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ORIGINAL PAPER

Predominance of *Lactobacillus plantarum* Strains in Peruvian Amazonian Fruits

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Abstract

The objective of this research was the identification and characterization of lactic acid bacteria (LAB) isolated from Peruvian Amazonian fruits. Thirty-seven isolates were obtained from diverse Amazonian fruits. Molecular characterization of the isolates was performed by ARDRA, 16S-23S ITS RFLP and rep-PCR using GTG_5 primers. Identification was carried out by sequencing the 16S rDNA gene. Phenotypic characterization included nutritional, physiological and antimicrobial resistance tests. Molecular characterization by Amplified Ribosomal DNA Restriction Analysis (ARDRA) and 16S-23S ITS RFLP resulted in four restriction profiles while GTG_5 analysis showed 14 banding patterns. Based on the 16S rDNA gene sequence, the isolates were identified as *Lactobacillus plantarum* (75.7%), *Weissella cibaria* (13.5%), *Lactobacillus brevis* (8.1%), and *Weissella confusa* (2.7%). Phenotypic characterization showed that most of the isolates were homofermentative bacilli, able to ferment glucose, maltose, cellobiose, and fructose and grow in a broad range of temperatures and pH. The isolates were highly susceptible to ampicillin, amoxicillin, clindamycin, chloramphenicol, erythromicyn, penicillin, and tetracycline and showed great resistance to kanamycin, gentamycin, streptomycin, sulfamethoxazole/trimethoprim, and vancomycin. No proteolytic or amylolytic activity was detected. *L. plantarum* strains produce lactic acid in higher concentrations and *Weissella* strains produce exopolymers only from sucrose. Molecular methods allowed to accurately identify the LAB isolates from the Peruvian Amazonian fruits, while phenotypic methods provided information about their metabolism, physiology and other characteristics that may be useful in future biotechnological processes. Further research will focus especially on the study of *L. plantarum* strains.

Key words: Peruvian Amazonian fruits, *Lactobacillus*, *Weissella*, ARDRA, 16S-23S ITS RFLP, GTG_5

Introduction

Lactic acid bacteria (LAB) are Gram-positive, non-sporulating, microaerophilic bacteria that produce mainly lactic acid as a product of carbohydrate fermentation product. LAB are among the most widespread group of microorganisms isolated from various sources in nature, most of which related to the presence of sugar (Liu et al. 2014). LAB isolated from the natural environments may possess special characteristics including phenotypic differences and high intraspecific variability compared with culture collection strains (Fortina et al. 1998).

Previous research has reported the isolation and identification of LAB from different fruits such as ripe mulberries, pineapples, wine grapes, cherries, apples, peaches, prickly pears, bananas and others (Bae et al. 2006; Trias et al. 2008; Chen et al. 2010; Di Cagno et al. 2010; Verón et al. 2017; Abubakr and Al-Adiwish 2017). The most commonly isolated LAB species in these studies were *W. cibaria*, *L. plantarum*, *Leuconostoc mesenteroides*, *Enterobacter* sp. and *Lactococcus* sp. The Peruvian Amazon is a source of a great diversity of fruits which in some cases are consumed by the population as fresh fruits or constitute raw materials for the preparation of different products (juices, ice cream, jams or desserts). They are offered in local markets and provide a great contribution to the regional economy. Peruvian Amazonian fruits grow in conditions of temperature, humidity, and rainfall that differ from those found in the rest of the country. These environmental conditions, in addition to other extrinsic and intrinsic factors, influence fruits microbiology making them an interesting source of microorganisms with unique characteristics of potential use in the industry as starter cultures, probiotics or the production of metabolites such as lactic

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acid or exopolysaccharides. Different studies have been performed in order to take advantage of the diversity of Peruvian Amazonian fruits, but there is neither relevant information on the microbiota that colonizes the surface of the fruits nor the potential of these microorganisms.

Selection of biotechnologically useful strains requires accurate identification and characterization. As many LAB show similar nutritional and growth requirements, the biochemical tests for identification sometimes fail, leading to erroneous species identification. Some of the most common physiological tests are included in commercially available systems, such as those specially designed for LAB identification, the API 50CHL (Biomerieux, Marcy l'etoile, France) kit, which tests for 49 carbohydrates and esculin. Other systems designed for Gram-positive or Gram-negative bacteria have been applied to LAB identification, such as the Biolog system, which includes the fermentation of 96 carbohydrates (Moraes et al. 2013). On the other hand, the development of molecular techniques has allowed more accurate identification of LAB. The wide method used for this purpose is based on ribosomal gene sequencing or restriction analysis of the amplified product. These genes are conserved among bacteria but show small variations that allow LAB species identification (Mohania et al. 2008). Using ARDRA of 16S rDNA it is possible to differentiate the main LAB present in wine fermentation (Rodas et al. 2003), but to ensure the identification many authors have used the sequencing of the complete 16S rDNA gene (Reginensi et al. 2013). Although the sequencing of 16S rRNA genes is still considered the gold standard for bacterial identification, in recent years laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has emerged as a useful technique for microbial identification. It has already been used in different investigations for the identification of pathogenic bacteria, viruses, and fungi. Although the technique has the advantage of being fast and sensitive, its main disadvantage is the high initial cost of equipment and reagents (Singhal et al. 2015).

Although the main phenotypic characteristics of the LAB are common to all strains in a species, the characteristics of interest generally are specific to a strain, and for this reason, a method of strain discrimination should be applied (Kingston et al. 2010). The method most widely used for strain discrimination in LAB has been PCR amplification using the primers M13 (Andrighetto et al. 2001) or GTG_5 (Gevers et al. 2001).

The main focus of the present study was the isolation of LAB from Peruvian Amazonian fruits and their identification and characterization by phenotypic and molecular methods.

Experimental

Materials and Methods

Fruits. Thirteen fresh fruits were collected in July 2016 in a small rustic market of Iquitos, a city located in the Amazonian region of Peru in the northeastern part of the country. The thirteen fruits were chosen for their abundance at the time of sampling. (Table I). Accord-

Table I
Peruvian Amazonian fruits used to isolate lactic acid bacteria. Scientific and Peruvian names have been included together with the LAB species isolated.

Scientific name	Peruvian name	No. LAB strains isolated	LAB Species (No. strains)
Anacardiun occidentale	Casho	2	*Lactobacillus plantarum* (1) *Weissella confusa* (1)
Averrhoa carambola	Carambola	0	–
Bactris gasipaes	Pijuayo	5	*Lactobacillus plantarum* (3) *Weissella cibaria* (2)
Genipa americana	Huito	5	*Lactobacillus plantarum* (5)
Mauritia flexuosa	Aguaje	0	–
Mauritiella aculeate	Aguajillo	3	*Lactobacillus plantarum* (3)
Myrciaria dubia	Camu camu	0	–
Oenocarpus bataua	Ungurahui	5	*Lactobacillus plantarum* (2) *Lactobacillus brevis* (3)
Passiflora edulis	Maracuyá	4	*Lactobacillus plantarum* (4)
Passiflora nitida	Granadilla	5	*Lactobacillus plantarum* (5)
Poraqueiba sericea	Umarí	2	*Lactobacillus plantarum* (2)
Psidium guajava	Guayaba	5	*Lactobacillus plantarum* (2) *Weissella cibaria* (3)
Solanum sessiliflorum	Cocona	1	*Lactobacillus plantarum*

ing to the size, 3 or 4 pieces of each fruit were used to perform the microbiological analysis. After selection of ripe fruits with no apparent spoilage, fruits were placed in sterile plastic bags. Samples were refrigerated and shipped to the laboratory for analysis.

Isolation and presumptive selection of lactic acid bacteria. Surface sampling of the entire fruits was done using swabs wet with 0.85% NaCl. After sampling the cotton part of the swabs were placed in Man Rogosa Sharpe (MRS) (Merck, Darmstadt, Germany) broth in anaerobic conditions at 30°C for 48 h using an Anaerocult system (Merck, Darmstadt, Germany). One hundred microliters of the enriched cultures were spread on MRS agar (Merck, Darmstadt, Germany) and incubated at 30°C for 48 h in anaerobiosis. Five colonies were randomly isolated from each fruit. To check the purity of the isolates, they were streaked out on MRS plates three times, after that, they were kept in 50% glycerol at –20°C. Further cultivation was done in MRS medium.

Cultures of 48 h were used to observe cell morphology of the presumptive LAB strains in a contrast microscope (Beltec Scientific). These cultures were also used to perform Gram staining and catalase activity with 3% hydrogen peroxide. Acid production was performed by adding 2% $CaCO_3$ to MRS plates. Gram-positive, catalase-negative and acid producer isolates were considered presumptive LAB.

Molecular characterization and identification. From an overnight culture, 1 ml of each culture was used for bacterial DNA extraction according to the procedure of Ausubel et al. (2003). DNA was resuspended in 50 µl of TE and stored at –20°C until use. Identification and characterization of the bacterial isolates were performed by ARDRA, 16S-23S ITS RFLP and rep-PCR using GTG_5 primers. ARDRA was done by amplification and digestion of the 16S rDNA gene, amplification was performed according to Rodas et al. (2003) using a Perkin Elmer 2400 (Norwalk, USA) thermal cycler and *Taq* DNA polymerase (Thermo Scientific, Massachusetts, USA). Digestion was carried out using the restriction enzymes *Alu*I, *Hae*III (Thermo Scientific, Massachusetts, USA) and *Mse*I (Biolabs, Massachusetts, USA) according to the manufacturer instructions. PCR products or restriction fragments were run in a 1% or 2.5% (respectively) agarose gels using TBE 1X. A 100 bp Marker (GeneRules 100 bp Plus Ladder) was used to estimate fragment size. Agarose gels were stained with ethidium bromide for 20 min and revealed using a UV transilluminator (UVP Ultra-violet Products). For 16S-23S ITS RFLP, amplification of 16S-23S ITS region was performed using a modification of the procedure of Zavaleta et al. (1996), but we shorten the annealing and elongation steps from 1 min in the original protocol to 45 sec. Then, sequential restriction digestion was performed with enzymes *Hae*III and *Taq*I (Thermo Scientific, Massachusetts, USA) according to the manufacturer instructions (first 16 h at 37°C and then 6 h at 65°C after *Taq*I addition). Gel electrophoresis and visualization was performed as described before. Strain characterization by rep-PCR using GTG_5 primer was done as described in Gevers et al. (2001). PCR products were run in 1% agarose gels using TBE 1X, and Lambda/*Eco*RI+*Hind*III was used as a molecular weight marker. LAB identification was performed by 16S rDNA gene sequencing of representative isolates of different profiles obtained by rep-PCR. The 16S rDNA gene sequencing analysis was done at Macrogen Inc. (Seoul, Korea) using an ABI3730 XL DNA sequencer. The sequence homology searching against databases was done using the BLAST software from NCBI database (http://blast.ncbi.nlm.nih.gov). Accession numbers were assigned to all the sequences deposited in the GenBank database (Table II). Information available on NCBI of the 16S rDNA nucleotide sequences was used to construct a phylogenetic tree using the Mega version 7.0 program (Biodesign Institute, Tempe, AZ, USA) using the Neighbor-joining method.

The species *L. plantarum*, *L. pentosus* and *L. paraplantarum* were differentiated using the amplification of the *rec*A gene as described by Torriani et al. (2001).

Phenotypic characterization. The different assays were in all cases performed at 30°C for 48 h under anaerobic conditions. Methods for LAB identification were used according to Sharpe (1979); in all assays, the inoculum was approximately $1–2 \times 10^8$ cells/ml. The growth capacity was evaluated in MRS medium under different conditions of pH (3.5 and 7.5), temperature (10°C and 45°C), and in the presence of NaCl (5%, 10%, and 12.5%). Bacterial growth was evaluated by measurement of the optical density at 620 nm in a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific, Waltham, USA).

The sugar fermentation pattern was done according to MacFaddin (2000) in phenol red broth with 1% of each sugar to be analyzed (glucose, fructose, galactose, maltose, lactose, cellobiose, and sucrose). Positive results were considered after the change from the red color of the medium to yellow. Additionally, only in glucose tubes, an inverted Durham tube was included to test for the production of CO_2. After incubation under the same conditions, a test was considered positive if gas was present inside the Durham tube.

Extracellular enzymes production was also analyzed. Proteolytic activity was measured in MRS with 1% skimmed milk medium (Jini et al. 2011). Positive production was considered when a clear area around a colony was produced. Amylolytic activity was tested on agar MRS after replacement of glucose with 1.5% starch (Díaz-Ruiz et al. 2003). Starch hydrolysis was revealed by Lugol staining.

Table II
Molecular characterization and identification of the representative LAB isolates from Peruvian Amazonian fruits.

Isolate	ARDRA (*Alu*I) Restriction profile	16S-23S ITS RFLP Restriction profile	GTG_5 Profile	16S GenBank Accession number	Final identification Species name
LBMBAL1	I	I_R	I.1	KY977384	*Lactobacillus plantarum*
LBMBAL 2			I.2	KY977388	*Lactobacillus plantarum*
LBMBAL 3			I.3	KY977397	*Lactobacillus plantarum*
LBMBAL 4			I.4	KY977386	*Lactobacillus plantarum*
LBMBAL 5			I.5	KY977393	*Lactobacillus plantarum*
LBMBAL 6			I.6	KY977394	*Lactobacillus plantarum*
LBMBAL 7			I.7	KY977399	*Lactobacillus plantarum*
LBMBAL 8			I.8	KY977398	*Lactobacillus plantarum*
LBMBAL 9	II	II_R	II.1	KY977400	*Lactobacillus brevis*
LBMBAL 10	III	III_R	III.1	KY977385	*Weissella confusa*
LBMBAL 11	IV	IV_R	IV.1	KY977390	*Weissella cibaria*
LBMBAL 12			IV.2	KY977391	*Weissella cibaria*
LBMBAL 13			IV.3	KY977392	*Weissella cibaria*
LBMBAL 14			IV.4	KY977395	*Weissella cibaria*

Susceptibility against some antimicrobials was tested using commercial paper discs (Oxoid) with the antimicrobial compound, as described in Bauer et al. (1966). According to the criteria of the European Food Safety Authority (EFSA, 2012) the antimicrobials selected were as follows: amoxicillin (10 μg), ampicillin (10 μg), bacitracin (10 μg), clindamycin (2 U), chloramphenicol (30 μg), erythromycin (15 μg), kanamycin (30 μg), gentamicin (10 μg), novobiocin (30 μg), penicillin (10 μg), rifampicin (30 μg), streptomycin (10 μg), sulfamethoxazole/trimethoprim (25 μg), tetracycline (30 μg), and vancomycin (30 μg). The bacterial susceptibility toward antibiotics was analyzed by the agar diffusion test on MRS or Kirby-Bauer disk-diffusion method. According to the presence or absence of bacterial growth around the antimicrobial disc, the colonies were classified as Resistant (R) or Sensitive (S) according to the criteria of Charteris et al. (1998).

Production of lactic acid and exopolymers (EPS) was also tested. Lactic acid production was evaluated according to Wakil and Ajayi (2013). EPS production was analyzed after 5 days of growth at 30°C in anaerobiosis on MRS plates supplemented with 2% of different sugars: glucose, maltose, fructose, and sucrose as described in Smitinont et al. (1999), development of mucoid colonies and precipitation of mucoid substance in cold absolute ethanol were considered positive for EPS production.

All phenotypic tests were carried out in duplicate to evaluate reproducibility according to the method proposed by Sneath and Johnson (1972). For acid lactic production, the mean of two measures was presented.

Results

Sixty-five isolates were obtained from the Amazonian fruits, of which thirty-seven Gram-positive, catalase-negative and acid producer isolates were selected as presumptive LAB (Table I). Colonies from these isolates were very small (1–3 mm), with creamy appearance, convex surface with entire margins and without pigments. Morphologically, 28 isolates were short bacilli and nine isolates were coccobacilli.

Molecular characterization of the LAB isolates by ARDRA showed three restriction profiles using the enzymes *Mse*I and *Hae*III and four profiles when performing the digestion with the enzyme *Alu*I. 16S-23S ITS RFLP analysis also showed four restriction profiles, clustering the strains in the same way that it was observed with the ARDRA *Alu*I analysis. Based on the 16S rRNA gene sequences, the 37 LAB isolates were identified as *L. plantarum* (28), *W. cibaria* (5), *L. brevis* (3) and *W. confusa* (1) (Table II). Multiplex PCR for *rec*A amplification confirmed the identity of *L. plantarum* strains by obtaining amplicons of approximately 318 bp. GTG_5 analysis showed a total of 14 different banding patterns, which corresponded to 14 LAB strains (Fig. 2). Among then, *L. plantarum* and *W. cibaria* strains showed the highest intraspecific diversity with eight and four profiles, respectively. The phylogenetic tree constructed on the basis of the 16S ribosomal gene sequences separated LAB isolates into two large groups, one corresponding to the genus *Lactobacillus* and the other to *Weissella*; additionally, each group consisted of two subgroups correspond-

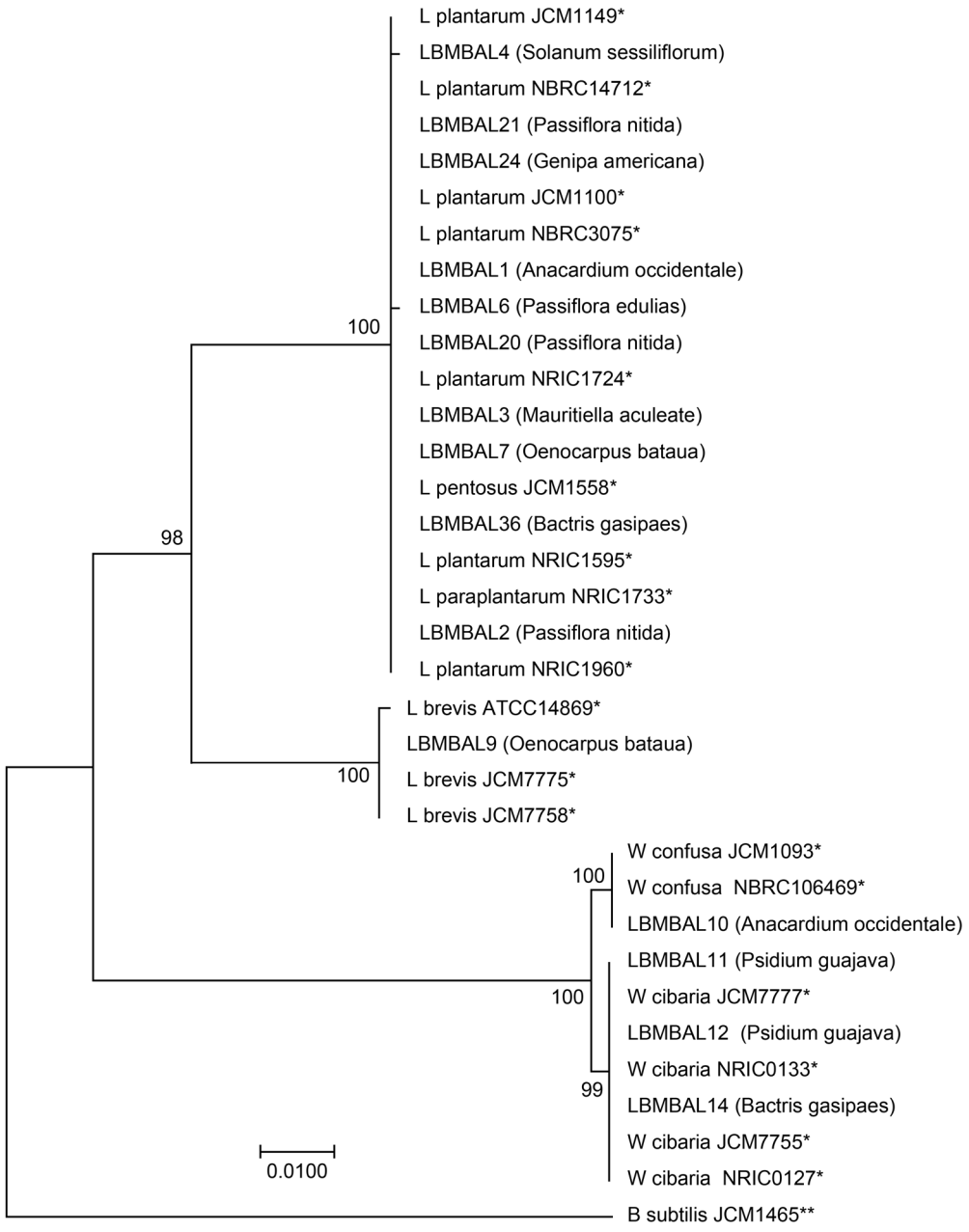

Fig. 1. Phylogenetic tree of LAB isolated from Amazonian Peruvian fruits based on the 16S rDNA sequences. Neighbor-Joining method and bootstrap 1000. Numbers in the nodes correspond to the percentage of bootstrap. The bar represents 1% divergence in the sequences.

* – LAB reference strains. ** – Outgroup. Parentheses include the name of the fruit from which the strain was isolated.

ing to *L. plantarum* and *L. brevis*, and *W. confusa* and *W. cibaria*, respectively. High bootstrap values (98–100) supported these groupings (Fig. 1).

Regarding phenotypic characterization (Table III), most of the isolates (92–100%) fermented glucose, fructose, cellobiose, and maltose but they showed differences in the uptake of the other sugars tested, being galactose and lactose the sugars with minor preference among the isolates. When CO_2 production was analyzed, 25 isolates showed homofermentative metabolism while 12 isolates were heterofermentatives. Growth evaluated at different conditions of temperature, pH and NaCl showed that 95% to 100% of the isolates were able to grow between 10°C to 45°C, pH 3.5 to 7.5 and 5% NaCl, but only 62% of the isolates grew at 10% NaCl and none of them grew at 12.5% NaCl. No amylolytic or proteolytic activity was detected in the LAB isolates. Concerning antimicrobial susceptibility, the isolates showed high susceptibility (95–100%) to ampicillin, amoxicillin, clindamycin, chloramphenicol, erythromycin, penicillin, and tetracycline, on the contrary, the high resistance (98–100%) was observed against kanamycin, gentamicin, streptomycin, vancomycin, and sulfamethoxazole-thrimethoprim. Only for novobiocin

Table III
Phenotypic characteristics of LAB strains isolated from Peruvian Amazonian fruits.

Specie isolated	*Lactobacillus plantarum*	*Lactobacillus brevis*	*Weissella confusa*	*Weissella cibaria*
No. strains	28	3	1	5
Cell morphology	Rods	Rods	Cocobacilli	Cocobacilli
Fermentation of:				
Glucose	+	+	+	+
Fructose	+	+	+	+
Galactose	23/5	–	+	–
Sucrose	26/2	–	+	+
Maltose	+	–	+	+
Cellobiose	+	+	+	+
Lactose	24/4	–	–	–
CO_2 from glucose	–	–	+	+
Growth at:				
10°C	+	+	+	+
30°C	+	+	+	+
45°C	+	+	+	3/2
5% NaCl	+	+	+	+
10% NaCl	22/6	1/2	–	–
12.5% NaCl	–	–	–	–
pH 3.5	+	+	+	4/1
pH 7.5	+	+	+	+
Susceptibility to:				
Ampicillin (10 µg)	S	S	S	S
Amoxicillin (10 µg)	S	S	S	S
Bacitracin (10 µg)	R	R	S	S
Clindamycin (2 U)	S	R	S	S
Chloramphenicol (30 µg)	S	S	S	S
Erythromycin (15 µg)	S	S	S	S
Kanamycin (30 µg)	93%	R	R	R
Gentamicin (10 µg)	93%	R	R	R
Penicillin (10 µg)	S	S	R	S
Novobiocin (30 ug)	S	S	R	R
Streptomycin (10 µg)	R	R	R	R
Sulfamethoxazole/ trimethoprim (25 µg)	R	R	R	R
Tetracycline (30 µg)	S	S	S	S
Vancomycin (30 µg)	R	R	R	R
EPS production from:				
Sucrose	–	–	+	+
Glucose	–	–	–	–
Maltose	–	–	–	–
Fructose	–	–	–	–
Lactic acid production (g/l)	20.1 – 23.6	13.1 – 14.6	14.4	14.2 – 16.0

and bacitracin, the differences between bacilli and coccobacilli were observed, while all bacilli were resistant to bacitracin and susceptible to novobiocin, the opposite was observed in coccobacilli.

About EPS production, according to our methodology only isolates belonging to *Weissella* species produce EPS from sucrose but negative results were obtained when glucose, fructose or maltose was used as single

carbon source in the medium. With regard to acid lactic production, a wide range of production was observed (13.1 g/l to 23.6 g/l) being *L. plantarum* strains the higher lactic acid producer (Table III).

Discussion

This research work was interested in the isolation and characterization of LAB from Amazonian Peruvian fruits. Of the 13 fruits studied in three of them (*Myrciaria dubia, Averrhoa carambola* and *Mauritia flexuosa*), LAB was not detected on their surfaces. It is possible that the enrichment method used was not appropriate for the development of LAB that inhabit the surface of these fruits or may be the presence of other microorganisms colonizing the fruit surface set up some kind of competence for nutrients available on the surfaces. It is also is possible that some intrinsic factors such as the great acidity given by the high vitamin C content, the waxy cuticle or the ripening period of these fruits, were conditions that made LAB survival on the external layer of these fruits difficult (Barrera and Hernández 2004; Leff and Fierer 2013; Azevêdo et al. 2015). As can be seen from Table I, *L. plantarum* was isolated from all the fruits analyzed, while *L. brevis*, *W. cibaria* and *W. confusa* were additionally isolated from only four fruits: *Bactris gasipaes*, *Psidium guajava*, *Anacardiun occidentale,* and *Oenocarpus bataua*. Therefore, the predominance of *L. plantarum* over the other LAB species in the Amazonian Peruvian fruits was evident.

In the present study, the presumptive LAB isolates were characterized by ARDRA using the restriction enzymes: *Mse*I, *Hae*III, and *Alu*I. *Mse*I and *Hae*III revealed three restriction profiles, while *Alu*I showed four restriction profiles demonstrating greater discriminatory power to differentiate the LAB isolates. 16S-23S ITS RFLP analysis also showed four restriction profiles similar to those formed with ARDRA A*lu*I, which confirmed the presence of at least four LAB species. These results are in agreement with previous studies which indicate that ARDRA and 16S-23S ITS RFLP are useful techniques for LAB differentiation at species level (Zeng et al. 2013) and agree with the results obtained by Jeyaram et al. (2010) who used both techniques obtained the same number of restriction profiles for LAB species of the genera *Carnobacterium, Lactobacillus* and *Enterococcus* isolated from fermented bamboo roots. It is important to bear in mind that the success of ARDRA or 16S-23S ITS RFLP techniques lies in the adequate selection of enzymes for the digestion. Thus, Rachman et al. (2003) showed that the digestion of 16S-23S ITS segment using the *Hind*III enzyme was not efficient to differentiate *L. sakei*, *L. curvatus*, *L. farciminis*, *L. alimentarius*, *L. plantarum* and *L. paraplantarum*; however, the use of *Taq*I allowed them to obtain different genetic profiles that differentiated most of these species except *L. plantarum* and *L. paraplantarum* due to their phylogenetic closeness. In this study, the discrimination between *W. cibaria* and *W. confusa* was possible by digestion with *Alu*I but not when *Mse*I or *Hae*III was used.

ARDRA and 16S-23S ITS RFLP are useful tools to determine the interspecific diversity of LAB; however, it is difficult to detect intraspecific variability when the strains are closely phylogenetically related. Using rep-PCR technique with the GTG_5 primer, it was possible to obtain 14 different patterns which correspond to 14 genotypes or strains demonstrating that among the LAB isolates there was intraspecific diversity that was neither revealed by ARDRA nor 16S-23S ITS RFLP. These results are similar to those reported by Silva et al. (2017) who used the 16S-23S ITS RFLP and the sequencing of the 16S ribosomal genes identified six LAB species from 33 isolates, but using GTG_5 fingerprinting they observed 18 genotypes. Similarly, Kingston et al. (2010) using ARDRA observed similar profiles for 16 LAB isolates identified as *L. paraplantarum* and *L. pentosus*, but using rep-PCR found eight genotypes, demonstrating the existence of intraspecific variability. In this study, the highest intraspecific diversity was observed among isolates of *L. plantarum* (eight patterns). *L. plantarum* is known for its genetic variability, which according to Pisano et al. (2010) is related to the existence of genomic islands composed of groups of the genes destined to the use of carbohydrates that can be acquired, combined, replaced or deleted depending on the characteristics of the medium. The great flexibility of these genomic islands favors the versatility of *L. plantarum* to different substrates and environmental changes. For this reason, Siezen and van Hylckama Vlieg (2011) consider *L. plantarum* a "natural metabolic engineer".

An interesting fact was the presence of the GTG_5 pattern I.1 in seven fruits analyzed, the imposition of a single strain on those fruits could be attributed to the production of antagonist compounds that limit the survival of other strains (Hibbing et al. 2010).

By sequencing the 16S ribosomal genes, the isolates were identified as *L. plantarum*, *L. brevis*, *W. cibaria* and *W. confusa*, being *L. plantarum* the most abundant LAB isolated from the Amazonian fruits analyzed. These results are in the agreement with different studies which indicates that *L. plantarum* is the most abundant LAB distributed in fruits and vegetables (Naeem et al. 2012, Emerenini et al. 2013, Franquès et al. 2017).

Identification of *L. plantarum*, *L. paraplantarum*, and *L. pentosus* based only on the sequence of the 16S ribosomal genes is not accurate because these species have a similarity greater than 99% in the sequence of these genes (Torriani et al. 2001; Agaliya and

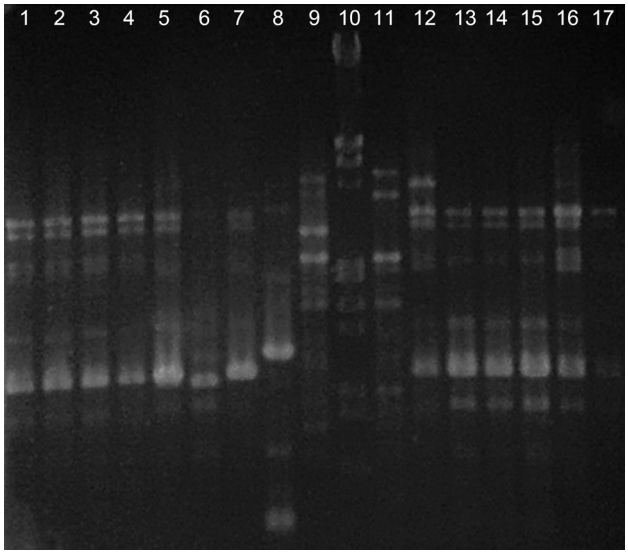

Fig. 2. Different GTG_5 profiles of LAB strains isolated from Peruvian Amazonian fruits. Lines 1–5: *L. plantarum*, 6: *L. brevis*, 7: *L. plantarum,* 8: *W. confusa*, 9: *W. cibaria,* 10: Lambda/*Eco*RI+*Hind*III, 11: *W. cibaria*, 12–17: *L. plantarum*.

Jeevaratnam 2013). Their closeness was corroborated when constructing the phylogenetic tree using the 16S rDNA sequences of the *L. plantarum* isolates and reference strains of *L. paraplantarum* and *L. pentosus*, where it was observed that the three species are located in the same group (Fig. 2). Because of this fact, the identity of the *L. plantarum* isolates had to be confirmed by a multiple PCR technique described by Torriani et al. (2001) that used specific primers to amplify the *rec*A gene and allows to differentiate the three species according to the amplicons size.

The phenotypic characterization demonstrated that LAB isolates were able to grow in a wide range of pH (3.5–7.5) and temperature (10°C–45°C), and tolerate up to 10% NaCl. These observations are in agreement with the described features of LAB, which indicate that they are robust microorganisms able to survive and adapt to different environmental conditions (Ludwig et al. 2009; Mazzoli et al. 2014). This feature gives LAB a great capacity to be used in diverse industrial processes.

The carbohydrate fermentation test showed that a large percentage of isolated LAB fermented both monosaccharides and disaccharides. As it was described, LAB are microorganisms with high energy requirements being able to obtain the necessary energy from the fermentation of a wide variety of carbohydrates (Mazzoli et al. 2014). Therefore, fruits that contain sugars such as fructose, sucrose, and glucose are favorite sources for LAB development (Serpen 2012). The results also showed that LAB strains belonging to the same species shared a similar carbohydrate fermentation pattern with some differences in the fermentation of galactose, sucrose, and lactose. These metabolic variations are typical of the intraspecific variation existing in the isolates, especially in *Lactobacillus* strains, such variability is manifested in strains that show atypical characteristics to those usually reported (Pot et al. 1994).

The LAB isolates did not hydrolyze casein or starch. There are different publications that report the isolation of proteolytic and amylolytic LAB from sources rich in proteins (dairy products) or starch (cereal based drinks), respectively (Díaz-Ruiz et al. 2003; Moulay et al. 2006; Hattingh et al. 2015; Kıvanç and Yapıcı 2015). Endo and Dicks (2014) noted that LAB have evolved to adapt to specific niches, gaining specific genes and losing others. In this sense, Kelly et al. (2010) provide evidence that defined dairy starter cultures have arisen from *Lactococcus lactis* strains that have plant origin, such adaptation to the dairy environment involved loss and acquisition of genes (usually plasmid associated) that favor growth in milk. Taking this information into account, it can be explained that LAB isolated from fruits and adapted to this habitat, in which the starch and protein contents are scarce have not developed enzymatic machinery to metabolize these compounds.

Regarding the antimicrobial susceptibility, all the isolates were sensitive to seven of the 14 antimicrobials tested, on the contrary, they were resistant against kanamycin, gentamicin, streptomycin, sulfamethoxazole/trimethoprim, and vancomycin. The high resistance observed, and the results of previous investigations would indicate that the observed resistance is intrinsic among LABs, which means that the possibility of being transferred to other bacteria by horizontal transfer is minimal (Abriouel et al. 2015; Sharma et al. 2016). Intrinsic resistance is typical of all strains of the same species. Some LAB, especially from the genus *Lactobacillus*, are used as probiotics, however a growing concern has arisen over the possibility that LAB may constitute a reservoir of antimicrobial resistance genes that could be transferred horizontally (via plasmids and conjugative transposons, integrons or insertion sequences) to pathogens during their passage through the gastrointestinal tract (Jose et al. 2015). This fact justifies the importance of previously determining antimicrobial resistance patterns before using a LAB strain as a probiotic. Due to their natural origin, LAB isolates from Amazonian fruits could be a safe alternative to be used as probiotics; however, it is necessary to confirm the genetic nature of the observed resistance.

Regarding lactic acid production, the results are in agreement with the type of metabolism observed, being the homofermentative strains (*L. plantarum*) those that produced lactic acid in higher concentrations. They are good candidates to be evaluated for industrial processes where homofermentative strains are preferred to avoid necessary further purification steps if heterofermentative strains are used. One of the advantages of microbial

production of lactic acid by microbial fermentation is that a product of high purity can be obtained when the strains were selected properly, while by chemical synthesis a racemic mixture of D and L lactic acid is obtained (Taskila and Ojamo 2013). Lactic acid is a compound with many industrial applications being one of the most interesting the manufacture of polylactic acid, a biodegradable plastic that can replace similar products derived from petroleum (Ilmen et al. 2007).

EPS production using different carbon sources was also evaluated. *Weissella* strains were able to produce EPS only using sucrose. Similar results were obtained by Smitinont et al. (1999), Van Geel-Schutten et al. (1999) and Di Cagno et al. (2006) who determined that sucrose was the best sugar for EPS production by LAB isolated from different samples. The preference for a particular carbon source has been attributed to the presence of different sugar transport systems in LAB strains or to variations in the activity of the enzymes involved in the precursor synthesis of the repeating units that make up the structure of EPS (Chervaux et al. 2000; Mozzi et al. 2001). LAB strain, medium composition and growth conditions (temperature, agitation, incubation time, pH, oxygen tension) are important factors that influence EPS production (Sanalibaba and Çakmak 2016). EPS production is a distinctive feature of the genus *Weissella* and currently *W. cibaria* and *W. confusa* are two species valued for the production of dextrans, fructans, heteropolysaccharides and non-digestible oligosaccharides, which have a large number of applications in biomedical, cosmetics, food, and feed industries; however, both species have also been reported as human opportunistic pathogens (Fusco et al. 2015). For this reason, the biotechnological use of these strains would have to be evaluated exhaustively.

In this work, 37 LAB isolates from Peruvian Amazon fruits were characterized and identified using molecular and phenotypic methods, which provided complementary information on the genetic diversity and physiology of the isolated strains being necessary to continue the study to determine their usefulness in the future biotechnological processes.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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