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TWO-STEP CONTROLLED FERMENTATION USING NISIN Z-PRODUCING *LACTOCOCCUS LACTIS* SSP. *LACTIS* AND SELECTED STRAINS OF THE GENUS *LACTIPLANTIBACILLUS* SPP.

Franco M. Sosa[1,2], Romina B. Parada[1,2], Emilio Marguet[1], and Marisol Vallejo[1]*

Address(es):
[1]Bacterial Biotechnology Laboratory (BBL). Faculty of Natural Sciences and Health Sciences, National University of Patagonia San Juan Bosco, Chubut, Argentina.
[2]BBL-CONICET, Chubut, Argentina.

*Corresponding author: franco.m.sosa94@gmail.com

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ABSTRACT

Spontaneous fermentation of *Brassica* vegetables is an ancient method that improves the foods' sensory properties, safety, and shelf life. However, in recent years, there has been a growing interest in studying controlled fermentation with lactic acid bacteria selected based on their technological properties. This study reported the inhibitory activity of a *Lactococcus lactis* ssp. *lactis* of marine origin and the evolution of a two-step controlled fermentation using the cited strain and two selected *Lactiplantibacillus* strains. *Lc. lactis* ssp. *lactis* was studied by phenotypic and genotypic methods. The inhibitory activity was assayed against common contaminants and pathogens. The strain behaviour in Chinese cabbage and white cabbage fermentation was studied by monitoring the cell count and inhibitory activity against *Listeria innocua* ATCC 33090. The two-step controlled fermentation of Chinese and white cabbage was conducted using, in the first step, the *Lactococcus* strain and, in the second step, the two *Lactiplantibacillus* strains. The evolution of the processes was monitored by cell counts and pH measurements. PCR amplification confirmed the presence of nisin Z gen in the *Lc. lactis* ssp. *lactis* strain. The bacteriocin exerted inhibitory activity against Gram-positive related species but not against Gram-negative ones. The results obtained in the controlled fermentation suggested that the combination of the selected strains is compatible and may improve the fermented matrices' safety.

Keywords: *Lactococcus lactis* ssp. *lactis*, *Lactiplantibacillus*, *Brassica* vegetables, controlled fermentation

INTRODUCTION

Functional foods are products used as part of a regular diet with effects beyond their essential nutritional functions, such as providing physiological benefits and reducing the risk of chronic disease (**Syngai *et al.*, 2011**) The government of Japan coined the term in the mid-1980s, and in 1991, the Ministry of Health, Labor and Welfare introduced a functional food regulation called "Foods for Specified Health Uses" (FOSHU) (**Iwatani and Yamamoto, 2019**).

These foods include components classified into different categories that can divide into nutraceuticals, prebiotics, and probiotics (**Pandey *et al.*, 2015**). The World Health Organization (WHO) defines probiotics as live microorganisms that, when supplied in adequate quantities, promote benefits to the host's health.

Dairy products are the most widely used form by the food industry to provide the market with functional foods containing probiotics (**Gao *et al.*, 2021**). However, this situation is inconvenient for vegetarian consumers and those requiring cholesterol-free diets or lactose intolerance (**Pimentel *et al.*, 2021; Küçükgöz and Trzaskowska, 2022**). This situation has led to deepening the study of fermentation processes on plant matrices (**Torres *et al.*, 2022**), within which Brassicaceae has become the model to investigate due to their high nutritional value (**Šamec and Salopek-Sondi, 2019**). Since ancient times, fermentation has been one of the oldest and simplest ways to extend shelf life, improve sensory quality, and increase the safety of plant-based foods (**Di Cagno *et al.*, 2013; Torres *et al.*, 2020**).

Raw vegetables and fruits may be subjected to spontaneous lactic acid fermentation when conditions are favorable (anaerobiosis, water activity, salt concentration, and temperature) to the growth of the autochthonous lactic acid bacteria (LAB) (**Di Cagno *et al.*, 2013; Torres *et al.*, 2020**). However, the final products of the process are random because they depend on numerous variables such as plant species, geographical area, climatic conditions, fermentation techniques, among others (**Di Cagno *et al.*, 2013; Šamec and Salopek-Sondi, 2019**). Starter ferments have begun to be marketed for vegetables with LAB selected on the basis of their physiological characteristics in order to avoid these drawbacks and achieve a product of uniform quality (**Di Cagno *et al.*, 2013; Šamec and Salopek-Sondi, 2019**).

There are technological, sensory, and nutritional criteria for selecting strains for controlled fermentation (**Di Cagno *et al.*, 2013, Parada *et al.*, 2023**). In addition to the ability to multiply in a plant environment, one of the most appreciated metabolic traits in the selection of strains is the synthesis of antimicrobial compounds. The inhibitory activity of these metabolites may be nonspecific, such as that exerted by organic acids, diacetyl, hydrogen peroxide, or specific, as exhibited by bacteriocins (**Siddeeg *et al.*, 2022**).

These natural antibiotics are proteins or peptides synthesized by the ribosome that generally exert inhibitory activity against closely related species (**O'Connor *et al.*, 2020**). Currently, nisin and pediocin are the only bacteriocins authorized and marketed for inclusion in food as inhibitory additives to pathogenic or contaminating flora (**Ünlü *et al.*, 2015; Silva *et al.*, 2018**).

The search for bacteriocinogenic LAB strains that could be used as bioprotective cultures in food or for bacteriocin synthesis has intensified in recent years. This trend has focused on isolating and selecting strains with inhibitory capacity from food or terrestrial environments. However, recent reports indicate that the marine environment could become a suitable habitat for searching for microorganisms with such properties (**Sala Gomez *et al.*, 2015; Rather *et al.*, 2017**). This biota is exposed to severe environmental conditions (low temperature, high salt concentrations, high competition for resources, etc.) and consequently exhibits physiological profiles different from terrestrial analogs. Recently, some works have reported the isolation of bacteriocinogenic LAB from fish and invertebrates on the Patagonian coast, resulting in a promising ecosystem for isolating microorganisms with potential biotechnological properties (**Schelegueda *et al.*, 2015; Delcarlo *et al.*, 2019; Sosa *et al.*, 2022**).

In the present work, we report the isolation, molecular studies, and antimicrobial spectrum of a nisin Z-producing *Lactococcus lactis* isolated from the intestinal tract of silverside (*Odontesthes platensis*). We also investigated the growth evolution and inhibitory activity using Chinese and white cabbage as fermentation matrices.

Moreover, a two-step controlled fermentation of Chinese and white cabbage was conducted to determine the effect of the inclusion and behavior of a starter culture with inhibitory activity. The first step started with the addition of the nisin Z-producing *Lc. lactis*. The second step was carried out by adding two *Lactiplantibacillus* strains previously isolated from *Brassica* vegetables and selected by technological properties (**Parada *et al.*, 2023**). The process was monitored by the evolution of pH, change in the bacterial population, and inhibitory activity.

MATERIALS AND METHODS

Microorganisms

Lc. lactis ssp. *lactis* Tw35 were isolated from the intestinal tract of *O. platensis*, captured on the northeast coast of Chubut Province, Patagonia, Argentina. A homogenate of the intestinal tract was inoculated into M17 broth (Biokar Diagnostics, France) and incubated at 25°C for 18 h. Phenotypic tests were based on the methods described by **Teuber (1995)**: growth at 10°C and 40°C, growth in NaCl at 4% but not 6%, growth with methylene blue at 0.1% in milk, ammonium production of arginine, and fermentation of maltose.
L. plantarum RBTw256 (accession number MT178442) and *L. argentoratensis* PCTw261 (accession number MT178443) were isolated in a previous work from spontaneous fermentation of *Brassica* vegetables and belong to the collection of Bacterial Biotechnology Laboratory (Faculty of Natural Sciences and Health Sciences, National University of Patagonia San Juan Bosco) (**Parada *et al*., 2023**).

Genotypic identification

Lc. lactis ssp. *lactis* Tw35 genomic DNA purification was carried out using Wizard Genomics kits (Promega, Madison, Wisconsin, USA) following the manufacturer's instructions. Molecular identification was conducted by amplification of the 16S rRNA gene using the following universal primers 27F (5′ -AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (**Hou *et al*., 2018**). Sequencing of PCR products was performed by the commercial services of Macrogen Inc. (Seoul, Korea). The 16S rRNA sequence homology search against the NCBI database was carried out using BLAST algorithm (https://www.ncbi.nlm.nih.gov/BLAST/).

Phylogenetic tree construction

Software package MEGA version 6.0 (**Tamura *et al*., 2018**) was used to construct a phylogenetic tree based on Neighbor-Joining method (**Saitou and Nai, 1987)**. The Tamura-Nei substitution model was used, with a bootstrap value of 1,000 replicates. The 16S rDNA sequence was aligned against sequences of collection strains obtained from the database Ribosomal Database Project (RDP) and the National Center for Biotechnology Information (NCBI). *Bacillus subtilis* ATCC 19659 (MN456847.1) sequence was chosen as an outgroup strain.

Screening for antimicrobial activity

Cell-free culture supernatants (CFS) were obtained by centrifugation of an overnight culture grown in MRS (Biokar Diagnostics, France) broth at 12,000 × *g* for 2 min. Neutralized cell-free culture supernatants (NCFS) were adjusted to pH 6.5 using 0.5 M NaOH (Anedra, Argentina). Afterward, CFS and NCFS were heated at 100 ºC for 5 min, filtered through a membrane 0.20 mm pore size (Sartorius, Stedim Biotech, Germany), and stored at -30 ºC until use.
The antimicrobial activity of *Lc. lactis* ssp. *lactis* Tw35 was assessed by the agar well diffusion assay (AWDA) described by **Rivas *et al*. (2012)**. Molten agar was seeded (1% v/v) with an overnight suspension of indicator strains and dispensed in sterile Petri dishes until solidification. Wells of 6 mm diameter were bored in the plates and filled with 50 µL of CFS or NCFS. The strains used as indicators, along with their respective culture media and conditions, were outlined in Table 1. Plates were incubated at optimum temperature according to the different indicator strains and examined after 24 h. The diameter of the inhibition zones was measured by a caliper and classified as follows: no inhibition (–), ≥ 10 mm (+), ≥ 15 mm (++), and ≥ 20 mm (+++).

Molecular identification of the structural gene of Nisin

Lc. lactis ssp. *lactis* Tw35 genomic DNA was obtained as described previously. PCR amplification was carried out using the primers F 5'-GGATAGTATCCATGTCTG-3' and R 5'-CAATGATTTCGTTCGAAG-3', following the protocol suggested by **Li and O'Sullivan (2002)**. Sequencing of PCR products was performed by the commercial services of Macrogen Inc. (Seoul, Korea).

Controlled Fermentation with *Lc. lactis* ssp. *lactis* Tw35

The Chinese cabbage (*Brassica rapa* L. var. *glabra*, Regel) and white cabbage (*B. oleracea* L. ssp. *capitata*, Metzg.) were purchased from a local farm of Valle Inferior del Río Chubut located in Patagonia, Argentina. The cleaned bulbs were chopped in a shredder into 2 mm thick strips, supplemented with NaCl 3% (w/w), and steamed in an autoclave for 5 min.
Lc. lactis ssp. *lactis* Tw35 was cultured in MRS broth for 18 h at 30°C and then centrifuged (4,000 *g*, 10 min). The cell pellets were washed twice and resuspended in distilled water. The vegetables were individually inoculated, reaching a final population of approximately 10^4 colony-forming units per gram ($CFU.g^{-1}$), and incubated at 18°C for 12 days. Samples were taken every 24 h.

Viable cell count and inhibitory activity

Viable lactic counts were determined by serially diluting the samples in distilled water and pour plating on M17 agar. The population was expressed as $\log_{10}$ colony-forming unit per gram ($\log_{10}$ $CFU.g^{-1}$) of the cabbage sample.
The antimicrobial activity of the samples was assessed by AWDA, as described previously. Briefly, ferment samples were centrifuged (12,000 *g*, 5 min), and supernatants were adjusted to pH 6.5 with 0.5 M NaOH. Molten MRS agar was seeded (1% v/v) with an overnight suspension of *Listeria innocua* ATCC 33090 as indicator strain and dispensed in sterile Petri dishes until solidification. Wells were filled with 50 µL of serial dilution of NCFS. Plates were incubated at 35°C and examined after 24 h. The antimicrobial activity was defined as the reciprocal of the last dilution, which exhibited a detectable inhibition zone and was expressed as arbitrary units per milliliter ($AU.mL^{-1}$) (**Sosa *et al*., 2022**).

Two-step controlled fermentation trial

The process was conducted according to the recommendations previously described by **Jagannath *et al*. (2012)** with some modifications. In the first step, Chinese and white cabbage were processed and inoculated with *Lc. lactis* ssp. *lactis* Tw35, as previously described. The second step starts at third day and involved the addition of *L. plantarum* RBTw256 and *L. argentoratensis* PCTw261. Both strains were cultured in MRS broth (18 h, 30°C), centrifuged, washed twice, and resuspended in distilled water. Both vegetables were inoculated; the former strains reached a population of approximately 10^4 $CFU.g^{-1}$. Samples were collected every 24 h throughout the 30 days of process.
The pH of the samples was measured using a pH meter (model Orion 410A). *Lc. lactis* ssp. *lactis* Tw35 growth was monitored on M17 agar using a serial dilution of ferment samples, and the plates were incubated at 30°C for 48 h. *Lactiplantibacillus* population was monitored similarly, using MRS agar supplemented with vancomycin (30 µg.mL^{-1}). The population was expressed as $\log_{10}$ $CFU.g^{-1}$ of cabbage sample.

RESULTS AND DISCUSSION

Phenotypic identification

Lc. lactis is specie closely related to dairy products. Most reference strains deposited in international collections have been isolated from milk and dairy products. Together with *Lc. cremoris*, they make up homofermentative mesophilic ferments, one of the most used combinations in cheese making (**Poudel *et al*., 2022**). Few studies have reported on the isolation of *Lc. Lactis* (**Sequeiros *et al*., 2010**) in environments outside the dairy industry, so it is curious to find the strain under investigation in the intestinal content of silverside.
The results of the phenotypic tests suggested by **Teuber (1995)** allowed presumptively classifying the strain under study as *Lc. lactis* ssp. *lactis*: growth at 10°C and 40°C, growth in NaCl at 4% but not 6%, growth with methylene blue at 0.1% in milk, ammonium production of arginine, and fermentation of maltose. However, the biochemical tests cited are insufficient to differentiate them from the other species and subspecies of the genus *Lactococcus*. Currently, the genus has 24 species and eight subspecies, and it is essential to resort to molecular methods for its definitive identification (**Parte *et al*., 2020**).

Genotypic identification and phylogenetic tree

The amplification of the 16S rRNA gene with universal primers allowed to obtain an amplicon of 1,332 bases that, compared with the sequences of the databases, showed a 100% homology with the collection strain *Lc. lactis* ssp. *lactis* NCDO 604T.

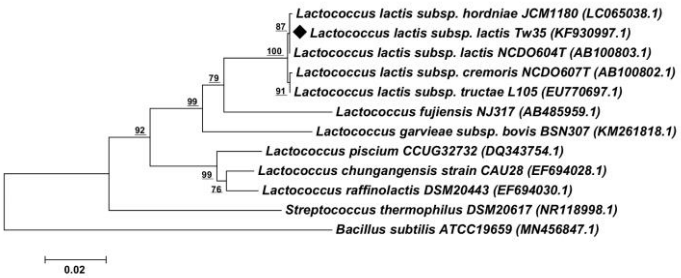

Figure 1 Phylogenetic tree constructed by Neighbor-Joining method based on the relationship between the 16S rRNA gene sequences of *Lactococcus lactis* ssp. *lactis* Tw35 and related type strains of the genus *Lactococcus*. The numbers at internal nodes are bootstrap support values. The 16S rRNA sequence of *Bacillus subtilis* was chosen arbitrarily as an outgroup sequence. (bar, 0.02 substitution per nucleotide position).

In the phylogenetic tree (Figure 1), the strain under study can be observed in the same clade of the 4 subspecies of *Lc. lactis*: *lactis*, *cremoris*, *hordniae* and *tructae*, separated from the other species of genus. The partial sequence of the 16S rRNA gene was deposited in the GenBank database under the name *Lc. lactis* ssp. *lactis* Tw35 and accession number KF930997.1.

Molecular identification of the structural gene of nisin

An amplicon of approximately 250 bp was obtained by the amplification of the genetic material using the specific primers for nisin A. Its subsequent sequencing and comparison with the sequences of the databases allowed determining a homology of 100% with the sequence of the gene coding for nisin Z. Nisin is a bacteriocin classified as a lantibiotic due to the presence of lanthionine, derived from the reaction between a cysteine residue and a dehydrated serine residue. We can also find in these molecules the presence of β-methylantionin, which is produced by the reaction between a cysteine and a dehydrated threonine (**Cheig and Pyun, 2005**). There are five variants of this bacteriocin, all of 34 residues, except for nisin U, which exhibits a sequence of 31 amino acids. The bactericidal activity of lantibiotics is based on forming a transmembrane pore that produces the depolarization of the cytoplasmic membrane of the target bacteria. Nisin A and nisin Z are the most frequent variants and only differ in amino acid position 27. Nisin A exhibits a histidine residue, while variant Z contains asparagine in that position. (**Cheig and Pyun, 2005**). Currently, nisin and pediocin are the only bacteriocins allowed as food additives (**Silva *et al*., 2018**).

Screening for antimicrobial activity

The inhibitory activity of CFS and NCFS and catalase-treated against related LAB, food spoilers, and food pathogens can be observed in Table 1. *Brochothrix thermosphacta* was sensitive to both supernatants. This microorganism is phylogenetic and closely related to the family *Listeriaceae*, and like the members of this group, they are tolerant to the action of organic acids. *Listeria monocytogenes* ATCC 7644, and *L. innocua* ATCC 33090 were sensitive to the activity of both supernatants. Like in the case of *B. thermosphacta*, inhibition was due to the action of nisin Z. Same results were obtained when *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ssp. *aureus* ATCC 25923 were used as target microorganisms. The remaining strains tested were resistant to both supernatants. These results agree with previous reports, revealing that nisin Z exerts inhibitory activity against closely related bacteria but not against Gram-negative ones (**Blay *et al*., 2007; Sadiq *et al*., 2014**). These results strongly suggested that the inhibitory peptide produced is nisin Z

Table 1 Inhibitory activity of cell-free supernatant (CFS) and neutralized cell-free supernatant (NCFS) of nisin Z-producing *Lactococcus lactis* ssp. *lactis* Tw35.

Indicator strains	Growth medium and temperature	Inhibition activity	
		CFS	NCFS
Brochothrix thermosphacta ATCC 11509	TSB, 25 ºC	+	+
Listeria innocua ATCC 33090	TSB, 30 ºC	++	++
L. monocytogenes ATCC 7644	TSB, 30 ºC	++	++
Enterococcus faecalis ATCC 29212	MRS, 35 ºC	+	+
Bacillus subtilis ATCC 6633	TSB, 30 ºC	+	+
Staphylococcus aureus ssp. *aureus* ATCC 25923	TSB, 35 ºC	+	+
Escherichia coli ATCC 25922	TSB, 35 ºC	-	-
E. coli ATCC 35218	TSB, 35 ºC	-	-
Pseudomonas aeruginosa ATCC 27853	TSB, 30 ºC	-	-

Size of the halo diameters: (–) no inhibition; (+) ≥ 10 mm; (++) ≥ 15 mm

Viable cell count and inhibitory activity in controlled fermentation with *Lc. lactis* spp. *lactis* Tw 35

In figures 2 and 3 can be observed the evolution of *Lactococcus* population and the antimicrobial activity against *L. innocua* ATCC 33090 in Chinese cabbage and white cabbage, respectively. At the start of the process in Chinese cabbage (Fig. 2), the cell count was 4.9 $\log_{10}$ CFU.g^{-1} and reached the maximum population after three days of incubation (8.2 $\log_{10}$ CFU.g^{-1}). Therefore, *Lactococcus* count dropped quickly, and after ten days of fermentation, no growth could be detected in M17 medium. In white cabbage (Fig. 3), the initial count was 4.3 $\log_{10}$ CFU.g^{-1} and after four days, it reached a peak level of 8.6 $\log_{10}$ CFU.g^{-1}. Then, the population decrease displayed a similar pattern to Chinese cabbage, and at 12 days of process, no growth was detected. The antimicrobial activity reached a maximum of 320 AU in both cases, on the third day in Chinese cabbage and the fourth day in white cabbage, in coincidence with the maximum population. Inhibitory activity disappeared after eight days of process in both cases.

Despite its marine origin, these results suggest that *Lc. lactis* ssp. *lactis* Tw35 can grow efficiently and produce antimicrobial compounds in vegetal matrices. The adaptation of strains of marine origin has been described in a previous work of our group (**Parada *et al*., 2022**). *Leuconostoc mesenteroides* ssp. *jonggajibkimchii* Tw234, isolated from the intestinal content of *Parona* leatherjacket, displayed comparable features with the cabbage isolated strain RCTw1.1 and the type of strain *Ln. mesenteroides* ssp. *jonggajibkimchii* DRC1506.

Adding bacteriocin-producing strains as protective cultures or co-culture may be the best resource for replacing chemical additives, warranting reducing or inhibiting food-borne pathogens and food spoilage organisms. However, it must be considered that applying strains with antimicrobial activities must not alter or negatively interfere with the wanted fermentation process and should not affect the final product's sensory properties (**Sadiq *et al*., 2014**).

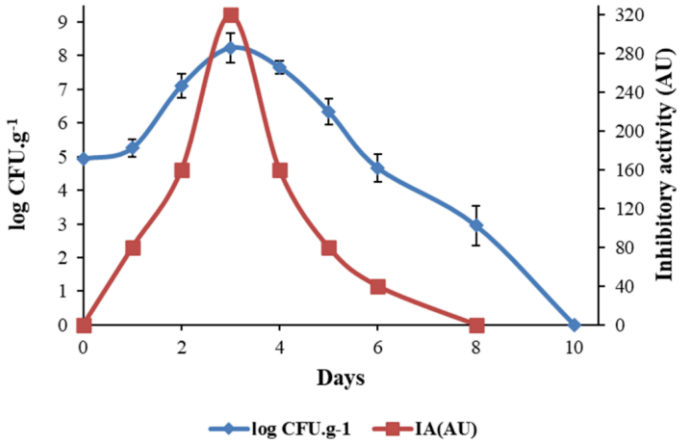

Figure 2 Evolution of *Lactococcus lactis* ssp. *lactis* Tw35 population and inhibitory activity throughout controlled fermentation of Chinese cabbage.

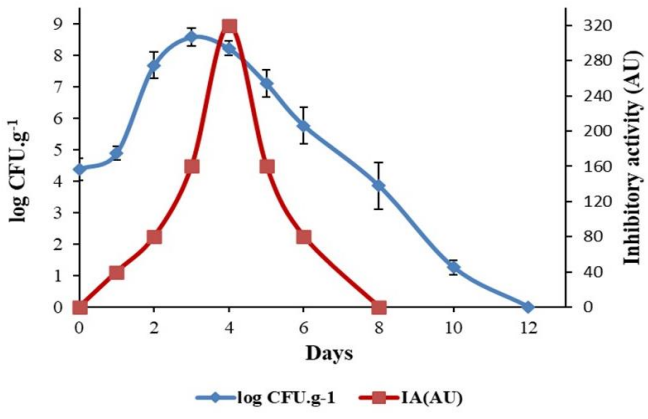

Figure 3 Evolution of *Lactococcus lactis* ssp. *lactis* Tw35 population and inhibitory activity throughout controlled fermentation of white cabbage.

Two-step controlled fermentation trial

Following these considerations, we assayed the behavior of a combination of *Lc. lactis* and two strains of *Lactiplantibacillus* in a two-step controlled fermentation of Chinese and white cabbage. *L. plantarum* RBTw256 and *L. argentoratensis* PCTw261 strains were isolated from the spontaneous fermentation of white cabbage and pak choi, respectively, and further selected based on their nisin resistance and vancomycin resistance (**Parada *et al.*, 2023**).

Figure 4 displays the changes in *Lactococcus* and *Lactiplantibacillus* counts and the drop in pH values in Chinese cabbage fermentation. The initial fermentation phase was carried out with the inoculation of *Lc. Lactis* ssp. *lactis* Tw35 (≈4.8 $\log_{10}$ CFU.g^{-1}). After 72 h, when *Lactococcus* count reached ≈7.4 $\log_{10}$ CFU.g^{-1} and the pH value dropped to 4.8, *L. plantarum* RBTw256 and *L. argentoratensis* PCTw261 were inoculated (≈4.2 $\log_{10}$ CFU.g^{-1}). Thereafter, the *Lactococcus* counts decreased sharply, and no growth was detected on the sixth day. After eight days of the process, the pH value decreased to 4.1 and remained stable until the end of the assay. The maximum *Lactiplantibacillus* population was reached on day 8 (≈8.2 $\log_{10}$ CFU.g^{-1}) and then diminished slowly until the end of the experience. Maximum antagonist activity was detected after three days of the process (320 AU) and disappeared on day five.

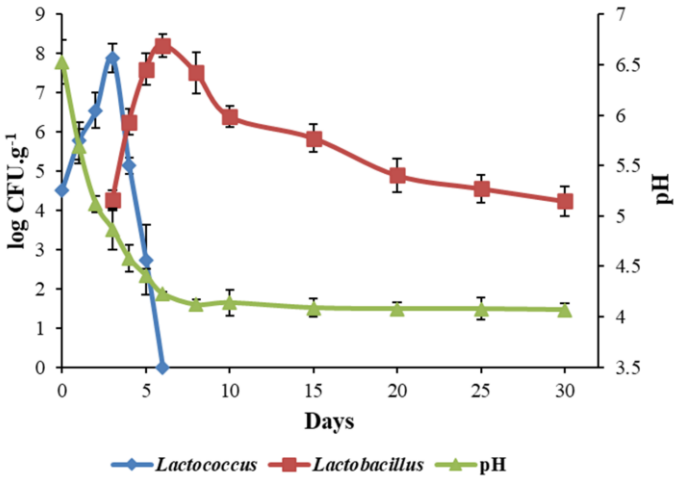

Figure 4 Evolution of *Lactococcus lactis* ssp. *lactis* Tw35 population, *Lactiplantibacillus* strains population, and pH changes throughout the two-step controlled fermentation of Chinese cabbage.

In the case of white cabbage (Fig. 5), the initial inoculation of *Lactococcus* was ≈4.5 $\log_{10}$ CFU.g^{-1}, and the pH was 6.7. After 72 h of fermentation, when *Lactococcus* population achieved ≈7.9 $\log_{10}$ CFU.g^{-1} and pH dropped to 4.8, *Lactiplantibacillus* strains were inoculated (≈4.8 $\log_{10}$ CFU.g^{-1}). Thereafter, the *Lactiplantibacillus* counts increased to 9.2 $\log_{10}$ CFU.g^{-1} on day eight and decreased until the end of the process (≈4.7 $\log_{10}$ CFU.g^{-1}). A minimal pH value of 3.9 was achieved on day ten, and it remained stable. Inhibitory activity displayed a maximum of 320 AU on day four and disappeared 48 h later.

The evolution of *Lactococcus* and *Lactiplantibacillus* populations, the pH decrease, and antimicrobial activity were comparable in both vegetables. Including the two *Lactiplantibacillus* strains on the third day speeded up the pH decrease and stopped the nisin production. This phenomenon might be due to the sharp drop in *Lactococcus* population as a response to its sensibility to low pH and the degradative activity of cabbage and *Lactiplantibacillus* proteases towards nisin (**Sun *et al.*, 2016; Pan *et al.*, 2020**).

The succession of hetero- and homo-fermentative lactic acid bacteria carries out natural fermentation. *Leuconostoc* species, mainly *Ln. mesenteroides*, start the first step of the process characterized by a sharp drop in pH. This change inhibits the *Leuconostoc* population growth due to its sensitivity to acid conditions and allows different *Lactiplantibacillus* species to continue the process (**Beganović *et al.*, 2014; Siddegg *et al.*, 2022**). However, it must be considered that spontaneous fermentation is random and depends on various factors, mainly the natural epiphytic biota of raw vegetables that may be responsible for undesirable sensory or nutritional properties or may fail the inhibition of spoilage or pathogen microorganisms.

The market offers several commercial/allochthonous starters; however, they were not previously selected to ferment a specific vegetable. Several reports have recommended using autochthonous starters to ensure better adaptation to a particular vegetable environment, which enhances sensory, nutritional, and shelf-life properties. To avoid these drawbacks, the use of starters selected on the basis of their technological properties, is recommended for controlled vegetable fermentation (**Jagannath *et al.*, 2012; Di cagno *et al.*, 2013**).

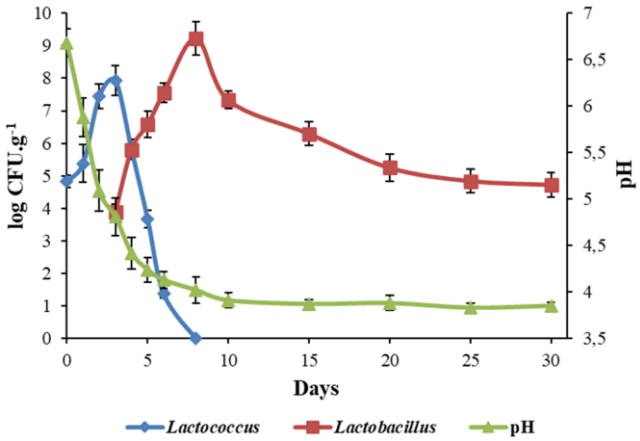

Figure 5 Evolution of *Lactococcus lactis* ssp. *lactis* Tw35 population, *Lactiplantibacillus* strains population, and pH changes throughout the two-step controlled fermentation of white cabbage.

The analysis of the fermentation evolution confirms the use of nisin-producing *Lc. lactis* ssp. *lactis* Tw35 to replace *Leuconostoc* strains, responsible for the first stage of spontaneous fermentation of *Brassica* vegetables, did not alter the homo-fermentative step. The strain displayed a comparable behavior to *Leuconostoc* strains in Chinese and white cabbage fermentation: rapid growth and high acid production. On the third day, the second step was initiated by adding two nisin-resistant *Lactiplantibacillus* strains, which displayed a sharp increase in growth and led to a further decline in pH and the disappearance of the *Lactococcus* population. Antagonist activity was detectable for four days in Chinese cabbage and five days in white cabbage.

CONCLUSION

In this work, we report the isolation of a *Lc. lactis* ssp. *lactis* strain from the intestinal tract of silverside. The behavior and production of nisin Z in vegetable matrices suggest that this strain can be used in controlled fermentation. The combination with selected *Lactiplantibacillus* strains in Chinese and white cabbage fermentation displayed comparable behavior to spontaneous fermentation (**Parada *et al.*, 2023**). The nisin production during the process's first stage may improve the fermented matrices' safety and shelf-life by inhibiting pathogenic or spoilage bacteria.

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Contribution: Franco Sosa is responsible for conceptualization, methodology, software, validation, formal analysis, investigation, reviewing, proofreading, and visualization. Romina Parada is responsible for conceptualization, methodology, formal analysis, investigation, writing, reviewing, and editing. Emilio Marguet is responsible for conceptualization, formal analysis, investigation, and drafting. Marisol Vallejo participated in conceptualization, methodology, writing, reviewing, editing and visualization.

Conflict of interest: The authors declare that there is no conflict of interests regarding the publication of this article.

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