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ORIGINAL ARTICLE

Lactobacillus pentosus ABHEAU-05: An *in vitro* digestion resistant lactic acid bacterium isolated from a traditional fermented Mexican beverage

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Lactic acid bacteria;
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Abstract The health benefits attributed to probiotics generate interest in the search of competent strains adapted to several ecological niches, especially those related to traditional beverages and foods of each country. Pineapple *tepache*, a traditional Mexican fermented beverage, was used for the isolation of lactic acid bacteria with probiotic potential, one of which withstood the *in vitro* tests. The isolated strain AB-05, which exhibited the tested probiotic functional properties, was designated as *Lactobacillus pentosus* ABHEAU-05. The sequence was registered in GenBank under access code MK587617. This study is the first report of a lactic acid bacterium with *in vitro* digestion resistance isolated from pineapple *tepache*. The survival of *L. pentosus* ABHEAU-05 in a symbiotic medium was proven using fermented milk enriched with inulin. The *in vitro* digestion-resistant probiotic activity of lactobacilli was measured through analysis of pH and proteolysis. Results showed that *L. pentosus* grew properly in fermented milk; therefore, this microorganism could be used in the manufacture of this kind of products. The concentration of *L. pentosus* reached up to 8.5 log CFU/ml after 40 h of fermentation. In addition, the production of peptides and the decrease in pH indicated the vigorous and active metabolic state of the lactic acid bacterium tested. The activity and the concentration of this microorganism were maintained during refrigeration. The results of this research conclude that *L. plantarum* ABHEAU-05 is an *in vitro* digestion-resistant microorganism that can be used as a starter culture for the production of functional foods of dairy origin.

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PALABRAS CLAVE
Bacteria ácido láctica;
Potencial probiótico;
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Leche fermentada

Lactobacillus pentosus ABHEAU-05: una bacteria ácido láctica resistente a la digestión _in vitro_ aislada de una bebida fermentada tradicional mexicana

Resumen Los beneficios a la salud atribuidos a los probióticos generan interés en la búsqueda de cepas competentes adaptadas a varios nichos ecológicos, especialmente los relacionados con bebidas y alimentos tradicionales de cada país. En este estudio, se aisló del tepache de piña, una bebida fermentada tradicional mexicana, una bacteria láctica resistente a la digestión *in vitro*. Entre 5 bacterias aisladas, una de ellas soportó las pruebas simuladas de digestión gastrointestinal. Se analizó la resistencia a sales biliares, a condiciones ácidas y al ataque enzimático con pepsina. La bacteria aislada, que exhibió las propiedades funcionales probióticas referidas, fue identificada como *Lactobacillus pentosus* y designada como *L. pentosus* ABHEAU-05. La secuencia fue depositada en GenBank (acceso MK587617). Se comprobó la supervivencia de *L. pentosus* ABHEAU-05 en una leche fermentada adicionada con inulina y su resistencia a la digestión *in vitro* mediante el análisis del pH y la proteólisis. Los resultados muestran que la leche fermentada es una matriz adecuada, donde *L. pentosus* ABHEAU-05 se desarrolla sin inconvenientes, alcanzando un título de 8,5 log ufc/ml después de 40 h de fermentación. Además, la producción de péptidos y el descenso del pH indicaron el estado metabólico vigoroso y activo del microorganismo probiótico. Se concluye que *L. pentosus* ABHEAU-05 es un microorganismo resistente a la digestión *in vitro*, que puede servir como cultivo iniciador para la producción de alimentos de origen lácteo. Este es el primer informe acerca del aislamiento de una bacteria ácido láctica resistente a la digestión *in vitro* a partir del tepache de piña.

Introduction

Tepache is a Mexican traditional fermented drink prepared from pineapple and brown sugar[49]. In Mexico, it is consumed as a refreshing drink and, despite the lack of reliable data; it is known that its origin goes back to pre-Hispanic times[24]. The microorganisms associated with the production of *tepache* include various species of lactic bacteria, acetic bacteria and yeasts, being part of a microbial consortium that turns this drink into a microbiologically complex system[32]. Several lactic acid bacteria (LAB) of interest for human health have been identified from some traditional fermented beverages[15]. Lactic acid bacteria are a broad group of microorganisms that have been considered an essential part of food biotechnology. These microorganisms are important because of their production of secondary metabolites during the fermentation processes as well as for their effect on human health[55]. Currently, research on LAB applications has been increasing on a daily basis; with the broadest approach in the area of probiotics, especially those belonging to the genera *Lactobacillus* and *Bifidobacterium*.

LAB potential use in foods as an ingredient or supplement is directed to the development of products with multiple benefits to consumer health[50,38]. Because consumers have become more aware of health issues and the effects that food has on it, there has been an increase in research related to the discovery of new probiotic microorganisms, mainly those that are isolated from matrices other than milk and dairy products[59,44,26,1]. Isolated LAB have been used as ingredients and starter cultures in the elaboration of functional foods[1,30].

Probiotic cultures have been used successfully in different food matrices such as dairy, meat, beverages, fruits, vegetables, cereals and bakery products[53]. However, there are several factors that can affect the viability of probiotics in the food such as the strain, pH, presence of hydrogen peroxide and oxygen supply, concentration of metabolites, mainly lactic acid and acetic acid, buffer capacity of the medium, storage temperature, origin of added ingredients and matrix, among others[10,14,48,7].

Survival in the gastrointestinal ecosystem and adhesion to the mucosa are considered fundamental requirements for probiotic bacteria to exert their activity[11]. For this reason, the *in vitro* tests used for the evaluation of susceptible microorganisms to be characterized as probiotics are based mainly on their ability to survive gastrointestinal transit[4]. These tests evaluate the capacity of microorganisms to tolerate the hostile conditions imposed by the gastrointestinal tract, such as extremely acidic pHs and the detergent action of bile salts present in the intestine. The ability to adhere to the epithelial surface, the antagonistic activity against pathogenic microorganisms and the potential to colonize the intestine temporarily are also tested through *in vitro* tests[17,22,9]. The analysis of those abilities can be qualitative or quantitative and will allow to correlate the strains with their probiotic capacity *in vivo*[51]. The objective of this research was to isolate and identify a microorganism with resistance of *in vitro* digestion from a Mexican traditional drink and to prove its survival in fermented symbiotic milk during refrigeration storage.

Methodology

Preparation of *tepache*

Tepache was prepared according to the methodology reported by Corona et al.[6] with some modifications. The beverage was elaborated using ripe pineapple peels (unwashed) and a previously pasteurized solution of 13% (w/v) of *panela* (brown cane sugar), in a pineapple/*panela* solution in the ratio 1:2 (w/w). The container was covered and sealed with self-adhesive plastic to simulate semiaerobic conditions. The fermentation was conducted in duplicate in 5 l-glass containers at 22 °C, for 54 h with an initial pH of 4.5.

Sampling and measurement of pH and acidity changes

Aliquots of 25 ml of *tepache* were taken at 2-h intervals from time 0 to 54 h. For the total pH and acidity measurement, a volume of 24 ml was used; the remaining milliliter was used for the viable lactic acid bacteria count. The pH was measured using a potentiometer and total acidity was determined by volumetry and results were expressed as mg of lactic acid per milliliter[25].

Determination of lactic acid bacteria viability during fermentation

The viable lactic acid bacteria count was carried out by the microdrop technique in the Man, Rogosa and Sharpe medium (MRS)[42]. Seeding was performed from consecutive dilutions of the sample from 1×10^{-1} to 1×10^{-5} in peptone water solution. Plates were incubated at 37 °C for 18–24 h.

Isolation and macroscopic characterization of LAB

LAB strains were selected from the plates used for the viable count. Subsequently, the morphological differentiation was carried out based on criteria such as color, consistency, shape, size and type of edge previously described by Winn and Koneman[60]. The selected colonies were isolated on MRS agar by the streaking technique. Using this same technique, the developed LAB strains were transferred to the M17 medium to differentiate lactic bacilli from lactococci and streptococci. In order to corroborate the gram positive character of the isolated strains, as well as to identify their microscopic morphology and verify the purity of the culture, stained smears under the Gram technique for each identified colony were performed.

In vitro digestion resistance tests

Three continuous *in vitro* tests (tolerance to bile salts, resistance to acidic pH and resistance to the action of pepsin) were carried out to test the survival capacity of the selected LAB strains under conditions imposed by the gastrointestinal tract. *Lactobacillus casei* Shirota was used as the reference strain.

Bile salt tolerance

Bile salt tolerance of the isolated strains was determined by the method proposed by Lee et al.[33] with slight modifications. MRS broth was enriched with 0.3% (w/v) of bile salts and adjusted at pH = 7.0 with 0.1 N NaOH. One hundred (100) μl of the fresh culture were inoculated into the enriched medium and incubated at 37 °C for 2 h. To know the initial count, 100 μl of fresh culture of each strain were inoculated in MRS broth without enrichment (control). The seeding of both samples was performed by the microdrop method on MRS agar and they were incubated for 24 h at 37 °C. Subsequently, the counting of each plate was carried out.

Resistance to acid pH

The resistance to acid pH was determined according to Park et al.[47] with some modifications, inoculating 100 μl of fresh culture into a culture tube containing 10 ml of MRS broth previously adjusted to pH 2.0 with concentrated HCl. Then, it was incubated at 37 °C for 2 h. Additionally, an initial culture count was conducted from a control tube containing 100 μl of fresh culture in MRS broth without acidification. To confirm the survival of the microorganisms after the incubation period, viable cells were counted by the microdrop technique.

Resistance to pepsin action

The *in vitro* capacity of isolated LAB to resist pepsin action was measured by inoculating 100 μl of culture into MRS broth supplemented with 1000 IU of pepsin and adjusted to pH 2.5[54]. The medium was incubated for a period of 2 h at 37 °C. The count of viable cells after incubation and the initial count were carried out on MRS agar using the microdrop technique.

Determination of survival rate

The number of viable cells was determined by plate count on MRS agar. The survival percentage was calculated using the following equation[5]:

$$\% \ \text{survival} = \left(\frac{\log \text{UFC}\, N_1}{\log \text{UFC}\, N_0} \right) \times 100$$

where

N_1 refers to the number of viable cells after treatments
N_0 represents the initial number of LAB inoculated

Identification of isolated LAB and phylogenetic analysis

The identification of isolated LAB was performed using the 16S rRNA sequence. After 24 h of incubation, the reaction broths were centrifuged and total bacterial DNA extraction was carried out using the Wizard Genomic DNA Purification Kit (Promega) following the manufacturer's instructions.

Genomic DNA was stored at −20 °C for an additional molecular study. The variable region V1–V3 of the 16S rRNA gene (approximately 510 bp) was amplified by the PCR technique using primers 4F and pD[29]. PCR reactions were carried out in a total volume of 50 ul in a thermocycler (Biorad). The reaction mixture consisted of 10 ng of genomic DNA, 1 U of *Taq* polymerase, *Taq* buffer (1×), 200 μM dNTP, 10 μM of each primer (forward and reverse) and 2 mM $MgCl_2$. The thermocycler protocol was an initial denaturation at 95 °C for 5 min, followed by 30 cycles of 95 °C for 1 min, 56 °C for 1 min and 72 °C for 1 min with a final extension of 5 min to 72 °C. The amplicons were purified using the Gel DNA Extraction Kit (Promega) and sequenced in an ABI PRISM 3100 AVANT. The variable region of 16S sequences obtained was sent to the BLAST algorithm with the aim of comparing them to the database of the National Biotechnology Information Center (www.ncbi.nih.gov). For the phylogenetic analysis, the sequences were aligned using the CLUSTAL X software[23]. Based on the implemented algorithm, a phylogenetic tree was constructed using the maximum likelihood method based on the General Reversible model. The stability or accuracy of each inferred topology was assessed by bootstrap analysis with 1000 replicates. The *Staphylococcus epidermidis* L22 strain was used as an external group (access number KU922485.1). Molecular Evolutionary Genetics Analysis Version 7.0 (MEGA7) was used to infer the maximum probability in order to generate the phylogenetic Tree[31].

Survival evaluation of *Lactobacillus pentosus* ABHEAU-05 in fermented milk

In order to assay the survival of *L. pentosus* ABHEAU-05 and to evaluate its behavior as a starter culture in fermented milk, this microorganism was grown in a solution of skimmed milk powder at 12% (w/v) (Dairy Gold®) added with 2% of inulin (E NATURE®) following the technique reported by Jaimez-Ordaz et al.[28]. The prepared milk solution was pasteurized at 90 °C for 10 min in autoclave, inoculated with 10^6 CFU/ml and incubated at 37 °C for 28 h. Samples were taken every 2 h, during fermentation until the end of the logarithmic phase (beginning of deceleration). At the end of the fermentation, the plate count was performed on MRS-agar by the microdrop technique. The fermented milk was stored at 4 °C for 21 days. Sampling was performed at 7, 14 and 21 days to measure the pH decrease and the proteolytic capacity, which was monitored through the analysis of free amino groups by the TNBS technique[2]. Both analyses were performed during lactic fermentation and during the refrigerated storage of fermented milk. The survival of *L. pentosus* ABHEAU-05 in refrigerated fermented milk was verified through plating on MRS medium by the microdrop technique.

Results and discussion

Fermentation of *tepache*

Changes in pH, acidity and viable LAB count were monitored. The initial pH of the fermentation system was 4.4 and at the end of the process the pH reached 3.3, which is within the pH range (3.22–3.66) obtained by Moreno-Terrazas[40] in a study on four *tepache* samples elaborated in Mexico City. The changes in pH during *tepache* fermentation are probably directly related to the production of organic acids, mainly lactic and acetic acid, which are generated by the native microbial consortium of this beverage (yeast, lactic and acetic bacteria), from available fermentable sugars[58]. With regard to acidity, values between 0.34 and 0.48% were obtained, which shows a significant increase, since the initial values were between 0.027 and 0.03%. Similar to pH, titratable total acid values obtained at the end of fermentation are in agreement with those reported by Moreno-Terrazas[40].

With respect to the viable LAB count, the results obtained showed a curve similar to the typical bacterial growth curve (Fig. 1). In the stationary phase, a straight line was not observed (which would respect the ideality of a typical bacterial growth curve). On the contrary, the points showed increases and decreases throughout this stage, approximately between 6 and 8 log CFU/ml; with a final viable account of 7.29 log CFU/ml. Despite the observed variations, the values observed during the stationary phase and the final viable account were similar (6.37–8.56 log CFU/ml) to those obtained by Moreno-Terrazas[40]. The observed variations are due to competition for the substrate or to a metabolic imbalance caused by the depletion of fermentable sugars, since each metabolic reaction is regulated by the concentration of nutrients in the environment[21].

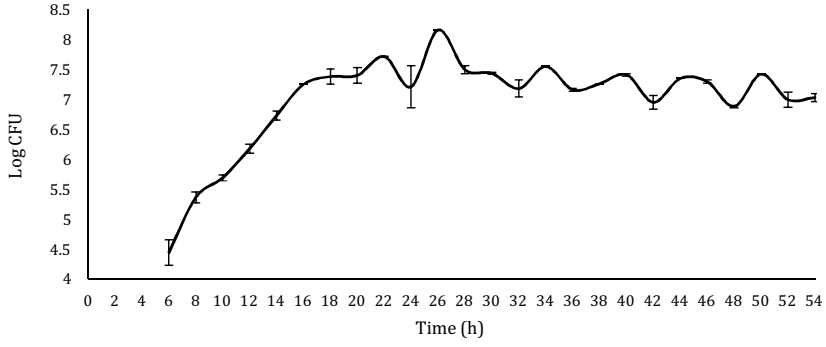

Figure 1 LAB concentration during pineapple *tepache* fermentation process.

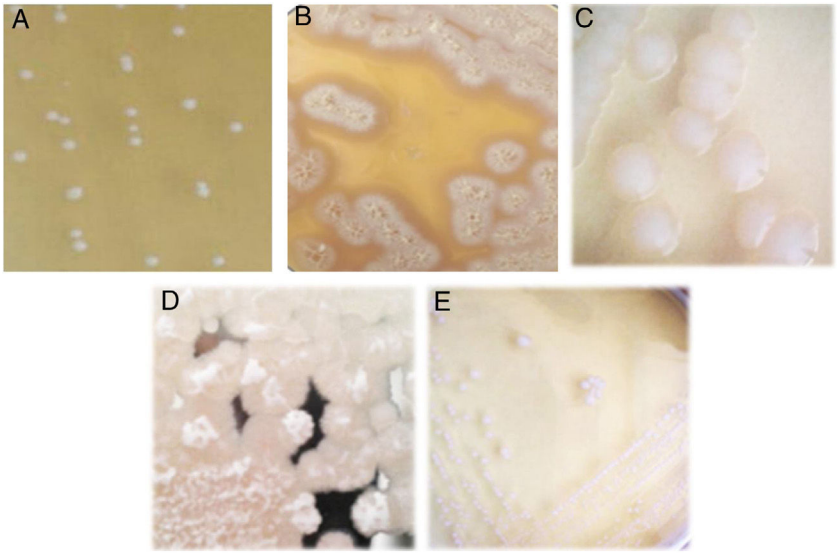

Figure 2 Colony morphology of lactic acid bacteria isolated from *tepache*. (A) Strain AB-1, (B) strain AB-2, (C) strain AB-3, (D) strain AB-4 and (E) strain AB-5.

Isolation of lactic acid bacteria

Five lactic acid bacteria were isolated from *tepache* (AB-1 to AB-5). Only isolated strain AB-1 showed growth in both culture media (MRS and M17). Its morphology on MRS medium was slightly white, circular, with defined edges, convex, 2 mm of approximate diameter and creamy consistency (Fig. 2A). The morphology observed for isolated strain AB-1 on M17 medium was similar to that described above, only differing in colony size (3 mm) and the intensity of the white color. Maximum growth for this isolated bacterium was observed between 28 and 36 h after fermentation.

Isolated strains AB-2 and AB-3 only grew on MRS agar. They showed colonies with a large extension on the surface of the plate due to the production of exopolysaccharides. Isolated strain AB-2 presented extended and filamentous colonies of beige color and a crystalline halo (Fig. 2B) while isolated strain AB-3 showed rhizoid acuminate white colonies, with dry consistency (Fig. 2C). Isolated strain AB-2 had a maximum prevalence between 36 and 46 h of fermentation. In contrast, isolated strain AB-3 grew during all the fermentation process from the first 4 h showing minimal and constant concentration compared to isolated strains AB-1 and AB-2.

Similar to isolated strains AB-2 and AB-3, isolated strains AB-4 and AB-5 only developed on MRS agar (Fig. 2D and E). Isolated strain AB-4 exhibited colonies of approximately 4 mm in diameter, irregular shape, flat, white, with a transparent halo around it, exopolysaccharide production, creamy consistency and development only in the first hours of fermentation. In the case of isolated strain AB-5, the colonies measured approx. 4–5 mm in diameter, were circular in shape with a defined edge, convex, exhibited intense white color, creamy consistency and its prevalent development occurred during the last hours of fermentation.

Once isolated on MRS medium, the five isolated bacteria obtained from *tepache* were analyzed microscopically by the Gram staining technique, with positive results. Using the observed morphology, an isolate corresponding to lactic streptococci and four bacillary isolates (Fig. 3) were identified. Strain AB-1 exhibited a morphology characterized by small cocci grouped in a chain (streptococci) (Fig. 3A) while strain AB-2 showed short bacilli with rounded edges (Fig. 3B). On the other hand, strain AB-3 presented a peculiar microscopic morphology in which it was possible to observe long bacilli, grouped in laterally joined chains. In addition, this isolate presented a halo around the cells due to the production of exopolysaccharides (Fig. 3C). Strain AB-4 showed long bacilli (Fig. 3D), for strain AB-5 the bacilli observed were of medium size, slightly curved and grouped in pairs at the ends. Some groups showed bacilli with curvatures so pronounced that appeared to join to form a circle (Fig. 3E).

Comparing the morphology described by several authors with the isolated bacteria in this investigation, it was found that isolated strains AB-3 and AB-5 presented morphological similarities to *Lactobacillus* species[15,12]. Isolated strain AB-3 also showed morphological similarities to a dextran-producing *Leuconostoc* species isolated from *pulque* samples[57]. On the other hand, isolated strains AB-2 and AB-4 were morphologically similar to 3 species of gram positive bacilli also isolated from *pulque* while isolated strains AB-1, AB-2 and AB-4 presented similarities to the gram positive cocci isolated from white *pozol* and with gram positive bacilli isolated from *sotol*[12,43].

Several native microorganisms, including lactobacilli species have been isolated from traditional Mexican fermented beverages such as *pulque*, *pozol*, *sotol*, *aguamiel* and *tepache*[15,12,43,13]. For these revealed facts, *tepache* has been a traditional beverage to research for the isolation and identification of novel potential probiotic species[49].

Several traditional Mexican fermented beverages such as *pulque*, *pozol*, *sotol*, *aguamiel* and *tepache* have been used for the isolation and identification of novel probiotics, including lactobacilli species[15,12,43,13,49].

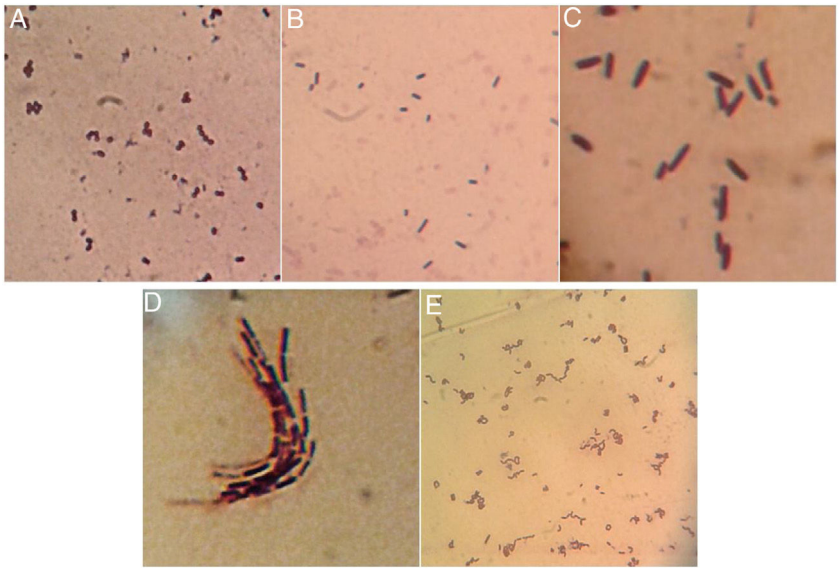

Figure 3 Microscopy morphology of lactic acid bacteria isolated from *tepache*. (A) Strain AB-1, (B) strain AB-2, (C) strain AB-3, (D) strain AB-4 and (E) strain AB-5.

Table 1 Percentage of viable cells measured by the *in vitro* probiotic capacity test.

Strain	Bile salt tolerance (%)	Acidic condition resistance (%)	Enzymatic condition resistance (%)
AB-1	89.5	No survival	No survival
AB-2	66.1	72.7	No survival
AB-3	80.8	No survival	No survival
AB-4	83.4	72.6	No survival
AB-5	92.8	82.8	97.6
Lactobacillus casei	96.3	91.3	95.5

In vitro digestion resistance tests

Table 1 shows the survival percentage of the five lactobacilli isolated from *tepache*, elaborated for the *in vitro* capacity tests conducted. These results were compared to those obtained for *L. casei* which was used as reference.

Bile salt tolerance

For bile salt treatment, the survival rates of the isolated bacteria were comparable to those of the reference probiotic, except for isolated strain AB-2. It is known that the ability to resist the bactericidal action of bile salts is directly related to the synthesis of hydrolases that cause their deconjugation, thus preventing the dissolution of cell membranes[41]. The isolated bacteria from *tepache* demonstrated strong tolerance to 0.3% bile salts, showing survival rates >80%, except for isolated strain AB-2 (Table 1). That concentration has been reported as critical for the evaluation of bile salt-resistant strains[18]. Bile salt-resistance of the isolated bacteria was similar to that reported for potential probiotic bacteria isolated from fermented food and assayed under the same experimental conditions including a *L. pentosus* strain isolated from mustard pickles[59,44,26,4,17,22,9,35,52,39,51].

Resistance to acid pH and pepsin action

None of the isolated bacteria, except for AB-5, showed resistance to the pepsin treatment, although isolated strains AB-1 and AB-4 were able to grow under acidic conditions (pH 2). It has been reported that each LAB species and even each isolate, exhibit different tolerance to acid conditions[52]. The results observed for the abovementioned isolated strains are in agreement with those reported in studies carried out with LAB isolated from traditional Mexican beverages such as *pulque*[13], *pozol*, *sotol* as well as *aguamiel*[49]. Those LAB have demonstrated their ability to survive to acidic conditions. The behavior observed for isolated strain AB-5 coincides with that shown by several species of *L. pentosus* isolated from green table olives[39], one lactobacillum isolated from *pulque*[51] and some lactic acid bacteria isolated from fermented food[9,39,51,56]. Some of those microorganisms also showed resistance to the *in vitro* treatment with artificial

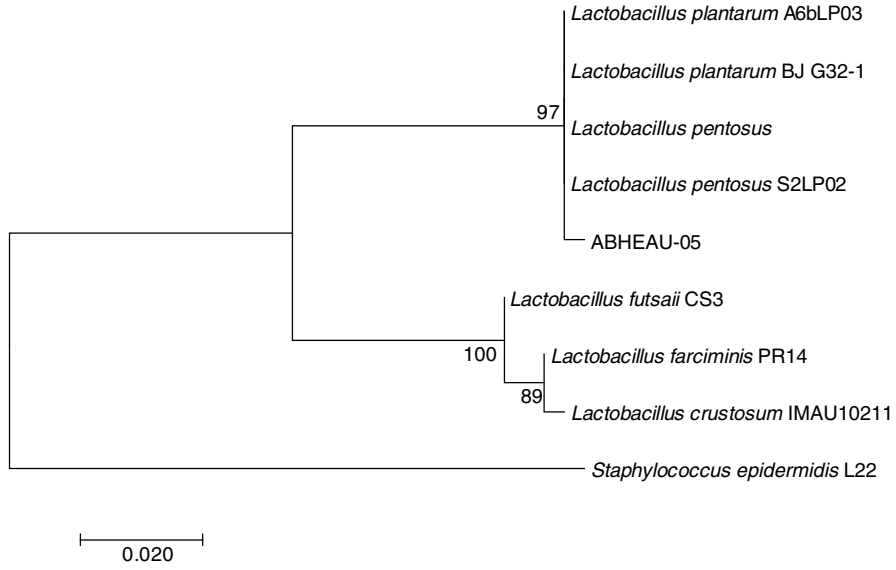

Figure 4 Phylogenetic tree showing the interrelationship of *Lactobacillus pentosus* ABHEAU-05 with closely related species of *Lactobacillus* and other related genera.

gastric juices (different combinations of acidic conditions and enzymes such as pepsin).

The resistance to low pH (2.0–3.0) is a critical factor for the selection of a probiotic due to the fact that microorganisms must be able to survive unfavorable conditions in the stomach during transit[39].

It is known that the ability of a microorganism to survive acidic conditions (which varies from one species to another) depends directly on the concentration of hydronium ions that accumulate inside the cell[27], which may affect the Mitchell's chemiosmotic mechanism[8], as well as the source and activity of H^+-ATPase[37]. Survival under acidic conditions is positively affected by adaptation to low pH, a behavior known as the acid-tolerance response[8].

With regard to resistance to pepsin, isolated strain AB-5 was the only one that was able to grow under the conditions of the *in vitro* tests conducted, showing a similar behavior to that of the *L. casei* strain used as reference. These results are also in agreement with viability reported for other lactobacilli species in the presence of pepsin at pH 2.0 for 3 h[34,36,37]. The action of pepsin consists in the selective hydrolysis between hydrophobic amino acid bonds with certain specificity for some of them[3], so that the resistance of each bacterial species to its proteolytic action is related to the content of amino acids of its membrane proteins.

Molecular identification of the selected LAB

Considering the *in vitro* digestion resistance of isolated LAB AB-5, which showed desirable characteristics compared to those of a commercial probiotic, its identification was performed using the 16S rDNA analysis sequence. The 16S rDNA sequence of the isolated LAB showed 99% similarity with several *L. pentosus* species available from GenBank (Fig. 4) (National Center for Biotechnology Information (NCBI)). Therefore, isolated strain AB-5 was designated as *L. pentosus* ABHEAU-05. The sequence was deposited in the GenBank under access number MK587617.

This study is the first to report the isolation of *L. pentosus* as a lactic acid bacterium resistant to *in vitro* digestion from a traditional Mexican fermented drink (*tepache*) prepared under standardized conditions. The results encourage the research on fermented beverages in Mexico as a potential source of probiotics of non-dairy origin.

Survival evaluation of *L. pentosus* ABHEAU-05 in symbiotic fermented milk

The pH decrease during milk fermentation demonstrated the activity of *L. pentosus* ABHEAU-05 during the process. The symbiotic fermented milk reached a pH of 5.0 after 28 h. In this kind of system, the pH decrease is directly related to the conversion of lactose into lactic acid[46].

With regard to survival, a two logarithmic cycles higher concentration of viable cells (8.68 ± 0.007 log of CFU/ml) was observed compared to the initial. It has been shown that some probiotic microorganisms that are developed in symbiotic milk as a fermentation medium reached similar values[45].

Table 2 *Lactobacillus pentosus* ABHEAU-05 survival during 21 days of refrigerated storage (4 °C).

Time (weeks)	0	1	2	3
log CFU/ml	8.65 ± 0.01	8.62 ± 0.02	8.46 ± 0.02	8.30 ± 0.07

Similar to the pH, the proteolytic capacity decreased through the fermentation process demonstrating the activity of the microorganism. The concentration of free amino groups reached a maximum of 269 ± 2.2 mg/l after 18 h of processing, getting a final value of 123.16 ± 0.83 mg/l at 28 h. This behavior has already been described by some authors[16,20].

Concerning the survival of *L. pentosus* ABHEAU-05 under refrigerated storage of fermented milk, there was no significant difference in the concentration of viable cells after 21 days at 4 °C (Table 2). The concentration of *L. pentosus* ABHEAU-05 remained at higher levels than those recommended for a probiotic in fermented milks (1×10^6 CFU/ml). These results are comparable to those reported by several authors for probiotic and non-probiotic lactic acid bacteria[10,28,35,19].

The evaluation of the survival of *L. pentosus* ABHEAU-05 during fermentation and refrigerated storage of a symbiotic fermented milk demonstrated that this microorganism isolated from a medium other than milk has the ability to grow, develop and remain viable under hostile conditions.

Conclusion

This is the first report of *L. pentosus* (ABHEAU-05) isolated from *tepache*, a Mexican traditional beverage, which has exhibited resistance to *in vitro* digestion, one of the preliminary analyses in order to select probiotic strains. However, further studies are required to consider this microorganism a potential promising starter culture in the manufacture of fermented dairy products in order to obtain a symbiotic functional food.

Conflict of interest

The authors have no conflict of interest to declare regarding the publication of this article.

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