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Increasing the folate content of tuber based foods using potentially probiotic lactic acid bacteria

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ABSTRACT

It is known that certain lactic acid bacterial (LAB) strains can produce folates, a B-group vitamin that cannot be synthesized by humans and must be exogenously obtained. The aim of this study was to select folate-producing LAB and evaluate their probiotic characteristics in order to obtain a tuber-based food with elevated folate content. Several LAB strains were isolated from a traditional Andean fermented potato product tocosh and cultured in folate-free culture medium. Five folate-producing strains (29–138 ng/mL) were selected to ferment three Andean tubers (potato *S. tuberosum* spp. *andigena*, oca *Oxalis tuberosa* and papalisa *Ullucus tuberosus*). Sterile purees were inoculated and samples were collected at 0, 6 and 24 h of fermentation and after 28 days of cold storage. Cell growth, pH and total folate were determined. All selected strains were able to grow and produce folates in the substrates and two *Lactobacillus sakei* strains, CRL 2209 and CRL 2210, produced the highest folate concentrations (730–1484 ng/g after 24 h fermentation). These strains were selected to ferment potato substrates supplemented with amaranth (*Amaranthus caudathus*) and chia (*Salvia hispanica*) flour to increase the nutritional value. This addition increased folate synthesis in 89–95%. Furthermore, the ability to survive under simulated gastrointestinal conditions was evaluated and cell counts of the 5 strains remained above the recommended for a probiotic candidate (8.0 log CFU/mL). In conclusion, the selected LAB could be considered potentially probiotic strains and could be used to produce novel tuber based products with elevated folate concentrations. These products could also be used as novel food matrixes for the delivery of probiotic microorganisms.

1. Introduction

Tetrahydrofolate and its derivatives, generally known as folates, is a B-group vitamin that cannot be synthesized by humans and must be obtained exogenously. They are involved, as cofactors of metabolic enzymes, in one-carbon transfer reactions participating in vital pathways such as DNA replication, repair and methylation, nucleotide biosynthesis and amino acid metabolism (Saubade, Hemery, Rochette, Guyot, & Humblot, 2018). Folate deficiency in humans can cause megaloblastic anaemia in all populations and neural tube defects in developing embryos. On the other hand, optimal folate intake has been associated with positive health impacts such as increased cognitive performance, and decreased risks of cardiovascular diseases and certain cancers (Iyer, Tomar, Singh, & Sharma, 2009).

Because of the high incidence of folate deficiencies, many countries have adopted mandatory fortification programs in foods of mass consumption such as flours and rice. The recommended dietary allowance (RDA) of folate in adults is 200–400 μg/day (FAO/WHO, 2002a). Folic acid is the synthetic form of folate that is commonly used in nutritional supplements and mandatory food fortification programs in most countries. Some reports have shown that high intakes of folic acid could exert some adverse secondary effects, such as masking symptoms of vitamin B12 deficiency and possibly promoting colorectal cancer (Fajardo, Alonso-Aperte, & Varela-Moreiras, 2012; Liew, 2016). These adverse effects do not occur when natural folates, such as those found in foods or produced by certain microorganisms, are consumed (LeBlanc et al., 2011).

In this context, the bio-enrichment of foods with natural folates produced by selected microorganisms during the fermentative process represents a promising alternative since they are not associated with these unwanted side effects (Kariluoto et al., 2006; Laiño, Juarez del Valle, Savoy de Giori, & LeBlanc, 2013). Certain strains of lactic acid bacteria (LAB) have been described as being folate producers (Pompei et al., 2007). Folate production depends on strains, growth time and cultivation conditions including the presence or absence of certain biomolecules such as carbohydrates or proteins (Albuquerque, Bedani,

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Vieira, LeBlanc, & Saad, 2016). Some of these LAB tolerate gastrointestinal barriers and can reach the colon where they can synthetize folates which may be absorbed and used by the host through specific mechanisms, and therefore could be considered as probiotics (Rossi, Amaretti, & Raimondi, 2011). According to Hill et al. (2014) probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. The term probioactive could be also be used to describe the folates produced by LAB since these are bioactive compounds influencing health which are synthesized by probiotics (Farnworth et al., 2007.).

It has been shown that different substrates such as milk, soy, wheat, among others, are suitable to be fermented by probiotic LAB that can result in an increase of the folate content (Saubade, Humblot, Hemery, & Guyot, 2017). However, because of the high incidence of people who are lactose intolerance, have high cholesterol levels and/or are vegetarians, there is an increasing demand for non-dairy probiotic products. In this regard, fruits, vegetables, grains and cereals have been studied as fermentation substrates for beneficial strains (Kim, Jang, & Yoon, 2012). Research on local and ancient crops and their use to develop new products have a worldwide renewed interest (Coda, Di Cagno, Gobbetti, & Rizzello, 2014) and may represent an opportunity to enhance the regional economies. Andean crops could be used to develop nutritionally balanced foods with beneficial properties. In a previous study it was shown that three Andean tubers-oca, papalisa and potato-could be used as substrates for lactic acid fermentation with a potentially probiotic strain (Mosso, Lobo, & Sammán, 2016). In another study many potentially probiotic LAB strains were isolated from tocosh -a typical Andean fermented potato food (Jimenez et al., 2018).

Other important local crops are amaranth (*Amaranthus caudatus*) and chia (*Salvia hispanica* L.). The increased interest in production and utilization of amaranth is due to its protein content (around 15%) with a good essential amino acid pattern, high lysine content and a biological value comparable to that of cheese (Bressani, 1979; Bressani, de Martell, & de Godinez, 1993). Furthermore this Andean grain hasdietary fiber and lipids rich in unsaturated fatty acids (Albuquerque et al., 2016). Alvarez-Jubete, Arendt, and Gallagher (2010) found that the replacement of potato starch with an Andean grain flour resulted in nutritionally balanced gluten-free products with an increased content of nutrients such as protein, fiber, calcium, iron, polyphenol compounds and vitamin E. Therefore, the addition of these grains can be used to increase the nutritional value of foods.

Thus, the aim of this study was to select potentially probiotic LAB in order to increase the folate content of tuber-based foods.

2. Materials and methods

2.1. Strains

Forty-one (41) strains from the genus Lactobacillus that were previously isolated from tocosh (Jimenez et al., 2018) were used in this study (Table 1). Strains were activated in MRS (Britania Co., Buenos Aires, Argentina) and incubated statically at 37 °C for 16 h.

2.2. Folate screening

The selection of folate-producing LAB was performed following previously established protocols (Carrizo et al., 2016; Carrizo et al., 2017; Laiño, LeBlanc, & Savoy de Giori, 2012); folate production was evaluated in strains able to grow in the absence of the vitamin. After activation in the above-mentioned conditions, these LAB were washed 3 times with saline solution (0.85% m/v NaCl), resuspended in this solution at the original culture volume, and used to inoculate at 2% (v/v) folate-free culture medium (Folic Acid Casei Medium- FACM) (Difco, Becton, Dickinson, and Co., Sparks, Maryland) that was then incubated without agitation at 37 °C for 18 h. After growth, this washing–resuspension procedure was repeated, and the resulting LAB solution was used to inoculate at 2% (v/v) fresh FACM. This last step (washing, inoculation, incubation) was repeated 7 times with the cultures showing good growth (observed by increased turbidity). Samples from the 7th subcultures were centrifuged (5.000*g*, 5 min) and supernatants were mixed with equal volumes of 1% ascorbic acid, this was considered the extracellular sample. Cell pellets were resuspended in the initial volume in the same solution; these were considered the intracellular samples. All samples (extracellular and intracellular) were then boiled (100 °C, 5 min) to both release folates bound to proteins and to sterilize samples. Samples were then centrifuged (5.000*g*, 5 min) and used to determine vitamin content.

Table 1
Folate production by tocosh isolated strains after growth in FACM.

Strains	Folate production		
	Extracellular (ng/mL)	Intracellular (ng/mL)	Total (ng/mL)
Lactobacillus sakei			
T2M3	8.8 ± 0.8	26 ± 2	35 ± 3
T3Y2	31 ± 3	32 ± 3	63 ± 6
T3Y3	7.3 ± 0.3	41 ± 4	48 ± 4
T3Y4	8.3 ± 0.8	41 ± 4	49 ± 4
T3Y7	31 ± 3	30 ± 3	61 ± 5
T3M1	25 ± 2	31 ± 3	56 ± 5
T3M2	19 ± 2	41 ± 4	60 ± 5
T3M7	23 ± 2	41 ± 4	64 ± 6
T3MM1	15 ± 1	41 ± 4	56 ± 5
T3MM2	10 ± 1	31 ± 3	41 ± 4
T3MM4	18 ± 1	41 ± 4	59 ± 5
T3MS2	27 ± 2	32 ± 3	59 ± 5
T3MS4	16 ± 1	28 ± 2	44 ± 4
T3MS5	35 ± 3	31 ± 3	67 ± 6
2T1MM5	92 ± 9	45 ± 4	137 ± 11
2T2Y6	92 ± 8	46 ± 4	137 ± 12
2T2M2	91 ± 8	46 ± 4	137 ± 12
2T2MM5	91 ± 8	46 ± 4	137 ± 12
2T2MM9	91 ± 7	46 ± 4	137 ± 11
2T2MM10	92 ± 9	46 ± 4	137 ± 13
2T2MS4	92 ± 8	46 ± 4	137 ± 12
2T3Y5	93 ± 9	46 ± 4	137 ± 13
2T3MM10	91 ± 8	46 ± 4	137 ± 12
2T3MS3	91 ± 8	46 ± 4	137 ± 12
2T3MS6	91 ± 8	46 ± 4	137 ± 12
2T3MS8	92 ± 8	46 ± 4	138 ± 10
2T3MS9	90 ± 7	47 ± 4	136 ± 11
3T1MS1	31 ± 3	41 ± 4	72 ± 7
Lactobacillus fermentum			
T3M3	7 ± 0.6	23 ± 2	29 ± 3
Lactobacillus sp.			
T2Y2	–	–	–
T3Y6	25 ± 2	33 ± 3	58 ± 5
Lactobacillus casei			
3T3M2	–	–	–
3T3M3	24 ± 2	40 ± 3	64 ± 5
3T3MS10	–	–	–
3T3MS11	–	–	–
3T3MS12	29 ± 2	41 ± 4	60 ± 6
3T3MS13	–	–	–
3T3MS2	28 ± 2	41 ± 3	69 ± 5
3T3MS7	9 ± 0.8	41 ± 3	50 ± 4
3T3R1	–	–	–
Lactobacillus brevis			
3T3R2	–	–	–

Results are expressed as means, standard deviation for all values are below 10% and have not been shown to facilitate viewing. Strains in bold were selected for further studies and renamed A–E in the following sections. (−) indicates that folate concentrations were below detection limits.
Results are expressed as means ± standard deviation.

2.3. Folate determination

Folate concentrations (intra- and extra-cellular and total) were determined by a previously described microbiological assay using *Lactobacillus* (L.) *rhamnosus* NCIMB 10463 as indicator strain (Laiño et al., 2012). Briefly, samples and different concentrations of HPLC-grade folic acid (FlukaBioChemica, Sigma-Aldrich, Switzerland) were placed with the indicator strain and incubated for 48 h at 37 °C in 96-well sterile microplates containing FACM. Measurements were done at A580nm using a microplate reader (EPOCH, BioTech). Folate concentration was determined by comparing the absorbance of samples with those obtained from the standard curve prepared using commercial folic acid.

2.4. Fermentation of tuber purees

Selected strains (5, based on folate production in their respective species, see results and discussion) were used to ferment three Andean tuber varieties: *Solanum tuberosum* ssp. *andigena* (collareja potato), *Oxalis tuberosa* (purple oca) and *Ullucus tuberosus* (pink papalisa). Tubers (skin and flesh) were boiled for 5 min, mashed with a mixer to obtain purees and sterilized in autoclave at 121 °C for 20 min. 50 g of each puree were inoculated with *ca.* 6.0 log CFU/mL of each strain. Fermentations were carried out for 24 h at 37 °C in plastic tubes and then placed at 4 °C for 28 days. Samples were collected at 0, 6, 24 h and 28 days to determine cell growth by serial dilutions and count in plates. The values of pH were measured using a digital pH meter (DALVO, MHS 400) and total folate content as described above.

2.5. Fermentation of tuber purees containing amaranth or chia flours

Selected strains were subcultured twice for 12 h at 37 °C in MRS broth (Britania Co., Buenos Aires, Argentina), washed twice and inoculated (ca 6.0. log CFU/mL) in two potato mixes which were previously sterilized (autoclave at 121 °C for 20 min). Mix 1 (PA) consisted of 50% collareja potato puree and 50% amaranth (*A. caudathus*) flour whereas Mix 2 (PAC) consisted of 45% potato puree, 45% amaranth flour and 10% chia (*Salvia hispanica* L.) flour. Fermentations were carried out for 24 h at 37 °C and then stored at 4 °C for 28 days. Samples were collected at 0, 6 and 24 h and 28 days to determine cell growth by serial dilutions and count in plates, pH and total folate content by microbiological assay.

2.6. Tolerance to gastrointestinal simulated conditions (TGISC)

Gastrointestinal resistance of selected strains was assessed *in vitro* with the technique described by Capra, Tibaldo, Vinderola, Reinheimer, and Quiberoni (2014) with slight modifications. The following components were used: (i) simulated gastric juice containing phosphate buffered saline (PBS) buffer solution and 3.0% (w/v) pepsin (Sigma, St. Louis, MO, USA) pH 2.5 adjusted with 6 N HCl, and (ii) simulated intestinal juice containing PBS, 1.0% (w/v) bovine bile (Sigma, St. Louis, MO, USA) and 0.1% (w/v) pancreatin (Sigma, St. Louis, MO, USA) pH 8.0 adjusted with 10 N NaOH. Fermented purees (5 g) were homogenized in 45 mL of distilled water and aliquots were suspended in gastric juice (dil 1:1). Strains grown in MRS broth at 37 °C for 12 h were also tested. Samples were incubated for 90 min at 37 °C and 150 rpm. Afterwards, cells were harvested (10.000*g*, 10 min), resuspended (dil 1:1) in the intestinal juice and incubated for 120 min at 37 °C and 150 rpm. Viable cell counts were obtained from the initial cultures and after simulation of each condition tested. Control was assessed with a commercial strain (*L. casei* DN-114 001) with proven gastrointestinal tolerance.

2.7. Cell surface hydrophobicity

The relative surface hydrophobicity was determined for the 5 selected strains using microbial adhesion to hydrocarbons, as described by Peres et al. (2014). Bacterial suspension in PBS (1 mL) was mixed with 0.25 mL of xylene by vortexing for 30 s. The two phases were allowed to separate for 15 min. The aqueous phase was carefully removed, and its A580 nm was recorded. The decrease in absorbance of the aqueous phase was taken as a measure of cell surface hydrophobicity, calculated as

$$[(A0–A)/A0] \times 100$$

where A_0 and A denote absorbance before and after extraction with xylene, respectively.

2.8. Statistic analysis

All experiments were conducted in triplicate and the results were expressed as mean ± SD (standard deviation). Statistical analyses were performed with the MINITAB 17 software (Minitab, State College, PA, USA) using ANOVA followed by a Tukey's post-hoc test, and differences were considered statistically significant at $P < 0.05$. The Pearson's correlation coefficient (r) was used to assess the relationship between bacterial growth and folate production using the same software.

3. Results and discussion

LAB strains isolated from tocosh are obvious promising candidates for tuber fermentation because it is their ecological niche and thus are adapted to this substrate for growth. Since some LAB can produce folates during fermentation processes, tocosh isolated strains could be used as starters to manufacture folate fortified tuber-based products with improved nutritional value. With this perspective in mind, strain selection, growth and folate synthesis in different tuber prototype products were evaluated. In addition, the ability to tolerate simulated gastrointestinal harsh conditions and hydrophobic surface adherence were also studied in order to evaluate the probiotic potential of these strains.

3.1. Selection of folate producing strains

From a total of 41 tested strains, 26 could grow in folate free culture media after 7 subcultures. Folate concentration was determined in cell free supernatants (extracellular) and after cell lysis (intracellular) and used to determine folate production by these 43 strains (Table 1). Folate production and partitioning between accumulation and excretion were both strain specific properties. Total folate production in this culture media varied from 29.4–137.9 ng/mL. From the 27 *L. sakei* strains that were evaluated, 13 synthesize > 136 ng folate/mL and excreted in average 67% of folates into the external medium, which is necessary for folate release if strains are able to colonize the gastrointestinal epithelium. In contrast, in the other species, cytoplasmic retention of folate was preponderant, *e.g.* 78% in *L. fermentum* and 59% in *L. casei*. According to Greppi, Hemery, Berrazaga, Almaksour, and Humblot (2017) from a total of 151 LAB strains (including *L. fermentum*, *L. plantarum*, *L. acidilactici* and *L. paraplantarum*) isolated from traditional cereal-based fermented food, several strains consumed folates from the media, 91 strains produced between 0.3 and 35 ng/mL and only two produce over 110 ng/mL. These levels are also similar to those of LAB that were isolated from raw cereal materials (Salvucci, LeBlanc, & Perez, 2016) and fermented dairy foods (Laiño et al., 2014). The 41 strains of lactobacilli that were previously isolated from tocosh belonged to 4 different species (*L. sakei*, *L. fermentum*, *L. casei*, and *L. brevis*). The 2 highest folate-producing strains from each species were chosen for further study (only 1 *L. fermentum* was isolated and no *L. brevis* were able to produce folate). Based on these results, 5 strains were selected

for further studies: *L. casei* 3T3MS2 (strain A), *L. casei* 3T3M3 (B), *L. fermentum* T3M3 (C), *L. sakei* 2T3MS8 (D) and *L. sakei* 2T2MM10 (E), which produced the highest folate concentrations in folate free media among strains in their corresponding species (between 29 and 138 ng/mL).

3.2. Fermentation in tuber purees

The selected strains, now referred to as strains A to E, were then used for the fermentation purees prepared with three Andean tuber varieties and several studies were carried out to determine cell growth, pH and folate production. In order for foods containing probiotics to have positive effects on health (FAO/WHO, 2002b), the number of viable beneficial microorganism at the time of consumption (corresponding to its shelf life) is crucial and must be determined in each food substrate and on a strain by strain basis. Some authors have suggested a minimum dose of 6–9 log CFU/g of probiotic microorganisms are required to provide beneficial effects (Meira et al., 2015). Viable cell counts of all 5 selected strains in the three tuber purees significantly increased (in a range of 1.1–1.8 log CFU/g = in the first 6 h of fermentation and reached between 8.15 and 8.96 log CFU/g after 24 h. After fermentation, purees were stored at 4 °C for 28 days and viable cell counts were maintained, indicating an active metabolism because pH levels dropped and folate levels increased after this period of cold storage, especially in the case of papalisa puree. The pH values in oca and papalisa purees after 24 h were around 4.0 and in potato it was 4.5. These results are in agreement with other studies that have shown that LAB can grow in different food matrices, a clear example is Albuquerque, Bedani, LeBlanc, and Saad (2017) who fermented soymilk supplemented with passion fruit and fructooligosaccharides with folate producing probiotic strains of LAB. Although all 5 selected strains in this study were able to grow and produce folates in the three tuber purees (Fig. 1), fermentation with strains D and E showed the highest folate production values; with folate concentrations reaching between 760 and 1484 ng/g after 24 h. The Pearson's coefficient shows that that there is a positive correlation between folate production and bacteria growth, which is in disagreement with other authors who observed that: i) growth of *L. rhamnosus* was similar in oat and barley fermented matrices but folate production was higher in the latter (Kariluoto et al., 2014), and ii) milk fermentation with *L. delbrueckii* spp. *bulgaricus* and *S. thermophilus* did not alter microbial growth but folate production was strain specific and not growth associated (Laiño et al., 2012). In the present study, the initial folate content of the tubers did not affect folate synthesis as was also observed by Laiño et al. (2012) in milk. From these results, strains D and E were selected for further studies due to their ability to produce elevated concentrations of folate and grew well in all tuber purees. Dry matter of Churqueña potato variety is mainly composed by carbohydrates (87% of dry matter), including starch, sugars and non-starch polysaccharides (Jiménez, Rossi, & Sammán, 2009) and this tuber showed the best sensorial properties in preliminary trials (data not shown), reason for which this tuber was used for further studies.

3.3. Fermentation in purees containing amaranth or chia

Supplementation with grains is an option to develop nutritionally balanced formulations. In this sense, amaranth is a local and ancient Andean grain with an excellent nutrient profile because of its protein content with good essential amino acid pattern, dietary fibers and unsaturated fatty acids (Alvarez-Jubete et al., 2010; Bressani, 1979). Chia seed is rich in polyunsaturated fatty acids, mainly linolenic and linoleic acids, proteins and fibers. It also contains a mucilague that can be used as a food thickener, gel former and chelator (Fernandes & Salas Mellado, 2017). In this study, two formulations (PA: 50% potato + 50% amaranth flour, and PAC: 45% potato + 45% amaranth flour + 10% chia flour) were fermented with the selected strains (D and E) to assess the impact of different dietary ingredients on microbial growth and folate production. Results (Fig. 2) show that viable cell counts of all the strains reached concentrations between 9.8 and 10.5 log CFU/g after 24 h of fermentation. This shows that the addition of the Andean grains was able to increase growth (1.73 log CFU/g in average) with respect to potato puree fermentation without their addition. This result might be associated with the protein and fatty acids contribution which complements the starchy matrix. The addition of amaranth and chia flours in potato purees increased folate synthesis in both strains (89% for strain D and 95% for strain E) after 24 h fermentation. Strain E produced the highest folate amounts (1.9 μg/g) when chia was added and its concentration was maintained ($P > 0.05$) during 28 days of cold storage. A 50 g serving of 24 h-fermented puree contains 80 μg of folate, which could represent a 40% of the RDA. Albuquerque et al. (2016) obtained similar results using modified MRS supplemented with amaranth flour where increased folate production by *B. longum* spp. *infantis* BB-02 and other lactobacilli and streptococci strains. The exact reason why the addition of amaranth and chia in the tuber purees is able to help increase folate concentrations is not clear, but it may be related with growth improvement and its direct relation. There were no losses in folate concentrations during 28 days storage at 4 °C and in some samples there was even a significant increase in this vitamin concentration (Fig. 2). Although there was a loss in strain viability after 28 days, the bacterial counts still remained above the log 8 CFU/g, which is traditionally used as the required amount of a probiotic in markets.

3.4. Tolerance to gastrointestinal simulated conditions

With the aim to produce folate-enriched fermented foods and/or to develop probiotics that accomplish folate biosynthesis *in vivo* within the colon, LAB have been studied for their ability to both produce vitamin and survive under GISC. The five strains grown in MRS were tested for their tolerance to GISC. Results (Fig. 3a) show that viable cell counts decreased between 0.4 log CFU/mL (strain A) and 1.02 log CFU/mL (strain D) after the complete treatment; but final concentrations were above the minimum recommended for a probiotic candidate (8.0 log CFU/mL). Gastric simulated treatment did not affect survival rate since all of the strains remained at similar level ($P > 0.05$). The critical point was treatment with bile salts and pancreatin during 120 min, after gastric phase. The survival of the selected strains D and E in fermented PA and PAC purees (24 h incubation) was evaluated in order to assess if food matrices improve the tolerance to GISC (Fig. 3b). Several authors point out the importance of food components in maintaining viability during storage and digestion process (Klu & Chen, 2014; Ranadheera, Evans, Adams, & Baines, 2012). Functional properties of probiotics may be influenced by the presence of some molecules in the matrix. For instance,Silva et al. (2017) assessed the viability after GISC of a *L. casei* strain in Prato cheese formulations, with different levels of NaCl y KCl and found a mean reduction of 2 log cycles, which ranged from 6.7 to 7.3 log CFU/g, showing the suitability of these matrixes as protective agents during gastrointestinal transit. Furthermore the same group studied viability and tolerance to GISC of this strain in the same formulations at 30 and 60 days of refrigerated storage and observed a mean reduction of about 1 log cycle, which was around 6–7 log CFU/g, suggesting this can be an effective food matrix to maintain survival to GISC during shelf life (Silva et al., 2018). Klu and Chen (2014) studied the effect of full fat and reduced fat peanut butter on survival throughout GISC using three commercial probiotics and found that viable cell counts decreased from 7.0 log CFU to 5.7, 3.5 and 3.0 log CFU in each case. Fat content did not significantly impact on survivability, although it has previously been reported to enhance protection. Similar results were found in the present work, chia seeds contain a relatively high amount of fat but

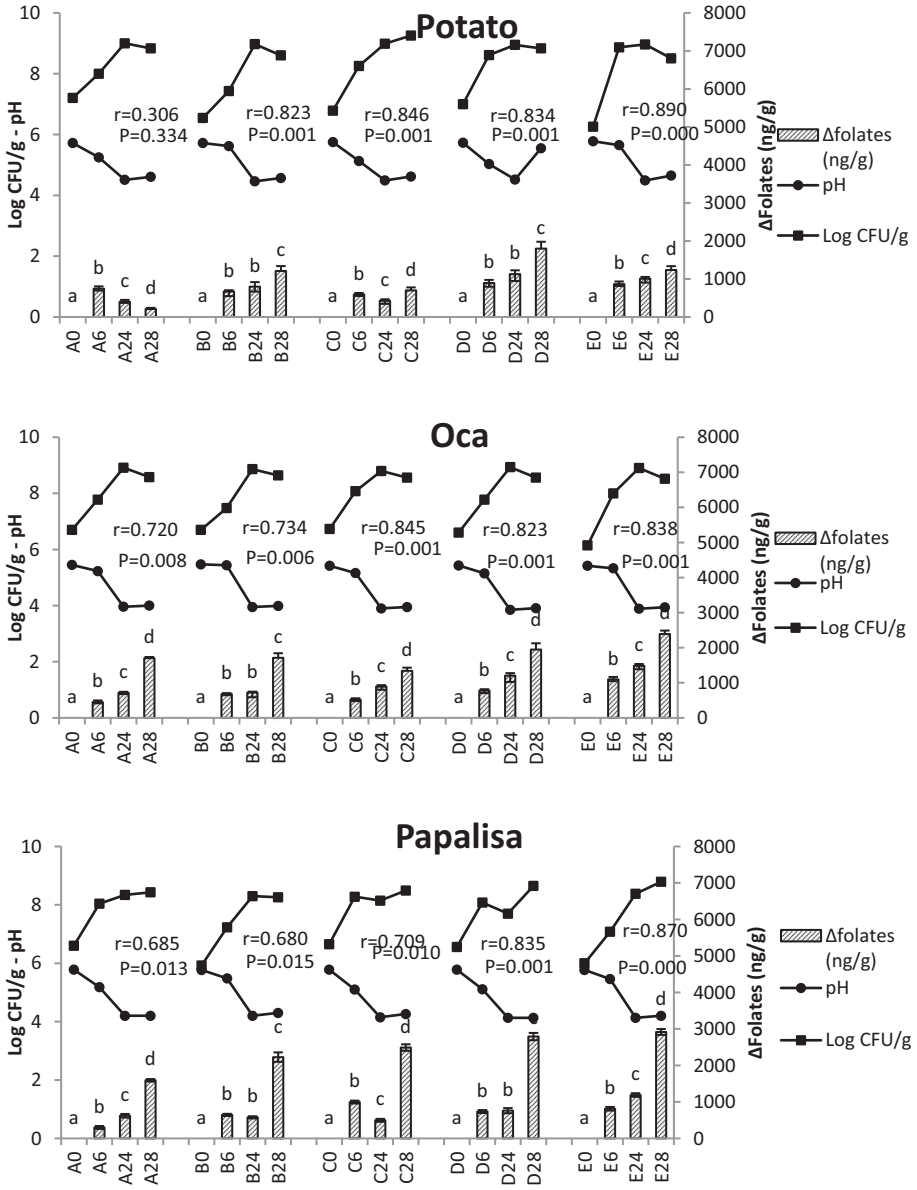

Fig. 1. Growth (CFU/ml and pH) and folate production of *L. casei* 3T3MS2 (A), *L. casei* 3T3M3 (B), *L. fermentum* T3M3 (C), *L. sakei* 2T3MS8 (D) and *L. sakei* 2T2MM10 (E) in three tuber purees. For folate production, data with different letters (a-d) statistically differ with each other ($P < 0.05$). The relationship between bacterial growth and folate production is shown for each strain using the Pearson coefficient (r) together with its significance level (P).

the same cell reductions were obtained after GISC in matrices with and without chia (PA and PAC, respectively). When placed in GISC, the substrates did not protect the strains since cell counts decreased between 1.36 and 2.78 log CFU/g which were similar to strains grown in MRS. It must be stated that in this trial the strains were tested after 24 h of fermentation, and at this time most of the bacteria are in a static growth state. Further studies must be performed when the strains are physiologically active to evaluate if this affects survivability, but would affect the total folate concentrations in the product. In addition, a control strain (*L. casei* DN-114 001) with proven gastrointestinal tolerance was tested and viable cell counts were similar ($P > 0.05$) to those obtain for strains D and E. This result suggests that even though matrices did not impart a protective effect against GISC, the selected strains have an inherent survival property and could be considered as probiotic candidates. Although dairy products are clearly the most commonly used carriers for probiotic strains (Lollo et al., 2015), there has been a few reports of novel carriers being used such as a dessert based on coconuts (Moura et al., 2016). To our knowledge, this is the first study where tuber based foods are being proposed to be used as probiotic carriers.

3.5. Cell surface hydrophobicity

Hydrophobicity plays an important role in adhesion that could confer a competitive advantage for bacterial colonization (Kos et al., 2003). The studied strains showed variable hydrophobicity, with values that ranged from 47% to 19% (Table 2). Ramos, Thorsen, Schwan, and Jespersen (2013) tested 51 LAB strains and found hydrophobicity values < 1.5% in all the strains except for one *L. brevis*. Peres et al. (2014) reported that from 10 Lactobacillus strains, only four presented > 20% of hydrophobicity.

4. Conclusion

In this study it was shown that selected LAB strains isolated from tocosh could increase folate concentrations and have properties that

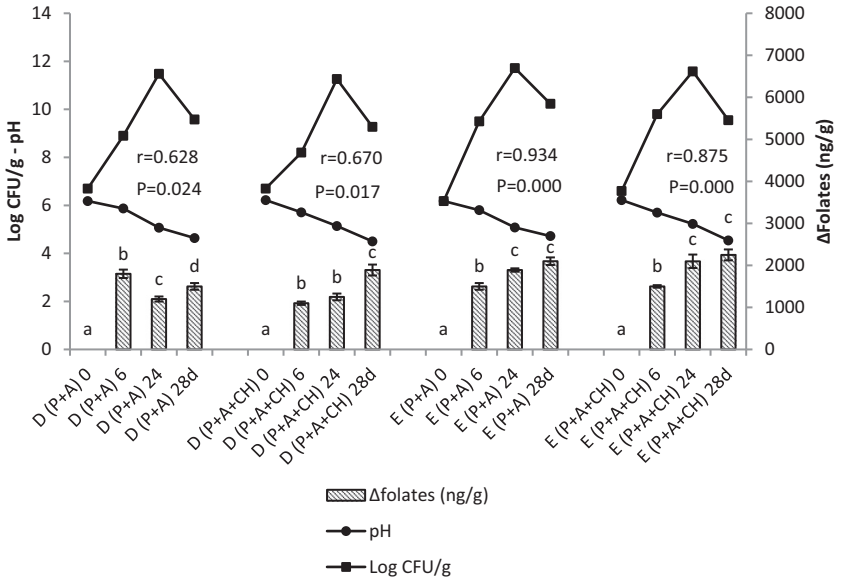

Fig. 2. Growth (CFU/ml and pH) and folate production of *L. sakei* 2T3MS8 (D) and *L. sakei* 2T2MM10 (E) in 2 different tuber mixes containing amaranth with or without chia. For folate production, data with different letters (a–d) statistically differ with each other ($P < 0.05$). The relationship between bacterial growth and folate production is shown for each strain/substrate combination using the Pearson coefficient (r) together with its significance level (P).

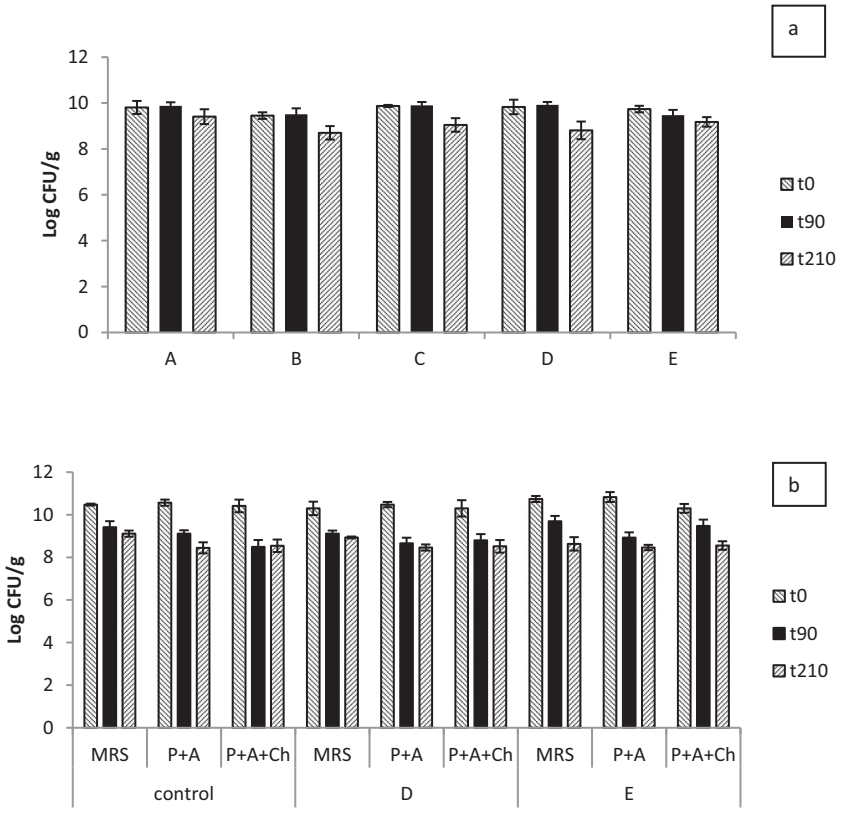

Fig. 3. Tolerance to GISC of *L. casei* 3T3MS2 (A), *L. casei* 3T3M3 (B), *L. fermentum* T3M3 (C), *L. sakei* 2T3MS8 (D) and *L. sakei* 2T2MM10 (E).

make them candidates to develop novel bioenriched tuber based foods. Studied strains tolerated simulated gastrointestinal conditions, have promising cell adherence properties and kept an active metabolism during 28 d of cold storage, therefore these could be considered potentially probiotics and could be used for further investigations. Potato substrate fortified with amaranth showed an increase of 89–95% in folate concentrations when added with *L. sakei* 2T3MS8 (D) and *L. sakei* 2T2MM10 (E), respectively. These two strains were deposited in the Culture Collection of the Centro de Referencia para Lactobacilos (CERELA-CONICET, Argentina) and are now denominated *L. sakei* CRL 2209 and CRL 2210, respectively. Their ability to survive in this matrix for at least 4 weeks at commercially critical levels and keep increased folate levels renders them suitable strains for probioactive foods development and their inclusion may enhance the potential market of this novel products especially aimed at nutritionally deprived populations.

Table 2
Hydrophobicity of *L. casei* 3T3MS2 (A), *L. casei* 3T3M3 (B), *L. fermentum* T3M3 (C), *L. sakei* 2T3MS8 (D) and *L. sakei* 2T2MM10 (E).

Strain	%H
A	47 ± 2
B	19 ± 1
C	46 ± 2
D	49 ± 2
E	37 ± 2

Results are expressed as means ± standard deviation.

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References

Albuquerque, M. A. C., Bedani, R., LeBlanc, J. G., & Saad, S. M. I. (2017). Passion fruit by-product and fructooligosaccharides stimulate the growth and folate production by starter and probiotic cultures in fermented soymilk. *International Journal of Food Microbiology, 261*, 35–41.

Albuquerque, M. A. C., Bedani, R., Vieira, A. D. S., LeBlanc, J. G., & Saad, S. M. I. (2016). Supplementation with fruit and okara soybean by-products and amaranth flour increases the folate production by starter and probiotic cultures. *International Journal of Food Microbiology, 236*, 26–32.

Alvarez-Jubete, L., Arendt, E. K., & Gallagher, E. (2010). Nutritive value of pseudocereals and their increasing use as functional gluten-free ingredients. *Trends in Food Science and Technology, 21*(2), 106–113.

Bressani, R. (1979). The potential of grain amaranth. *Food Reviews International, 5*, 13–38.

Bressani, R., de Martell, E., & de Godinez, C. (1993). Protein quality evaluation of amaranth in adult humans. *Plant Foods for Human Nutrition, 43*, 123–143.

Capra, M. L., Tibaldo, M. M., Vinderola, G., Reinheimer, J. A., & Quiberoni, A. (2014). Technological and probiotic characterisation of *Lactobacillus casei/paracasei* strains and their phage-resistant mutants. *International Dairy Journal, 37*(1), 39–47.

Carrizo, L. S., Montes de Oca, C., Laiño, J. E., Suarez, N. E., Vignolo, G., LeBlanc, J. G., & Rollan, G. (2016). Ancestral Andean grain quinoa as a source of functional lactic acid bacteria. *Food Research International, 89*, 488–494.

Carrizo, S. L., Montes de Oca, C. E., Hebert, M. E., Saavedra, L., Vignolo, G., LeBlanc, J. G., & Rollan, G. (2017). Lactic acid bacteria from andean grain amaranth: Source of functional value enzymes and vitamins. *Journal of Molecular Microbiology and Biotechnology, 27*, 289–298.

Coda, R., Di Cagno, R., Gobbetti, M., & Rizzello, C. G. (2014). Sourdough lactic acid bacteria: Exploration of non-wheat cereal-based fermentation. *Food Microbiology, 37*, 51–58.

Fajardo, V., Alonso-Aperte, E., & Varela-Moreiras, G. (2012). Lack of data on folate in convenience foods: Should ready-to-eat products considered relevant for folate intake? The European challenge. *Journal of Food Composition and Analysis, 28*, 155–163.

FAO/WHO (2002a). Human vitamin and mineral requirements. In Food and Agriculture Organization of the United Nations and World Health Organization (Ed.). *Human Vitamin and Mineral Requirements* (Bangkok, Thailand. Accessed 19 March 2018).

FAO/WHO (2002b). Guidelines for the evaluation of probiotics in food. *Food and Agriculture Organization of the United Nations and World Health Organization Working Group Report*http://www.fao.org/es/ESN/food/foodandfood_probio_en.stmS, Accessed date: 23 December 2017.

Farnworth, E. R., Mainville, I., Desjardins, M. P., Gardner, N., Fliss, I., & Champagne, C. (2007). Growth of probiotic bacteria and bifidobacteria in a soy yogurt formulation. *International Journal of Food Microbiology, 116*, 174–181.

Fernandes, M. S., & Salas Mellado, M. (2017). Addition of chia seed mucilage for reduction of fat content in bread and cakes. *Food Chemistry, 227*, 337–344.

Greppi, A., Hemery, Y., Berrazaga, I., Almaksour, Z., & Humblot, C. (2017). Ability of lactobacilli isolated from traditional cereal-based fermented food to produce folate in culture media under different growth conditions. *LWT - Food Science and Technology, 86*, 277–284.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., ... Sanders, M. E. (2014). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology, 11*, 506–514.

Iyer, R., Tomar, S. K., Singh, R., & Sharma, R. (2009). Estimation of folate in milk by microbiological assay using tri-enzyme extraction method. *Milk Science International, 64*, 125–127.

Jimenez, E., Yepez, A., Perez-Cataluña, A., Ramos Vasquez, E., Zúniga Dávila, D., Vignolo, G., & Aznar, R. (2018). Exploring diversity and biotechnological potential of lactic acid bacteria from tocosh – Traditional Peruvian fermented potatoes – By high throughput sequencing (HTS) and culturing. *LWT - Food Science and Technology, 87*, 567–574.

Jiménez, M. E., Rossi, a. M., & Sammán, N. C. (2009). Phenotypic, agronomic and nutritional characteristics of seven varieties of Andean potatoes. *Journal of Food Composition and Analysis, 22*, 613–616.

Kariluoto, S., Aittamaa, M., Korhola, M., Salovaara, H., Vahteristo, L., & Piironen, V. (2006). Effects of yeasts and bacteria on the levels of folates in rye sourdoughs. *International Journal of Food Microbiology, 106*(2), 137–143.

Kariluoto, S., Edelmann, M., Nyström, L., Sontag-Strohm, T., Salovaara, H., Kivelä, R., & Piironen, V. (2014). In situ enrichment of folate by microorganisms in beta-glucan rich oat and barley matrices. *International Journal of Food Microbiology, 176*, 38–48.

Kim, N. J., Jang, H. L., & Yoon, K. Y. (2012). Potato juice fermented with *Lactobacillus casei* as a probiotic functional beverage. *Food Science and Biotechnology, 21*(5), 1301–1307.

Klu, Y. A. K., & Chen, J. (2014). Effect of peanut butter matrices on the fate of probiotics during simulated gastrointestinal passage. *LWT - Food Science and Technology, 62*(2), 983–988.

Kos, B., Šušković, J., Vuković, S., Šimpraga, M., Frece, J., & Matošic, S. (2003). Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. *Journal of Applied Microbiology, 94*, 981–987.

Laiño, J. E., Juarez del Valle, M., Savoy de Giori, G., & LeBlanc, J. G. J. (2013). Development of a high folate concentration yogurt naturally bio-enriched using selected lactic acid bacteria. *LWT - Food Science and Technology, 54*(1), 1–5.

Laiño, J. E., LeBlanc, J. G., & Savoy de Giori, G. (2012). Production of natural folates by lactic acid bacteria starter cultures isolated from artisanal Argentinean yogurts. *Canadian Journal of Microbiology, 58*, 581–588.

LeBlanc, J. G., Laiño, J. E., Juarez del Valle, M., Vannini, V., van Sinderen, D., Taranto, P., ... Sesma, F. (2011). Vitamin production by Lactic Acid Bacteria - current knowledge and potential applications. *Journal of Applied Microbiology, 11*, 1297–1309.

Liew, S. C. (2016). Folic acid and diseases - supplement it or not? *Revista Da Associacao Medica Brasileira, 62*, 90–100.

Lollo, P., Morato, P., Moura, C., Almada, C., Felicio, T., Esmerino, E., ... Cruz, A. (2015). Hypertension parameters are attenuated by the continuous consumption of probiotic Minas cheese. *Food Research International, 76*, 611–617.

Meira, Q. G. S., Magnani, M., de Medeiros Júnior, F. C., Queiroga, R. D., Madruga, M. S., Gullón, B., & de Souza, E. L. (2015). Effects of added *Lactobacillus acidophilus* and *Bifidobacterium lactis* probiotics on the quality characteristics of goat ricotta and their survival under simulated gastrointestinal conditions. *Food Research International, 76*, 828–838.

Mosso, A. L., Lobo, M. O., & Sammán, N. C. (2016). Development of a potentially probiotic food through fermentation of Andean tubers. *LWT - Food Science and Technology, 71*, 184–189.

Moura, C., Lollo, P., Morato, P., Esmerino, E., Margalho, L., Santos-Junior, V., ... Amaya-Farfán, J. (2016). Assessment of antioxidant activity, lipid profile, general biochemical and immune system responses of Wistar rats fed with dairy dessert containing *Lactobacillus acidophilus* La-5. *Food Research International, 90*, 275–280.

Peres, C. M., Alves, M., Hernandez-Mendoza, A., Moreira, L., Silva, S., Bronze, M. R., & Malcata, F. X. (2014). Novel isolates of lactobacilli from fermented Portuguese olive as potential probiotics. *LWT - Food Science and Technology, 59*, 234–246.

Pompei, A., Cordisco, L., Amaretti, A., Zanoni, S., Matteuzzi, D., & Rossi, M. (2007). Folate production by bifidobacteria as potential probiotic property. *Applied and Environmental Microbiology, 73*, 179–185.

Ramos, C. L., Thorsen, L., Schwan, R. F., & Jespersen, L. (2013). Strain-specific probiotics properties of *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus brevis* isolates from Brazilian food products. *Food Microbiology, 36*(1), 22–29.

Ranadheera, C. S., Evans, C.a., Adams, M. C., & Baines, S. K. (2012). In vitro analysis of gastrointestinal tolerance and intestinal cell adhesion of probiotics in goat's milk ice cream and yogurt. *Food Research International, 49*(2), 619–625.

Rossi, M., Amaretti, A., & Raimondi, S. (2011). Folate production by probiotic bacteria. *Nutrients, 3*, 118–134.

Salvucci, E., LeBlanc, J. G., & Perez, G. (2016). Technological characterization of Lactic Acid Bacteria isolated from raw cereal material. *LWT- Food Science and Technology, 70*, 185–191.

Saubade, F., Hemery, Y. M., Rochette, I., Guyot, J.-P., & Humblot, C. (2018). Influence of fermentation and other processing steps on the folate content of a traditional African cereal-based fermented food. *International Journal of Food Microbiology, 266*, 79–86.

Saubade, F., Humblot, C., Hemery, Y. M., & Guyot, J.-P. (2017). PCR screening of an African fermented pearl-millet porridge metagenome to investigate the nutritional potential of its microbiota. *International Journal of Food Microbiology, 244*, 103–110.

Silva, H., Balthazar, C., Esmerino, E., Neto, R., Rocha, R., Moraes, J., ... Cruz, A. (2018). Partial substitution of NaCl by KCl and addition of flavor enhancers on probiotic Prato cheese: A study covering manufacturing, ripening and storage time. *Food Chemistry, 248*, 192–200.

Silva, H., Balthazar, C., Esmerino, E., Vieira, A., Cappato, L., Neto, R., ... Cruz, A. (2017). Effect of sodium reduction and flavor enhancer addition on probiotic Prato cheese processing. *Food Research International, 99*, 247–255.