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Lactic acid bacteria strains isolated from Jerusalem artichoke (Helianthus tuberosus L.) tubers as potential probiotic candidates

Carolina Iraporda

ciraporda@fio.unicen.edu.ar

Universidad Nacional del Centro de la Provincia de Buenos Aires, Provincia de Buenos Aires

Irene A. Rubel

Universidad Nacional del Centro de la Provincia de Buenos Aires, Provincia de Buenos Aires

Guillermo D. Manrique

Universidad Nacional del Centro de la Provincia de Buenos Aires, Provincia de Buenos Aires

Analía G. Abraham

Universidad Nacional de La Plata, Provincia de Buenos Aires

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Abstract

The search for probiotic candidates is an area that accompanies the world trend of development of novel probiotic strains and new products. In recent years, unconventional sources of potential probiotic bacteria have been studied. Furthermore, nowadays there has been a growing interest in non-dairy probiotic products and fermented plant-based foods, which has led to the development of probiotic foods currently being presented as a research priority for the food industry. The aim of this work was to evaluate the probiotic potential of lactic acid bacteria (LAB) isolated from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers. The results proved that the selected isolated LAB strains exhibited a high survival rate in the simulated gastrointestinal treatment, with non-hemolytic nor DNAse activity and antibiotic sensitivity. The isolated strains also showed antimicrobial activity against pathogen microorganisms, due to their acidification capacity. The molecular identification of the bacilli strains showed a high similarity with the genus *Lentilactobacillus* and, within this genus, with the species *kosonis* and *curieae*. Hence, these strains revealed potential probiotic *in vitro* characteristics that position them to be used in plant-based functional food.

Introduction

Probiotic microorganisms are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Hill et al., 2014). The scientific community has grown increasingly the interest in probiotic research over the years due to the wide range of health benefits they provide for people and consumer demand. In this sense, the search for probiotic candidates is an area that accompanies the global trend of development of novel probiotic strains for their application in new products. Most of the microorganisms used as probiotics include bacteria and yeast. The microorganisms that have been traditionally considered as probiotics are those that belongs to the family *Lactobacillaceae* or the genus *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Propionibacterium*, *Enterococcus*, *Bifidobacterium*, *Bacillus* or yeast such as *Saccharomyces boulardii, Saccharomyces cerevisiae*, *Candida pintolopesii*, *Aspergillus niger* and *A. oryzae* (Fuller & Fuller, 1992). Lactic acid bacteria (LAB) belonging to the genus formerly called *Lactobacillus* and *Bifidobacterium* are considered a major group of probiotic bacteria commonly used for both animals and human nutrition (Nousiainen et al., 2004; Soccol et al., 2010). It should be noted that since 2020, the *Lactobacillus* genus has been reclassified into more than 20 new genera (Zheng et al., 2020). These microorganisms are commonly isolated from traditional fermented foods and dairy products. Much research suggests that LAB strains from animal and human origin may have a good survival against gastric and intestinal stress factors; making them promising probiotic candidates (Panwar et al., 2021; Thakur et al., 2016). The high carbohydrate content, nutrient availability and the acidic environment of fruits and vegetables may favor the growth of LAB. In recent years, unconventional sources of potential probiotic bacteria have been studied, including some of non-intestinal origin and from non-dairy products, such as traditional fermented foods, grains (Pedersen et al., 2004; Sáez, Saavedra, Hebert, & Zárate, 2018), honey-comb (Tajabadi et al., 2013), air and soil (Chen et al., 2005; Yanagida, Chen, Shinohara, 2006; Zielińska & Kołożyn-Krajewska, 2018), and vegetables (Cele et al., 2022; Naeem et al., 2012; Patel et al., 2014;

Somashekaraiah et al., 2019). Furthermore, nowadays there has been a growing interest in non-dairy probiotic products and fermented plant-based foods, which has led to the development of probiotic foods currently being presented as a research priority for the food industry (Betoret et al., 2012). The isolation, identification, and evaluation of safety and probiotic properties of ´new´ or ´wild´ strains of microorganisms from different sources require a systematic approach consisting of sequential evaluations to select candidate strain. The methodology and criteria used to evaluate probiotic candidates include assessing their ability to tolerate stressful conditions exerted by the human body, ability to interact with host epithelial cells, safety attributes (such as β-hemolysis, gelatinase, and DNAse enzyme activities), and sensitivity to antibiotics, antimicrobial activity, and competition with pathogens (Choudhary et al., 2019; Falah et al., 2019; Gharbi et al., 2019; Wang et al., 2018).

Jerusalem artichoke (*Helianthus tuberosus* L.) is an annual plant native from North America that is characterized by its high adaptability and resistance to adverse environmental conditions. Jerusalem artichoke tubers (JAT) store inulin as carbohydrate reserve, reaching up to 20% of its fresh weight (Kays & Nottingham, 2008; Rubel et al., 2021). The plant rhizosphere is an interesting environmental niche enriched with root secretions and various plant-associated bacteria and fungi. Thus, it can be a good source for isolating probiotics exhibiting competence for survival in the microbe-rich human gastrointestinal tract (Singhal et al., 2021). As suggested by Chen et al. (2005) the rhizospheres of fruit trees are good sources of LAB. Many traditional preparations are made from vegetables that are fermented by the bacteria that naturally exist on the raw material (Wacher et al., 2010). Moreover, adding LAB as starter cultures plays an important role in food fermentation, avoiding variations and inconstant properties of the naturally fermented final products, and reaching desirable organoleptic properties and preservative effect. Naturally fermented Jerusalem artichoke tubers added or not with LAB as starters showed that *Weissella soli* and *Lactococcus lactis* were the predominant group of LAB in the microbial community of the brine (Yokoi et al., 2006).

The aim of this work was to evaluate the probiotic potential of LAB isolated from an unconventional source such as the Jerusalem artichoke tubers. The isolated LAB strains were characterized by *in vitro* tests for their biochemical and probiotic properties (acid and bile tolerance, survival after simulated gastrointestinal treatment, inhibition of pathogen microorganisms) and safety aspects (antibiotic susceptibility, hemolytic and DNAse activity) promoting their use for the development of vegetal-fermented food or other potential technological applications.

Materials and methods

Isolation of bacterial strains from Jerusalem artichoke tubers

Lactic acid bacteria were isolated by culture enrichment, according to Endo et al. (2009), with minor modifications. Fresh Jerusalem artichoke tubers (JAT) were harvested and washed with sterile water. Then, small pieces of JAT were incubated aerobically at 37°C for 72 h, in a formulated medium

containing (g/L): yeast extract (10), polypeptone (5), sodium acetate (2), tween 80 (0.5), $MgSO_4.7H_2O$ (0.2), $MnSO_4.4H_2O$ (0.01), $FeSO_4$ (0.01), NaCl (0.01), cycloheximide (0.05) with fructose (FYP) or inulin (IYP) as carbon source (10). After incubation, 100 µL of the enriched cultures were inoculated into fresh FYP or IYP broth and further incubated at 37°C until visible growth detection. Subsequently, serial dilutions of the cultures were plated onto FYP or IYP agar containing 5 g/L of $CaCO_3$. All plates were incubated aerobically at 37°C. Colonies, randomly selected according to morphological differences (colony size and shape), were picked and purified by streaking on the suitable agar media and further characterized. Overnight cultures of the isolates were preliminarily assayed for Gram staining, microscopic morphology, catalase and oxidase activity. Gram-positive, catalase and oxidase-negative cocci and rods were selected, and stored in MRS broth containing skim milk (1:1) at -20°C.

Genotypic characterization of selected bacteria

Genomic DNA used as template was extracted according to Ruiz Rodríguez et al. (2016). Oligonucleotide primers 27F 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R 5'-GGTTACCTTGTTACGACTT-3' were used to amplify the 16S ribosomal RNA gene. The obtained fragment of each isolate was purified and sequenced by the Sequencing Service of CCT-CONICET- Tucuman (San Miguel de Tucumán, Argentina). The sequences were analyzed using BLAST (The Basic Local Alignment Search Tool) from NCBI (http://www.ncbi.nlm.nih.gov/BLAST). The percentage of similarity was calculated as the product of the query cover and the percentage identity obtained.

Morphological and biochemical tests

The LAB isolates were studied according to their morphological and biochemical characteristics.

Cell morphology

Bacterial cultures in stationary phase grown in MRS agar and broth were mounted on microscopic slides and examined under a light microscope using oil immersion objectives. Cell morphology and cell arrangements were observed.

Growth at 45°C and at high NaCl concentrations

The selected bacterial cultures were transferred into MRS broth and incubated for 5 days at 45°C and into MRS broth containing NaCl (4 and 6.5%w/v) for 5 days at 37°C. The final pH and optical density (OD) at 600 nm of the medium were measured to verify the growth of the strains under each condition, and the growth was registered as positive or negative.

Gas production from glucose

In order to determine the homofermentative or heterofermentative metabolism of the selected LAB, CO_2 production from glucose was determined in MRS broth containing inverted Durham tubes. The presence of gas in Durham tubes during 48 h of observation indicates CO_2 production from glucose.

Sugar fermentation

The ability to use different sugars, oligo, and polysaccharides or sugar alcohols, including D-glucose, D-fructose, D-xylose, lactose, sucrose, D-raffinose, fructooligosaccharides, inulin with low or high polymerization degree, sorbitol, and mannitol, were evaluated according to He et al. (2021). These carbohydrates were used as the primary carbon source in MRS broth without glucose and meat extract. The 1%v/v cell suspension (10^8 cells/mL) was inoculated into the medium containing 2%w/v carbohydrate. The OD at 600 nm and pH were recorded and compared with the growth obtained in MRS without carbon source after 48 h of incubation at 37°C.

Proteolytic capacity

The selected LAB strains grown in MRS broth at 37°C during 48 h were streaked in milk agar and incubated for 72 h at 37°C. The appearance of a clear zone around the bacterial colonies confirms the ability of the strains to hydrolyze caseins to peptides and amino acids (Olajuyigbe & Ajele, 2005).

Cell surface: autoaggregation

The autoaggregation assay was performed according to Del Re et al. (2000). Briefly, the selected LAB grown for 48 h at 37°C in MRS broth, were then centrifuged for 10 min at 10000 xg. Subsequently, cells were washed three times with phosphate saline buffer (PBS). Further, obtained cells were suspended in sterile PBS to obtain a 10^8 CFU/mL concentration. A suspension of 5 mL was vortexed for 10 s and incubated at room temperature for 24 h. After this time, the OD at 600 nm was measured. The percentage of autoaggregation was expressed as follows:

$$\text{Autoaggregation (\%)} = [(OD_1 - OD_2)/OD_1] \times 100$$

Where OD_1 and OD_2 are the optical densities at the initial time and after 24 h, respectively. All experiments were performed in triplicate.

Resistance to gastrointestinal passage and antimicrobial properties of the strains

Acid tolerance

The acid tolerance of the isolated strains was evaluated by inoculating active cultures in in MRS broth with pH adjusted to 2.5 with HCl 1N. Cell viable counts were done initially and followed by incubation at 37°C for 1.5 and 3 h. At each time, decimal dilutions were plated in MRS agar to determine the colony-forming units per mL (CFU/mL). The survival percentage was calculated as the relation between the final Log CFU/mL and the initial Log CFU/mL.

Bile tolerance

The bile tolerance of the selected LAB strains was evaluated by incubating bacterial culture in MRS broth containing 0.3%w/v bile salts (Sigma-Aldrich, USA) at 37°C for 24 h. An initial and final viable count was

done in MRS agar. The survival percentage was calculated as the relationship between the final Log CFU/mL with respect to the initial Log CFU/mL.

Tolerance to simulated gastrointestinal conditions

The selected LAB strains grown in MRS at 37°C for 48 h were harvested and resuspended in a simulated gastric juice (NaCl 125 mM, KCl 7 mM, $NaHCO_3$ 45 mM, pepsin 3 g/L, pH adjusted to 2.5) at OD 600 nm 0.5 (10^8 CFU/mL). The suspensions were incubated at 37°C for 1.5 h and then centrifuged 10 min 10000 x*g*. The bacterial pellet was then resuspended in simulated intestinal fluid (NaCl 22 mM, KCl 3.2 mM, $NaHCO_3$ 7.6 mM, pancreatin 0.1%w/v, bovine bile salts 0.15%w/v, final pH adjusted to 8.0) and incubated at 37°C for 2.5 h. After treatment, samples were diluted in saline solution and plated on MRS agar to determine bacterial viability (Grimoud et al., 2010). The percentage of survival of the strains was calculated as the relation between Log CFU/mL after and before the sequential gastrointestinal treatment.

Antimicrobial activity

Double layer agar method

The antimicrobial activity of the selected LAB strains against the foodborne pathogens *Escherichia coli* ATCC 11229 and *Bacillus cereus* ATCC 10876 was determined using the double-layer agar method, as described by Ripamonti et al. (2011). Overnight test cultures were spotted (5 µL) on the surface of MRS agar and incubated for 24 h at 37°C, and two different assays were carried out. In the first one, the LAB colonies grown in the surface of MRS agar plates were inactivated with chloroform for 30 min. Then 10 mL of brain heart infusion (BHI, Britania, Argentina) soft agar (0.7%w/v) inoculated with 50 µL of an overnight culture of each pathogen indicator grown in BHI was poured onto MRS agar plates. In the second one, the LAB colonies were not inactivated. The plates with double-layer agar were incubated aerobically at 37°C for another 24 h and the inhibition zones were subsequently measured (cm).

Diffusion agar assay

Evaluation of the antagonic activity of the LAB culture supernatants was screened by the agar well diffusion assay reported by Tejero-Sariñena et al., (2012) with some modifications. Briefly, Petri dishes were overlaid with 20 mL of BHI agar previously inoculated with 100 µL of an overnight culture of the foodborne pathogen indicator microorganisms grown in BHI broth (*E. coli* or *B. cereus*). Subsequently, cell-free supernatant, sterilized by filtration with 0.22 µm pore size membrane (Gamafil, Argentine), were placed into agar wells (40 µL). Also, cell-free supernatants neutralized with NaOH 3N and 20-fold concentrated by lyophilization were analyzed. The plates were incubated aerobically for 24 h at 37°C and the inhibition zones were subsequently measured (cm).

Safety evaluation of selected LAB strains

Antibiotic susceptibility

The following antibiotics were tested at the recommended minimal concentration (mg/L) for homofermentative/heterofermentative *Lactobacillus* strains: ampicillin (1/4), gentamicin (16/16), streptomycin (16/64), erythromycin (1/1), clindamycin (1/1), tetracycline (4/8) and chloramphenicol (4/4) (EFSA FEEDAP Panel, 2012). All antibiotics were dissolved in the appropriate diluent for preparing concentrated stock solutions (10X). Then, stock solutions were diluted 1/5 in LSM broth (90% Iso-Sensitest plus 10% MRS). Bacterial cultures growth 48 h at 37°C were diluted 1:50 in LSM broth for inoculation of microdilution plates. Then, 100 µL of diluted inoculum in LSM were added to each well containing 100 µL of an antibiotic solution in LSM (2X). After incubating plates under aerobic conditions at 37°C for 48 h, OD at 600 nm was measured. The susceptibility of LAB strains was categorized as resistant (R) when the growth was higher than 10% concerning the control media without antibiotic, moderately sensitive (MS) when the growth was lower than 10%, or susceptible (S) when no growth was observed.

Hemolytic activity

The selected LAB cultures were streaked on blood agar (Britania, Argentine) and incubated for 72 h at 37°C under aerobic conditions. Green zones around the colonies suggested α-hemolysis, clear zones around the bacterial colonies indicated the presence of β-hemolysis, whereas no clear zones were recorded as non-hemolytic.

DNAse activity

The selected LAB isolates were streaked onto a deoxyribonuclease (DNAse) agar medium (Britania, Argentine) to test for the production of the DNAse enzyme. The plates were incubated at 37°C for 48 h under aerobic conditions and observed for the zone of DNAse activity after the addition of HCl 1N. A clear zone around the colonies was considered as positive (+) DNAse activity, whereas no clear zones were recorded as negative (-) (Shuhadha et al., 2017).

Results

Isolated bacterial strains

After seeding FYP $CaCO_3$ agar plates with serial dilutions of the enriched medium, many colonies surrounded by a clear zone due to the local solubilization of $CaCO_3$ by presumptive lactic acid bacteria from the rhizosphere of the tubers, were obtained. Forty-eight colonies were isolated, 35 cocci and 13 bacilli, Gram positive, catalase and oxidase negative. Considering the acidification power in FYP broth, 3 cocci (named as A, E, J) and 3 rods (named as F, G, H) were selected for their further characterization and assays of their probiotic capacity as a preliminary step for their potential use in the development of vegetal-fermented food. Neither of the selected isolated strains were able to grow at 45°C nor with NaCl 4,5 or 6%w/v. The 3 strains with coccus morphology did not produced gas from glucose, while the 3 rod-shaped strains produced gas from glucose, indicating homofermentative and heterofermentative metabolism, respectively. The colony macroscopic aspect in MRS agar and microscopic morphology

subjected to a Gram stain of the selected strains grown at 37°C are shown in Fig. 1. A difference in the bacterial morphology in liquid and solid medium was observed for the 3 selected rod-shaped strains. They presented a typical bacilli shape in the broth, while in solid medium they grew forming shorter bacilli cells. Meanwhile, the coccus-shaped strain presented the same bacterial morphology when they grew in MRS broth or solid media. All the strain grew in MRS broth at 37°C, the pH decreased during bacterial growth, and they reached the stationary phase in 24 h with concentrations around 9 Log CFU/mL (Figs. 2 and 3). The acidification in the MRS broth of the cocci was slightly higher than that of the bacilli, reaching final pH values of 3.6–3.8 and 4.1–4.3 after 48 h, respectively (Figs. 2 and 3). The three rod-shaped strains were able to ferment glucose and fructose, while the three coccus-shaped strains, in addition to these carbohydrates, they also fermented sucrose and mannitol. The isolated strains revealed no considerable casein degradation during their aerobic growth in milk agar plates. The isolated strains A, E and J showed autoaggregation capacity after 24 h of 62.8 ± 1.5; 58.3 ± 4.8 and 71.3 ± 3.9% respectively; whereas the strains F, G and H presented values of 77.7 ± 9.7; 61.2 ± 11.4 and 81,5 ± 10.2%, respectively.

In this instance the focus was placed in the rod-shaped strains. The amplification products corresponding to the 16S RNA gene gave an approximately 1400 bp amplicon. The analysis of the sequences obtained, revealed similarity of the strains F, G and H with isolates belonging to the Phylum ´Firmicutes´, class ´*Bacilli*´, order ´*Lactobacillales*´, family ´*Lactobacillaceae*´, genus ´*Lentilactobacillus*´. Within this genus, both strains F and H, showed high similarity with strains belonging to the species *Lentilactobacillus kosonis*, with a percentage of similarity 94.2% (percentage of identity of 96.1%) and 96.4% (percentage of identity 97.3%), followed by *Lentilactobacillus curieae* percentage of similarity 92.3% (percentage of identity of 96.1%) and 91.8% (percentage of identity 95.6%), for the strains F and H, respectively. Meanwhile the percentage of similarity of the strain G with strains belonging to the species *Lentilactobacillus kosonis* and *Lentilactobacillus curieae* was 91.2% (percentage of identity 92.1%). Likewise, percentage of similarity higher than 90% were also obtained concerning other species from the genus *Lentilactobacillus*, such as *Lentilactobacillus senioris* and *Lentilactobacillus fungorum* for the strain G, and *Lentilactobacillus sunkii*, *Lentilactobacillus otakiensis*, *Lentilactobacillus buchneri* and *Secundilactobacillus odoratitofui* for the strain H.

Probiotic properties

All the strains survived in acidic conditions, in presence of bile salt, and after the simulated sequential gastrointestinal treatment (GIT) (Table 1). After 1.5 h of exposure at pH 2.5, the percentage of survival were higher than 94.8%, except for the strain J, which presented a significantly lower survival. Meanwhile, after 3 h, the rod-shaped strains presented a significantly higher percentage of survival than the coccus-shaped strains. After the bile salt exposure, the rod-shaped strains presented high resistance (> 94.2%) without significant differences in their percentage of survival. Among the isolated coccus, the strain A show survival percentage after bile salt treatment of 80.2%, with no significant differences with the strain J, and significantly higher than the strain E. A high resistance was also observed after the

sequential simulated GIT challenge for all the selected strains. The rod-shaped strains presented significantly higher survival than the coccus-shaped strains.

Table 1
Percentage of bacterial survival after acidic treatment (pH 2.5, 37°C, 1.5 h or 3 h), bile salt exposure (0.3%w/v, 37°C, 24 h) and simulated sequential gastrointestinal treatment.

Strain		Acid treatment 1.5 h	Acid treatment 3 h	Bile salt treatment	GIT
Coccus-shaped	A	96.7 ± 1.4 $^{B\,b}$	63.4 ± 4.1 $^{A\,a}$	80.2 ± 2.0 $^{AB\,b}$	74.1 ± 0.9 $^{a\,A}$
	E	94.8 ± 0.6 $^{B\,b}$	58.5 ± 1.6 $^{A\,a}$	65.6 ± 8.4 $^{A\,a}$	73.7 ± 2.6 $^{a\,A}$
	J	83.2 ± 5.2 $^{A\,a}$	76.6 ± 2.2 $^{B\,b}$	75.0 ± 9.2 $^{A\,ab}$	71.2 ± 2.1 $^{a\,A}$
Rod-shaped	F	97.8 ± 1.44 $^{B\,a}$	96.1 ± 0.9 $^{C\,a}$	108.1 ± 8.8 $^{C\,a}$	94.6 ± 1.1 $^{b\,C}$
	G	96.8 ± 1.5 $^{B\,a}$	95.8 ± 2.0 $^{C\,a}$	108.5 ± 11.9 $^{C\,a}$	93.2 ± 0.2 $^{b\,BC}$
	H	97.2 ± 0.8 $^{B\,a}$	96.4 ± 3.1 $^{C\,a}$	94.2 ± 4.3 $^{BC\,a}$	88.9 ± 2.6 $^{a\,B}$

GIT: Gastrointestinal treatment. Values are represented as mean ± SD of at least three independent replicates. Superscripts with different capital letters indicate significant differences between strains, for each column. Lowercase superscripts indicate significant differences between the cocci-shaped or rod-shaped strains, for each column. ANOVA followed by Tukey test (α = 0.05).

Both viable cells and the metabolites of all the selected strains exerted an inhibitory effect on the pathogenic microorganisms *E. coli* and *B. cereus*. Moreover, this antimicrobial activity was significantly higher for the rod-shaped strains than that observed for the coccus-shaped strains. The agar plates with inhibition zones produced by the strains F, G, and H against *E. coli* and *B. cereus* are shown in Fig. 4. The strain F exerted an inhibitory effect significantly higher ($p < 0.05$) against both pathogens than the rest of the strains, with diameters of inhibition of 3.0 ± 0.1 cm and 3.7 ± 0.1 for *E. coli* and *B. cereus*, respectively. The lowest inhibition was exerted by the strain A against *B. cereus* (diameter of inhibition 1.73 ± 0.1 cm) and strains A and J with *E. coli* (diameters of inhibition 2.1 ± 0.1 cm). The inhibition exerted by metabolites of rod-shaped strains against *B. cereus* did not show significant differences (diameters of inhibition between 4.1 and 4.4 cm). Finally, the inhibition against *E. coli* exerted by the rod-shaped strains produced diameters between 3.9 and 4.3 cm. On the other hand, the concentrated and neutralized culture supernatants of any of the selected strains show inhibitory effect against the pathogens analyzed (data not shown).

Safety aspects

None of the selected strains showed hemolytic capacity or DNAse activity. These attributes indicated safety aspects for these strains (Table 2). Moreover, the isolated strains were sensitive to the antibiotic ampicillin, streptomycin, erythromycin, clindamycin, gentamycin, tetracycline and chloramphenicol, at concentrations below the cut-off limit established by the EFSA (2012), for heterofermentative and homofermentative *Lactobacillus* strains and therefore they could be classified as sensitive (Table 2), however a moderate sensitivity was observed for the three rod-shaped strains against tetracycline.

Table 2
Antibiotic sensibility, hemolytic and DNAse activity of the isolated strains.

Strain		AMP	STR	ERT	CLI	GEN	TET	CLO	Hemolytic capacity	DNAse activity
Coccus-shaped	A	S	S	S	S	S	S	S	-	-
	E	S	S	S	S	S	S	S	-	-
	J	S	S	S	S	S	S	S	-	-
Rod-shaped	F	S	S	S	S	S	MS	S	-	-
	G	S	S	S	S	S	MS	S	-	-
	H	S	S	S	S	S	MS	S	-	-

S: sensitive. MS: moderately sensitive. R: Resistant. (-): Negative. (+): Positive.

Discussion

The rhizosphere is an interesting environmental niche enriched in plant secretions and soil-associated bacteria and fungi. Thus, it can be thought as a potential source for isolating competitive bacterial strains exhibiting probiotic properties in the human gastrointestinal tract (Singhal et al., 2021). The strains A, E and J resulted Gram positive coccus, catalase and oxidase negative, homofermentative, unable to growth at 45°C nor with NaCl 6%w/v, and gamma-hemolytic, so they could be classified as *Streptococcus*.

The genus formerly called *Lactobacillus* displays a great level of genetic diversity. The reclassification of the formerly *Lactobacillus* genus in 23 new genera proposed in 2020 by Zheng et al. (2020) was considered for classifying the rod-shaped isolated strains. The molecular analysis of the strains F, G and H allowed them to be classified as members of the genus *Lentilactobacillus*. In recent years, strains with probiotic potential that were identified within the genus *Lentilactobacillus* have been isolated and from vegetable and fermented food sources (Carasi et al., 2022; Ebrahimi et al., 2017; Wu et al., 2021). The genus *Lentilactobacillus* is mainly represented by Gram-positive, rod-shaped, catalase negative, heterofermentative strains, that mostly grow at 15°C and some also grow at 45°C. Most of the strains

that constitute this genus were isolated from silage, fermented vegetables, wine and cereal mashes (Zheng et al., 2020). All these sources present environmental characteristics that can be considered similar to those of the Jerusalem artichoke tubers. Moreover, strains in this genus lead a free-living lifestyle, and generally metabolize a broad spectrum of pentoses, hexoses, and disaccharides. The highest percentage of similarity found in this work was 96.4% for the isolate referred as H with the strain *Lentilactobacillus kosonis* strain C06.No73T, isolated from a traditional Japanese fermented beverage called kôso (Chiou et al., 2021), closely related also with the previously described *Lentilactobacillus curieae* CCTCC M 2011381T from stinky tofu (Lei et al., 2013). The similarity percentage of the isolated strains F and G were below 96%, being the highest 94.2% with *Lentilactobacillus kosonis* and 91.2% with *Lentilactobacillus kosonis* and *Lentilactobacillus curieae*, for F and G, respectively. Considering that a percentage of similarity higher than 90% was observed concerning several species, such as *Lentilactobacillus senioris*, *L. fungorum*, *L. sunkii*, *L. otakiensis*, *L. buchneri* and *Secundilactobacillus odoratitofui*, with which they also share ecological and metabolic properties, however other molecular studies could contribute to specify the identification of the strains at the species level (Zheng et al., 2020). In the base of the 16S rRNA gene sequence Lei et al. (2013) showed that the strain *L. cuerieae* CCTCC M 2011381T was closely related to *L. senioris* JCM 17472T, similar results with the obtained in the present work for rod-shaped strains isolated form Jerusalem artichoke tubers. It is worth to mention that the strain *L. curieae* CCTCC M2011381 showed a great potential as starter culture in fermentation of plant foodstuff (Liu et al., 2019). In another hand, *Lentilactobacillus buchneri subsp. buchneri, Levilactobacillus brevis*, and *Limosilactobacillus fermentum* were isolated and identified from Jordanian traditional pickled and fermented foods (Abudoleh et al., 2021).

The autoaggregation capacity can favor the bacterial adhesion to intestinal epithelial cells, allowing bacteria to form a barrier and prevent the adhesion of undesirable microorganisms (Saito et al., 2019). Therefore, a high autoaggregation capacity is desirable for potential probiotic candidates. As suggested by Kos et al. (2003) the autoaggregation capacity is related to the type of proteins on the cell surface. The percentages of autoagregation obtained for the strains isolated from Jerusalem artichoke tubers in this work were high (> 50%). Among the isolated strains, the highest autoaggregation capacity was observed for the strain H (81,5 ± 10.2%) followed by the strain F (77.7 ± 9.7%). In general, lower percentage of autoagreggation were reported for LAB strains isolated from different vegetable sources. Fonseca et al. (2021) reported that LAB strains isolated from food and fermented vegetable foods, presented percentage of autoaggregation from 16.5 to 52.6%. Various LAB strains isolated from ethnic pickled bamboo shoot products displayed values of autoaggregation between 20 and 55%, in particular the strains *Lv. brevis* FS2.1, *P. pentosaceus* FS36.2, *Lb. argentoratensis* FS40.1, and *P. pentosaceus* FS34.1, showed the highest values (Kanpiengjai et al., 2022). Also, percentage of autoaggregation ranging from 2 to 28%, were reported for LAB strains isolated from fermented grains of Chinese baijiu (Fan et al., 2022). The high autoggregation obtained for LAB strains isolated from the Jerusalem artichoke tubers represent a positive aspect for their survival in adverse conditions resulting from a lesser exposure and also favorable for their intestinal adhesion capacity. On another hand, the selected

LAB strains isolated in this work were able to ferment only a few carbohydrates, and as expected, the strains do not show proteolytic capacity.

The survival of strains after the passage through the gastrointestinal tract (GIT), high acidic and bile salt levels (Han et al., 2021; Urdaneta & Casadesús, 2017), as well as the adhesion to epithelial cells capacity (Garcia-Gonzalez et al., 2018; Petrova et al., 2019), are important preliminary tests to claim about potential probiotic properties of a microorganism (Krawczyk et al., 2021; Qi et al., 2021). Moreover, no scientific consensus exists on the pH and bile concentration to which probiotic strains should be tolerant (Zago et al., 2011). The survival rates after acidic, bile salts, and simulated gastrointestinal treatments for the isolated strains were high. This assay demonstrated the resistance of the strains against adverse environments and their potential ability to reach the colon in high concentrations when consumed as part of food. Similar results were reported by other authors for LAB strains isolated from different natural sources (Delgado et al., 2007; Ramos et al., 2013; Vinderola et al., 2008; Zago et al., 2011). In particular, Singhal et al. (2021) reported that *Lactiplantibaciullus plantarum* strains isolated from rhizospheric soil remained viable in the presence lysozyme, after simulated gastric juice, and moderate bile salts exposure. Also, the strain *Leuconostoc mesenteroides* subsp. *mesenteroides* isolated from fermented carrot ang ginger brine exhibited high tolerance to acid, bile, and lysozyme (Cele et al., 2022). Abudoleh et al. (2021) reported only 1–2 Log CFU/mL reduction after simulated GIT for LAB strains isolated from plant-based fermented foods. Also, LAB strains isolated from a naturally fermenting coconut palm nectar could resist the simulated GIT without losing viability (Somashekaraiah et al., 2019). In line, many LAB strains isolated from Neera, resisted to simulated GIT and presented good antimicrobial activity (Somashekaraiah et al., 2019).

The selected LAB strains of the present work showed a remarkable antimicrobial activity against the pathogenic bacteria *E. coli* and *B. cereus*, without producing bacteriocin-like substances, like most of the probiotic candidates isolated from unconventional sources. The inhibition results were attributed to the effect of the organic acids produced during bacterial growth, since no inhibition was exerted by the concentrated and neutralized culture supernatants, suggesting that they did not produce bacteriocin-like substances. In concordance, Fhoula et al. (2013) reported that culture supernatants of LAB isolated from rhizosphere exhibited strong inhibitory activity against several pathogenic bacteria, but only a few neutralized supernatants showed antagonistic activity against the tested pathogens. Bacterial strains isolated from traditional fermented Indian food, identified as *Lactiplantibacillus plantarum* 86 and *Weissella cibaria* 92, showed considerable antimicrobial activity against Gram-positive and Gram-negative pathogens (Patel et al., 2014). In contrast, Min Hsiu et al. (2016) informed that beneficial LAB isolated from fresh fruits and vegetables produced bacteriocin and other antimicrobial substances active against pathogenic bacteria and fungi.

Safety aspects are essential when selecting probiotic candidates to support their potential use in food applications. In the present work, the selected LAB strains were sensitive to antibiotics for human and animal use, complying with European regulated cut-off values. Other authors reported that LAB isolates from Neera were resistant to some of the antibiotics tested; however, it is accepted that it is rarely their

risk of infection outside healthcare situations (Sanders et al., 2010). Moreover, Arias and Murray, (2012) and Hanchi et al. (2018) reported that plant-origin strains generally display low virulence levels. It is important to remark that before using new isolates in food or feed formulations, their virulence and antimicrobial resistance genes must be verified to prevent the horizontal gene transfer for antibiotic resistance (EFSA FEEDAP Panel, 2018). Probiotic properties, safety aspects, and beneficial health effect must be reinforced by other *in vitro* and *in vivo* studies, if strains are intended to be used for food applications. Finally, this research confirms that the plant rhizosphere might be explored as a valuable ecological niche representing a source of strains with essential and desirable probiotic potential, that promote their use in plant-based foods.

Conclusion

In summary, in this work, lactic acid bacteria were isolated from an unexplored source, such as the Jerusalem artichoke tubers, for the first time, and results showed that they were a reservoir of promising probiotic candidates. The results proved that the selected isolated LAB exhibited a high survival rate in the simulated gastrointestinal treatment, with non-hemolytic activity and antibiotic sensitivity. The isolated strains also showed antimicrobial activity against pathogen microorganisms, mainly attributed to their acidification capacity. The molecular identification of the bacilli strains showed a high similarity with the genus *Lentilactobacillus* and, within this genus, with the species *kosonis* and *curieae*. Hence, these strains revealed potential probiotic *in vitro* characteristics that position them to be used in plant-based functional food. The promising results promote further studies on the *in vivo* probiotic potential.

Declarations

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by C. Iraporda and I.A. Rubel. The first draft of the manuscript was written by C. Iraporda and I.A. Rubel all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval

This work did not require ethics approval.

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Figures

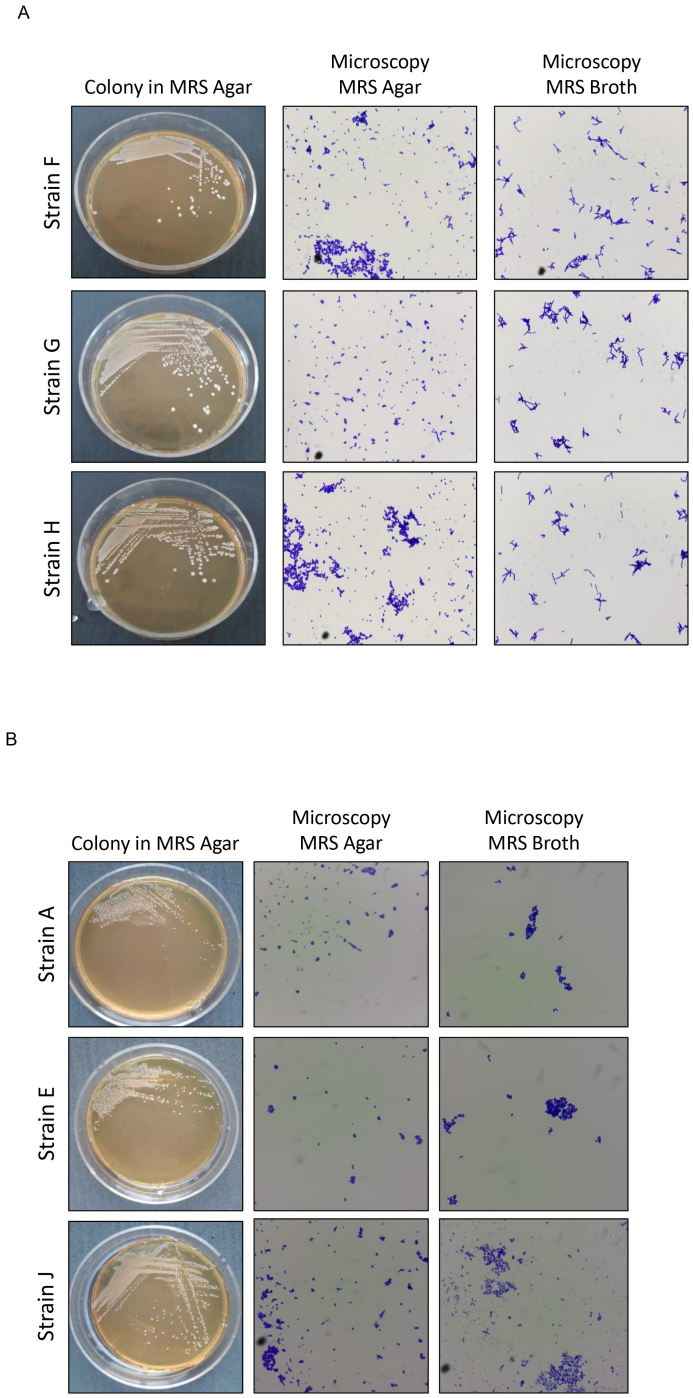

Figure 1

Colony aspects and Gram stain of isolated selected strains. **(A)** Colony aspect in MRS agar 48 h 37 °C (left column), Gram stain of bacteria grown in MRS agar (middle column) and MRS broth, 48 h 37 °C (right column). Rod-shaped strains F (top row) G (middle row) and H (bottom row). **(B)** Colony aspect in MRS agar 48 h 37 °C (left column), Gram stain of bacteria grown in MRS agar (middle column) and MRS broth, 48 h 37 °C (right column). Rod-shaped strains A (top row) E (middle row) and J (bottom row).

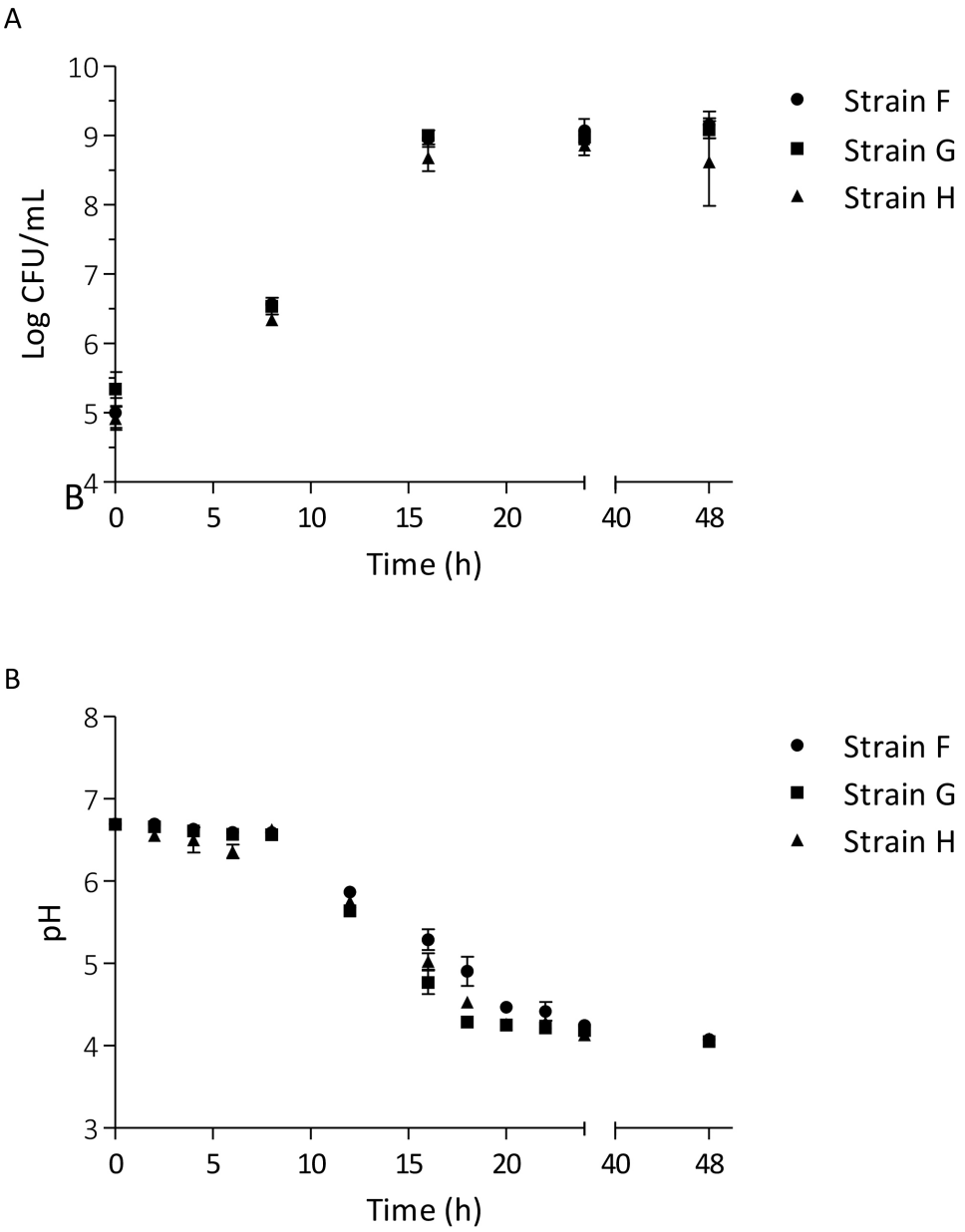

Figure 2

pH and bacterial growth of rod-shaped selected strain in MRS broth. Bacterial growth (CFU/mL) in MRS broth at 37 °C. **(B)** pH of MRS broth during bacterial growth at 37 °C. (●) Strain F (■) Strain G and (▲) Strain H.

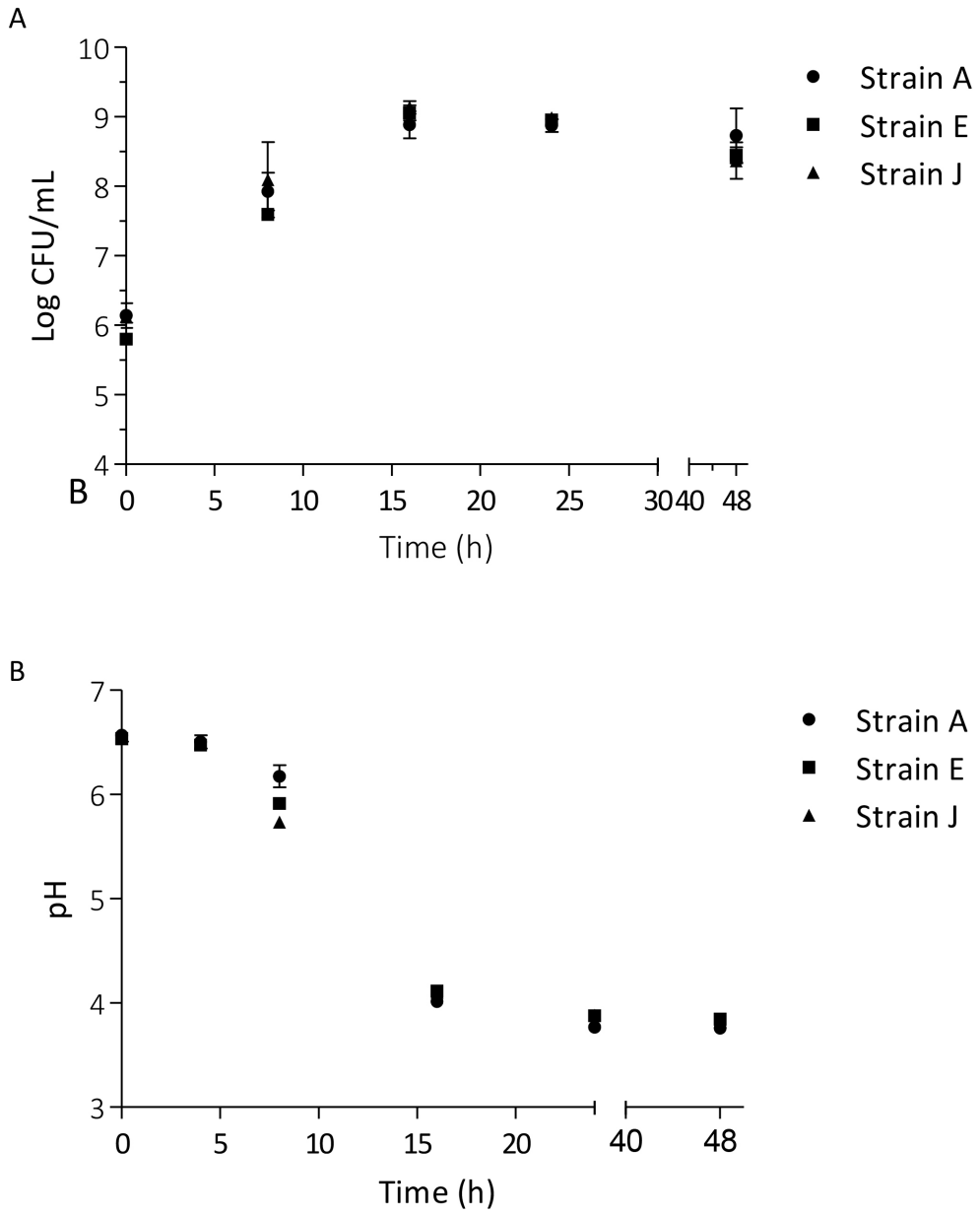

Figure 3

pH and bacterial growth of coccus-shaped selected strain in MRS broth. (A) Bacterial growth (CFU/mL) in MRS broth at 37 °C. **(B)** pH of MRS broth during bacterial growth at 37 °C. (●) Strain A (■) Strain E and (▲) Strain J.

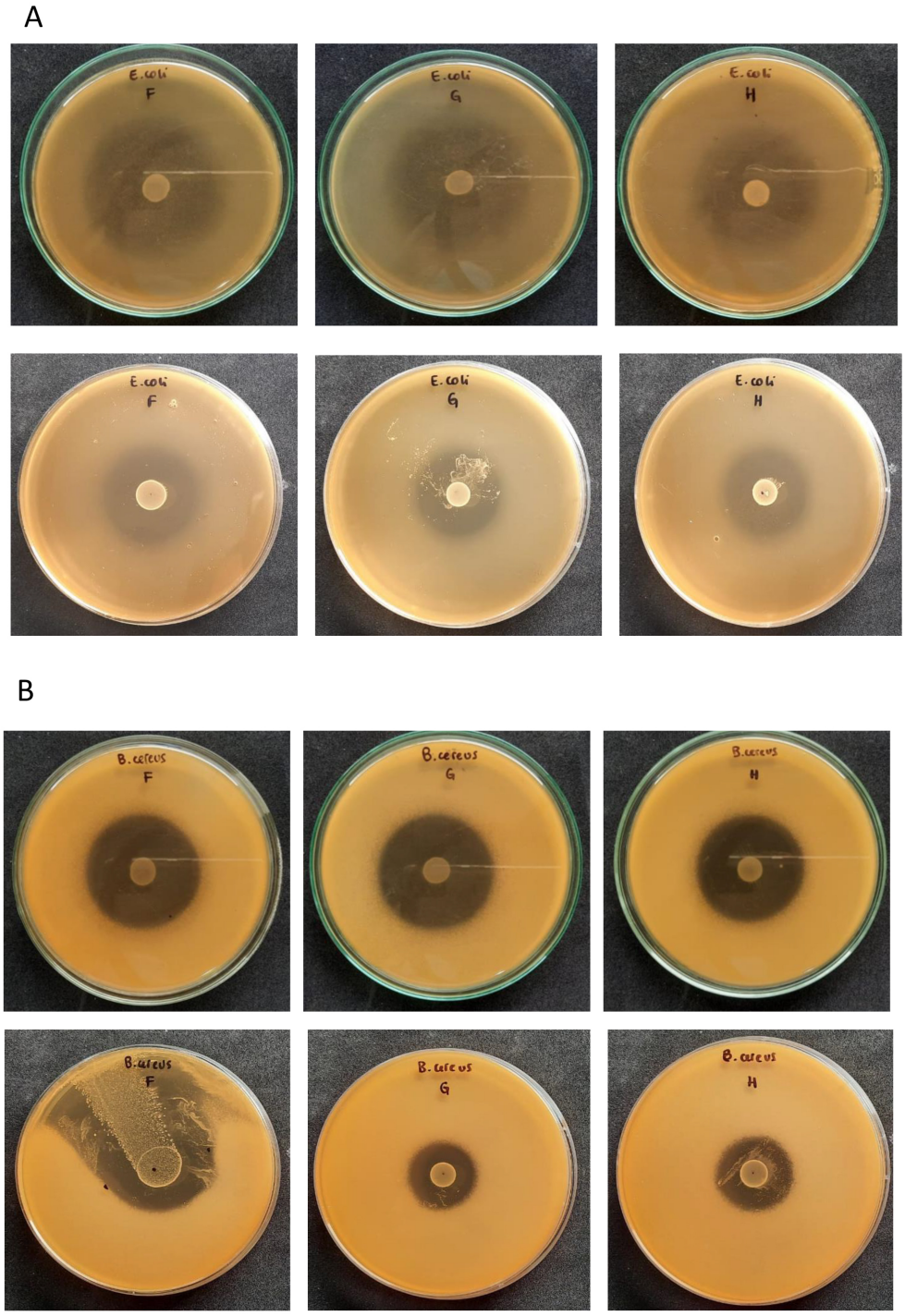

Figure 4

Antimicrobial activity of isolated strains and their metabolites. (A) Inhibitory effect against *E. coli* of metabolites (top row) and vegetative cells (bottom row) of the strain F (left column), G (middle column) and H (right column). **(B)** Inhibitory effect against *B. cereus* of metabolites (top row) and vegetative cells (bottom row) of the strain F (left column), G (middle column) and H (right column).

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