International Journal of Food Science and Technology, 2025, 60(1), vvae003
https://doi.org/10.1093/ijfood/vvae003
Advance access publication 30 March 2025
Original Article

Exploring the potential of lactic acid bacteria to produce postbiotics with antimicrobial and antioxidant properties: focus on the probiotic strain *Pediococcus pentosaceus* RC007 for industrial-scale production

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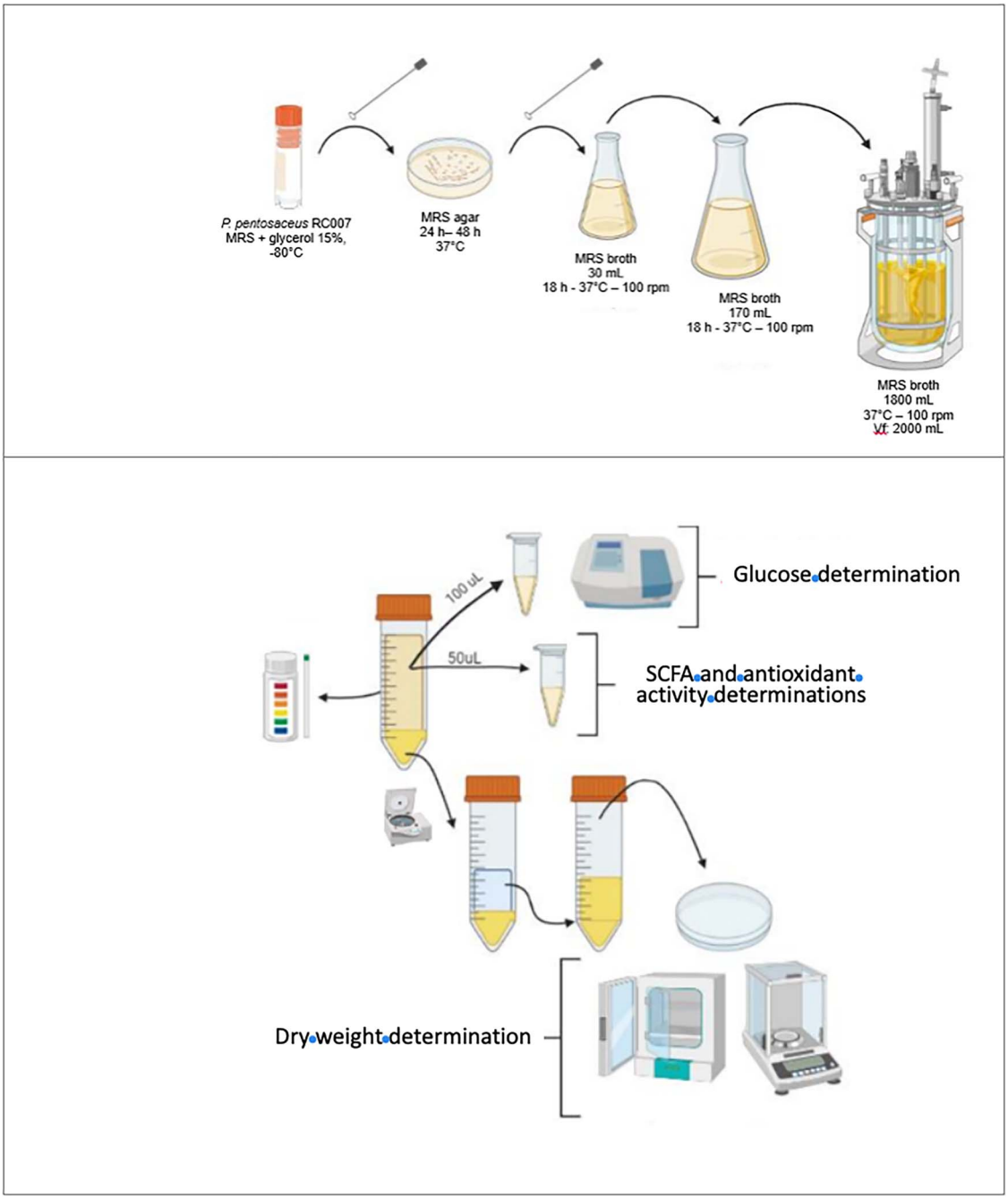
Abstract

Bioprospection of natural compounds by next-generation probiotic strains holds immense promise for promoting beneficial effects on both animal and human health. The aim of this work was to study the antimicrobial activity of lactic acid bacteria (LAB) postbiotics on multidrug-resistant pathogenic *Salmonella* sp. and *Escherichia coli*. Moreover, short-chain fatty acids (SCFAs) production and the postbiotics antioxidant activity were evaluated. Additionally, the potential for industrial-scale production to ensure feasibility and scalability of postbiotic-based applications was determined. LAB strains inhibited *E. coli* O157:H7 and *Pediococcus pentosaceus* showed the strongest inhibition. Among SCFA, the highest acetic and lactic acid concentrations were 41.91 and 181.52 mM, respectively. The highest $ABTS^{+}$ Trolox equivalent and ferric-reducing antioxidant power values were 31.47 μg Trolox/g after 24 hr and 11.38 μmol/g after 48 hr, respectively. There was no statistically significant difference between fermentation times. Industrial-scale production of *P. pentosaceus* RC007 showed the specific growth rate as a kinetic parameter and the biomass production as a productive parameter; they were 1.99 hr^{-1} and 1.1 g/L, respectively. The maximum production point was reached at pH 4. *P. pentosaceus* RC007 can be considered a good candidate to scale up considering its probiotic properties and the large amount of postbiotic metabolites of interest in health and industrial purposes.

Keywords: *Pediococcus pentosaceus*, postbiotics, antimicrobial and antioxidant properties, industrial-scale production

Received: 21 August 2024. **Accepted:** 24 August 2024

Graphical abstract



Introduction

Recently, advanced genetic sequencing tools have provided new insights into the composition of the gut microbiota. A specific group of microorganisms, known as next-generation probiotics (NGPs), has been identified and has garnered significant scientific interest due to their potential health benefits for humans. The number of scientific studies highlighting the importance of NGPs in maintaining the balance of the human microbiome and their positive effects on disease prevention and therapy is increasing. The bioprospection of natural compounds by NGP strains holds immense promise for promoting beneficial effects on both animal and human health. These innovative probiotics are poised to revolutionize the fields of biotechnology and medicine by harnessing the power of naturally occurring compounds (Sionek et al., 2023). Through meticulous exploration of diverse ecosystems, these strains are sourced with the aim of uncovering novel bioactive molecules that can positively influence various physiological processes including their role in immunomodulation (Shweta et al., 2021), on meat composition, carcass characteristics, and fatty acids profile of farm animals (Parada et al., 2024), reduction of the incidence of respiratory tract infections and the management of lactose intolerance (Otunba et al., 2021), resistance to infections (Li et al., 2021), anti-obesity properties (Barathikannan et al., 2022) and post-COVID-19 recovery of the patience (Gutiérrez-Castrellón et al., 2022). The integration of cutting-edge bioprospecting techniques with advanced genetic engineering allows for the creation of probiotics with targeted

health benefits such as bioengineered probiotic lactic acid bacteria (LAB) that represent a part of the next generation in whole-cell-mediated biotherapies for the treatment of human diseases (Charbonneau et al., 2020). As these NGPs continue to be developed and refined, they offer a glimpse of a healthier future where personalized and sustainable solutions may play a pivotal role in enhancing our well-being (Tagliazucchi et al., 2019).

Probiotic microorganisms are live microorganisms that, administered in adequate amounts, confer a health benefit on the host (FAO, 2001). Probiotic strains comprise different genus of microorganism, as LAB strains, *Bifidobacterium* sp., *Saccharomyces* sp. and *Bacillus* sp. (Staniszewski & Kordowska-Wiater, 2021). LAB include a heterogeneous group that comprises species belonging to *Streptococcus*, *Lactococcus*, *Lactobacillus*, and *Leuconostoc* genera. *Bifidobacterium* and *Lactobacillus* are the most prominent probiotic bacteria along with the yeast *Saccharomyces boulardii* (Maske et al., 2024). Nevertheless, it is crucial that novel probiotic strains such as *Pediococcus* sp. are fully investigated in order to prove their beneficial effect. *P. pentosaceus* is a lactic acid bacterium that has garnered significant attention due to its potential beneficial effects on animal and human health. As a probiotic strain, *P. pentosaceus* has been studied for its ability to exert positive influences on the gastrointestinal system and immune function. Research has indicated that *P. pentosaceus* strains can produce bioactive compounds, such as antimicrobial peptides and bacteriocins, which have demonstrated inhibitory effects against various pathogenic bacteria. These antimicrobial properties make it a promising candidate for potential use as an antibiotic replacer in animal husbandry and agriculture (Shiman et al., 2021).

A postbiotic is a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host (Salminen et al., 2021). They can include exopolysaccharides (EPSs), short-chain fatty acids (SCFAs), enzymes, cell-free supernatants (CFSs), cell wall fragments, bacterial lysates, and other metabolites produced by the gut microbiota. Among them, SCFA and antioxidant molecules have garnered significant attention for their potential health benefits. SCFAs, such as acetate, propionate, and butyrate, are by-products of the fermentation of dietary fibres by gut microbiota. They play essential roles in maintaining gut health, modulating the immune system, and exerting antimicrobial effects against pathogenic bacteria (Liu et al., 2023). Additionally, postbiotics derived from probiotic metabolism, such as antioxidants, demonstrated the ability to scavenge free radicals and reduce oxidative stress, which is implicated in various diseases, and ageing processes (Lin et al., 2022). They have shown that certain *Lactobacillus* sp. and *Bifidobacterium* sp. strains and their fermented metabolites (postbiotics) exhibited antioxidative activities that regulated oxidative stress and protected cells from oxidative damage. The incorporation of these postbiotics into functional foods and supplements holds promise in promoting overall well-being and preventing certain health conditions.

Some researchers in the field conducted *in vitro* experiments to explore the beneficial effects of *Pediococcus* sp. strains. Martínez et al. (2017) isolated *Pediococcus* sp. strains from the gastrointestinal tract of healthy animals and evaluated their probiotic potential. The results showed that *P. pentosaceus* RC007 exhibited strong adherence to intestinal epithelial cells, suggesting its ability to persist in the gut environment and potentially confer beneficial effects. This study also revealed that strains exhibited probiotic characteristics, including resistance to acidic conditions and bile salts, as well as adhesion to intestinal cells, indicating their potential to survive and colonize the gut. Additionally, the researchers observed that these strains possessed ABF_1 adsorbing/degrading abilities *in vitro*. Furthermore, Parada et al. (2024) used the same *P. pentosaceus* RC007 strain to evaluate its probiotic abilities used alone or in combination with *S. cerevisiae* var. *boulardii* RC009, as an in-feed additive to replace the non-therapeutic use of antibiotics in the diet of post-weaning pigs. The results demonstrated that pigs consuming both probiotic treatments tended to improve the indicators for carcass quality. Furthermore, the administration of probiotics without the addition of antibiotics in the feed of post-weaning pigs was able to maintain the productive performance and health of the animals.

Salmonellosis stands out as one of the two most prevalent food-borne illnesses worldwide. The rise in antibiotic resistance among both pathogenic and non-pathogenic bacteria has been attributed to the increased use of antibiotics, particularly prophylactic in-feed antibiotics, in animal agriculture. The European Antimicrobial Resistance Surveillance Network has recently reported a growing presence of antibiotic-resistant *Salmonella* strains, including resistance to quinolones, ampicillin, sulphonamides, and tetracyclines. This escalation in antibiotic-resistant strains of *Salmonella enterica* in food animals poses a significant risk to public health (Alessiani et al., 2022).

Several *Escherichia coli* strains are food-borne pathogens and one of the most prevalent causal agents of bloodstream infection; for the period of 2013–2016, its prevalence has risen compared with previous periods (Sano et al., 2023). The European Antimicrobial Resistance Surveillance Network has reported a high third-generation cephalosporin resistance (cefotaxime/ceftriaxone/ceftazidime) along with fluoroquinolone resistance (ciprofloxacin/levofloxacin/ofloxacin) of *E. coli* strains. Carbapenem-resistant strains still remain rare compared with other antibiotic-resistant strains (EFSA, 2018). Enterohemorrhagic *E. coli* O157:H7 is the most common protoserotype within this classification that seriously threatens human health due to its capability to produce Shiga toxins, thereby causing the severe diseases as hemolytic uremic syndrome (Vidovic & Korber, 2016). Consequently, comprehensive epidemiological understanding of zoonotic pathogens, such as *Salmonella* sp. and *E. coli*, and their antibiotic resistance becomes crucial in adopting a One Health approach (EFSA, 2018).

While investigations on probiotic strains have expanded, the specific bioactive compounds present in the supernatants and their potential applications in promoting health have not been extensively explored for *P. pentosaceus* RC007 postbiotics. Addressing this research gap could unveil novel insights into the therapeutic and preventive properties of *P. pentosaceus* RC007 free-cell supernatants, paving the way for innovative approaches in functional food development and human health enhancement.

Therefore, the aim of this work was to study the antimicrobial activity of LAB postbiotics on multidrug-resistant pathogenic strains of *Salmonella* sp. and *E. coli*. Moreover, SCFA production and the postbiotics antioxidant activity were evaluated. Additionally, it was essential to determine the potential for industrial-scale production of the microorganism involved in postbiotics production to ensure the feasibility and scalability of postbiotic-based applications for widespread commercial use.

Materials and methods

Microorganisms

LAB strains and inoculum preparation

LAB probiotic strains were acquired from the culture Collection of Microbiology and Immunology department of National University of Río Cuarto, Córdoba, Argentina (*Lacticaseibacillus rhamnosus* RC007, *Lactiplantibacillus plantarum* RC009, *P. pentosaceus*

RC007, *P. acidilactici* RC004 and *Enterococcus faecium* RC001). *Pediococcus* sp. strains and *E. faecium* were isolated from gastrointestinal content of juvenile rainbow trout from a fish farm (Martínez et al., 2017). *Lacticaseibacillus rhamnosus* and *Lactiplantibacillus plantarum* were isolated from maize silage samples (Dogi et al., 2015).

LAB were maintained at −20 °C in 50% (v/v) skim milk. A transfer was made from the frozen stock to a Man, Rogosa, and Sharpe (MRS) broth and incubated for 24 hr at 37 °C to obtain the working LAB culture.

Pathogenic strains

Salmonella enterica subsp. *enterica* serovars and *E. coli* strains were procured from the collection of Agronomy and Veterinary Department of National University of Rio Cuarto.

From the total amount of *Salmonella*'s collection, 22 strains were chosen owing to their level of multi-resistance to different antibiotics (from 0% to 47.6%). The antimicrobial susceptibility evaluation was assessed using the Kirby–Baüer method (diffusion in Mueller–Hinton agar) according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2013) and Nassar et al. (2019) for the following antibiotics: ampicillin (Amp), amoxicillin—clavulanic acid (Amc), piperacillin–tazobactam (Tzp), cephalothin (Cef), cefepime (Fep), cefotaxime (Ctx), cefoxitin (Fox), ceftazidime (Caz), ertapenem (Etp), imipenem (Imp), gentamicin (Gen), amikacin (Amk), azithromycin (Azm), tetracycline (Tet), ciprofloxacin (Cp), levofloxacin (Lvx), nalidixic acid (Nal), trimethoprim–sulfamethoxazole (Sxt), chloramphenicol (Chl), fosfomycin (Fof), and nitrofurantoin (Nit). Although there were strains that did not present any antibiotic resistance, each of them was isolated from relevant sources: pig farms (F), slaughterhouses (STh), and clinical cases (C) (Parada et al., 2022).

From the total amount of *E. coli*'s strain collection, 22 were chosen from different environmental sources (11 of them with non-β-haemolytic activity and 11 of them with β-haemolytic activity). β-haemolytic *E. coli* strains were tested under analytical determinations such as RapidChek®*E. coli* O157 lateral flow assay, including H7 and isolated in selective and differential media (MacConkey Agar Sorbitol and CHROM Agar O157) to confirm *E. coli* O157:H7 strains.

RapidChek®*E. coli* O157 lateral flow assay was conducted as follows. Dehydrated commercial medium (85.05 ± 0.02 g) were added to deionized water (3.375 L) and autoclaved. Sample (25 g) was added to the prepared medium and incubated at 42 °C for 8 hr. Enriched sample (1 ml) was transferred into a sample tube, and after 10 min, the results were read. One line represents a negative result, and two lines represent a positive result.

Mac Conkey Agar sorbitol medium was used to isolate *E. coli* O157:H7 and separate it from other *E. coli* strains because it does not ferment sorbitol. A reddish-pink colour colony represents a positive result, and a colourless colony represents a negative result.

CHROM Agar O157 is composed with a chromogenic mix. Sample was grown and enriched and a direct streaking was done on the medium and incubated at 37 °C for 24 hr. Violet colonies were considered positive for *E. coli* O157:H7, and blue or colourless colonies were considered negative for *E. coli* O157:H7.

In vitro antimicrobial activity screening

Antimicrobial activity of selected LAB strains was performed by cross-streak method in MRS agar (Britania, Buenos Aires, Argentina) from working cultures. A single central line of each LAB culture grown in MRS broth (adjusted to 1.5 McFarland scale equivalent to < 3.109 UFC m L $-$ 1) was seeded in the middle of the agar plates containing MRS agar. Calibrated loops were used. Plates were cultivated for 24 hr at 37 °C and 5% CO_2-air atmosphere. After cultivation time, LAB were inactivated using UV for 30 min.

On the other hand, pathogenic *Salmonella* spp. and *E. coli* strains were cultured in nutritive broth (Britania, Buenos Aires, Argentina) for 18 hr at 37 °C, and inocula were adjusted to 0.5 McFarland scale. Then, a second layer of nutritive agar was poured over the first MRS layer and left to dry at room temperature for 2 hr to allow LABs metabolites diffusion. After that, perpendicular streaks of pathogenic strains were seeded by triplicate and incubated for 24 hr at 37 °C. Controls without LABs and pathogenic strains, separately, were included. The width zone of inhibition (mm) extending from the central line of LAB culture to the pathogenic bacteria growth was measured. Inhibition zones larger than 5 mm were considered as "inhibition" and lesser than 5 mm were considered "no inhibition" according to Fernandez Juri et al. (2013) criterion.

P. pentosaceus RC007 growing conditions for SCFAs extraction

100 ml of MRS broth was poured in each of two 250 ml Erlenmeyer flasks. Subsequently, 1 ml of the working culture of *P. pentosaceus* was inoculated into each flask, and then both were incubated at 37 °C, the first one for 24 hr and the second one for 48 hr. After incubation time, 50 ml of the sample was collected in sterile tubes and then centrifuged at 10,000×*g* (Beckman Coulter Avanti J-25 Centrifuge) for 10 min at 4 °C to separate bacterial cell debris from the CFS. CFS then was used to determinate SCFA. Each experiment was repeated twice.

SCFAs detection

The supernatant (1 ml) was centrifuged at 5,000×*g* for 20 min at 4 °C. After that, 0.2.ml meta-phosphoric 25% was mixed with the supernatant using a vortex mixer. This stage facilitated the separation of proteins and fatty acids. Finally, the samples were centrifuged at 5,000×*g* for 15 min at 4 °C. The samples were then transferred to gas chromatography vials. Acetic, propionic, butyric, and lactic acids were determined using a gas chromatograph (GC) (Agilent 7890A, USA) equipped with a flame ionization detector (FID), an automatic injector (Agilent 7693A, United States), and a DB-FAPP capillary column (30 m × 0.25 mm × 0.25 μm). Helium was used as the carrier gas at a flow rate of 14.4 ml/min. The initial oven temperature was 110 °C, which was maintained for 5 min, increased to 180 °C at 8 °C/min, held for 1.0 min, increased to 200 °C at 20 °C/min, and finally held at 200 °C for 5 min. The injector and detector temperatures were 200 and 240 °C, respectively (Park et al. 2024). The post-run was performed for 2 min at 1.5 ml/min. The flow rates of hydrogen, air, and nitrogen as makeup gases were 30, 300, and 20 ml/min, respectively. The injected sample volume for GC analysis was 1 μl, with a 55:1 split ratio, and the run time for each analysis was 20.75 min. Data handling was performed using the ChemStation. SCFA concentrations were expressed as millimoles (mM) per ml.

Antioxidant capacity by ABTS and FRAP

CFS was prepared as previously described and lyophilized for antioxidant capacity. The antioxidant activity was evaluated as free radical scavenging activity by using the cation radical ABTS ($ABTS^{\bullet+}$) decolorizing assay and ferric reducing antioxidant power (FRAP).

Activity of scavenging ABTS radicals

This assay was performed according to the method described by Re et al. (1999). Briefly, the radical cation was prepared by mixing 7 mM of ABTS with 2.45 mM potassium per-sulphate and leaving the mixture in agitation for 12 hr at room temperature in the dark. $ABTS^{+}$ solution was diluted in distilled water until the absorbance at 734 nm reached 0.70 (± 0.05). The reaction mixture contained 1 ml $ABTS^{++}$ and 15 μl of sample and absorbance were determined after 16 min. Scavenging activity percentage (SA [%]) was determined according to Equation (1).

$$SA\ (\%) = \frac{A_0 - A_x}{A_0} \times 100 \quad (1)$$

where A_x is the absorbance of the sample solution and A_0 is the absorbance of the control solution. The antioxidant activity was expressed in Trolox equivalent (TEAC) (ug Trolox/g sample) according to Equation (2).

$$TEAC = \frac{\text{Slope sample } (g/L)}{\text{Slope Trolox } (ug/L)} \quad (2)$$

Here, the slope of each sample corresponds to the linear regression fit of ABTS+ SA (%), Equation (1) versus concentration of lyophilized supernatant g/L, and the slope for Trolox corresponds to the regression fit of SA (%) versus Trolox concentration expressed as μg/L.

Reducing power determination

FRAP determination were carried out according to Benzie and Devaki (2017). A working reagent was prepared by mixing 50 ml of 300 mM sodium acetate buffer with pH 3.6 with 5 ml of 10 mM TPTZ (2,4,6-tri-pyridyl-s-triazine) prepared in 40 mM HCl and 5 ml of 20 mM $FeCl_3$ $6H_2O$. A 3 ml-aliquot of this reagent was added to 100 μl of sample, blank, or standard solution. The reaction mixture was homogenized and incubated at 25 °C. Finally, the absorbance of the solution was measured at 593 nm after 6 min of reaction. Results were calculated as follows (Equation 3):

$$FRAP = \frac{A_{sample}}{A_{Fe^{2+}}} \times Fe^{2+} standard concentration\ (\mu mol/L) \quad (3)$$

where A_{sample} is the absorbance of the sample solution and $A_{Fe}{}^{2+}$ is the absorbance of the 1000 μM ferrous sulphate standard. Antioxidant activity was expressed as the FRAP value in μmol/g of sample.

Scale up of *P. pentosaceus* RC007 production in bioreactor.

Kinetic and productive parameters determination

Preparation of inoculum

Inoculum of *P. pentosaceus* RC007 was prepared from the working culture, and it was seeded with the streak method in a plate with MRS medium. Once developed, colonies were recovered in 30 ml MRS broth and incubated at 37 °C with shaking (100 rpm) for 18 hr. Subsequently, the total volume of the culture was inoculated in 170 ml MRS broth with a final volume of 200 ml and incubated at 100 rpm and 37 °C for 18 hr (Figure 1A).

Fermentation in batch conditions and sampling

Laboratory-scale fermentation of *P. pentosaceus* RC007 was carried out in a 7-L stirred bioreactor (New Brunswick 7 L BioFlo™ 2000). A culture previously obtained was inoculated (10% v/v) into the fermenter tank containing 1800 ml of MRS broth, ending up with a working volume of 2 L. It was stirred at 50 rpm at 37 °C for 24 hr. Dissolved oxygen concentration at the beginning of the experiment was 0%, and the pH was 5. Fermentation process was performed without aeration and without maintaining of stable pH, though pH was measured throughout the process. The samples were taken in Falcon tubes every hour for 12 hr and then each 3 hr for 12 hr else, starting 30 min after the inoculation, to ensure a correct homogenization of the medium (Figure 1B).

Estimation of biomass concentration, sugar content, pH, and total titratable acidity

Biomass was determined in triplicate with a Spectrum SP-1104, UV–visible spectrophotometer by monitoring absorbance of the fermentation broth. Cell dry weight (CDW) was obtained from a calibration curve relating optical density at 600 nm to dry cell weight (g/L). A series of dilutions of samples obtained after fermentation were performed to obtain a linear relationship between OD660 and biomass concentration. The CDW was determined as the difference in the masses obtained after centrifuging the broth sample (100 ml) and blank sample of equal quantity used at 6700 rpm for 12 min at 4 °C and then placing those in an oven at 65 °C for 72 hr before weighing. Reducing sugars were quantified by DNS (3,5-dinitrosalicylic acid) method (Miller et al 1954). pH change was tested by using a pH metre *(Brand and model of the pH instrument)*. Total titratable acidity was determined using Alan (2019) methodology with slightly modifications. One millilitre of the sample was centrifuged at 10,000×*g* for 10 min at 4 °C to obtain the CFS. Then, 10 μl of the CFS were taken and diluted in 20 μl of distilled water. Drops (2 or 3) of phenolphthalein (1%) were added at the solution. Then, the solution was titrated with 0.1 N NaOH until a slightly pink colour appeared and persisted for 15–30 s. Total acidity was calculated in terms of acetic acid plus lactic acid according to the formula:

$$\text{Total acidity } (g/L) = (V \times N \times 150)/M$$

where V = volume of 0.1 N NaOH solution (ml); N = normality of the NaOH; M = volume of the sample (ml); 150 = Equivalent of lactic acid and acetic acid.

Growth kinetic and productive parameter determinations

Five growth kinetic parameters were calculated using experimental data, i.e., cultivation time (hr), cell biomass concentration x (g/L), and substrate use, s (g/L). (a) The specific growth rate, μ (hr^{-1}), is calculated from, $\mu = \frac{dlnx}{dt}$ where the differential natural log of *x* is divided by the time change; (b) The productivity or production rate of cell biomass, r_x (g/L/hr) is $r_x = \frac{dx}{dt}$ at 18 hr; (c) The utilization rate of substrate, r_s (g/L/hr) is from $r_s = \frac{ds}{dt}$, where *s* is the glucose uptake (Glu); (d) The cell yield coefficient, $Y_{x/s}$ (g/g), is from $\%\ Y_{x/s} = \frac{\Delta x}{\Delta s} \times 100$, where Δ_x is the cell biomass produced (g/L) and Δ_s is the substrate utilized (g/L).

Statistical analysis

All experiments and analyses were conducted in triplicate. The results were expressed as mean ± *SD* (standard deviation). All statistical analyses were carried out using statistical differences among different factors, which were determined using ANOVA.

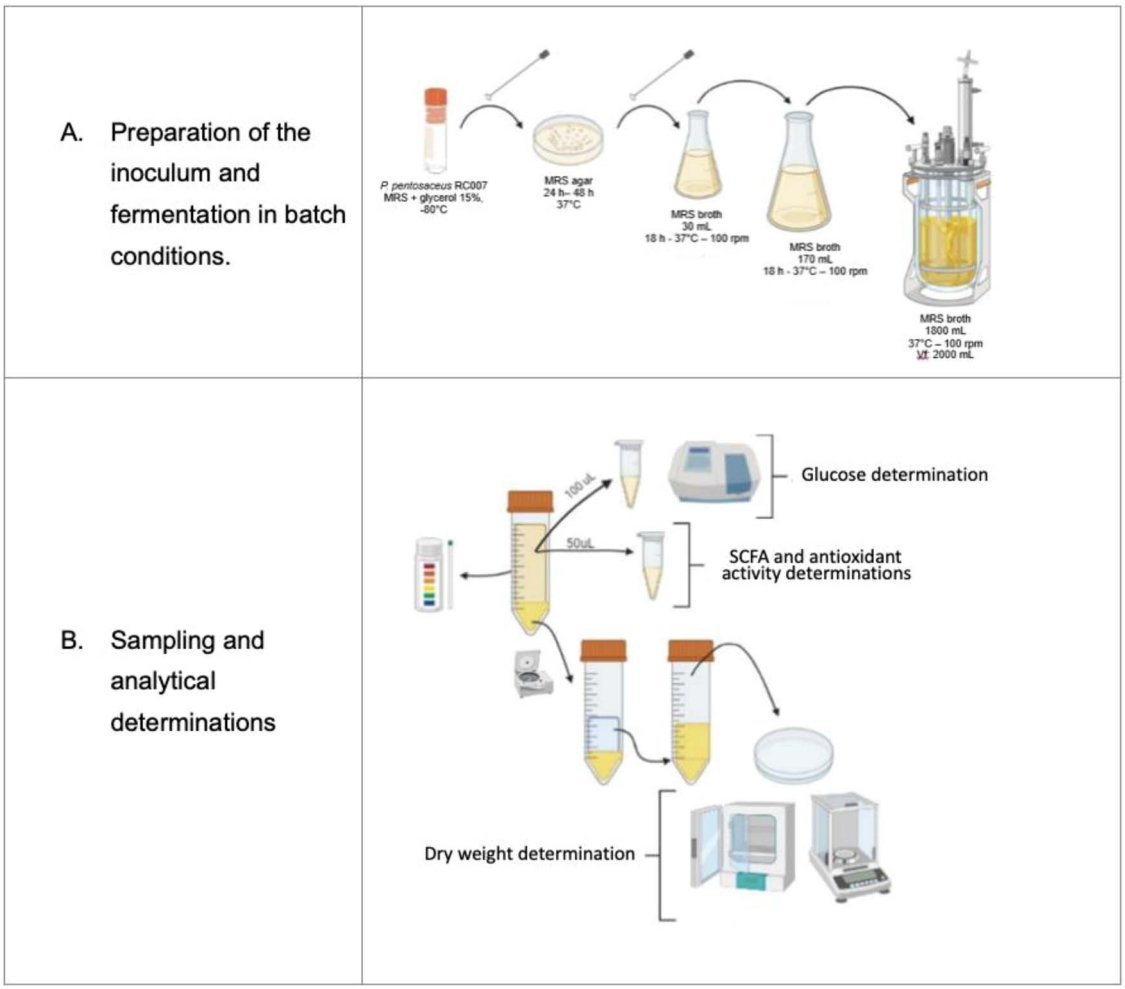


Figure 1. *Pediococcus pentosaceus* RC007 production. A. from laboratory to pilot scale. B. determinations to obtain kinetic and productive parameters.

Results

Antibiotic resistance profile of *Salmonella* sp. serovars

Table 1 shows the resistance profile of *Salmonella enterica* subsp. *enterica* serovars. Tetracycline exhibited the highest resistance among *Salmonella* strains (63.9%), followed by ampicillin and nalidixic acid resistance (50% each), and chloramphenicol (40.9%). Gentamicin resistance was present in *S. typhimurium* strains, being the only aminoglycoside resistance observed. There was high susceptibility from serovars to different tested β-lactamases, except to ampicillin (50%) and, to a much lesser extent, cephalothin, in which only four serovars were susceptible with extended exposition to the antibiotic.

Antibiotic resistance profile of *E. coli* strains

Table 2 shows the results of analytical determinations to confirm the *E. coli* O157:H7 identity from β-haemolytic *E. coli* strains assayed. Two of them were confirmed as *E. coli* O157-H7.

In vitro antimicrobial activity screening

Table 3 shows the antimicrobial activity of LAB against multidrug-resistant *Salmonella enterica* subsp. *enterica* serovars and *E. coli* pathogenic strains.

The largest inhibitory activity was given by *L. plantarum* RC009 against *Salmonella enterica* subsp. *enterica* serovars with an inhibition zone of 72.33 mm, followed by *E. faecium* RC001 (68.70 mm), *P. acidilactici* RC004 (61.33 mm), *L. rhamnosus* RC007 (56.35 mm), and *P. pentosaceus* RC007 (52.97 mm) ($p \leq .05$).

Lactiplantibacillus plantarum RC009 also had the largest inhibitory activity on *E. coli* with 73.56 mm, followed by *P. acidilactici* RC004 (73.56 mm), *E. faecium* RC001 (73.40 mm), and *P. pentosaceus* RC007 (72.03 mm) ($p \leq .05$).

Variability was also evaluated for the LAB inhibition zones. The standard deviation (*SD*) of *Salmonella* strains showed that inhibition was between 2.82 and 16.83 mm, whereas *SD* of *E. coli* strains inhibition was between 2.71 and 5.27 mm. These results showed that *Salmonella* strains had a higher variability of inhibition than that observed in *E. coli* strains. Table 3 also shows that LAB strains were able to inhibit *E. coli* O157:H7 strains and *P. pentosaceus* was the strain with the strongest inhibition zone.

The five LAB strains tested for antimicrobial activity exhibited high *Salmonella* spp. and *E. coli* antimicrobial capabilities. Among these strains, *P. pentosaceus* RC007 was selected to continue being used for further experiments owing its novel properties as a probiotic and as a potential postbiotic producer.

P. pentosaceus RC007 SCFAs production

Table 4 shows SCFA from *P. pentosaceus* at different production times. Specifically, SCFA sought were acetic, butyric, propionic, and lactic acid. Neither propionic nor butyric acid was detected, while acetic and lactic acids were found. The highest acetic and

Table 1. Antibiotic resistance profile of *Salmonella* sp. serovars.

Strain	Serovar	Origin	Amp	Amc	Tzp	Cef	Fep	Ctx	Fox	Caz	Etp	Imp	Gen	Amk	Azm	Tet	Cp	lvx	Nal	Sxt	Chl	Fof	Nit
S17	S. Anatum	F																					
S33	S. Anatum	STh																					
S2	S. Anatum	F																					
S22	S. Branderburg	F																					
S10	S. *Choleraesuis*	C																					
S24	S. *Choleraesuis*	C																					
S27	S. *Choleraesuis*	C																					
S32	S. *Choleraesuis*	C																					
S11	S. Derby	F																					
S16	S. Derby	F																					
S20	S. Derby	F																					
S51	S. Derby	F																					
S29	S. Glostrup	C																					
S40	S. Glostrup	C																					
S43	S. Heidelberg	F																					
S55	S. Heidelberg	F																					
S1	*Streptococcus infantis*	F																					
S14	S. Livingston	F																					
S3	S. Montevideo	F																					
S7	S. Oranienburg	F																					
S8	S. Oranienburg	F																					
S18	S. *Panama*	F																					
S4	S. Rissen	F																					
S9	S. *Typhimurium*	F																					
S13	S. *Typhimurium*	F																					
S21	S. *Typhimurium*	F																					
S41	S. *Typhimurium*	STh																					
S46	S. *Typhimurium*	F																					
S48	S. *Typhimurium*	F																					
S50	S. *Typhimurium*	F																					

Note. Black = resistant, Grey = susceptible with extended exposition, White = susceptible F = field, C = clinical case, STh = slaughterhouse Ampicillin (Amp), Amoxicillin – Clavulanic acid (Amc), Piperacillin – Tazobactam (Tzp), Cephalothin (Cef), Cefepime (Fep), Cefotaxime (Ctx), Cefoxitin (Fox), Ceftazidime (Caz), Ertapenem (Etp), Imipenem (Imp), Gentamicin (Gen), Amikacin (Amk), Azithromycin (Azm), Tetracycline (Tet), Ciprofloxacin (Cp), Levofloxacin (Lvx), Nalidixic acid (Nal), Trimethoprim – Sulfamethoxazole (Sxt), Chloramphenicol (Chl), Fosfomycin (Fof) and Nitrofurantoin (Nit).

Table 2. Analytical determinations to confirm *E. coli* O157:H7 identity from β-haemolytic *E. coli* strains.

E. coli strain number	RapidChek®*E. coli* O157 lateral flow including H7	Selective and differential media		Results
		MacConkey Agar Sorbitol	CHROM Agar O157	
1	Negative	——	——	Negative
2	Positive	Sorbitol (–)	Violet colonies	Positive
9	Positive	Sorbitol (+)	Blue colonies	Negative
11	(+)	Sorbitol (–)	Violet colonies	Positive
13	(+)	Sorbitol (+)	Blue colonies	Negative
15	(–)	——	——	Negative
16	(–)	——	——	Negative
17	(–)	——	——	Negative
18	(–)	——	——	Negative
20	(–)	——	——	Negative
31	(+)	——	——	Negative

Table 3. Antimicrobial activity of lactic acid bacteria against *Escherichia coli* and *Salmonella spp.* pathogenic strains with multidrug resistance.

Pathogenic strains	Lactic acid bacteria				
	P. pentosaceus RC007	*P. acidilactici* RC004	*L. plantarum* RC009	*L. rhamnosus* RC007	*E. faecium* RC001
	Inhibition zone (mm) (mean ± SD)				
Salmonella sp. (*n* = 22)	52.97 ± 13.44[b]	61.33 ± 16.03[b]	72.33 ± 2.82[a]	56.35 ± 9.78[b]	68.70 ± 10.01[a]
E. coli (*n* = 22)	72.03 ± 5.27[a]	73.56 ± 2.71[a]	74.52 ± 3.8[a]	66.87 ± 3.49[b]	73.40 ± 4.41[a]
E. coli O157:H7 (*n* = 2)	74.18 ± 5.38[a]	72.95 ± 4.16 [a]	73.35 ± 4.71[a]	66.41 ± 3.39[b]	71.47 ± 3.82[a]

Note. Letters in common indicate non-significant differences according to Fisher's protected Least Significant Difference test ($p \leq .05$). Statistical letters must be compared horizontally.

Table 4. Volatile fatty acid content in cell-free supernatants of *P. pentosaceus* RC007 at different times of production.

P. pentosaceus RC007 cell-free supernatants	Concentration (mM)	
	Acetic acid	Lactic acid
24 h	39.64 ± 5.66[a]	121.89 ± 34.39 [a]
48 h	41.91 ± 5.67[a]	181.52 ± 45.92 [b]

Note. Letters in common indicate not significant differences according to Fisher's protected Least Significant Difference test ($p \leq .05$).

Table 5. Antioxidant capacity evaluated by the ability to deactivate the radical cation $ABTS^+$ and by FRAP assays at two fermentation times.

Incubation time(h)	FRAP(μmol/g)	TEAC ($ABTS^{\bullet+}$)
24 h	9.63 ± 0.43[a]	34.97 ± 2.05[a]
48 h	11.38 ± 0.59[a]	31.467 ± 1.08[a]

Note. Letters in common indicate not significant differences according to Fisher's protected Least Significant Difference test ($p \leq .05$).

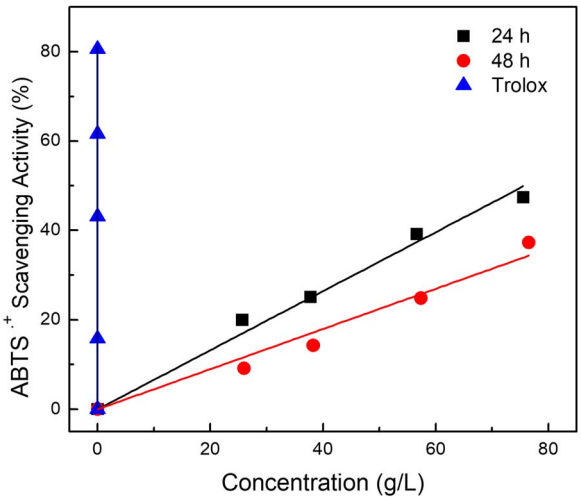


Figure 2. Antioxidant activity expressed as percentage of scavenging activity (SA%) of ABTS cation radical.

lactic acids concentration was 41.91 and 181.52 mM, respectively. Lactic acid production exceeded acetic acid production by over fourfold. Even though the maximum concentration of acetic acid was reached at 48 hr, there was no statistically difference comparing it with 24 hr production. In contrast, lactic acid production at 48 hr was statistically different compared with at 24 hr, being 1.5 times more.

Antioxidant capacity of *P. pentosaceus* RC007 postbiotics

Antioxidant capacity was evaluated by the ability to deactivate the radical cation ABTS• + and by FRAP assays. Two fermentation times were evaluated (Table 5 and Figure 2). The highest ABTS• + TEAC and FRAP values were 31.467 μg Trolox/g after 24 hr and 11.38 μmol/g after 48 hr, respectively. Although there was a slight difference between 24 and 48 hr of fermentation, there was no statistically significant difference between times.

Determination of kinetic and productive parameters of P. pentosaceus RC007 biomass production in bioreactor

Kinetic and productive parameters of *P. pentosaceus* RC007 produced in bioreactor at sub-pilot scale production were obtained. Specific growth rate (μ) led to a growing of 1.99 hr^{-1} (Figure 3). Biomass production (r_x) was 1.1 g/L at 7 hr fermentation and reached its maximum production point when the pH decreased to a 4 value, and from so on a stationary phase was maintained until the end of the experiment. The substrate consumption rate (r_s) was 1.6 g/L/hr. The production rate of lactic acid (r_p) was 0.82 g/L/hr. Lactic acid was detected from the first hour of production and it steadily grew until the 11th hour of the experiment, when its production reached a stationary phase. Yield of the production ($Y_{x/s}$) was 68.8%.

Discussion

In this study, the capabilities of LAB for the generation of postbiotics that possess antimicrobial and antioxidant qualities, with a particular emphasis on the industrial-scale production potential of the probiotic strain *P. pentosaceus* RC007, were assessed.

Pathogenic bacteria become widely resistant to antibiotics due to the increasingly administration of antibiotics, particularly prophylactic in-feed doses (Ricker et al., 2020). In Argentina, *Salmonella* and *E. coli* are becoming a noteworthy issue due to their multidrug resistance profile (Faccone et al., 2019; Parada et al., 2022).

In vitro antimicrobial screening aimed to perform a broad number of multidrug resistant *Salmonella* sp. strains and β-haemolytic *E. coli* strains, owing to their clinical relevance. In this work, antimicrobial screening showed larger zones of inhibition, proving

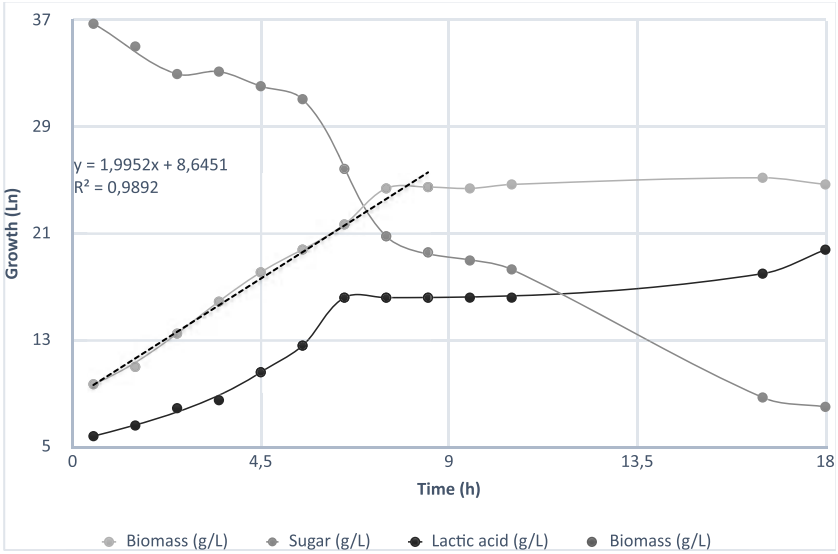


Figure 3. *Pediococcus pentosaceus* RC007 growth and lactic acid production.

that all tested LAB were highly capable of inhibiting clinically relevant pathogenic strains. This activity could be attributed to the ability of *P. pentosaceus* RC007 to ferment sugars to produce organic acids, such as lactic acid and acetic acid. These acids lower the pH of the environment, creating an acidic condition that is hostile to many pathogenic bacteria, including *E. coli* and *Salmonella*. The acidic environment disrupts the internal pH homeostasis of these pathogens, impairing their growth and survival.

Our findings align with the study by Digută et al. (2020), where the antimicrobial properties of *P. pentosaceus* and *P. acidilactici* strains, isolated from an industrial Kombucha for their biotechnological potential, against *Salmonella* sp. and *E. coli* were evaluated. Their research revealed inhibitory activity against both pathogens, albeit with a more substantial inhibition observed against *Salmonella* sp. However, there were some contrasts with our results. We observed greater inhibition zones for *Salmonella* sp. compared with *E. coli*. These authors considered (+) an inhibition halo of 1–5 mm diameter; (++) halo of 6–17 mm diameter and (+++) halo of 17–29 mm diameter. All tested *P. pentosaceus* strains exerted the lowest inhibition over *E. coli* strain (+) and a high inhibition over *Salmonella* sp. tested strain (+++). In this study, inhibition halos were over 52 mm diameter for *Salmonella* sp. strains, over 72 mm diameter for *E. coli* strains, and over 74 mm diameter for *E. coli* O157:H7 strains.

The agar diffusion method showed BLIS inhibition halos against LAB strains with diameter in the range 11.0–19.5 mm.

de Azevedo et al. (2019) also examined the antimicrobial activity of *P. pentosaceus* strains against *E. coli ATCC25922* and *Salmonella enterica CECT 724*. They reported inhibition of both pathogenic strains from 0 to 8 mm at different aeration and agitation conditions (shake flasks under 150 rpm; shake flasks under 200 rpm; anaerobic jar without agitation; anaerobic jar in rotary shaker under 200 rpm). These inhibition zones were smaller than those observed in our study for both *E. coli* and *Salmonella* sp. strains. İncili et al. (2023) demonstrated antimicrobial activity against *E. coli O157:H7* and *S. typhimurium* strains using a strain of *L. plantarum*. Their research indicated effective inhibition of both pathogenic strains, although the observed inhibition zones were smaller than those in our work. Moreover, *L. rhamnosus* exhibited inhibition against both *Salmonella and E. coli* strains, but the inhibitory effects were less pronounced than those of *L. plantarum*.

Enterococcus faecium proved to be a good inhibitory strain, but our results were different compared with those obtained by Ökzan et al. (2021), where no inhibition against strains of *S. typhimurium* and *E. coli* were observed. Similar results were obtained by Ahmadova et al. (2013), where three strains of *Salmonella* and one of *E. coli* were tested, and no inhibitory effects were observed.

Probiotic strains comprise a different genus of microorganisms that have been widely studied in animals. Metabolic by-products or bioactive molecules produced by probiotics during its growth are known as postbiotics (Salminen et al., 2021). Postbiotics are less susceptible to environmental conditions, which makes them stable and safer for food application unlike the probiotics they are derived from and allows them to be produced not only at laboratory scale but also potentially in mass at industrial scale (Barros et al., 2020; Cuevas-González et al., 2020). Although its stability compared with probiotics, postbiotics are still in need of further investigation regarding the industrial scale-up process (Cuevas-González et al., 2020). Probiotic and postbiotic capabilities of *P. pentosaceus* strains have not been well characterized yet. Great evidence of probiotic abilities *in vivo* of *P. pentosaceus* showed to maintain healthy and productive performance of post-weaning pigs without in-feed antibiotic addition (Parada et al., 2023) improvement in productive parameters of shrimps (Wanna et al., 2021) and cholesterol-lowering activity in mice (Damodharan et al., 2015).

Parada et al.'s (2022, 2024) studies with *P. pentosaceus* RC007 justified the interest in the search of its postbiotic production ability to confer health benefits, especially SCFA and antioxidant metabolites. Zaki et al. (2024) isolated a *P. pentosaceus* strain from traditional Malaysian food that was capable to produce different volatile compounds, being lactic acid the most prevalent compared to acetic acid, butyric acid, and propionic acid. Our results partially agree with these findings, as neither butyric acid nor propionic acid production was found. However, lactic acid concentration was higher than acetic acid, up to +4 times higher. Even though these findings are similar, their production of lactic acid was almost 250 mM while ours was 181.5 mM. The same occurred with acetic acid, while their production was 110 mM and ours 41.9 mM. Fugaban et al. (2022) made a deeper analysis of organic acids produced by *P. pentosaceus* strains isolated from silage, among which lactic acid but no other volatile acids as

acetic, butyric, or propionic were found. Production of organic acids could vary among *P. pentosaceus* strains. Environmental factors other than genetic differences, metabolic pathways, and substrate availability could influence the organic acids biosynthesis, most probably via the moderating expression of related genes involved in production and expression processes.

The antioxidant proprieties of LAB are well documented among the various effects of probiotic strains, and antioxidant activity may have the strongest beneficial effect, which help to mitigate oxidative damage in the body resulting from stress and potentially improving host health. The cultivation in MRS produces diverse metabolites that can confer antioxidant capacity, such as EPS, bioactive compounds, and antioxidant enzymes (Ayyash et al., 2020; Łepecka et al., 2023). Ayyash et al. (2020) has shown the antioxidant capacity of EPS from *P. pentosaceus* M41 in MRS. Łepecka et al. (2023) evaluated the antioxidant capacity of supernatants (CFCS—Cell-Free Culture Supernatant) of some LAB. Furthermore, Zhang (2020) evaluated the reducing activity of intact cells and CFCS, showing a higher reducing activity for CFCS. In this work, the antioxidant capacity of postbiotics could be attributed to combination of EPS and diverse metabolites produced in MRS. *Pediococcus* strains have been informed among the most prominent EPS-producing LABs. Ayyash et al. (2020) informed antimicrobial, antioxidant, and antitumor activities produced by *P. pentosaceus* M41 EPS. Other mechanism is probably the activation of the Nrf2-Keap1 signalling pathway, a cellular antioxidant system that regulates redox homeostasis, cellular metabolism, and the body's cryoprotective response to oxidative and eletrophilic stress, that was informed by Yu and Xiao (2021). According to Yang et al. (2023) probiotic treatment with LAB strains and supernatant free cell might impact oxidative stress in the host both directly, through ROS scavenging, and indirectly, by altering the community structure of intestinal microbiota.

Taking account probiotic properties of *P. pentosaceus* and the large amount of postbiotic metabolites of interest in health and industry that it produces, it is relevant to evaluate the possibility of achieve industrial culture scale. There are not many studies in relation to the optimization of *P. pentosaceus* production on an industrial scale. de Souza de Acevedo et al. (2019) studies bacteriocins-like inhibitory substances produced by *P. pentosaceus* and described its growth in bioreactor at sub-pilot scale (1.5 L) under similar conditions of this work (MRS medium, 24 hr fermentation, 200 rpm at 30 °C). They obtained a biomass production at 6 hr fermentation similar to us at 7 hr fermentation (1.06 ± 0.04 g/L). However, they were able to optimize growth conditions from shake flasks to bioreactor, obtaining 3.41 g/L biomass at 24 hr fermentation. In this work, kinetic and productive parameters obtained in bioreactor at sub-pilot scale compared to other authors were considered acceptable. Tadi et al. (2024) studied *P. pentosaceus* production on economical and sustainable media using low-cost renewable feedstocks and industrial by-products. They obtained μ (hr^{-1}) and ($Y_{x/s}$) 0.346 hr^{-1} and 25.7% yield in Palmira Palm Jaggery medium and 0.204 hr_{-1} and 14.1% yield in whey protein hydrolysate medium. Both parameters were lower than ours; however, it was an optimization using low-cost substrates. These results demonstrate the potential of *P. pentosaceus* RC007 to be industrially scaled and the need to carry out studies to optimize its production.

Conclusion

The antimicrobial and antioxidant properties of LAB, with a focus on the probiotic strain *P. pentosaceus* RC007 were evaluated for their potential in industrial-scale postbiotic production. The high antibiotic resistance among *Salmonella* and *E. coli* strains emphasizes the need for alternative antimicrobial strategies. *In vitro* screening demonstrated significant inhibition of these pathogens by LAB strains, particularly *P. pentosaceus* RC007, attributed to its production of organic acids such as lactic and acetic acids, which create a hostile environment for pathogens.

Compared with previous studies, our findings showed larger inhibition zones for both *Salmonella* and *E. coli* strains, indicating a strong antimicrobial capacity of *P. pentosaceus* RC007. The antioxidant properties of *P. pentosaceus* RC007 were notable, with high ABTS• + TEAC and FRAP values, suggesting its potential in reducing oxidative stress.

Kinetic and productive parameters in a bioreactor demonstrated efficient biomass production and lactic acid yield, highlighting the feasibility of scaling up *P. pentosaceus* RC007 for industrial applications. Overall, *P. pentosaceus* RC007 shows great promise as a probiotic and postbiotic producer, with significant health benefits and industrial potential. Further optimization studies are recommended to enhance its production efficiency on an industrial scale.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

Lilia Cavaglieri (Conceptualization [equal], Formal analysis [equal], Funding acquisition [equal], Project administration [equal], Resources [equal], Writing—original draft [equal], Writing—review & editing [equal]), Maite Corti Isgro (Investigation [supporting], Methodology [supporting]), Carla Aminahuel (Investigation [supporting], Methodology [supporting]), Julián Parada (Formal analysis [equal], Investigation [equal], Methodology [equal], Supervision [equal]), Valeria L. Poloni (Formal analysis [equal], Investigation [equal], Methodology [equal], Supervision [equal]), Mariana Montenegro (Investigation [equal], Supervision [equal], Visualization [equal]), Verónica Alonso (Investigation [equal], Methodology [equal], Validation [equal]), Ruben D. Falcone (Formal analysis [equal], Investigation [equal], Supervision [equal]), and Lorenzo A. Rosales Cavaglieri), Conceptualization [equal], Investigation [equal], Methodology [equal], Validation [equal], Visualization [equal], and Writing—original draft [equal]).

Funding

This study was supported by grants from SECyT-UNRC, PIP-CONICET, and FONCYT-PICT 3089/18.

Conflicts of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work; there is no professional or other personal interest of any nature or kind in any product, service, and/or company that could be construed as influencing the content of this paper.

Ethical guidelines

Ethics approval was not required for this research.

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International Journal of Food Science and Technology, 2025, **60(1)**, vvae003
https://doi.org/10.1093/ijfood/vvae003
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