LWT - Food Science and Technology 87 (2018) 567–574

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Exploring diversity and biotechnological potential of lactic acid bacteria from *tocosh* - traditional Peruvian fermented potatoes - by high throughput sequencing (HTS) and culturing

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ARTICLE INFO

Article history:
Received 9 June 2017
Received in revised form
21 September 2017
Accepted 22 September 2017
Available online 25 September 2017

Keywords:
Lactic acid bacteria
Bacterial diversity
Tocosh
High throughput sequencing (HTS)
Biotechnological potential

Chemical compounds studied in this article:
Sucrose (PubChem CID: 5988)
Calcium phytate (PubChem CID: 24495)
Sodium phytate (PubChem CID: 66391)
Riboflavin (PubChem CID: 493570)
Folic acid (PubChem CID: 6037)
L-Tyrosine (PubChem CID: 6057)
L-Histidine (PubChem CID: 6274)
L-Lysine (PubChem CID: 5962)
L-Arginine (PubChem CID: 6322)

ABSTRACT

Lactic acid bacteria (LAB) diversity associated with *tocosh*, Peruvian traditional fermented potatoes, was for the first time investigated by culturing and high throughput sequencing (HTS) approaches. They were applied on three samples i.e. freshly harvested potatoes, one-month and eight-months production. While by culture-dependent approach a few *Lactobacillus (Lb)* species (*Lb. sakei*, *Lb. casei*, *Lb. farciminis*, *Lb. brevis*, *Lb. fermentum*) and *Leuconostoc (Ln) mesenteroides* were identified, twenty-four OTUs belonging to six LAB genera were considered in *tocosh* samples by HTS, being *Lactobacillus* dominant in all three samples. LAB predominated on fresh potatoes, while *Clostridium, Zymophilus* and *Prevotella* were the most abundant genus in 1- and 8-months *tocosh* samples. When biotechnological features were investigated, amylase and phytate-degrading abilities as well as EPS and group B vitamin (riboflavin and folate) production were exhibited by several *Lb. sakei* and *Ln. mesenteroides* strains. Safety traits of major LAB species from *tocosh* showed antibacterial activities as well as biogenic amines production capacity. The molecular inventory achieved by HTS approach provided information on LAB population composition during fermentation of this ancestral potato fermented product while culturing allowed the selection of LAB strains suitable for novel functional cultures design for the production of fermented starchy products.

1. Introduction

Household fermentation of foods has a long and very important tradition in Latin American countries (Londoño-Zapata, Durango-Zuleta, Sepúlveda-Valencia, & Moreno Herrera, 2017; Tamang, Watanabe, & Holzapfel, 2016). Andean communities have managed to preserve native crops as well as their production, harvesting and storage during centuries. Among them, potatoes, originated approximately 8000 years ago in the South American Andes, Perú lodging one of the most important reservoirs of varieties and wild relatives (Goldner, Pérez, Pilosof, & Armada, 2012; Mosso, Lobo, & Sammán, 2016; Velásquez-Milla, Casas, Torres-Guevara, & Cruz-Soriano, 2011). Numerous ingenious ways of preserving potatoes in order to maintain adequate stocks for survival have been developed, such as sun-drying or natural freeze-drying to obtain white or black *chuño* and fermentation to obtain *tocosh*.

Potatoes *tocosh* is an ancestral fermented food product that is

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https://doi.org/10.1016/j.lwt.2017.09.033
0023-6438/

still prepared in small communities from the highlands of Central Peruvian Andes by local peasants (Horkheimer, 1973). The traditional preparation method consists of digging a well (0.70 × 1.50 m deep) in the ground near a water spring in which large amounts of potatoes (normally discarded potatoes) are placed between straw layers. Rocks are used to cover the pile in order to prevent the tubers to be washed away by the slight water current that passed through the ditch; the potatoes are then left to ferment in this running water up to 12 months. After this time, potatoes suffer an enzymatic browning (Zvitov-Ya'ari & Nussinovitch, 2014) and are laid in a dry shaded area to allow the water to drain. The obtained product is kept for consumption, sale or most commonly, as a sun-dried and ground fine flour-type product that is used to prepare different broths, stews and "mazamorra" which is a semi-liquid food with thick consistency (De Moreno de LeBlanc, Todorov, Vignolo, Savoy de Giori, & LeBlanc, 2014). From a microbiological point of view, only preliminary studies have been performed demonstrating that tocosh results from microbial fermentation, mainly by lactobacilli. Besides being an important staple food for local population, the compounds generated by these beneficial microorganisms are thought to be responsible for the large diversity of medicinal properties attributed to this product as such being known as the "natural antibiotic of the Incas". Although no scientific articles supporting these claims were found, its probiotic potential was demonstrated using an experimental animal model and compared with a recognized probiotic *Lactobacillus acidophilus* LA-5® strain (Prentice & Milka, 2005). Lactic acid bacteria (LAB) are usually involved in traditionally fermented food of vegetal origin and many LAB species have been described as vitamin producers or phytate degraders (Anastasio et al., 2010; Juarez del Valle, Laiño, Savoy, de Giori, & LeBlanc, 2014; Ruiz-Rodríguez et al., 2016).

Recently environmental and food microbiology have benefited from the advances in molecular biology and adopted novel strategies to detect, identify, and monitor microbes. An in-depth study of the microbial diversity in food can now be achieved by using high-throughput sequencing (HTS) approaches after direct nucleic acid extraction from the sample to be studied; the current scenario of this metagenomic approach to study food microbiota was described by Ercolini (2013). Therefore, the aim of this study was to evaluate LAB populations present in *tocosh* and to disclose their biotechnological potential for future applications. Fresh potatoes and fermented samples from two wells corresponding to two storage times were analysed by combining both, culture-dependent and HTS approaches.

2. Material and methods

2.1. Bacterial strains and growth conditions

LAB reference cultures used in this work were supplied by the Spanish Type Culture Collection (CECT) as follows: *Lactobacillus sakei* subsp. *sakei* CECT 906^T, *Leuconostoc mesenteroides* subsp. *cremoris* CECT 872^T, *Leuconostoc mesenteroides* subsp. *dextranicum* CECT 912^T, *Leuconostoc mesenteroides* subsp. *mesenteroides* CECT 219^T, *Lactobacillus amylophilus* CECT 4133^T. LAB strains were routinely grown on MRS (De Man, Rogosa and Sharpe) medium (Oxoid) at 28 °C and stored in growth liquid medium containing 20% (v/v) glycerol at −80 °C. *Listeria monocytogenes* FBUNT (Facultad de Bioquímica, Quíimica y Farmacia, UNT, Argentina) and *Bacillus subtilis* 168 (PROIMI-CONICET) were grown overnight on Trypticase Soy broth (TSB, Britania, Argentina) at 30 °C.

2.2. Sample processing, microbiological analysis and LAB isolation

Potato *tocosh* samples were obtained from ground wells traditionally prepared by local producers from the community of Tambogán, (Huánuco, Perú) located at 2500 m above sea level (Fig. 1). The region presents average annual temperatures of 12 °C, rainy summers and winters with strong frost, temperature during the day being largely variable (22 to −3 °C) but keep constant along the year. Due to difficult access to this place, all samples were collected at once in Summer 2012, corresponding to freshly harvested potatoes (before placing in the ground well), 1-month and 8-months *tocosh* storage wells. Samples (5 g) were analysed as previously described for microbial counts (Elizaquível et al., 2015): total mesophilic counts on Plate Count Agar, incubated aerobically at 30 °C for 72 h; LAB on MRS containing glucose (MRS), maltose (MRS-M) or starch (MRS-S) at 0.5% (w/v) and Yeast Glucose Lactose Peptone (YGLP), incubated anaerobically at 30 °C for up to 7 days; total yeasts and molds on Yeast and Molds agar, incubated aerobically at 30 °C for 72 h. Counts were performed in triplicate. For each sample, up to six colonies per plate and LAB medium, representing different morphologies, were randomly picked from plates with 30–300 colonies and sub-cultured on the corresponding medium. Isolates that were Gram positive and catalase negative were considered as presumptive LAB and were stored at −20 °C in the same liquid media containing 20% (v/v) glycerol for further analysis. Samples pH was determined from homogenized samples using a digital pH meter (PT-10 Sartorius).

2.3. Culture-dependent analysis of LAB populations

2.3.1. PCR-based LAB identification

DNA extraction and identification of pure isolates into species was approached following a three steps schedule as previously described (Elizaquível et al., 2015), with some modifications: isolates from each ISR group, were subjected to RAPD-PCR analysis using universal primers P2 (5′-GATCGGACGG-3′) and P16 (5′-TCGCCAGCCA -3′) as described by Samaržija, Sikora, Redzepović, Antunac, & Havranek, 2002, In addition, the 16S rRNA sequences of selected isolates representing the different clusters were compared with the Ribosomal Database Project (RDP) (http://rdp.cme.msu.edu/) for species identification.

2.3.2. Cluster analysis of ISR-PCR and RAPD-PCR electrophoretic profiles

Digitized images were converted, normalized, analysed and combined using the Software package BioNumerics 4.61 (Applied Maths, Kortrijk, Belgium). Identification of profiles was carried out by comparison with a database previously generated with the aid of the BioNumerics software, containing ISR and RAPD profiles corresponding to 132 reference strains (Chenoll, Macián, Elizaquível, & Aznar, 2007).

2.4. Culture-independent analysis of bacterial populations

2.4.1. Tocosh DNA isolation, 16S rRNA gene amplification and pyrosequencing

For DNA isolation, 10 ml of each homogenized sample was taken from the upper liquid phase and centrifuged (5000 × *g*, 10 min). Total DNA was extracted using the Bacterial DNA preparation kit (Jena Biosciences, Germany) according to the manufacturer's instructions.

Amplicon library preparation and pyrosequencing were carried out by LifeSequencing Inc. (Valencia, Spain). The DNA isolated from *tocosh* samples was used as template for the amplification of the V3–V5 hypervariable region of the bacterial 16S rRNA genes with primer set 357F/926Rb (Sim et al., 2012). Amplicon library preparation and pyrosequencing was carried out by LifeSequencing Inc. (Valencia, Spain) as previously described in Elizaquível et al. (2015).

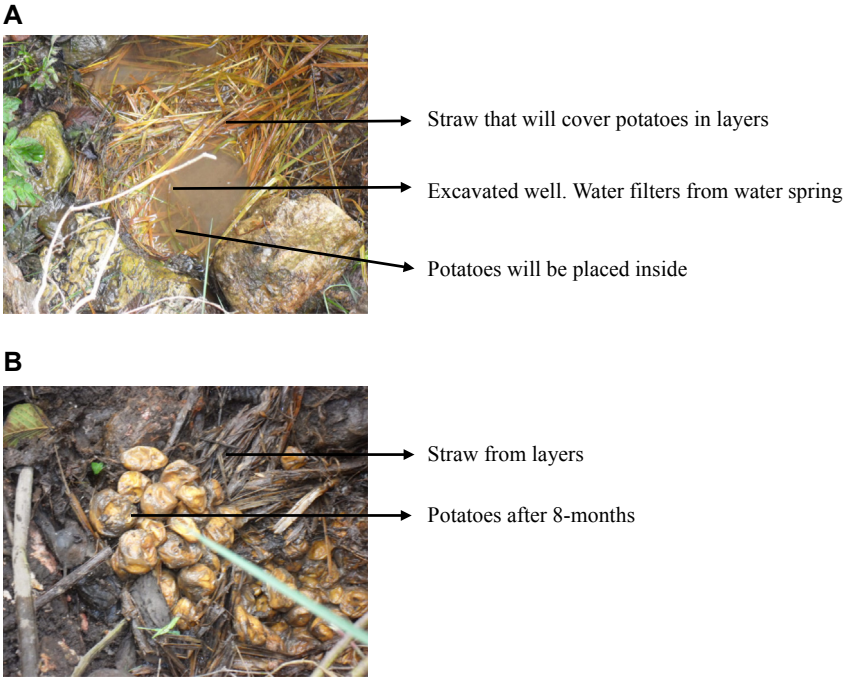

Fig. 1. Traditional *tocosh* production. (A) Ground well dug near a water spring where potatoes are placed between straw layers; (B) Potatoes removed from a well after 8-months storage – *tocosh*.

2.4.2. Bioinformatics and pyrosequencing data analysis

Sequences shorter than 300 bp and those of average quality score lower than 20 were removed using Galaxy server tools (Goecks, Nekrutenko, & Taylor, 2010), while UCHIME algorithm Edgar, Haas, Clemente, Quince, and Knight (2011) was applied using RDPII 16S rRNA database (http://rdp.cme.msu.edu/) as reference to remove chimera sequences. To estimate species richness in each sample, the freeware program aRarefactWin by Holland (http://strata.uga.edu/software/anRareReadme.html) was used and rarefaction curves were obtained. Then, sequences were clustered into OTUs (operational taxonomical units) at 97% sequence similarity to the sequences deposited in RDPII and were clustered as described by Cole et al., (2005).

2.5. Functional and safety characterization of LAB

2.5.1. Exopolysaccharides (EPS) production

EPS production was assigned to strains that formed mucoid colonies in MRS-sucrose (Notararigo et al., 2013).

2.5.2. Phytate-degrading activity

Phytate-degrading activity was tested using the method described by Anastasio et al. (2010).

2.5.3. Amylolytic activity

Starch degradation was investigated by spot inoculation of active LAB isolates according to the proceeding carried out by Díaz-Ruiz, Guyot, Ruiz-Teran, Morlon-Guyot, & Wacher, (2003). *Lb. amylophilus* CECT 4133^{T} was used as positive starch degrading control strain.

2.5.4. Group B vitamin production

The production of folates and riboflavin was evaluated following the methods already described (Laiño, Juarez del Valle, Savoy de Giori, & LeBlanc, 2013; Juarez del Valle, Laiño, Savoy de Giori, & LeBlanc, 2014) and based on microbiological bioassays.

2.5.5. Biogenic amines production

The ability to decarboxylate amino acids used as precursors was tested according to Bover-Cid and Holzapfel (1999) methodology.

2.5.6. Antimicrobial activity

Antibacterial activity of LAB isolates was determined against *L. monocytogenes* FBUNT and *B. subtilis* 168 (PROIMI-CONICET) as sensitive strains by the agar spot test (Fontana, Cocconcelli, Vignolo, & Saavedra, 2015). Inhibitory activity was expressed as + (halo presence) or - (no halos) around the spot.

3. Results

3.1. Identification of the bacterial community as determined by HTS

Identification of bacterial populations associated to *tocosh* was performed by high-throughput 454 pyrosequencing. Partial 16S rRNA gene sequencing was obtained from DNA directly extracted from each three-*tocosh* samples. After quality control, 42,530 reads with an average length of 541 bp were obtained and analysed (19,224; 9254 and 14,052 in fresh potatoes, 1- and 8-months samples, respectively); reads distribution and Chao/Shannon values are shown in Table 1. Rarefaction analysis and diversity indexes indicated that there was satisfactory coverage of the diversity within the three batches analysed. Relative abundance of the taxonomic levels calculated at phylum level indicates that *Firmicutes* predominated in *tocosh* production, followed in less proportion by *Bacteroidetes*. As it can be observed in Fig. 2A, LAB predominated in fresh potatoes, while they were below 25% in 1- and 8-eight months samples. Sequence assignment at genus level (Fig. 2B and C) showed that *Lactobacillus* and *Leuconostoc* were detected in all samples, while *Lactococcus* were only found in one-month fermentation sample. *Carnobacterium*, *Weissella* and *Pediococcus* were present in low percentages. Eight-months sample showed the greatest diversity at genus level with five genera represented (*Lactobacillus*, *Carnobacterium*, *Pediococcus*, *Leuconostoc*,

Table 1
Number of sequences analysed, observed diversity richness (OTUs), estimated OTU richness (Chao1) and diversity index (Shannon) for 16S rRNA amplicons from *tocosh*.

Sample	No. of sequences	OTUs	Chao index	Shannon Diversity
Fresh potatoes	19,224	74	97.75 (81.98; 144.61)	0.92 (±0,00015)
1-month[a]	9254	330	439.20 (396.23; 510.02)	4.06 (±0.00025)
8-months	14,052	295	402.50 (356.43; 483.10)	3.76 (±0,00018)

[a] Storage time in the sampled well.

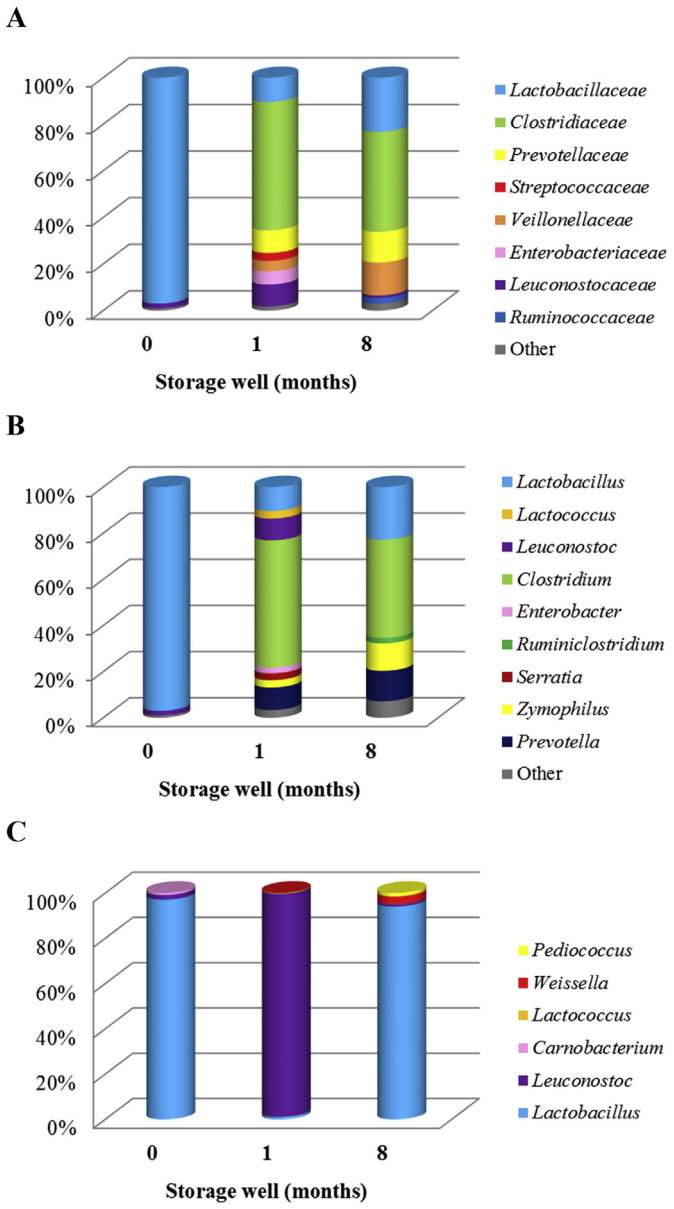

Fig. 2. Identification of bacterial populations in *tocosh* derived from HTS analysis corresponding to each sampled batch, fresh potatoes, and *tocosh* from 1- and 8-months storage wells dug near a water spring. Relative abundance (%) of families (A), genera (B) and LAB genera (C) are shown, considering sequences that exceeded 1% in at least one of the samples.

and *Weissella*). Besides LAB, other genera were evidenced such as *Clostridium, Zymophilus* and *Prevotella*, the former being the most abundant in 1- and 8-months samples (Fig. 2A and B). *Clostridium acetobutylicum* and *Clostridium tyrobutyricum* were found in a greater proportion (Fig. 3).

The composition of the LAB community was analysed by the construction of a heat-map representing the relative abundance of the OTUs with an abundance level above 0.1% in at least one *tocosh* sample (Fig. 3). Sixty-three OTUs were identified, of them twenty-four belonging to six LAB genera, were considered. Diversity in *Lactobacillus* species increased from fresh potatoes to 1- and 8-months samples with 3, 7 and 12 species identified, respectively; *Lb. sakei* being the only species present in all three samples. *Lactobacillus* showed the greatest species diversity with 16 identified OTUs, followed by *Leuconostoc* with three species recovered; one of them *Ln. mesenteroides* was present in the three analysed samples. Two species were identified belonging to the genus *Lactococcus* and one to *Weissella*, *Carnobacterium* and *Pediococcus*. The most abundant species in *tocosh* production were *Lb. sakei* in fresh potatoes (96.6%) and 1-month sample (9.5%) and *Lb. casei* in 8-months sample (7.1%).

3.2. Culture dependent microbiological analysis

Results on the microbiological analysis of *tocosh* samples are shown in Table 2. The trend of the total counts paralleled that of LAB population in the three samples. Total mesophilic bacteria in PCA varied from 4.3×10^6 to 1.9×10^8 CFU/g from the initial stage to the 8-months batch, while LAB counts in MRS and YGLP media showed similar levels, which increased from 7.4×10^2 and 1.1×10^3 CFU/g to 5.5×10^7 and 1.1×10^7 CFU/g, respectively, for fresh potatoes and 8-months samples. No difference in LAB counts was observed in MRS-M or MRS-S except a one-log reduction for 8-months sample in MRS-S. Total molds and yeasts decreased from 7.1×10^3 (fresh potatoes) to 1.0×10^2 CFU/g (8-sample). A decrease in pH value from 5.5 to 3.8 was recorded in 1-month sample, in coincidence with higher LAB counts, while a final value of 4.2 was found in 8-months sample.

3.3. Identification of LAB isolates and species distribution among tocosh samples

A total of 151 colonies from *tocosh* samples recovered from MRS and YGLP plates were considered as presumptive LAB because they were Gram-positive and catalase-negative. First, 16S–23S ISR amplification yielded one to five bands of molecular sizes ranging from 300 to 1000 bp corresponding to the genera *Leuconostoc* and *Lactobacillus*. Sixty-six 16S rRNA gene sequences were analysed showing similarity levels >99% when compared with public sequences of LAB species. Distribution of LAB species among *tocosh* samples is shown in Table 3. In fresh potatoes and 1-month samples, only *Lb. sakei* and *Ln. mesenteroides* were recovered, with a clear dominance of *Ln. mesenteroides*. In 8-months sample, *Lactobacillus* species were more abundant, additionally involving *Lb. brevis, Lb. casei*, *Lb. farciminis* and *Lb. fermentum,* while *Leuconostoc* was less represented with just one *Ln. mesenteroides* isolate recovered.

3.4. Functional and safety characterization of LAB strains

In order to search out LAB strains with biotechnological potential, a total of 62 out of 151 *tocosh* isolates were selected representing different species and batches, *Lb. brevis* (1), *Lb. casei* (9), *Lb.*

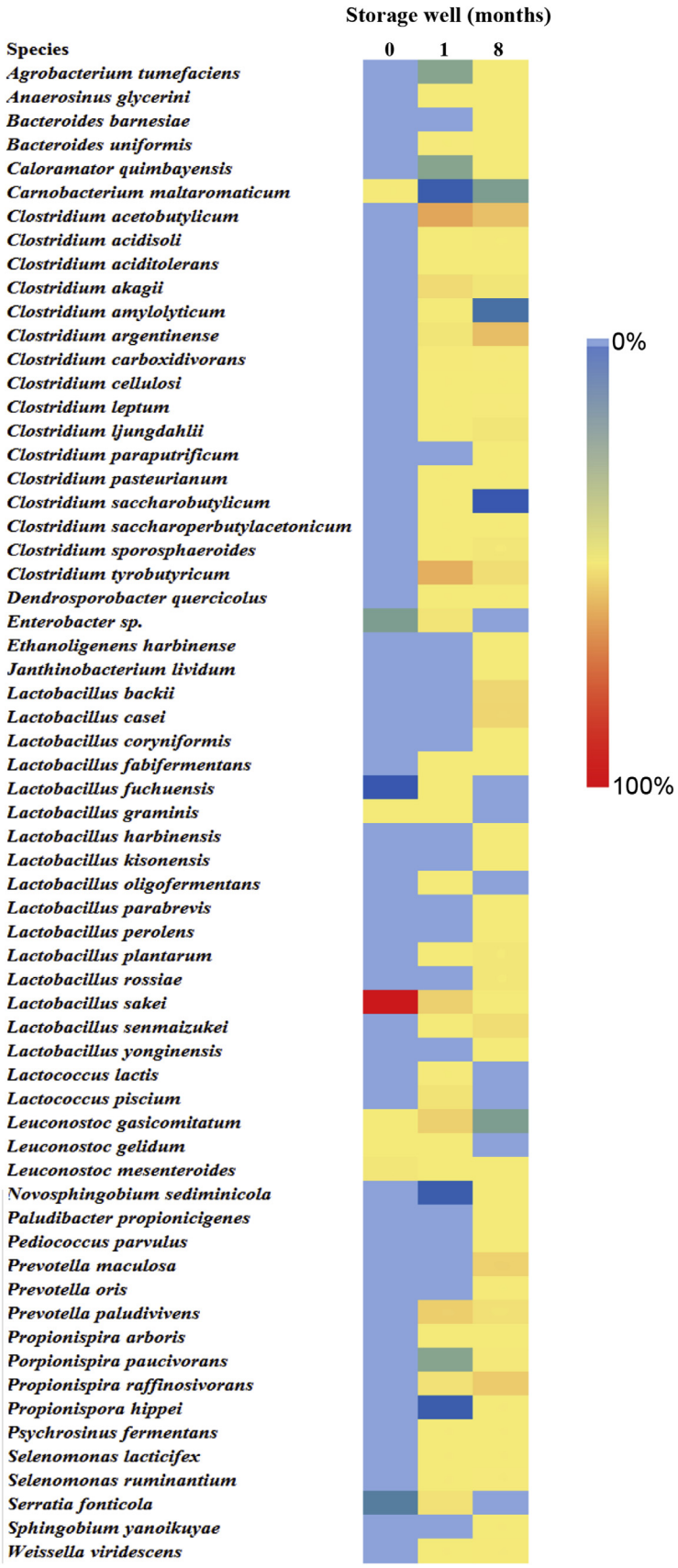

Fig. 3. Heat-map showing bacterial species abundance and distribution at each sampled batch, fresh potatoes, and *tocosh* from 1- and 8-months storage wells. Species accounting for more than 0.1% are represented.

Table 2
Microbiological analysis of fresh potatoes, and *tocosh* batches from 1- and 8-months storage wells.

	Fresh potatoes	1-month	8-months
Total mesophilic counts	4.3×10^6	5.8×10^7	1.2×10^8
LAB (MRS)	7.4×10^2	2.1×10^5	5.5×10^7
LAB (YGLP)	1.1×10^3	1.7×10^5	1.1×10^7
LAB (MRS-M)	1.6×10^2	2.5×10^5	5.7×10^6
LAB (MRS-S)	1.5×10^2	6.2×10^4	4.2×10^6
Total yeast and molds	7.1×10^3	2.7×10^3	1.0×10^2
pH	5.5	3.8	4.2

Table 3
Recovery of LAB isolates from fresh potatoes, and *tocosh* batches from 1- and 8-months storage wells.

Species identification	Storage well (months)			Total
	0	1	8	
Number of isolates	41	46	64	151
Lactobacillus				
Lb. brevis			1	1
Lb. casei			16	16
Lb. fermentum			1	1
Lb. sakei	10	19	44	73
Lb. farciminis			1	1
Leuconostoc				
Lc. mesenteroides	31	27	1	59

farciminis (1), *Lb. fermentum* (1), *Lb. sakei* (28), *Ln. mesenteroides* (22), that were screened for several relevant functional and safety features (Table 4). Among functional properties, amylolytic activity was only detected in *Ln. mesenteroides* (23%) and *Lb. sakei* (7%) strains, whereas noticeable phytate-degrading activity (>100 U/ml) was found among strains belonging to all three species. As expected, EPS producing strains belonged to *Ln. mesenteroides* (100%) and *Lb. sakei* (18%). Furthermore, all *Lb. sakei* produce folates and almost half of them above 70 ng/ml, whereas *Ln. mesenteroides* mainly produce riboflavin; 32% of them were above 100 ng/ml. On the other hand, safety traits indicated the production of tyramine mainly for lactobacilli, particularly *Lb. sakei* (50%) and *Lb. casei* (30%). When antimicrobial activity was screened, *Ln. mesenteroides* (9%) and *Lb. sakei* (11%) showed anti-*Listeria* activity, whereas inhibition against *Bacillus* was variously distributed among the three LAB species, these two indicator strains representing industrial environment contaminants (Table 4). The antibacterial compounds were active after treatment with proteinase K evidencing their proteinaceous nature (data not shown).

4. Discussion

To provide a global overview of the microbial communities of *tocosh,* and particularly LAB populations, both culture-dependent and -independent approaches were applied in order to disclose the LAB species and to recover isolates to further be tested for relevant biotechnological abilities. Culture methods showed that LAB were present in 8-months storage *tocosh* at somewhat lower level (10^6–10^7 CFU/g) than other traditionally fermented products such as African and Andean amylaceous foods/beverages (Elizaquível et al., 2015; Sekwati-Monang & Gänzle, 2011). In fresh potatoes, LAB were a small part of the autochthonous microbiota (10^2–10^4 CFU/g) in coincidence with results reported by Di Cagno, Coda, De Angelis, & Gobbetti, 2013, for raw vegetables and fruits. HTS analysis provided a snapshot of bacterial populations revealing a noticeable presence of *Clostridium* surpassing LAB in 1- and 8-months samples. Both approaches evidenced a higher LAB species diversity at 1- and 8-months samples than in fresh potatoes, which might be explained by changes in nutrients under fermentation in the wells. Potato tubercles contain about 18–20% carbohydrates of which 16% is starch (Burlingame, Mouillé, & Charrondière, 2009) and therefore bacterial growth depends on starch degradation. Most of LAB lack amylolytic activity; which is in agreement with the infrequent occurrence of amylase genes in lactobacilli (Gänzle, 2014). However, clostridia include saccharolytic species, which might have contributed to provide low-molecular weight malto-oligosaccharides to support non-amylolytic microorganism's growth.

Fresh potatoes were dominated by *Lactobacillus*, in coincidence with that previously described for amylaceous spontaneous fermentations (De Vuyst et al., 2014). *Leuconostoc*, represented a minor proportion in *tocosh* samples this being in accordance to starchy flours and beverages fermentations (Elizaquível et al., 2015; Ruiz-Rodríguez et al., 2016). At species level, HTS approach showed the dominance of *Lb. sakei* followed by *Ln. gasicomitatum*, *Ln. gelidum* and *Ln. mesenteroides,* these species being also described during kimchi fermentation (Jung, Lee, & Jeon, 2014).

One-month sample showed a bloom of *Clostridium* of which *C. acetobutylicum* and *C. tyrobutyricum* constituted major species. None of the clostridia species detected in *tocosh* is pathogenic to humans or produce toxins; therefore, they do not compromise

Table 4
Functional and safety traits of LAB isolates representing the majority species recovered from *tocosh.*

	Ln. mesenteroides (22 strains[a])	*Lb. sakei* (28 strains[a])	*Lb. casei* (9 strains[a])
Functional traits			
Amylolytic activity	5 (23%)	2 (7%)	–
Phytate-degrading activity[b]	9 (41%)	5 (18%)	2 (22%)
EPS production from sucrose	22 (100%)	5 (18%)	–
Vitamins production			
Folates (B9)	6 (27%) (77–102 ng/ml)	12 (43%) (72–100 ng/ml)	–
Riboflavin (B2)	9 (41%) (106–465 ng/ml)	2 (7%) (113–127 ng/ml)	–
Food safety-related traits			
Tyramine production	2 (9%)	14 (50%)	3 (30%)
Antimicrobial activity against:			
L. innocua	2 (9%)	3 (11%)	–
B. subtilis	6 (27%)	7 (25%)	3 (33%)

[a] Strains recovered from different samples and differentiated by RAPD profiles.
[b] Positive >100 U/ml.

tocosh safety. Clostridia are ubiquitous in soil and its presence in *tocosh* correlates with the anaerobic, low temperature and carbohydrates rich environment (Wiegel, Tanner, & Rainey, 2006). Their saccharolytic metabolism would probably have conferred a competitive advantage to the LAB population. Results showed similar proportions of lactobacilli and leuconostoc at this *tocosh* sample; even in a decreased proportion *Lb. sakei* was present together with *Lb. plantarum*, *Lb. oligofermentans* and *Lb. senmaizukei* in agreement with their plant origin as previously described (Hiraga, Ueno, Sukontasing, Tanasupawat, & Oda, 2008; Elizaquível et al., 2015). Among leuconostoc, *Ln. gasocomitatum* dominated at this fermentation stage. Moreover, *Weissella viridescens* was also detected by HTS approach; its presence during amylaceous fermentations has been previously described (De Vuyst et al., 2014; Elizaquível et al., 2015).

Eight-months sample was dominated by *Lactobacillus* with *Lb. casei, Lb. backii* and *Lb. senmaizukei* as major species, this being in coincidence to that described from African cereal doughs, orchardgrass silage and Japonese pickle, (Hiraga et al., 2008). *Lb. sakei* was found in lesser proportion as well as *Lb. rossiae*, *Lb. plantarum, Lb. kisonensis*, *Lb. yonginensis*, *Lb. parabrevis*, *Lb. perolens*, *Lb. harbinensis* and *Lb. coryniformis* were, commonly found in amylaceous raw materials such as cereal flours, sourdoughs and traditional Asiatic fermented vegetables (De Vuyst et al., 2014). Moreover, although detected in minor proportion, the presence of *Ln. mesenteroides, W. viridescens* and *P. parvulus* were also reported from other vegetable fermentations (De Vuyst et al., 2014.)

Discrepancies between both approaches were found in *Lb. sakei*, which dominated in fresh potatoes by HTS, while by culturing was majority in 8-months sample. Similarly, *Ln. mesenteroides* dominated in fresh potatoes while was equally present in the three samples by HTS. *Lb. farciminis* and *Lb. brevis* were detected by culturing despite of their sequences accounted for less than 0.1% and therefore, they were not considered in the HTS analysis. These results confirm that, both methods are still fundamental and complementary in order to gain satisfactory pictures of bacterial ecology in foodstuffs and to fully explore LAB biotechnological potential.

LAB features exploited for functional, safe and healthy foods design have been demonstrated to be strain-dependent more than a species character (Capozzi et al., 2011a). Several *Ln. mesenteroides* and *Lb. sakei tocosh* isolates exhibited extracellular amylase and EPS production, in agreement with the activities described for isolates of these species from tubers and cereals (Reddy, Altaf, Naveena, Venkateshwar, & Kumar, 2008) and the amylase secretion recently described for *Ln. mesenteroides* during cassava fermentation (Ramos et al., 2015); and their ability to synthesize α-glucan polymers (Badel, Bernardi, & Michaud, 2011). Phytate-degrading activity was highly distributed among major species recovered from *tocosh* as described for *Ln. mesenteroides* and *Lb. sakei* from kimchi and sourdough (Oh & In, 2009) and from some probiotic LAB and bifidobacteria (González-Córdova et al., 2016; Khodaii, Natanzi, Naseri, Goudarzvand, & Dodsons, 2013). Phytates are considered antinutrients due to the affinity of phytic acid for food components positively charged such as minerals and proteins, thus phytate-degrading LAB will improve nutritional and quality characteristics of fermented foods. Furthermore, many of the *tocosh* isolates produced group B vitamins and therefore would be suitable for industrial applications in bio-enriched products as it has been proposed for other LAB species (Capozzi et al., 2011a, Capozzi, Russo, Dueñas López, & Spano, 2012; Juarez del Valle et al., 2014). From a safety point of view, LAB can negatively affect food safety by amino acid-decarboxylase activity (Capozzi et al., 2011b). Among *tocosh* isolates production of tyramine was mostly found in *Lb. sakei* strains in accordance with strains of meat origin (Suzzi & Gardini, 2003); aminogenic potential of *Ln. mesenteroides* and *Lb. casei* was also described from wine and cider (Coton et al., 2010). On the other hand, LAB are widely known for their ability to inhibit food pathogens by the production of antimicrobial compounds such as organic acids, oxygen peroxide and bacteriocins, constituting a sustainable alternative as food preservatives. The persistence of antibacterial activity when supernatants of *Ln. mesenteroides*, *Lb. sakei* and *Lb. casei* were treated with proteinase K indicated that a proteinaceous compound i.e. a bacteriocin, could be responsible for *L. monocytogenes* and *B. subtilis* inhibition, as previously described (Fontana et al., 2015). In addition, a high proportion of strains recovered from *tocosh* exhibit antifungal activity and fulfil the EFSA requeriments as it was already described (Russo, Spano, & Capozzi, 2017; Yépez et al., 2017); they are sensitive to antibiotics or exhibit single resistances, therefore a number of them being suitable for food applications.

5. Conclusions

To the best of our knowledge, this is the first study focusing on the LAB community structure of the Andean traditional *tocosh* by using culture-dependent and HTS approaches. Species identified from the analysed samples and their abundances clearly reflected the particular environmental conditions encompassing the fermentation process. The microbial profile revealed *Lb. sakei* and *Ln. mesenteroides* as the main LAB species occurring during fermentation. Based on their functional and safety characterization, strains with valuable features for further design of functional starchy fermented foods were selected. Maintenance and promotion of indigenous Andean culture is crucial for ensuring protection of traditional agroecological systems and agrobiodiversity.

Acknowledgements

The research leading to these results has received funding from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme (FP7/2007–2013) under REA grant agreement no. 247650, and grants PICT 2010 0655 (ANPCyT) from Argentina, CSD 2007-00063 from the Spanish Ministry of Science and Innovation and PROMETEO/2012/040 from the Generalitat Valenciana. AYL is the recipient of a PhD grant from the Spanish Ministry of Education, Culture and Sports (FPU13/03398).

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