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Ancestral Andean grain quinoa as source of lactic acid bacteria capable to degrade phytate and produce B-group vitamins

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The lactic acid bacteria (LAB) microbiota of quinoa grains (QG) and spontaneous sourdough (QSS) was evaluated. Different strains of *Lactobacillus (L.) plantarum* (7), *L. rhamnosus* (5), *L. sakei* (1), *Pediococcus (Ped.) pentosaceus* (9), *Leuconostoc (Leuc.) mesenteroides* (1), *Enterococcus (E.) casseliflavus* (2), *E. mundtii* (3), *E. hirae* (1), *E. gallinarum* (12), *Enterococcus* sp. (1), and *E. hermanniensis* (2) were isolated, identified and characterized. Only four strains isolated from QSS and eight strains isolated from QG showed amylolytic activity. *L. plantarum* CRL 1973 and CRL 1970, *L. rhamnosus* CRL 1972 and *L. sakei* CRL 1978 produced elevated concentrations of folate with strain CRL 1973 producing the highest concentration (143 ± 6 ng/ml). *L. rhamnosus*, isolated from QSS, was the LAB species that produced the most elevated concentrations of total riboflavin (>270 ng/ml) with strain CRL 1963 producing the highest amounts (360 ± 10 ng/ml). Phytase activity, evaluated in forty-four LAB strains from quinoa, was predominantly detected in L. *rhamnosus* and Enterococci strains with the highest activities observed in *E. mundtii* CRL 2007 (957 ± 25 U/ml) followed by *E. casseliflavus* CRL 1988 (684 ± 38 U/ml), *Leuc. mesenteroides* CRL 2012 (617 ± 38 U/ml) and L. *rhamnosus* CRL 1983 (606 ± 79 U/ml). In conclusion, this study shows that a diverse LAB microbiota is present in quinoa with important properties; these microorganisms could be used as potential starter cultures to increase the nutritional and functional properties of Andean grains based foods.

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1. Introduction

Cereals are staple foods that form part of the normal human diet and have a significant effect on health, reasons for which they are of important for the food industry (Arendt & Zannini, 2013). While cereal grains and their by-products are known to be good sources of protein and fiber, there is a newfound interest in the use of alternative grains. Quinoa is an ancient grain of Andean origin that was an important food crop for the Incas civilizations that is still used by people of rural regions. Botanically, quinoa belongs to the class Dicotyledoneae, genus *Chenopodium*, family *Chenopodiaceae* and species Quinoa (Bonifacio, 2003). It is mainly grown and cultivated in the Andean region of South America. Its grain is commonly classified as a pseudocereal since it resembles the function and composition of true cereals but they are non-grasses (Alvarez-Jubete, Arendt, & Gallagher, 2010). Research on quinoa has mainly focused on the composition of seeds, protein quality, dietary fiber, as well as its incorporation into food products together with cereal flours (Lamothe, Srichuwong, Reuhs, & Hamaker, 2015). This Andean grain has a high worldwide potential as a crop with excellent nutritional food quality, and is now being recognized as a health-promoting food; reasons for which there is increasing interest in its study (Nascimento et al., 2014; Peñas et al., 2014; Repo-Carrasco-Valencia, Hellstrom, Pihlava, & Mattila, 2010).

Pseudocereals do not contain any prolamins that are toxic for people who suffer from celiac disease so the integration of these grains into gluten-free (GF) diets could be a valuable contribution since they could increase their nutritional and functional properties (Peñas et al., 2014).

Lactic acid bacteria (LAB) have been at the core of microbial fermentation of foods and beverages as far back as 6000 BCE and is one of the oldest and most economically and ecologically friendly methods of producing and preserving foods. LAB reside in a variety of habitats ranging from plants to multiple mammalian niches; their ability to thrive in a multitude of environments can be perceived as a sign of metabolic diversity (Gaspar, Carvalho, Vinga, Santos, & Neves, 2013). Sourdough (SD) fermentation is a complex and dynamic process with permanent changing ecological conditions. One important aspect to be considered is that the adaptability of LAB to different SDs depends on the type of flour used, which in turn determines the microbiota of the resulting

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SD. These findings indicate that exogenous starter cultures would not be suitable for the fermentation of GF materials, and the selection of adequate strains within the indigenous LAB microbiota of GF cereals is indispensable to guarantee optimal performance during fermentation (Moroni, Arendt, Morrissey, & Dal Bello, 2010; Vogelmann, Seitter, Singer, Brandt, & Hertel, 2009). Research on the LAB microbiota of non-wheat cereals of African and American origins has already been performed (Coda et al., 2011; Moroni et al., 2010).

Whole-grain foods play an important role in human diets since they are relatively rich in minerals; however, the absorption of minerals by humans is reduced in the presence of the mineral binding phytate (Kumar, Sinha, Makkar, & Becker, 2010). Phytase, an enzyme present in plants and microorganisms, including certain strains of LAB and yeasts, decreases phytate content and improves mineral bioavailability (Sandberg & Andlid, 2002).

Cereals and pseudocereals are also important sources of folates; however, B group vitamins, normally present in grain-derived products, are easily removed or destroyed during milling, food processing or cooking. Although LAB are usually auxotrophic for several vitamins, certain strains have the ability to synthesize water-soluble vitamins such as those included in the B group (Capozzi, Russo, Dueñas, López, & Spano, 2012; LeBlanc et al., 2011).

Considering that non-wheat cereal flours often possess different nutritional, technological, and sensory characteristics from wheat, the use of selected autochthonous LAB to ferment sourdough is a promising biotechnological tool to exploit the potential of pseudocereal-based fermented foods (Coda et al., 2010; Moroni et al., 2010; Sterr, Weiss, & Schmidt, 2009). Based on this, the aims of this study were to isolate, identify and characterize the LAB microbiota from quinoa grains and spontaneous sourdough, select riboflavin, folate, phytase and amylase producing LAB that could be used as potential starter cultures for novel Andean grains based foods.

2. Materials and methods

2.1. Sourdough fermentation

Type I sourdoughs were made and propagated using traditional protocols, without the use of starter cultures or baker's yeast. Two different commercial quinoa flours obtained from local supermarket were used: Q_1 and Q_2. Doughs were prepared using 100 g quinoa flour and 100 ml of sterile water containing 2 g of glucose. The dough having dough yield (DY, dough weight × 100/flour weight) 200, were incubated at 30 °C for 24 h. After this first fermentation, back-slopping was performed mixing 10% of the previously fermented dough with flour and water (DY = 200). This back-slopping step was repeated every 24 h during ten days. Samples of this QSS (50 g) were drawn every 24 h for determination of pH-value using a portable introductory pHmeter (Sartorius PT-10, Berlin, Germany) and for microbiological analysis.

2.2. Lactic acid bacteria isolation

2.2.1. Quinoa sourdough

The pH values, microbial counts and isolation of LAB from Q_1SS and Q_2SS were determined as described previously by Meroth, Walter, Hertel, Brandt, and Hammes (2003). Samples were withdrawn at 0, 1, 3, 6, 8, and 10 days. The MRS-5 agar plates were incubated under anaerobiosis (AnaeroGen and AnaeroJar, Oxoid, Hampshire, UK) at 30 °C for 48 h. Isolates that are Gram-positive, catalase-negative, non-motile rods and cocci were selected for further identification and characterization and stored at −80 °C in glycerol (20% *v*/v final concentration) for further analysis.

2.2.2. Quinoa grains

LAB were isolated from three varieties of quinoa grains (QG) obtained from two cooperatives situated in different locations in the northwestern region of Argentina: CHQ, RCQ and RHQ. Briefly, 2.5 g of grains was weighed and mixed with 22.5 ml of MRS-5 broth containing 0.1 g/l of cycloheximide (Meroth et al., 2003). The samples were homogenized in a stomacher (Stomacher 400, Seward, UK) and incubated for 30 min at 30 °C. The contents were transferred into a sterile container, after passing through the filter bag to remove seeds. Samples were incubated under anaerobiosis (AnaeroGen and AnaeroJar, Oxoid, Hampshire, UK) for 10 days at 30 °C. The samples were taken on 0, 2, 7 and 10 days for microbial counts on MRS-5 agar and pH measuring. The plates were incubated at 30 °C for 48 h under anaerobiosis (AnaeroGen and AnaeroJar, Oxoid, Hampshire, UK). Between thirty-five to forty LAB colonies of each grain variety were picked and transferred to fresh MRS-5 plates to achieve pure cultures. Tentative LAB isolates were examined for their morphology cells and colonies, Gram test, catalase test and stored at −80 °C in glycerol (20% final concentration) for further analysis and identification using different molecular techniques.

2.3. Genetic typing and identification of LAB

Species identification of isolates was performed as follows. DNA was isolated from pure cultures and used in RAPD-PCR as described below. Briefly, cultures in MRS medium (5 ml) from single colonies were prepared and incubated at 30 °C for 16 h; then 2 ml were centrifuged (10 min, 3000 *g)* and the resulting cell pellet was used for DNA-isolation according to Pospiech and Neumann (1995). RAPD-PCR analysis was carried out with a My cycler™ thermal cycler (BIO-RAD Laboratories, Inc., Hercules, CA, USA), using single primers XD9 and M13b as previously described by Fontana, Cocconcelli, and Vignolo (2005). RAPD-PCR profiles were analyzed with Scientific Image Processing (Image J 1.47v) software. LAB isolates were subjected to RAPD-PCR analysis twice. One representative of each RAPD-type profiles (see supplementary data) was subjected to sequencing of the first 550 bp of the 16S rRNA gene with primers PLB16 and MLB16 according to Hébert, Raya, Tailliez, and de Giori (2000). After purification, products were sequenced using an ABI 3130 DNA sequencer (Applied Biosystems, Foster, CA, USA). rRNA gene sequence alignments were performed using the multiple sequence alignment method (Edgar, 2004) and identification queries were fulfilled by a BLAST search (Altschul, Gish, Miller, Myers, & Lipman, 1990) in GenBank (http://www.ncbi.nlm.nih.gov/GenBank/) and in the Ribosomal Database Project (Cole et al., 2009).

2.4. Characterization of LAB from quinoa

2.4.1. Acidification capacity

The acidification capacity by LAB was evaluated in sterile flour extract (SFE) as described previously (Alfonzo et al., 2013). LAB were individually inoculated (1%, *v*/v) in 20 ml of SFE and incubated for 72 h at 30 °C. The pH measurements were made at 0, 4, 8, 24, 48, and 72 h after inoculation.

2.4.2. Amylolytic activity

The capacity of LAB from QSS and QG to hydrolyze starch was determined as described by Lee, Gilliland, and Carter (2001) with some modifications. MRS medium (de Man, Rogosa, & Sharpe, 1960) was used except that glucose was replaced by 1% soluble starch (*w*/*v*, BD Difco, Franklin Lakes, NJ, USA). Cultures of each isolate (5 μl) were spotted on modified MRS agar plates and incubated at 30 °C for 72 h, then, the plates were flooded with iodine solution (5 mM I_2 and 5 mM KI) to check haloes around the colonies. *Lactobacillus amylovorus* CRL887 was used as positive control.

2.4.3. Functional properties of LAB from quinoa

2.4.3.1. LAB producing riboflavin (B2) and folate (B9). The selection of LAB that produce vitamins B2 and B9 was determined by previous described methods (Juarez et al., 2014; Laiño, LeBlanc, & Savoy de Giori, 2012),

where vitamin production was evaluated in strains that were able to grow in culture media without these respective vitamins.

Folate concentrations were determined by a previously described microbiological assay using L. *rhamnosus* NCIMB 10463 as indicator strain (Laiño et al., 2012). Briefly, samples or different concentrations of HPLC-grade folic acid (FlukaBioChemica, Sigma-Aldrich, San Luis, MI, USA) or samples were placed with the indicator strain and incubated statically during 48 h at 37 °C in 96-well sterile microplates containing folate-free culture medium (Difco, Franklin Lakes, NJ, USA). The optical density was read at 580 nm (OD580nm) using a microplate reader (VERSAmax tuneable microplate reader, Molecular Devices, USA). Folate concentration was determined by comparing the OD of samples with those obtained from the standard curve prepared using commercial folic acid. Riboflavin concentration was determined in the same manner using L. *rhamnosus* ATCC 7469 as indicator strain grown in riboflavin-free medium. To confirm the validity of this latter method, some samples were also analyzed by HPLC using a previously described method (Juarez et al., 2014). No significant differences were obtained for riboflavin quantification using either method.

2.4.3.2. Phytate-degrading LAB. LAB were inoculated in Chalmers broth (Vanos & Cox, 1986) with 1% (*w*/*v*) of sodium phytate (Sigma-Aldrich Chemical Co., USA) and incubated at 30 °C to exponential growth phase (8 to 10 h approximately when strains reached between 10^9 and 10^{10} log CFU/ml. The cell-free extracts were prepared according to Nuobariene, Hansen, Jespersen, and Arneborg (2011). The enzyme activity was determined by measuring the concentration of liberated inorganic phosphate from sodium phytate (Shimizu, 1992). Results were compared to a standard curve prepared with inorganic phosphate (K_2HPO_4). One unit of phytase activity was defined as the amount of phytase that liberates 1 nmol ml^{-1} inorganic phosphate per minute from a 3 mM Na-phytate solution at pH 5.0 and a temperature of 45 ° C. Volumetric activity of intracellular phytase was determined as described by Nuobariene et al. (2011).

2.5. Statistical analysis

All values were expressed as means ± standard deviations (SD). Statistical analyses were performed with the software package Infostat using ANOVA GLM followed by a Tukey's post-hoc test, and differences were considered statistically significant at $p \leq 0.05$.

3. Results and discussion

3.1. Isolation, identification and selection of LAB

Microbiological analysis of lactic acid microbiota of spontaneous quinoa sourdough (QSS) and pH values were determined during fermentation. The LAB cell counts from Q_1SS, 10^9–10^{10} CFU/g dough were determined at 24 h; these values were approximately constant to the end of fermentation. There were no significant differences in cell counts obtained for Q_2SS with respect to those obtained for Q_1SS (Fig. A.1).

The LAB cell counts from QG were between 10^3 and 10^5 CFU/ml at the beginning of fermentation. At day 2, the number of presumptive LAB varied from 5×10^4 to 8.4×10^4 CFU/ml, 5.3×10^3 to 3.8×10^7 CFU/ml and 4.1×10^2 to 1.7×10^8 CFU/ml for CHQ, RCQ and RHQ, respectively. No significant differences were found after 7 and 10 days of fermentation (Fig. A.2).

During the fermentation, the pH values of Q_2SS and Q_1SS decreased from 6.3 ± 0.3 to 4.2 ± 0.1 (Δ = 2.2) and 6.0 ± 0.2 to 3.9 ± 0.2 (Δ = 2.1), respectively after 5 days. These values remained constant until the end of fermentation (Fig. A.1). For CHQ, RCQ and RHQ, pH values were 5.0 ± 0.2, 5.4 ± 0.3 and 4.5 ± 0.2, respectively at the end of fermentation (Fig. A.2).

Based on the preliminary characterization by Gram staining and catalase activity, 63 isolates (48% cocci and 52% rods) from Q_1SS and Q_2SS samples, and 54 (100% cocci) from QG were selected as presumptive LAB. Molecular identification of isolates revealed 44 different patterns or RAPD-types corresponding to different strains of *Lactobacillus* (*L.*) *plantarum* (7); *L. rhamnosus* (5); *L. sakei* (1), *Pediococcus pentosaceus* (9), *Leuconostoc mesenteroides* (1), *Enterococcus* (*E.*) *casseliflavus* (2), *E. mundtii* (3), *E. hirae* (1), *E. gallinarum* (12), *Enterococcus* sp. (1), and *E. hermanniensis* (2) in QSS and QG (Table A).

Q_2SS harboured higher LAB microbiota diversity consisting of L. *plantarum, L. rhamnosus, E. mundtii, P. pentosaceus,* and *E. casseliflavus* where the highest number of isolated strains belonged to *P. pentosaceus*; whereas in QSS_1 and more L. *plantarum* strains were identified.

On the other hand, LAB isolated from QG were principally, *E. gallinarum* in the three quinoa grains evaluated (Table A). These results show that the LAB microbiota was variable and dependent of the quinoa flour and grain variety.

According to literature, *P. pentosaceus*, L. *plantarum* and *L. sakei* are typical sourdough LAB (De Angelis et al., 2002; Gobbetti et al., 1999; Van der Meulen et al., 2007). *P. pentosaceus* was detected in spontaneous fermentations with sorghum (Mohammed, Steenson, & Kirleis, 1991), whereas L. *plantarum* was found in wheat, buckwheat and spelt (De Vuyst & Neysens, 2005; Moroni, Arendt, & Dal Bello, 2011; Van der Meulen et al., 2007) and is also frequently present in barley sourdough ecosystems (Harth, Van Kerrebroeck, & De Vuyst, 2016). This fact could be explained by its highly adapted carbohydrate metabolism during cereal fermentations (Kleerebezem et al., 2003) and its versatile metabolism (Coda et al., 2010). Vogelmann et al. (2009) reported that L. *plantarum* strains were highly competitive in buckwheat and amaranth sourdoughs, while L. *paralimentarius* dominated in quinoa sourdoughs demonstrating that the capability to adapt to a specific substrate is highly strain specific and depends on the composition of the substrate itself. In addition to the type of substrate, interactions amongst microorganisms or the substrate quality may also be of importance for the competitiveness of LAB in sourdoughs. It has been recently suggested that autochthonous strains would be preferable for use as starters for sourdoughs, since they would have less competition from the contaminants normally present in the flours (Minervini et al., 2010). These results indicate that GF flours, like quinoa flours are important reservoirs of novel and competitive LAB species that can be exploited as starter culture for the production of GF sourdoughs to be used for making novel and healthy fermented foods.

3.2. Characterization of LAB from quinoa

Based on the above mentioned result, 44 LAB were isolated and identified from QSS and QG and employed for further studies.

3.2.1. Acidification capacity

The results of the acidification capacity of the 44 LAB strains evaluated in SFE for 72 h are shown in Fig. B. L. *plantarum* CRL 1966, 1967 and 1969 from QSS and CRL 1993 from CHQ were able to decrease the pH below 4.0 and 5.0, respectively after 8 h (Fig. B.1 and 2). In contrast, the remaining strains from QSS and QG reached these pH values after 24 h. Rapid acidification during sourdough fermentation is an important technological characteristic of LAB (Corsetti & Settanni, 2007). The acidification capacity and viability of LAB isolated from quinoa were similar to that reported by other authors for other pseudocereals (Vogelmann et al., 2009; Moore, Juga, Schober, & Arendt, 2007).

3.2.2. Amylolytic activity

Starch is the major (32%–69%) component of quinoa carbohydrates (Wright, Pike, Fairbanks, & Huber, 2002). The capacity to hydrolyze soluble starch by LAB (44) isolated from QSS and QG was evaluated. The results demonstrated that only four strains isolated from QSS (*L. rhamnosus* CRL 1963 and CRL 1984, *L. plantarum* CRL 1964 and CRL 1968) and eight strains isolated from QG (*E. gallinarum* CRL 1989, CRL 1990, CRL 1991, CRL 1992, CRL 1999, CRL 2004, CRL 2005 and CRL 2014) showed amylolytic activity (Table A). Although most LAB are

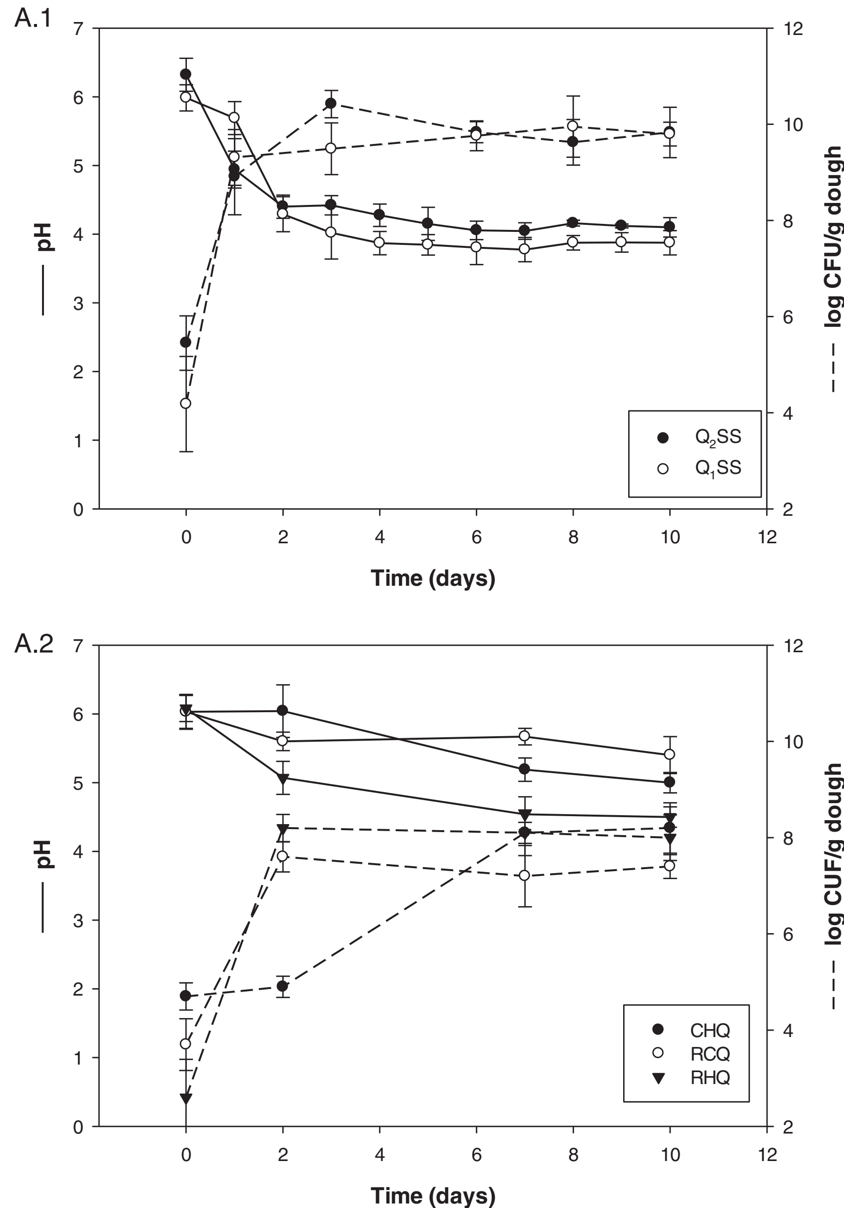


Fig. A. Changes in viable cell-counts (— — — —) and pH-values (———) during the fermentation of: (1) quinoa spontaneous sourdoughs, Q_1SS (₀) and Q_2SS (•) or; (2) quinoa grains, CHQ (•), RCQ (₀) and RHQ (▼). Results are expressed as mean ± SD.

unable to degrade starch, few possess amylolytic activity that allow them to decompose starchy material during the fermentation processes. *L. amylovorus*, *L. plantarum*, *L. manihotivorans*, and L. *fermentum* are some of the LAB exhibiting amylolytic activity which has been studied (Calderon Santoyo, Loiseau, Sanoja, & Guyot, 2003; Sanni, Morlon-Guyot, & Guyot, 2002; Songré-Ouattara et al., 2008).

3.3. Functional properties of LAB from quinoa

3.3.1. Production of folate and riboflavin

Several LAB, from the genera *Lactobacillus*, *Pediococcus* and *Enterococcus*, were screened for total, intra and extra folate production. In QSS, 14 of the 26 LAB strains evaluated produced B9 from which 4 (*L. plantarum* CRL 1973 and CRL 1970, *L. rhamnosus* CRL 1972 and L. *sakei* CRL 1978) produced high concentrations of vitamin (>100 ng/ml). *L. plantarum* CRL 1973 produced the highest concentration (143 ± 6 ng/ml). The other LAB strains produced variable concentrations between 16 and 143 ng/ml. (Fig. C.1). The degree to which the cells excreted the folate produced to the culture medium was strain dependent. Approximately 36% of the strains showed extensive secretion of folate (>70 ng/ml), whereas 29% showed high accumulation of intracellular folate (> 20 ng/ml). *E. hirae* CRL 1993 was the only strain isolated from QG that produced vitamin B9 (9 ± 0.5 ng/ml) (Fig. C.2).

L. rhamnosus from QSS, was the LAB species that produced the highest concentrations of total riboflavin (>270 ng/ml). Seven strains did not produce this vitamin and the others produced variable concentrations (6–360 ng/ml) (Fig. C.1). *L. rhamnosus* CRL 1963 produced the highest concentration of extracellular riboflavin (360 ± 10 ng/ml) from the L. *rhamnosus* species, whereas *L. plantarum* CRL 1967 and *P. pentosaceus* CRL 1974 were the highest extracellular riboflavin-producing strains from their respective species (>200 ng/ml). With respect to the intracellular riboflavin concentration, *L. plantarum* CRL 1964 retains the highest amount of this vitamin.

E. hirae CRL 1993, *E. casseliflavus* CRL 2016, *E. gallinarum* CRL 1992 and CRL 2005 produced the highest levels of B2 (>20 ng/ml) amongst the strains isolated from QG (18). The remaining strains produced between 13 and 20 ng/ml, except *E. mundtii* CRL 2006 and CRL 2007, which did not produce this vitamin (Fig. C.2).

Table A
Characteristics of LAB isolated from quinoa.

Lactic acid bacteria	Source	Amylolytic activity	Phytase activity (U/ml)
L. rhamnosus CRL 1963	Q_1SS	+	584 ± 48^{k}
L. plantarum CRL 1964	Q_1SS	+	114 ± 30^{abcdefg}
L. rhamnosus CRL1965	Q_1SS	–	284 ± 63^{hij}
L. plantarum CRL 1966	Q_1SS	–	80 ± 21^{abcdef}
E. mundtii CRL 1971	Q_1SS	–	448 ± 82^{jk}
L. plantarum CRL 1967	Q_1SS	–	140 ± 69^{abcdefghi}
L. plantarum CRL 1968	Q_1SS	+	156 ± 38^{abcdefghi}
L. plantarum CRL 1969	Q_1SS	–	214 ± 25^{abcdefghi}
L. rhamnosus CRL 1983	Q_1SS	–	606 ± 79^{k}
L. rhamnosus CRL 1984	Q_1SS	+	60 ± 16^{abcde}
E. casseliflavus CRL 1988	Q_2SS	–	684 ± 38^{k}
L. plantarum CRL1970	Q_2SS	–	294 ± 53^{fghij}
L. rhamnosus CRL 1972	Q_2SS	–	314 ± 67^{ghij}
L. plantarum CRL 1973	Q_2SS	–	105 ± 35^{abcdefg}
E. hermanniensis CRL 1976	Q_2SS	–	272 ± 25^{cdefghij}
E. hermanniensis CRL 1977	Q_2SS	–	250 ± 34^{defghij}
L. sakei CRL 1978	Q_2SS	–	336 ± 67^{ij}
P.pentosaceus CRL 1979	Q_2SS	–	177 ± 50^{abcdefghi}
P.pentosaceus CRL 1974	Q_2SS	–	189 ± 46^{abcdefghi}
P.pentosaceus CRL 1975	Q_2SS	–	179 ± 54^{abcdefghi}
P.pentosaceus CRL 1980	Q_2SS	–	223 ± 49^{abcdefghi}
P.pentosaceus CRL 1981	Q_2SS	–	152 ± 41^{abcdefghi}
P.pentosaceus CRL 1982	Q_2SS	–	221 ± 33^{bcdefghi}
P.pentosaceus CRL 1985	Q_2SS	–	270 ± 33^{efghij}
P.pentosaceus CRL 1986	Q_2SS	–	7 ± 2^{ab}
P. pentosaceus CRL 1987	Q_2SS	–	44 ± 15^{abcd}
E. gallinarum CRL 1991	CHQ	+	62 ± 10^{abcde}
E. gallinarum CRL 1992	CHQ	+	0^{a}
E. gallinarum CRL 1989	CHQ	+	0^{a}
E. gallinarum CRL 1990	CHQ	+	30 ± 10^{abc}
E. hirae CRL 1993	CHQ	–	579 ± 30^{k}
E. gallinarum CRL 1999	RCQ	+	0^{a}
E. gallinarum CRL 2000	RCQ	–	116 ± 30^{abcdefgh}
Enterococcus sp. CRL 2001	RCQ	–	9 ± 1^{ab}
E. gallinarum CRL 2002	RCQ	–	0^{a}
E. gallinarum CRL 2003	RCQ	–	234 ± 45^{cdefghij}
E. gallinarum CRL 2004	RCQ	+	0^{a}
E. gallinarum CRL 2005	RCQ	+	0^{a}
E. mundtii CRL 2006	RCQ	–	441 ± 67
E. mundtii CRL 2007	RCQ	–	957 ± 25^{l}
Leu. mesenteroides CRL 2012	RHQ	–	617 ± 38^{k}
E. gallinarum CRL 2014	RHQ	+	172 ± 35^{abcdefghi}
E. gallinarum CRL 2015	RHQ	–	91 ± 30^{abcde}
E. casseliflavus CRL 2016	RHQ	–	70 ± 35^{abcde}

Q_1SS and Q_2SS: Commercial quinoa flours. CHQ, RCQ and RHQ: Three varieties of Quinoa grains.
Symbols indicate the capacity (+) or not (−) of the strains to degrade starch.
Data presented as mean ± standard deviation.
$^{a-l}$Different letters indicate significant differences ($p < 0.5$ Tukey's test).

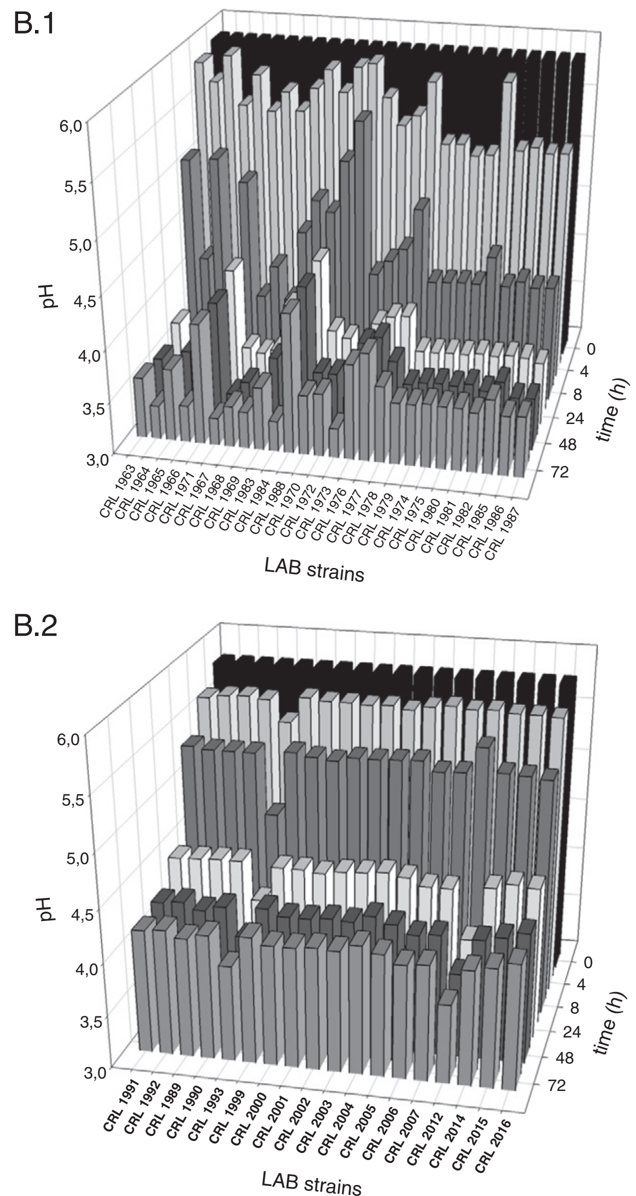


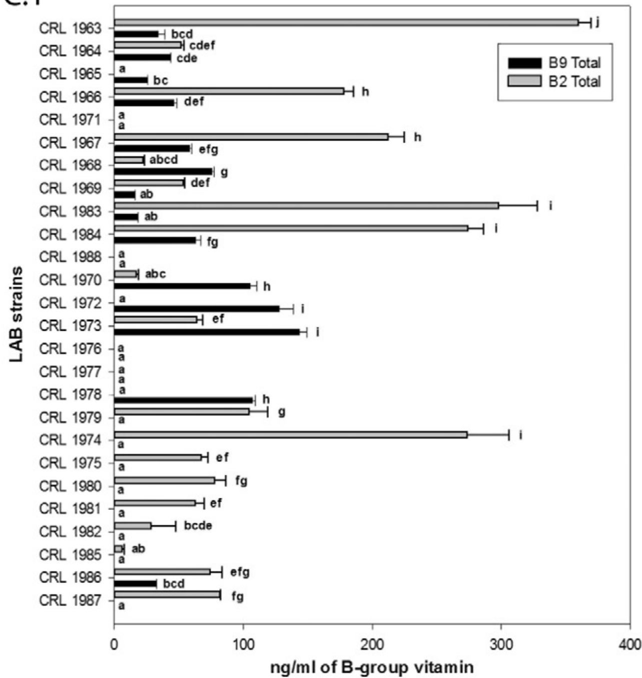
Fig. B. Acidification capacity of LAB isolated from (1) quinoa sourdough and (2) quinoa grains.

Vitamin production was strain specific and some strains were able to produce both vitamins; which would be important in order to develop novel quinoa based fermented foods biofortified in folates and riboflavin.

Humans cannot synthesize most vitamins and these have to be provided exogenously through diet or pharmaceutical products. Although most vitamins are present in a variety of foods, vitamin deficiencies still exist in many countries. In spite that most LAB are auxotrophic for several vitamins, it is now known that certain strains have the capability to synthesize B-group vitamins (LeBlanc et al., 2011). Although B-group vitamin producing strains have been identified previously from other ecological niches such as dairy products (Juarez del Valle, Laiño, de Giori, & LeBlanc, 2014; Laiño, del Valle, de Giori, & LeBlanc, 2014), oat bran (Kariluoto et al., 2014), sourdough (Russo et al., 2014), there have not been any reports of strains possessing this ability isolated from quinoa.

3.3.1.1. Phytase activity. In this study, 44 LAB isolated from quinoa were screened for their phytate degrading ability. The highest phytase values were determined in *E. mundtii* CRL 2007 (957 ± 25 U/ml) followed by *E. casseliflavus* CRL 1988 (684 ± 38 U/ml), *Leuc. mesenteroides* CRL 2012 (617 ± 38 U/ml) and L. *rhamnosus* CRL 1983 (606 ± 79 U/ml). The other strains showed lower phytase activity ranging between 7 and 579 U/mL (Table A). These results are similar to those obtained by Anastasio et al. (2010), where L. *plantarum* produced (710 U phytase/ml. In another study, it was shown that *Pediococcus pentosaceus* KTU05-8 and KTU05-9 isolated from spontaneous rye sourdough showed much lower phytase activities (32 and 54 U/ml, respectively), under conditions similar to leavening of bread dough (Cizeikiene, Juodeikiene, Bartkiene, Damasius, & Paskevicius, 2015).

The *anti*-nutritional factor phytic acid decreases the dietary bioavailability of minerals such as Ca^{2+}, Mg^{2+}, Fe^{2+}, and Zn^{2+}, and basic amino acid group of protein. Fermentation by LAB may result in a more suitable pH to activate flour endogenous phytases besides LAB possess somewhat phytase activity (Coda, Di Cagno, Gobbetti, & Rizzello, 2014; De Angelis et al., 2003; Fischer, Egli, Aeberli, Hurrell, & Meile, 2014). Nuobariene et al. (2015) showed phytate degradation by 90% and 70% in whole-wheat dough fermented with L. *panis* or L. *fermentum*, respectively.



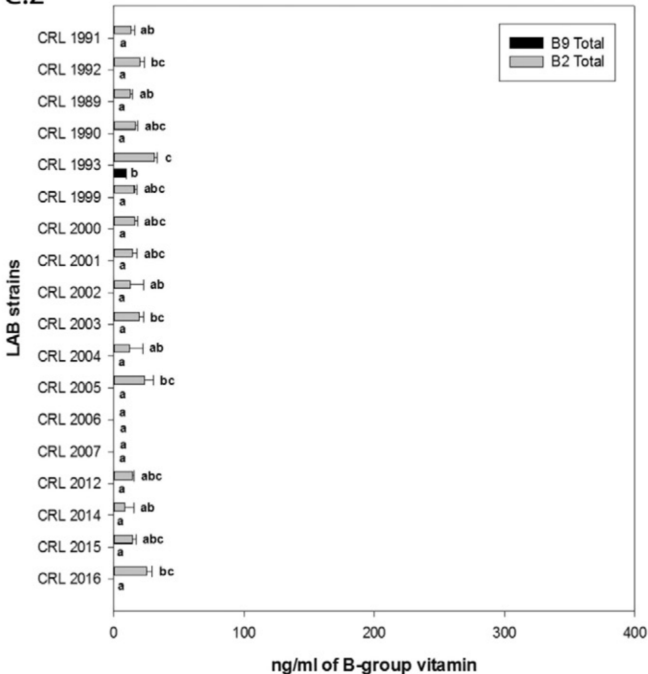


Fig. C. Riboflavin (B2) and folate (B9) production (ng/ml) by LAB isolated from (1) quinoa sourdough or (2) quinoa grains. Data presented as mean ± standard deviation. [a–j]Different letters indicate significant differences ($p < 0.5$ Tukey's test).

In this study, between the 44 LAB isolated from quinoa, *L. rhamnosus* CRL 1963, showed the highest riboflavin production, high phytase activity, produces folate and has amylolytic activity making it an ideal candidate for quinoa sourdough starter cultures.

In conclusion, this study shows that autochthonous LAB isolated from non-conventional grains seemed to be a viable technologic alternative for the production of new quinoa-based food with additional benefits such as an increase in their nutritional value suitable for celiac patients.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.foodres.2016.08.013.

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