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Screening and characterization of potential probiotic and starter bacteria for plant fermentations

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ABSTRACT

Probiotics are mostly consumed as fermented or fortified food products in Europe. There are two important factors in the selection of probiotic candidates regarding the potential health benefit; their viability and number when consumed, and their survival and persistence in the gastrointestinal tracts. This study focusses on the selection of potential probiotics to be used as starter culture in plant-based fermented foods. Lactic acid bacteria isolated from quinoa and amaranth were tested *in vitro* for their sensitivity to antibiotics, tolerance to gastrointestinal stress factors and adhesion to gut epithelial cells. Only five strains had suitable antibiotic profile to be used as probiotics and all of them were tolerant to lysozyme, bile salts, and had similar adhesion capacity. *Lactobacillus plantarum* Q823, administered as starter culture in a fermented quinoa drink, was selected for the human *in vivo* tests, because of its best *in vitro* tolerance to low pH. This strain was able to survive and persist at detectable levels (5–7 Log_{10} CFU/g feces) in the gastrointestinal tracts for at least seven days after the end of administration. Thus, *L. plantarum* Q823 has been identified as a suitable starter and a potential probiotic in fermented quinoa-based products.

1. Introduction

Probiotics are defined as living microorganisms which, when administered in sufficient numbers, confer a health benefits on the host (FAO/WHO, 2002). Probiotics are associated with the prevention or alleviation of several intestinal disorders such as antibiotic-associated diarrhea, irritable bowel disease, colon cancer, lactose intolerance, food allergies and more (Alander et al., 1999; Rijkers et al., 2010). These beneficial effects are related to one or more mechanisms, such as modulation of the intestinal microbiota, blockage of pathogen adhesion sites, modulation of the host immune responses and competition for nutrients (De Champs, Maroncle, Balestrino, Rich, & Forestier, 2003). Regardless of the mechanisms, probiotic bacteria should be able to survive the passage through the gastrointestinal (GI) tracts in order to provide health benefits, as well as persist in sufficient numbers in the gut to exclude pathogens and interact with the host epithelial and immune cells (Balgir, Kaur, Kaur, Daroch, & Kaur, 2013; Jacobsen et al., 1999; Sathyabama, Vijayabharathi, Devi, Kumar, & Priyadarisini, 2012).

Probiotic microorganisms can be introduced through consumption of fermented foods, as fortified food products or as pharmaceuticals. In Europe, because of the general attitude against medication, probiotics are mostly consumed components in food products, mainly as fermented milks (Yerlikaya, 2014). However, several factors have to be considered when using probiotics in fermented products, in particular, their viability and presence in high numbers at the time of consumption (Muller et al., 2013; Vinderola, Binetti, Burns, & Reinheimer, 2011). The strains mostly used as probiotics represent lactic acid bacteria (LAB) and bifidobacteria (Sathyabama et al., 2012; Vinderola et al., 2011). The recommended effective dose should be higher than 100 million CFU/

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dose. The numbers currently found in probiotics products and the numbers associated with significant outcomes in clinical trials are in the range of 1–10 billion CFU/dose (Naidu, Adam, & Govender, 2012; Reid, 2006; WGO, 2008).

Increasing rates in milk allergy and lactose intolerance, and the high content of saturated fatty acids are the major drawbacks associated with the consumption of dairy foods, including probiotic dairy products. Consequently, food industry is currently very interested in producing fermented or functional foods other than traditional milk-based products (Gupta & Abu-Ghannam, 2012; Rivera-Espinoza & Gallardo-Navarro, 2010). Also modern consumers are increasingly interested in the consumption of functional foods based on cereals, fruits and vegetables, because of their perceived beneficial nutritional values. Consequently, probiotic food products made from plant materials are nowadays in the focus of functional food product development (Mridula & Sharma, 2015).

In our search for novel plant materials suitable as functional foods quinoa (*Chenopodium quinoa* Willd) and amaranth (*Amaranthus* spp.), two Andean traditional crops with high nutritional values (Nascimento et al., 2014), were selected in this study, because these gluten-free crops could be used as a food matrix for new types of probiotic products.

Traditionally, most probiotic bacteria are of intestinal origin. However, probiotics used in plant-based fermented products should preferentially be isolated from plant materials, because plant-derived substrates present significant technological and physiological challenges to which strains of intestinal origin might not be well adapted (Gupta & Abu-Ghannam, 2012; Rivera-Espinoza & Gallardo-Navarro, 2010). However, for probiotic action, it is essential that plant-based potential probiotics are screened for their survival and persistence in the GI tracts.

There are several *in vitro* assays that are commonly used to check the intestinal survival and persistence of a potential probiotic strain based on exposing it to simulated gastrointestinal stress factors (low pH, digestive enzymes, bile acids) and checking its adhesion capacity to cultured gut epithelial cells or isolated mucus (Papadimitriou et al., 2015; Tuomola, Crittenden, Playne, Isolauri, & Salminen, 2001). Although these tests are important for a preliminary selection of potential probiotic strains, *in vivo* trials with the probiotic are needed in order to make a credible claim on the survival and persistence (De Champs et al., 2003; Oozeer et al., 2006). The aim of this study was to find LAB with potential probiotic properties from plant origin to be used as starter cultures in plant fermented food products. Therefore, indigenous isolated LAB were tested *in vitro* and *in vivo* to assess their ability to cope with the intestinal challenges and temporarily colonize the human gut.

2. Material and methods

2.1. Microorganisms and growth conditions

The LAB strains used in this study were isolated from different quinoa varieties and amaranth seeds obtained from INTA (Instituto Nacional de Tecnología Agropecuaria), Hornillos (Jujuy, Argentina) (Ruiz Rodriguez et al., in press). Seeds (25 g) were suspended in 225 ml MRS broth (LabM, UK) supplemented with cycloheximide (0.1 g/L) (Sigma–Aldrich, Germany) and incubated at 30 °C during 24 h. Then, decimal dilutions were prepared and plated on MRS-5 agar (Meroth, Walter, Hertel, Brandt, & Hammes, 2003) incubated at 30 °C for 48 h, aerobically and under anaerobiosis (AnaeroGen and AnaeroJar, Oxoid, UK) for LAB isolation. Gram-positive and catalase negative (determined by transferring fresh colonies from a Petri dish to a glass slide and adding H_2O_2 5%, v/v) were considered as presumptive LAB and further purified by successive subculturing (2–3 times) in MRS-5 broth, harvested and stored in MRS-5 containing 20% of glycerol at −80 °C for further experiments. The isolated strains were deposited at CERELA Culture Collection (Tucumán, Argentina).

For the fingerprinting of the strains, LAB isolates (212 from quinoa varieties and 32 from amaranth seeds) were subjected to RAPD-PCR analysis using primers RAPD2 (5′-AGCAGCGTCG-3′) and M13 (5′-GAGGGTGGCGGTTCT-3′) in separate reactions. The PCR conditions used for amplification experiments are those reported by Fontana, Cocconcelli, and Vignolo (2005). DNA extraction from isolates and reference strains (*Lactobacillus sakei* CRL1463, *Lactobacillus curvatus* CRL1465 and *Lactobacillus plantarum* CRL1481 from CERELA, as well as *Lactobacillus paracasei* UC8808, *Leuconostoc lactis* UC8020, *Leuconostoc mesenteroides* UC8232 from UCSC culture collections) was performed using Microlysis (Labogen, UK).

A total of 18 isolated strains were selected for further studies based on the genomic profiling. Taxonomical identification was carried out by species-specific PCR and by sequencing the 16S rDNA genes. Primer sequences, conditions and references are shown in Table 1. The sequences of the 16S rDNA gene amplicons have been deposited in the Gen Bank database under accession numbers listed in Table 2. Two commercial probiotic strains were used as controls in various *in vitro* assays; *Lactobacillus rhamnosus* GG (LGG) and *L. rhamnosus* LC705 (LC705). The strains and their source of isolation are listed in Table 2. All strains were routinely grown in MRS agar or broth (Oxoid, Italy) at 30 °C for 24–48 h.

2.2. Antibiotic resistance

The minimum inhibitory concentrations (MICs) of nine antibiotics (gentamicin, kanamycin, streptomycin, erythromycin, chloramphenicol, tetracycline, ampicillin, clindamycin and vancomycin) were determined for all strains according to ISO 10932:2010 standard. Epidemiological break point values were based on the recommendation of the committee on Antimicrobial Susceptibility Testing (EUCAST) and EFSA Panel on Additives and Products or Substance used in Animal Feeding (FEEDAP).

2.3. In vitro screening of probiotic properties

2.3.1. The potential to survive the passage through the gastrointestinal tracts

The resistance of the strains to some host defense mechanisms, namely lysozyme, bile and low pH, was determined for the isolates and controls. The resistance of the strains to lysozyme was tested as described by Kimoto-Nira, Suzuki, Kobayashi, and Mizumachi (2008). Briefly, overnight cultures of the strains grown in MRS broth were harvested and washed. One mL of cells was suspended in phosphate-buffering saline (PBS) supplemented with 100 μg/mL of lysozyme (Sigma–Aldrich) for 1 h. The degree of cell lysis was measured spectrophotometrically by calculating the change in absorbance at OD 620 nm.

The tolerance to bile and low pH was determined by standard plating of broth cultures onto MRS plates after different exposure times. For bile tolerance, 1% of overnight cultures of the bacterial strains were inoculated into MRS broth supplemented with 0.3% oxgall (Sigma–Aldrich) and incubated at 37 °C for 4 and 24 h pH tolerance was determined by adjusting the MRS broth to pH 2.5 (with 10 M HCl), inoculating 1% of the overnight culture cells and incubated for 1, 2 and 4 h. After the bile or pH challenge, the cells were plated on MRS plates. The plates were incubated for 48 h at 30 °C. All tests were done in duplicates.

2.3.2. Adhesion capacity to a human epithelial cells

The capacity of the isolates to adhere to a human epithelial colorectal adenocarcinoma cell-line, Caco-2 (ATTC HTB-37), was

Table 1
Sequence and amplicon size (bp) of the primers used for the strain identification.

Target	Primer	Primer sequence (5′-3′)	Amplicon size (bp)[a]	Reference
16S rRNA	P0	GAGAGTTTGATCCTGGCTCAG	1500	Di Cello, Pepi, Baldi, & Fani, 1997
	P6	CTACGGCTACCTTGTTACGA		
Lb. plantarum	Lp1f	AATTGAGGCAGCTGGCCA	250	Quere, Deschamps, & Urdaci, 1997
	Lp2r	GATTACGGAGTCCAAGC		
Leuconostoc lactis	Llacf	AGGCGGCTTACTGGACAAC	742	Lee, Park, & Kim, 2000
	Llac-r	CTTAGACGGCTCCTTCCAT		
Leuconostoc mesenteroides	Lmes-f	AACTTAGTGTCGCATGAC	1150	Lee et al., 2000
	Lmes-r	AGTCGAGTTACAGACTACAA		

Table 2
Lactic acid bacteria strains, source of isolation and accession numbers.

Microorganism	Strain	Source of isolation	Accession numbers
Lactobacillus casei/paracasei	Q11	Quinoa – Inca Pirce	KT601499
Leuconostoc lactis	Q127	Quinoa – Inca Pirce	*ns*
Lactobacillus reuteri	Q221	Quinoa – Chilena	KT601498
Lactobacillus sakei	Q41	Quinoa – Puno	KT601495
Leuconostoc lactis	Q61	Quinoa – Nerino Pesto	*ns*
Leuconostoc lactis	Q64	Quinoa – Nerino Pesto	*ns*
Leuconostoc lactis	Q615	Quinoa – Nerino Pesto	*ns*
Lactobacillus reuteri	Q722	Quinoa – CICA Hornillo	KT601497
Lactobacillus sakei	Q82	Quinoa - A26	KT601496
Lactobacillus plantarum	Q823	Quinoa - A26	*ns*
Lactobacillus plantarum	Q825	Quinoa - A26	*ns*
Lactobacillus plantarum	Q8212	Quinoa - A26	*ns*
Lactobacillus casei/paracasei	A1220	Quinoa – Kiwicha	KT601500
Leuconostoc lactis	A125	Amaranth - Kiwicha	*ns*
Leuconostoc lactis	A1210	Amaranth - Kiwicha	*ns*
Leuconostoc mesenteroides subsp. mesenteroides	CRL1901/60	Quinoa YY sourdough	*ns*
Pediococcus pentosaceus	CRL1902/68	Amaranth sourdough	KJ402413
Lactobacillus brevis	123	Quinoa RH sourdough	KF545926
Lactobacillus rhamnosus	GG	*control*	–
Lactobacillus rhamnosus	LC705	*control*	–

ns, not sequenced. Identification based on species-specific primers (Table 1).

investigated. Caco-2 cells were grown in 75 cm^3 cell culture bottles (Sarstedt, Inc., Newton, NC, USA) using Dulbecco's modified Eagle's minimal essential medium (DMEM) (EuroClone, Siziano, Italy) supplemented with 10% (v/v) heat inactivated fetal bovine serum, 2 mM L-glutamine, 1% (v/v) non-essential amino acids 100 IU penicillin/mL and 100 μg streptomycin/mL (all supplements from Sigma–Aldrich). The culture medium was replaced every 2–3 days. Caco-2 cells were subsequently seeded to 24-well culture plates at a concentration of 2.5×10^5 cells per well. Cells were differentiated for 2 weeks, changing medium every 2–3 days. Cells were always incubated at 37 °C in a 5% CO_2-enriched atmosphere.

Bacterial strains were grown overnight at 37 °C in MRS broth and washed with PBS. Bacterial strains diluted in DMEM to a concentration of 1×10^8 CFU/mL, were added to each well and incubated for 2 h. After incubation, the cells were washed four times and lysed with 0.1% Triton X-100 (Sigma–Aldrich). Cell lysates were serially diluted and plated in duplicates on MRS agar plates. Plates were then incubated at 37 °C for 2 days and the colony forming units counted. The adhesion capacity of the strains is calculated as percentage of the bacteria counted from the cell lysates of the total bacteria added to the well. Five biological repeats made in different days and three subsamples for each biological repeat were used for this test.

2.4. *In vivo* colonization by a potential probiotic strain

L. plantarum Q823 was chosen for the *in vivo* colonization human trial based on the results of the *in vitro* tests described above.

2.4.1. Preparation of the quinoa-based beverage with the potential probiotic

The mode of administration of the potential probiotic bacteria was through the consumption of a quinoa-based beverage fermented with *L. plantarum* Q823 and prepared as reported by Ludena et al. (unpublished). Briefly, 15% (w/w) of quinoa flour was mixed with water and gelatinized at 80 °C for 10 min in a water bath with continuous mixing. After cooling down, 1% of *L. plantarum* Q823-rifampicin resistant mutant (prepared as described by Plumed-Ferrer, Kivela, Hyvonen, & von Wright, 2005) was inoculated into the quinoa-based beverage and incubated for 8 h at 30 °C. After fermentation, the beverage was stored at 4 °C for 7 days. The viability of the *L. plantarum* Q823 was evaluated on day 1, 2, 5 and 7 by serial dilutions and plating onto MRS agar plates supplemented with 100 μg/mL of rifampicin (Sigma–Aldrich). The pH of the beverage was also monitored during the storage period. Additionally, the microbiological safety of the product was monitored by plating a sample of the beverage on blood agar plates and violet red bile glucose (VRBG) agar plates in order to detect fastidious and pathogenic bacteria, *Bacillus* spores and coliform bacteria.

2.4.2. Feeding trial and sample collection

A total of seven healthy female volunteers with ages ranging from 21 to 38 years and consuming a standard mixed western diet were included in the trial. No antibiotic had been taken by any participant during the 2 weeks before the study or during the intervention. No other probiotic products or fermented milks and yogurts were allowed during the test period. Volunteers were

asked to report any adverse side effects they might feel during the experimental trial and washout period.

Twenty mL of the quinoa-based beverage containing 9.19 Log_{10} CFU/mL of *L. plantarum* Q823 was given daily during a period of 7 days. Fecal samples from the participants were collected, if possible, once a day during the intake period (7 days) and for the subsequent (washout) period for another 7 days. Fecal samples were homogenized in peptone saline solution, serially diluted and plated into MRS plates containing 100 μg/mL of rifampicin. Plates were incubated at 30 °C for 48 h.

In order to confirm that the bacteria in the MRS-rifampicin plates were *L. plantarum* Q823, sixteen randomly selected colonies from different plates were selected and their genomic fingerprints were compared with that from Q823 as performed by Plumed-Ferrer et al., (2013). Briefly, total genomic DNA from overnight cultures of each selected colony was isolated using a DNA extraction kit (NucleoSpin Tissue, Macherey–Nagel, Germany). Randomly amplified polymorphic DNA (RAPD-PCR) analysis was carried out using primer P16 (5′TCGCCAGCCA-3′). PCR reaction was performed in 25-μl total volume containing 1 unit of GoTaq DNA polymerase (Promega), 10 pmol of primer, 200 μM of each dNTP, 3 mM $MgCl_2$ and 50 ng of genomic DNA. PCR amplifications were performed as follows: initial denaturation at 94 °C for 5 min; 30 cycles of 94 °C for 30 s, 24 °C for 2 min and 72 °C for 2 min; final extension at 72 °C for 5 min. Amplification products were electrophoretically separated in 1% agarose gels containing SYBR safe DNA gel staining (Life Technologies Ltd, UK). Gels were visualized and photographed using the Gel Doc UV transilluminator 2000 (Bio-Rad Laboratories Inc., USA).

2.5. Statistical analysis

The results were evaluated using analysis of variance (ANOVA) and Tukey test, both considering a significance level of $P < 0.05$. The analysis were performed with GraphPad Prism 6 software (GraphPad Software, Inc).

2.6. Ethics

The protocol for the colonization trial was approved by the Regional Health Authority Ethics Committee. Moreover, written consent from all participants was obtained before the colonization trial.

3. Results

3.1. Bacterial identification

Eighteen LAB isolates were successfully identified by sequencing the 16S rRNA genes. The isolates were mostly lactobacilli including species of *Lactobacillus casei, Lactobacillus reuteri, L. sakei, L. plantarum* and *Lactobacillus brevis*, *L. lactis* and *Pediococcus pentosaceus* (Table 2).

3.2. Antibiotic resistance

The antimicrobial susceptibilities of the isolates are shown in Table 3. Due to the internal variability of the method MICs within one dilution step of the break point value are considered to be within the normal fluctuation of the test and thus acceptable. From eighteen strains, eleven were resistant (having MICs at least four times higher than the break point value) to one or more antibiotics. Particularly, *L. lactis* strain was resistant to gentamycin, kanamycin, streptomycin, erythromycin and clindamycin. *L. casei* A1220 showed resistance to kanamycin and clindamycin, and *L. brevis* 123 to ampicillin and clindamycin. Strains L. *reuteri* Q221, *L. sakei* Q41 and *L. plantarum* Q8212 had single resistance to gentamycin, streptomycin and kanamycin, respectively. Seven strains show no antibiotic resistance and thus were safe to use as potential probiotics and/or starter cultures.

3.3. In vitro screening of probiotic properties

A total of five isolates were selected and examined for their potential as probiotic bacteria after showing no resistance to any antibiotic (i.e. Q11, Q615, Q82, Q823, and Q825). Strains Q722 and CRL1 902/68 were excluded for showing limited growth in quinoa fermentations (data not shown).

All isolates showed good tolerance to lysozyme with survival ratios similar to the control strains, LGG and LC705 (Table 4). All isolates also showed good tolerance to bile after 4 and 24 h, Q11 and the control LC705 being the least tolerant (Table 4). Strains Q82, Q11 and Q615 were sensitive to low pH during the 4 h exposure, Q82 showing no survival already after 1 h and Q11 marginal survival after 2 h (Table 4).

All selected strains had a good adhesion capacity to Caco-2 cells. Strain Q82 was not tested due to its lack of survival at low pH. In comparison to the positive control LGG, Q11 showed better and Q823 and Q825 nearly equal adhesion properties (Fig 1).

3.4. In vivo colonization of a potential probiotic strain

Based on the antibiotic resistance profile, *in vitro* tolerance to the GI-tracts stress conditions and adhesion capacity, strain *L. plantarum* Q823 was chosen for the *in vivo* colonization experiment.

The potential probiotic bacteria (*L. plantarum* Q823) was used to prepare a quinoa-based fermented beverage that was subsequently stored for 7 days. After fermentation, *L. plantarum* Q823 counts were 10.4 Log_{10} CFU/mL and the pH of the product was 4.15. During the storage time, pH decreased progressively to 3.85 although *L. plantarum* counts remained almost constant (Table 5). The quinoa-based beverage was also tested for safety aspects and no growth of any fastidious or pathogenic bacteria was detected.

The consumption of *L. plantarum* Q823 was monitored, and its survival through the GI-tracts is shown in Table 5. An average consumption of 10.46 Log_{10} CFU/mL of strain Q823 (20 mL of quinoa-based beverage containing 9.19 Log_{10} CFU/mL) daily for 7 days resulted in fecal counts of 5–7 Log_{10} CFU/g from the third day onwards. The counts started to decrease after the final dose. *L. plantarum* Q823 was still present in all subjects four days after the end of administration and was still present after 7 days in three of the subjects (Table 5).

All the selected 16 colonies from MRS-rifampicin plates obtained after culturing the fecal samples had identical RAPD fingerprints with that of *L. plantarum* Q823 control, confirming the identity of the fecal isolates (data not shown).

4. Discussion

The aim of this study was the selection of LAB with a dual function: as probiotic bacteria and as starter for plant-based fermented food products.

The total number of isolated LAB strains from quinoa and amaranth samples was surprisingly small according to our experience with other plant-based materials. Only 18 different strains were obtained after the cultivation of several samples. One possible reason for this could be the presence of saponins (naturally occurring toxic glycosides) in both crops. Saponins have been reported to be natural antimicrobial compounds and part of the

Table 3
The antimicrobial susceptibility testing of all isolates (in μg/mL). MICs indicating resistance (more than twice the break point value) in bold.

Genus/Species	Strain		AMP	VAN	GEN	KAN	STR	ERY	CLI	TET	CHL
L. casei/paracasei		*Break point*	*4*	*nr*	*32*	*64*	*64*	*1*	*1*	*4*	*4*
	Q11	MIC	2	>128	8	128	34	0.5	0.064	1	4
	A1220		2	>128	32	**256**	128	0.5	**>16**	1	8
Leuconostoc		*Break point*	*2*	*nr*	*16*	*16*	*64*	*1*	*1*	*8*	*4*
	Q127	MIC	1	2	**128**	**256**	**>256**	**>8**	**>16**	0.5	8
	Q61		1	1	**64**	**256**	**256**	**8**	0.5	0.25	4
	Q64		2	1	**128**	**512**	**>256**	**8**	0.5	0.25	8
	Q615		0.25	2	<0.5	2	16	1	0.25	<0.125	2
	A125		2	1	32	**256**	**256**	0.5	**>16**	0.25	4
	A1210		1	>128	16	**256**	64	0.25	0.125	1	4
	CRL1901/60		2	>128	4	**512**	64	0.5	0.064	8	8
L. reuteri		*Break point*	*2*	*nr*	*8*	*64*	*64*	*1*	*1*	*16*	*4*
	Q221	MIC	2	1	**32**	128	64	0.25	0.25	2	4
	Q722		1	>128	4	128	32	0.5	0.125	8	4
L. sakei		*Break point*	*4*	*nr*	*16*	*64*	*64*	*1*	*1*	*8*	*4*
	Q41	MIC	8	>128	32	128	**256**	1	1	4	4
	Q82		4	>128	8	32	64	0.25	<0.032	2	2
L. plantarum		*Break point*	*2*	*nr*	*16*	*64*	*nr*	*1*	*2*	*32*	*8*
	Q823	MIC	1	>128	4	128	32	0.5	1	32	4
	Q825		1	>128	8	128	64	0.5	1	16	8
	Q8212		1	>128	8	**256**	64	1	2	16	8
Pediococcus		*Break point*	*4*	*nr*	*16*	*64*	*64*	*1*	*1*	*8*	*4*
	CRL1902/68	MIC	4	>128	16	128	128	1	0.032	16	4
L. brevis		*Break point*	*2*	*nr*	*16*	*32*	*64*	*1*	*1*	*8*	*4*
	123	MIC	**8**	>128	2	32	32	0.5	**>16**	8	4

nr, not required.

Table 4
Lysozyme, bile and pH tolerance of the isolated lactic acid bacteria.

Strain	Lysozyme tolerance	Bile tolerance			pH tolerance			
	Survival score (%)	*Log_{10}CFU/mL*			*Log_{10}CFU/mL*			
	1 h	0 h	4 h	24 h	0 h	1 h	2 h	4 h
Q11	78.03^{b}	7.11^{a}	6.48^{a}	5.32^{c}	7.22^{a}	4.20^{c}	1.54^{d}	1.79^{b}
Q615	94.00^{a}	7.30^{a}	7.58^{b}	7.87^{a}	7.23^{a}	6.14^{b}	4.03^{c}	2.86^{c}
Q82	$83.04a^{b}$	7.29^{a}	5.49^{c}	5.83^{bc}	7.35^{a}	$<1^{d}$	$<1^{e}$	$<1^{d}$
Q823	$84.63a^{b}$	7.29^{a}	5.22^{c}	7.39^{a}	7.33^{a}	7.16^{ab}	7.23^{a}	6.83^{a}
Q825	$89.58a^{b}$	7.39^{a}	5.29^{c}	7.39^{a}	7.36^{a}	6.90^{ab}	6.52^{ab}	5.75^{e}
GG	78.09^{b}	7.11^{a}	6.32^{a}	6.27^{b}	7.20^{a}	7.50^{a}	7.44^{a}	7.29^{a}
LC705	94.62^{a}	6.87^{a}	1.94^{d}	1.30^{d}	6.96^{a}	6.00^{b}	5.45^{b}	5.13^{f}

The results sharing the superscript letter (within each column) are not significantly different ($P < 0.05$).

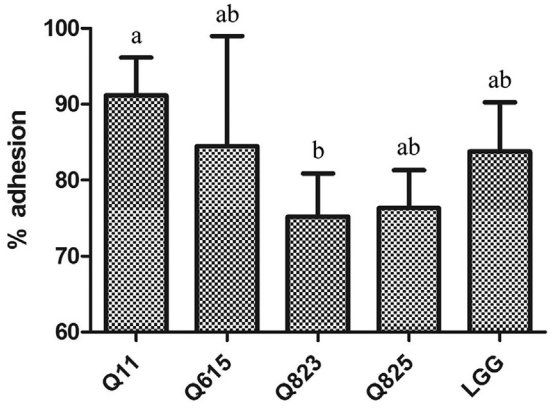

Fig. 1. The adhesion of the selected lactic acid bacteria strains to Caco-2 cells. Five biological replicates were used for this test. The results sharing the superscript letter are not significantly different ($P < 0.05$).

defense mechanisms of many plants (Hassan, Byrd, Cartwright, & Bailey, 2010; Miranda et al., 2014). From the 18 isolates, eleven were excluded as they were resistant to one or more of the antibiotics according to the EFSA criteria.

The *in vitro* screening tests were performed on five strains having a suitable antibiotic resistance profile. The final selection of *L. plantarum* Q823 was basically based on pH resistance since all five strains were tolerant to lysozyme, bile salts and had good adhesion to epithelial cells. Only the control *L. rhamnosus* GG was more resistant to low pH than strain Q823. Strains of plant origin (environmental strains) have more genetic diversity than industrial dairy strains (domesticated strains) (Bull et al., 2014; Passerini et al., 2010; Rahman et al., 2014). Because of that and in line with our previous experience in characterizing lactic acid bacteria, it was not surprising that all five strains were tolerant to most of the stress-factors of the GI-tract. Although there are alternative ways to protect the bacteria against intestinal stresses such as encapsulation (Huq, Khan, Khan, Riedl, & Lacroix, 2013), these solutions would not allow the use of the potential probiotics as starter culture on plant-based fermentations.

The *in vivo* consumption of a fermented drink containing *L. plantarum* Q823 was performed in order to verify that this strain is able to survive during the passage through the human GI-tracts and to evaluate its persistence after the discontinuation of its administration. The strain was administered in a quinoa-based beverage. *L. plantarum* Q823 was able to successfully grow in this

Table 5
Detection of *Lactobacillus plantarum* Q823 in the human faces of 7 volunteers and in quinoa-based beverage before consumption.

Subject	Log10CFU/mL													
	During the feeding period							After the feeding period						
	D-1	D-2	D-3	D-4	D-5	D-6	D-7	D-8	D-9	D-10	D-11	D-12	D-13	D-14
Dose	*10.4*	*10.5*	*nt*	*nt*	*10.6*	*nt*	*10.4*							
A	$<2^{a}$	3.9^{c}	4.1^{b}	6.5^{b}	5.0^{a}	6.4^{b}	6.7^{d}	*ns*	4.4^{b}	2.9^{a}	*ns*	$<2^{a}$	$<2^{a}$	$<2^{a}$
B	*ns*	$<2^{a}$	2.3^{a}	*ns*	5.8^{b}	6.5^{b}	6.4^{c}	*ns*	3.4^{a}	*ns*	3.3^{b}	2.0^{b}	*ns*	2.3^{b}
C	$<2^{a}$	*ns*	2.3^{a}	6.5^{b}	5.5^{b}	6.3^{b}	6.0^{c}	4.9^{a}	3.4^{a}	*ns*	2.7^{a}	$<2^{a}$	$<2^{a}$	$<2^{a}$
D	3.2^{b}	4.4^{d}	6.9^{e}	*ns*	6.5^{c}	6.2^{b}	5.0^{a}	*ns*	5.1^{c}	*ns*	3.3^{b}	2.8^{c}	$<2^{a}$	$<2^{a}$
E	*ns*	$<2^{a}$	6.2^{f}	5.1^{a}	6.3^{c}	6.5^{b}	5.5^{b}	6.0^{a}	3.4^{a}	2.8^{a}	4.8^{c}	4.8^{e}	4.2^{c}	$<2^{a}$
F	*ns*	2.3^{b}	5.3^{c}	6.7^{b}	7.0^{d}	5.5^{a}	5.4^{b}	5.8^{a}	3.6^{a}	2.7^{a}	4.8^{c}	ns	2.8^{b}	2.8^{c}
G	*ns*	2.3^{b}	5.7^{d}	*ns*	5.2^{a}	*ns*	5.5^{b}	5.0^{a}	6.4^{d}	5.9^{b}	4.6^{c}	3.7^{d}	*ns*	2.8^{c}

nt, not tested.
ns, no sample.
The results sharing the superscript letter (within each column) are not significantly different ($P < 0.05$).

food matrix and reach the dose generally recommended to obtain any potential health effects (Naidu et al., 2012; Reid, 2006; WGO, 2008).

The survival and persistence of *L. plantarum* Q823 proved to be comparable to those of established human probiotics in earlier trials, with recorded survival for at least 5–7 days after the washout period (Alander et al., 1999; De Champs et al., 2003; Jacobsen et al., 1999; Oozeer et al., 2006). Relatively stable numbers of Q823 were recovered in feces during the test period, the counts progressively decreasing during the washout period, being, however, detectable for a week after the last intake.

Although it is not possible to state at this stage that *L. plantarum* Q823 has beneficial health effects on the host, we have demonstrated that it complies with the Qualified Presumption of Safety (QPS) standards of the European Food Safety Authority (Leuschner et al., 2010) and that it can survive and persist in the human GI tracts without any obvious adverse side effects (reported by the volunteers) and that the strain might be suitable for the production of microbiologically safe quinoa-based fermented foods.

5. Conclusion

The global market for probiotic food products keeps growing at a very fast rate. Extensive research is ongoing to develop products in which probiotics could be incorporated other than those already established by the dairy industry. However, the research and technology is still underdeveloped in the area of plant functional food and thus, offers a great opportunity for the development of new products. Further research is also needed in order to screen for different types of probiotic bacteria that have the ability to grow in plant-based products (Rivera-Espinoza & Gallardo-Navarro, 2010).

In this study, strain *L. plantarum* Q823 was successfully identified as a potential probiotic bacteria for its use as a starter culture in the fermentation of a quinoa-based beverage. This strain is able to successfully grow in a quinoa-based product, and survive and colonize the GI-tract of humans. It is proven to be safe and thus, gives the opportunity for its successful use in functional food application in the future. Although extensive research should continue based on the products development technology, evaluation of the shelf-life properties and sensory attributes, as well as the actual health benefits in long-term human clinical trials, this study provides the first steps to follow on the development of new types of functional foods.

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