG1, are isolates described in this paper, other sequences were obtained from GenBank. The corresponding bootstrap values (100 replications) are shown on each branch.

SIERRA													
ID	Origin	Source	Acidifying activity pH		EPS	Bacteriocin production		Proteinase	Gram	Cell	Catalase	Identified species	GenGank Accesion Numbers
			37°C	45°C	production	*L. Lactis*	*L. Paracasei*	activity	Strain	Shape			
QAm	Ambato	Cheese	5.5	5.4	-	-	-	+	+	Rods	-	*Lb. casei*	JQ446490
Qcay	Cayambe	Cheese	5.9	6	-	-	-	+	+	Rods	-	*Lb. casei*	JQ446494
QRio	Riobamba	Cheese	5	5	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446489
YCu2.2	Cuenca	Yogurt	5.5	5.5	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446492
QBi2.1	Biblián	Cheese	5.9	5.6	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446497
QCu3	Cuenca	Cheese	6	5.7	-	-	-	+	+	Rods	-	*Lb. paraplantarum*	JQ446510
YCu1	Cuenca	Yogurt	6.2	6	-	-	-	+	+	Rods	-	*Lb. casei*	JQ446507
QAm2.2	Ambato	Cheese	6	5.6	+	-	-	-	+	Ovoid cocci	-	*Ln. mesenteroides*	JQ446504

QBi2	Biblián	Cheese	5.5	5.1	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446495
QCot2	Cotacachi	Cheese	6.2	5.5	-	-	-	-	+	Rods	-	*W. paramesenteroides*	JQ446511
QLat2	Latacunga	Cheese	5.7	5.6	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446502
QBi1	Biblián	Cheese	5.7	5.5	-	-	-	-	+	Rods	-	*Lb. zeae*	JQ446505
Qcu	Cuenca	Cheese	5.8	5.6	-	-	-	+	+	Cocci	-	*E. faecalis*	JQ446509
QCay2	Cayambe	Cheese	5.8	5.5	-	-	-	-	+	Ovoid cocci	-	*Ln. mesenteroides*	JQ446515
YCay1	Cayambe	Yogurt	5.8	5.3	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446491
QX2	Cuenca	Cheese	6	5.3	-	+	+	+	+	Rods	-	*Lb. plantarum*	JQ446498
QCa	Cañar	Cheese	5.5	4.9	-	-	-	+	+	Cocci	-	*Lc. lactis*	JQ446499
QAm2	Ambato	Cheese	5.9	5.2	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446500
YCu2	Cuenca	Yogurt	6.2	6	-	-	-	-	+	Rods	-	*Lb. plantarum*	

													JQ446506
QGu5 an	Guaranda	Cheese	5.9	4.8	-	-	-	-	+	Rods	-	*W. paramesenteroides*	JQ446512
QCay1 an	Cayambe	Cheese	6.3	6.1	-	-	-	+	+	Cocci	-	*E. faecalis*	JQ446508
QRio2 an	Riobamba	Cheese	6	6	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446501
QCu6.1 an	Cuenca	Cheese	5.6	4.5	-	-	-	+	+	Rods	-	*Lb. rhamnosus*	JQ446520
QX5 an	Cuenca	Cheese	6.3	6.2	-	+	+	-	+	Ovoid cocci	-	*Ln. mesenteroides*	JQ446513
QIb3 an	Ibarra	Cheese	5	4.9	-	-	-	-	+	Rods	-	*Lb. zeae*	JQ446493
QGCot3 an	Guaytacama	Cheese	6	5.4	-	-	-	-	+	Ovoid cocci	-	*Ln. mesenteroides*	JQ446518
QLat3 an	Latacunga	Cheese	6.4	5	+	-	-	-	+	Ovoid cocci	-	*Ln. mesenteroides*	JQ446514
QCay2 an	Cayambe	Cheese	4.9	4.5	-	-	-	+	+	Rods	-	*W. paramesenteroides*	JQ446496
YCay2 an	Cayambe	Yogurt	6.2	6.1	+	-	-	-	+	Ovoid cocci	-	*Ln. mesenteroides*	JQ446519

QRio3 an	Riobamba	Cheese	6	5.5	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446516
QIb1.3 an	Ibarra	Cheese	6.2	6.1	-	-	-	+	+	Rods	-	*W. paramesenteroides*	JQ446522
Qaz an	Azogues	Cheese	4	4	-	-	-	+	+	Cocci	-	*Lc. lactis*	JQ446517
QAm 5	Ambato	Cheese	6.2	5.6	-	-	-	+	+	Cocci	-	*E. faecium*	JQ446521
QGu	Guaranda	Cheese	6.1	5.7	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446503

ORIENTE

ID	**Origin**	**Source**	**Acidifying activity pH**		**EPS**	**Bacteriocin production**		**Proteinase**	**Gram**	**Cell**	**Catalase**	**Identified species**	**GenGank Accesion Numbers**
			37°C	**45°C**	**production**	**L. Lactis**	**L. Paracasei**	**activity**	**Strain**	**Shape**			
ChchCo2an	El Coca	Chicha Chonta	6	5.9	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446523
QTen9 an	Tena	Cheese	6	6	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446524
Chchp2.1an	El Puyo	Chicha Chonta	6.1	4.9	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446525

QTen2an	Tena	Cheese	6	5.4	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446527
QSuc an	Sucúa	Cheese	6.3	5.7	-	-	-	+	+	Rods	-	*Lb. fermentum*	JQ446528
QLag1.3an	Lago Agrio	Cheese	6	5.6	-	-	-	-	+	Rods	-	*Lb. plantarum*	JQ446529
QLag3an	Lago Agrio	Cheese	6	5.7	-	-	-	+	+	Rods	-	*Lb. fermentum*	JQ446530
Qmac	Macas	Cheese	5.9	5.9	-	-	-	-	+	Cocci	-	*E. durans*	JQ446531
Qla1	Lago Agrio	Cheese	4.5	4.5	-	-	-	+	+	Cocci	-	*Lc. lactis*	JQ446532
Qmac3	Macas	Cheese	6	6	-	-	-	+	+	Cocci	-	*E. faecalis*	JQ446533
Qlag3.2an	Lago Agrio	Cheese	5.7	5.4	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446534
Chchm7.1an	Macas	Chicha Chonta	6	6	-	-	-	+	+	Rods	-	*Lb. fermentum*	JQ446535
QTen2.1 an	Tena	Cheese	6	5.9	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446536
QTen6.3an	Tena	Cheese	5.8	5.8	-	-	-	+	+	Rods	-	*Lb. plantarum*	

													JQ446537
Chyco1.7an	El Coca	Chicha Yuca	5.7	5.7	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446538
Qlim4 an	Limón	Cheese	6.3	6.3	-	-	-	+	+	Cocci	-	*P. pentosaceus*	JQ446539
Chyco1.5an	El Coca	Chicha Yuca	5.6	5.6	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446540
Qla1.1	Lago Agrio	Cheese	5	4.8	-	-	-	+	+	Cocci	-	*Lc. lactis*	JQ446541
QCo4.1an	El Coca	Cheese	5.5	5	-	-	-	+	+	Cocci	-	*E. italicus*	JQ446542
QTen3	Tena	Cheese	5.9	5.8	-	-	-	+	+	Cocci	-	*E. italicus*	JQ446543
Qlag5 an	Lago Agrio	Cheese	5.8	5.6	-	-	-	+	+	Rods	-	*W. paramesenteroides*	JQ446544
QCo3	El Coca	Cheese	6	6	-	-	-	+	+	Cocci	-	*E. faecium*	JQ446545
QCo2.1an	El Coca	Cheese	6.4	5.8	-	-	-	+	+	Rods	-	*Lb. paraplantarum*	JQ446546
QTen3an	Tena	Cheese	6.2	5.7	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446547

Chyco1.3an	El Coca	Chicha Yuca	6.1	5.6	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446548
Qmac1.5	Macas	Cheese	6	5.7	+/-	-	-	+	+	Cocci	-	*E. faecalis*	JQ446549
QTen1.5	Tena	Cheese	6	5.6	-	-	-	+	+	Rods	-	*Lb. fermentum*	JQ446550
Qmac3.2	Macas	Cheese	5.9	5.5	-	-	-	+	+	Rods	-	*Lb. fermentum*	JQ446551
Chyla6.6	Lago Agrio	Chicha Yuca	5.5	5.3	-	-	-	+	+	Rods	-	*Lb.plantarum*	JQ446552
Qla3	Lago Agrio	Cheese	6.1	6	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446553
QCo1.3	El Coca	Cheese	5	4.8	-	-	-	+	+	Rods	-	*Lb. rhamnosus*	JQ446554
Qlim1.3	Limón	Cheese	6	5.8	-	-	-	+	+	Cocci	-	*P. acidilactici*	JQ446556
Qmac4	Macas	Cheese	5.8	5.5	-	-	-	+	+	Rods	-	*Lb. fermentum*	JQ446557
Chyla2	Lago Agrio	Chicha Yuca	6.2	6	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446558
Chchp3	Puyo	Chicha Chonta	6.3	5.6	-	-	-	+	+	Rods	-	*Lb. plantarum*	

													JQ446559
Qpuy3.3	Puyo	Cheese	5	4.7	-	-	-	+	+	Rods	-	*Lb. rhamnosus*	JQ446560
Chyco	El Coca	Chicha Yuca	6	6	-	-	-	+	+	Rods	-	*Lb. fermentum*	JQ446561
Chyp6	El Puyo	Chicha Yuca	6	6	+	-	-	-	+	Cocci	-	*E. lactis*	JQ446562
QMac2.5	Macas	Cheese	5.3	5	-	-	-	+	+	Rods	-	*Lb. casei*	JQ446563
Qmac1.1	Macas	Cheese	5.8	5.7	-	-	-	+	+	Rods	-	*Lb. fermentum*	JQ446564
Chyp1	El Puyo	Chicha Yuca	6.3	6.3	+/-	-	-	+	+	Rods	-	*Lb. fermentum*	JQ446565
Qmac3.1	Macas	Cheese	6	5.9	-	-	-	+	+	Rods	-	*Lb. fermentum*	JQ446566
Chchx	El Coca	Chicha Chonta	5.7	5.7	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446567
Chyla6	Lago Agrio	Chicha Yuca	6	6	-	-	-	+	+	Rods	-	*Lb. fermentum*	JQ446571
Chchp6	El Puyo	Chicha Chonta	6	5.7	-	-	-	+	+	Rods	-	*Lb. fermentum*	JQ446568

QCo1	El Coca	Cheese	4.5	4.1	-	-	-	+	+	Rods	-	*Lb. rhamnosus*	JQ446569
Qlim	Limón	Cheese	6	5.7	-	-	-	+	+	Cocci	-	*P. acidilactici*	JQ446570
Qlim	Limón	Cheese	5.6	5.5	-	-	-	+	+	Cocci	-	*P. acidilactici*	JQ446555
Qlim an	Limón	Cheese	6	6	-	-	-	+	+	Cocci	-	*P. stilesii*	JQ446526

COSTA

ID	**Origin**	**Source**	**Acidifying activity pH**		**EPS**	**Bacteriocin production**		**Proteinase**	**Gram**	**Cell**	**Catalase**	**Identified species**	**GenGank Accesion Numbers**
			37°C	**45°C**	**production**	**L. Lactis**	**L. Paracasei**	**activity**	**Strain**	**Shape**			
QP3B	Portoviejo	Cheese	5.8	5.5	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446453
QP1	Portoviejo	Cheese	5	5.4	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446454
QG2A	Guayaquil	Cheese	5	4.8	-	-	-	+	+	Rods	-	*Lb. rhamnosus*	JQ446455
CJG1A	Guayaquil	Chicha de Jora	6.2	6.1	-	-	-	+	+	Rods	-	*Lb. paracasei*	JQ446456
QP4D an	Portoviejo	Cheese	5.5	5.4	-	-	-		+	Rods	-	*Lb. pentosus*	JQ446457
LG3	Guayaquil	Sausage	5.6	5.3	-	-	-	+	+	Cocci	-	*Lc. lactis*	JQ446458
QCH3	Chone	Cheese	5.5	5.2	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446459

LG2	Guayaquil	Sausage	5.5	5.5	-	-	-		+	Cocci	-	*Lc. garviae*	JQ446460
VGG1	Guayaquil	Banano Vinegar	6	6	++			+	+	Rods	-	*Lb. fermentum*	JQ446461
QG5	Guayaquil	Cheese	5.2	4.8	-	-	-	+	+	Rods	-	*Lb. rhamnosus*	JQ446462
QP6	Portoviejo	Cheese	4.4	4.2	-	-	-	+	+	Rods	-	*Lb. curvatus*	JQ446463
VGS1A	Sto Domingo	Banano Vinegar	6.2	6	++	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446464
CJG2B	Guayaquil	Chicha de Jora	6.2	6	++	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446465
CJG1	Guayaquil	Chicha de Jora	6	5.8	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446466
QG2	Guayaquil	Cheese	6.2	6	-	-	-		+	Rods	-	*W. paramesenteroides*	JQ446467
QN1	Nobol	Cheese	6	5.6	-	-	-	+	+	Rods	-	*P. pentosaceus*	JQ446468
QG1S	Guayaquil	Cheese	5.9	5.7	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446469
LS1B	Sto Domingo	Sausage	5.5	5.5	-	-	-	+	+	Cocci	-	*P. pentosaceus*	JQ446470
QP7A	Portoviejo	Cheese	5.5	5	-	-	-	+	+	Cocci	-	*P. pentosaceus*	JQ446471
QCh1	Chone	Cheese	4.9	4.8	-	-	-		+	Cocci	-	*E. italicus*	JQ446472
QG1	Guayaquil	Cheese	5.6	5.5	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446473
LG3A	Guayaquil	Sausage	5.8	5.6	-	-	-	+	+	Cocci	-	*Lc. lactis*	JQ446474
QN2J	Nobol	Cheese	5	4.9	-	-	-	+	+	Cocci	-	*Lc. lactis*	JQ446475

LG2A	Guayaquil	Sausage	5.7	5.5	-	-	-	+	+	Cocci	-	*P. pentosaceus*	JQ446476
QP1A	Portoviejo	Cheese	5.5	5.3	-	-	-		+	Cocci	-	*E. casseliflavus*	JQ446477
QCH2A	Chone	Cheese	4.5	4.5	-	-	-	+	+	Rods	-	*Lb. rhamnosus*	JQ446478
LS1A	Sto Domingo	Sausage	6	5.8	-	-	-	+	+	Cocci	-	*P. pentosaceus*	JQ446479
QP1	Portoviejo	Cheese	6	5.5	-	-	-	+	+	Rods	-	*Lb. casei*	JQ446480
LG1	Guayaquil	Sausage	6.4	6.2	-	-	-	+	+	Rods	-	*Lb. sakei*	JQ446481
QG1A	Guayaquil	Cheese	6	5.4	+	-	-	-	+	Ovoid cocci	-	*Ln. mesenteroides*	JQ446482
QP7D	Portoviejo	Cheese	5.9	5.7	-	-	-	+	+	Cocci	-	*P. pentosaceus*	JQ446483
VGG1	Guayaquil	Banano Vinegar	6.4	6.4	-	-	-	+	+	Rods	-	*Lb. plantarum*	JQ446484
QN1D	Nobol	Cheese	6	6	-	-	-	+	+	Cocci	-	*P. pentosaceus*	JQ446485
QP2	Portoviejo	Cheese	5.4	5.4	-	-	-	+	+	Cocci	-	*Lc. lactis*	JQ446486
LG1B	Guayaquil	Sausage	5.5	5.5	-	-	-	+	+	Cocci	-	*Lc. garviae*	JQ446487

QG4A	Guayaquil	Cheese	5.9	5.8	-	-	-	+	+	Cocci	-	*E. faecalis*		JQ446488

Lb: Lactobacillus. Ln: Leuconostoc. Lc: Lactococcus. E: Enterococcus. W: Weissella. P: Pediococcus