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Original Article

Evaluation of *Saccharomyces cerevisiae* yeast cell wall as a microbial-derived prebiotic substrate for enhancing the functional properties of lactic acid bacteria

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Abstract

Saccharomyces cerevisiae yeast cell wall (SC), a fermentation by-product rich in β-glucans, mannans, and mannoproteins, was evaluated as a sustainable microbial-derived prebiotic for *Lacticaseibacillus paracasei*, *Lacticaseibacillus rhamnosus*, and *Levilactobacillus brevis*. Fourier transform infrared confirmed the characteristic structure of SC. Its effects on probiotic functionality were assessed through growth kinetics, biomass production, antimicrobial, antioxidant, enzymatic, and organic acid analyses, using de Man, Rogosa, and Sharpe (MRS) and inulin as controls. SC significantly enhanced biomass production, with the highest yield obtained for *L. paracasei* in 10% SC (5.85 g), compared with 3.75 g in MRS. *Lacticaseibacillus rhamnosus* showed superior antimicrobial activity in SC supplemented medium and inhibited *Escherichia coli* and *Staphylococcus aureus* (up to 4 mm), while *L. paracasei* exhibited 76.06% 2,2-diphenyl-1-picrylhydrazyl scavenging activity. *Lacticaseibacillus rhamnosus* showed the highest lipase (5.80 U/ml) and lactic acid (11.80 g/L) production, whereas *L. brevis* produced the highest acetic (0.56 g/L) and butyric acids (0.0050 g/L). These findings demonstrated that SC is a promising sustainable microbial-derived prebiotic for synbiotic fermentation and valorisation of industrial yeast by-products.

Keywords *Saccharomyces cerevisiae* yeast cell wall, microbial-derived prebiotic, synbiotic, lactic acid bacteria, biomass production, antioxidant activity, antimicrobial activity, enzyme activity, organic acids, circular bioeconomy

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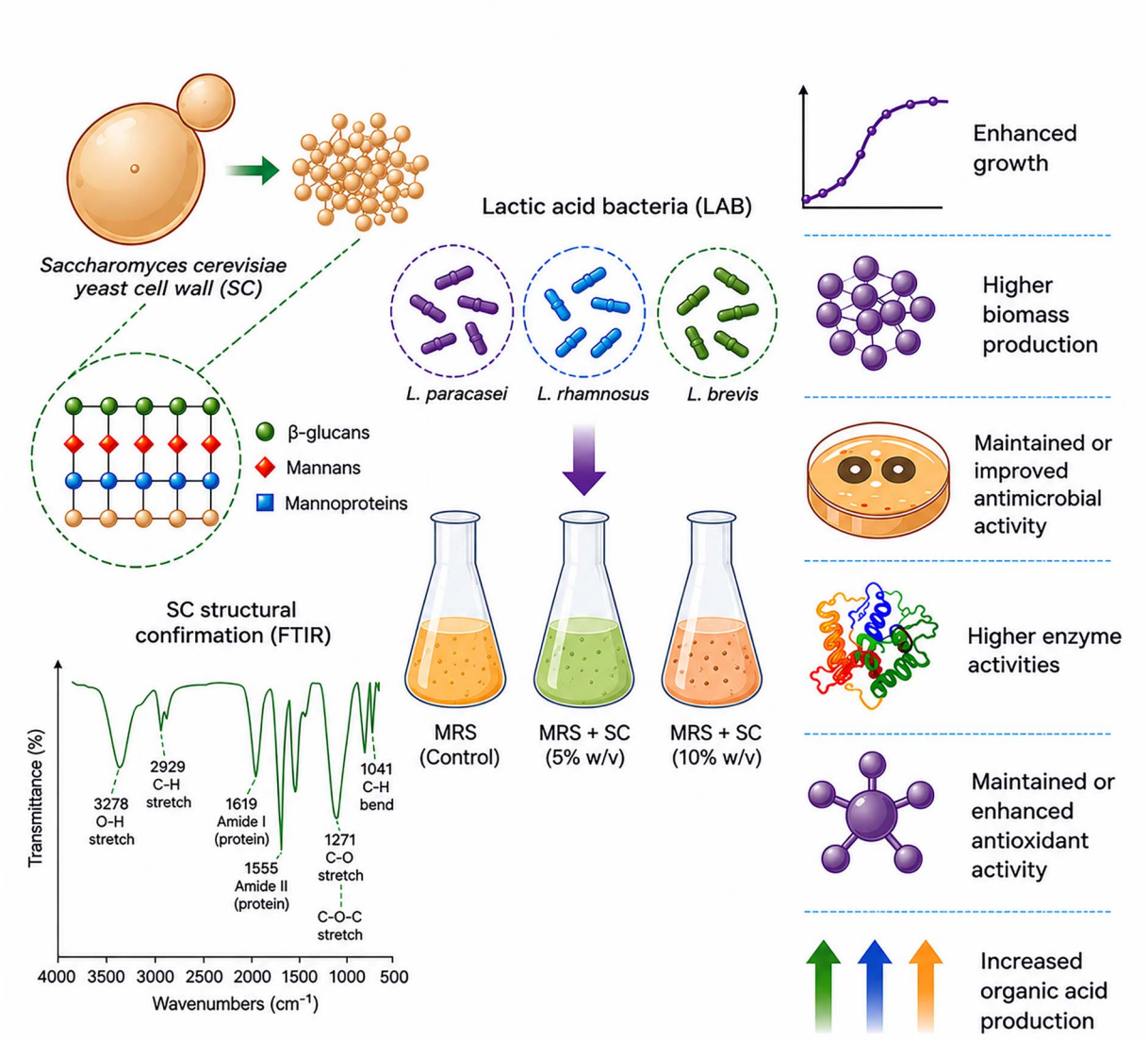
Graphical Abstract



Introduction

In recent decades, probiotics, prebiotics, and synbiotics have attracted considerable scientific and commercial attention due to their multifaceted contributions to human health, functional foods, pharmaceuticals, and nutraceutical formulations. This interest has been further amplified by the rapidly expanding field of microbiota-targeted therapies, which recognise the gut microbial ecosystem as a critical regulator of host metabolism, immunity, and overall physiological homeostasis (Hill et al., 2014). The Food and Agriculture Organization and the World Health Organization define probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit to the host". Among probiotic candidates, members of the lactic acid bacteria (LAB) group particularly species belonging to the genera *Lactobacillus, Lacticaseibacillus, Levilactobacillus,* and *Bifidobacterium* have been most extensively investigated owing to their well-documented capacity to restore intestinal microbial equilibrium, produce bioactive metabolites, antagonise enteric pathogens, modulate host immune responses, and reinforce epithelial barrier integrity (Fijan, 2014; Gibson et al., 2017; Plaza-Diaz et al., 2019). Nevertheless, the functional efficacy of probiotics is critically dependent on bacterial viability, metabolic competence, and persistence within the harsh conditions of the gastrointestinal tract. These attributes are profoundly influenced by the availability of suitable dietary substrates capable of supporting bacterial proliferation and stimulating the biosynthesis of beneficial metabolites (Markowiak & Ślizewska, 2017; Tripathi & Giri, 2014). In this context, the strategic combination

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of probiotics with selected fermentable substrates namely prebiotics has emerged as a powerful approach to enhance probiotic performance both in vitro and in vivo. According to the International Scientific Association for Probiotics and Prebiotics, a prebiotic is defined as "a substrate that is selectively utilised by host microorganisms conferring a health benefit" (Gibson et al., 2017). Historically, the most thoroughly characterised prebiotic compounds include lactulose, fructooligosaccharides, galactooligosaccharides, and inulin (IN). These non-digestible carbohydrates promote the selective proliferation of beneficial microbial populations, stimulate the production of short-chain fatty acids such as acetate, propionate, and butyrate, enhance mineral bioavailability, and contribute to the maintenance of intestinal homeostasis (Davani-Davari et al., 2019; Saville et al., 2025). However, the rising global demand for functional ingredients, combined with concerns regarding the cost, scalability, and sustainability of conventional prebiotic production, has driven a paradigm shift toward the exploration of alternative, sustainable, and microbial-derived prebiotic sources that align with the principles of the circular bioeconomy and agro-industrial waste valorisation (Aguilar–Toalá et al., 2018). Among such alternative sources, microbial biomass particularly that of yeasts has emerged as a particularly promising candidate. *Saccharomyces cerevisiae* is one of the most extensively employed microorganisms in the food, beverage, fermentation, and biotechnology industries, with vast quantities of spent yeast biomass generated annually as a by-product of bread, wine, beer, and bioethanol production (Parapouli et al., 2020). This abundant, low-cost, and renewable resource represents an underexploited reservoir of bioactive compounds with significant prebiotic potential. The cell wall of *S. cerevisiae*, which accounts for approximately 15%–30% of the cell's dry weight, is structurally composed of β-glucans, mannans, mannoproteins, and minor amounts of chitin. Structurally, the inner layer is mainly composed of β-(1→3)- and β-(1→6)-glucans, conferring mechanical strength to the wall, while the outer layer is enriched in mannoproteins and mannan-containing oligosaccharides that mediate interactions with the external environment (Klis et al., 2002). These polysaccharide components exhibit a remarkable spectrum of biological activities, including immunomodulatory, antioxidant, antimicrobial, cholesterol-lowering, and prebiotic effects (Liu et al., 2018). In particular, mannan-oligosaccharides (MOS) and β-glucans derived from yeast have been widely studied as microbiota-modulating compounds and functional feed additives. MOS contribute to the improvement of intestinal barrier function, competitive exclusion of pathogenic bacteria (*Salmonella, Escherichia coli*) through type-1 fimbriae binding, enhancement of microbial fermentation, and the selective stimulation of beneficial taxa (Rungrassamee et al., 2014). Likewise, yeast-derived β-glucans exert pronounced immunostimulatory effects *via* interactions with pattern recognition receptors such as Dectin-1 and TLR-2/6 on gut-associated immune cells, while simultaneously enhancing the viability and metabolic activity of probiotic strains (Pandey et al., 2015). Owing to these collective properties, yeast cell wall preparations are increasingly recognised as multifunctional microbial-derived prebiotic ingredients.

Recent studies have further strengthened the potential of yeast cell wall-derived components as functional ingredients in prebiotic and synbiotic systems. Yeast cell wall biopolymers, particularly β-glucans and mannoproteins, have been investigated as functional matrices capable of supporting probiotic viability and delivery, highlighting their relevance for food and nutraceutical applications (Utama et al., 2023). More recent studies have also emphasised the biological functionality of *S. cerevisiae* cell wall mannans and β-glucans, including their effects on gut health, antioxidant activity, and immune regulation (Baek, Lee et al., 2024; Baek, Rani Ramakrishnan et al., 2024). Importantly, yeast cell wall-derived preparations are no longer limited to experimental applications; commercial prebiotic and synbiotic preparations containing yeast-derived cell wall components, particularly mannan-oligosaccharides and β-glucans, are already available and have been evaluated in applied nutritional settings (Araba et al., 2024; Fornazier et al., 2024). Therefore, the novelty of yeast cell wall-based prebiotic systems should not be attributed simply to the use of yeast-derived polysaccharides themselves, but rather to the specific manner in which these materials are characterised, combined with probiotic strains, and evaluated for their effects on microbial functionality.

Despite these advances and the existing commercial use of yeast-derived prebiotic ingredients, important knowledge gaps remain regarding the direct effects of whole *S. cerevisiae* yeast cell wall preparations on probiotic bacterial functionality. Recent investigations have predominantly focused on isolated yeast cell wall fractions, host-related biological effects, probiotic encapsulation, or animal nutrition applications rather than on the simultaneous assessment of probiotic growth and metabolic outputs during fermentation (Baek, Rani Ramakrishnan et al., 2024; Fornazier et al., 2024; Utama et al., 2023). In particular, limited information is available on whether whole yeast cell wall material can differentially influence biomass production, antimicrobial and antioxidant activities, extracellular enzyme production, and organic acid formation among distinct lactic acid bacterial strains under identical fermentation conditions. Such strain-dependent responses are important because probiotic functionality cannot be inferred solely from growth promotion and may vary substantially according to substrate utilisation and metabolic capacity. Despite these advances and the existing commercial use of yeast-derived prebiotic ingredients, important knowledge gaps remain regarding the direct effects of whole *S. cerevisiae* yeast cell wall preparations on probiotic bacterial functionality. Recent investigations have predominantly focused on isolated yeast cell wall fractions, host-related biological effects, probiotic encapsulation, or animal nutrition applications rather than on the simultaneous assessment of probiotic growth and metabolic outputs during fermentation (Baek, Rani Ramakrishnan et al., 2024; Fornazier et al., 2024; Utama et al., 2023). In particular, limited information is available on whether whole yeast cell wall material can differentially influence biomass production, antimicrobial and antioxidant activities, extracellular enzyme production, and organic acid formation among distinct lactic acid bacterial strains under identical fermentation conditions. Such strain-dependent responses are important because probiotic functionality cannot be inferred solely from growth promotion and may vary substantially according to substrate utilisation and metabolic capacity. Based on the complex composition of the yeast cell wall and the strain-specific metabolic characteristics of lactic acid bacteria, we hypothesised that *S. cerevisiae* yeast cell wall supplementation would support probiotic growth while differentially modulating functional metabolite production among bacterial strains. We further hypothesised that these effects would not necessarily parallel those observed with IN, which was included as an established prebiotic reference.

Moreover, the interaction between probiotic bacteria and prebiotic substrates extends beyond mere growth stimulation. Nutrient composition strongly governs microbial metabolic pathways, thereby

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influencing the biosynthesis of physiologically active metabolites. Lactic acid bacteria are known producers of a wide array of bioactive compounds, including organic acids (lactic, acetic, propionic, butyric, succinic, and citric acids), antimicrobial peptides and bacteriocins, antioxidant molecules (glutathione, peptides, exopolysaccharides [EPS]), extracellular enzymes (β-galactosidase, protease, amylase, esterase), and EPS that collectively contribute to their probiotic functionality (Feng & Wang, 2020; Mounir et al., 2022). Alterations in available carbon and nitrogen sources can substantially reshape these metabolic pathways, leading to measurable changes in organic acid profiles, enzyme activity, antioxidant capacity, and antimicrobial efficacy. Consequently, a comprehensive evaluation of any novel prebiotic substrate should encompass not only its capacity to support bacterial growth but also its impact on the functional and metabolic phenotype of the probiotic strain. Despite the growing interest in yeast-derived prebiotics, comprehensive comparative studies examining the influence of whole *S. cerevisiae* cell wall material on probiotic growth performance, metabolic outputs, and functional traits remain scarce. Furthermore, comparative data on its effects across phylogenetically and physiologically distinct LAB species namely *Levilactobacillus brevis, Lacticaseibacillus rhamnosus,* and *Lacticaseibacillus paracasei* are notably lacking. To the best of our knowledge, comparative studies simultaneously examining growth performance, biomass production, antimicrobial and antioxidant activities, extracellular enzyme profiles, and organic acid production across multiple LAB strains cultivated with whole *S. cerevisiae* yeast cell wall remain limited. Accordingly, the novelty of the present study lies in the integrated, strain-level comparison of whole *S. cerevisiae* yeast cell wall supplementation across *L. brevis*, *L. rhamnosus*, and *L. paracasei*, using IN as a conventional prebiotic reference and simultaneously evaluating growth, biomass accumulation, antimicrobial and antioxidant functionality, extracellular enzyme production, and organic acid profiles. Therefore, the present study aimed to evaluate whole *S. cerevisiae* yeast cell wall as a microbial-derived prebiotic substrate and to determine its strain-dependent effects on the growth and functional metabolism of *L. brevis*, *L. rhamnosus*, and *L. paracasei*. Standard MRS and IN-supplemented media were used as reference conditions. Growth kinetics and biomass production were evaluated together with antimicrobial and antioxidant activities, extracellular lipase, phytase, and protease activities, and lactic, acetic, and butyric acid production. Through this integrated approach, the study aimed to determine whether whole yeast cell wall supplementation could support probiotic functionality beyond simple growth promotion and to assess its potential as a sustainable ingredient for synbiotic fermentation systems.

Materials and methods

Microorganisms, culture media, and chemicals

Three LAB strains were used in this study: *L. brevis* (GenBank accession number: PZ213757), *L. rhamnosus* (GenBank accession number: PV055690), and *L. paracasei*. These strains were previously isolated from bovine colostrum and identified by 16S rRNA gene sequencing in our previous projects (Soyer et al., 2023). The strains were obtained from the culture collection of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Anadolu University (Eskişehir, Türkiye). *Saccharomyces cerevisiae* ATCC 208288 was used for the preparation of yeast cell wall material. LAB strains were routinely cultured in de Man, Rogosa, and Sharpe broth (MRS broth) and maintained on MRS agar (Merck, Darmstadt, Germany). *Saccharomyces cerevisiae* ATCC 208288 was cultivated in Sabouraud Dextrose Broth (SDB) and maintained on Sabouraud Dextrose Agar (SDA) (Merck, Darmstadt, Germany). MRS broth, MRS agar, Sabouraud Dextrose Broth, Sabouraud Dextrose Agar, Mueller Hinton Agar, IN, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, sodium phytate, soluble starch, skim milk powder, tributyrin, bile salts, hydrochloric acid, sodium hydroxide, methanol, and all other analytical-grade chemicals were purchased from Merck (Darmstadt, Germany) or Sigma-Aldrich (St. Louis, MO, USA).

Preparation of *Saccharomyces cerevisiae* cell wall as a prebiotic substrate

The yeast cell wall preparation used as a microbial-derived prebiotic substrate in this study was obtained from *S. cerevisiae* ATCC 208288, following the protocol described by Zarei et al. (2016) with minor modifications, and in accordance with the general principles of yeast cell wall recovery reported by Liu et al. (2018) (Liu et al., 2021; Zarei et al., 2016). The *S. cerevisiae* strain was first reactivated by streaking into SDA plates and incubated aerobically at 30 °C for 48 hr. A single pure colony was then transferred into SDB and incubated under the same conditions for 24 hr to obtain a pre-culture. Subsequently, 1% (v/v) (10^8 CFU/ml) of the activated pre-culture was inoculated into fresh SDB and incubated at 30 °C for 48 hr under continuous agitation (150 rpm) in order to maximise biomass production. Following incubation, the yeast biomass was harvested by centrifugation at 9,000 rpm for 15 min at 4 °C (Hettich Universal 320R, Germany). The supernatant was discarded, and the resulting cell pellet was washed twice with sterile distilled water to remove residual culture medium components, followed by re-centrifugation under the same conditions. To release and recover the cell wall fraction, the washed yeast pellet was resuspended in sterile distilled water (1:5, w/v) and subjected to thermal autolysis by autoclaving at 121 °C for 15 min, which simultaneously inactivated the cells and facilitated the disruption of the intracellular content, as recommended for efficient separation of insoluble cell wall polysaccharides from soluble cytoplasmic constituents (Borchani et al., 2016). The autoclaved suspension was subsequently centrifuged at 9,000 rpm, for 30 min at 4 °C to separate the insoluble cell wall fraction from the soluble intracellular components. The supernatant was discarded, while the insoluble pellet primarily composed of β-(1→3)- and β-(1→6)-glucans, mannoproteins, MOS, and minor amounts of chitin was collected as the crude yeast cell wall fraction. The pellet was washed twice with sterile distilled water and re-centrifuged (9,000 rpm, 30 min, 4 °C) to remove residual soluble compounds. The recovered cell wall fraction was then resuspended in sterile distilled water and filtered through a 0.45 μm membrane filter to eliminate any residual particulates, followed by a final centrifugation step (9,000 rpm, 30 min, 4 °C). The obtained pellet was transferred into sterile glass petri dishes, sealed, and dried in a hot-air oven at 45 °C until a constant weight was achieved (approximately 48 hr). The resulting dry material, hereafter referred to as the yeast cell wall preparation, was ground into a fine powder using a sterile mortar and pestle, passed through a 250 μm sieve to ensure particle-size homogeneity, and stored in airtight, light-protected containers at 4 °C until further use as a prebiotic substrate in the subsequent fermentation experiments (Liu et al., 2021; Zarei et al., 2016).

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Fourier transform infrared analysis of yeast cell wall preparation

The chemical structure of the prepared *S. cerevisiae* yeast cell wall (SC) was characterised using Fourier transform infrared (FTIR) spectroscopy. Prior to analysis, the dried SC powder was finely ground and analysed directly using an attenuated total reflectance accessory. FTIR spectra were recorded using a Spectrum Two FTIR spectrometer (PerkinElmer, Waltham, MA, USA) over the spectral range of 4,000–650 cm^{-1} at a resolution of 4 cm^{-1} with 32 scans per sample. Background spectra were collected before each measurement and automatically subtracted from the sample spectra. The obtained spectra were processed and visualised using SpectraGryph software (version 1.2.16.1). Characteristic absorption bands corresponding to polysaccharides, proteins, and glycosidic structures were identified according to previously published studies on *S. cerevisiae* cell wall components, including β-glucans, mannans, and mannoproteins (Liu et al., 2021).

Preparation of synbiotic fermentation media and evaluation of probiotic growth

Synbiotic cultures were established by cultivating selected probiotic strains in the presence of *S. cerevisiae* yeast cell wall as a microbial derived prebiotic substrate, following a modified version of the method described by Iraporda et al. (2019). Modified MRS broth was used as the base fermentation medium and supplemented with SC powder at two different final concentrations, namely 5% (w/v) and 10% (w/v). For the preparation of the 5% SC-supplemented medium, 2.5 g of SC powder was added to 50 ml of sterile MRS broth, whereas 5 g of SC powder was incorporated into 50 ml of sterile MRS broth to obtain the 10% (w/v) formulation. Conventional MRS broth without SC supplementation was used as the control medium. All media were thoroughly mixed prior to inoculation to ensure the homogeneous dispersion of the SC substrate. Each medium was inoculated with 1% (v/v) (10^8 CFU/ml) of an overnight active culture of *L. brevis*, *L. rhamnosus*, or *L. paracasei*, and incubated at 37 °C under aerobic conditions for 48 hr. Bacterial growth was monitored spectrophotometrically by measuring the optical density (OD) at 600 nm (OD_{600}) at 2 hr intervals throughout the incubation period in order to construct growth curves. For each strain and culture condition, the onset of the exponential growth phase, the maximum OD_{max}, and the overall growth kinetics were evaluated. At the end of the incubation period, biomass production was quantitatively determined to compare bacterial growth across the different media. Cultures were centrifuged at 10,000 rpm for 10 min, the supernatants were discarded, and the resulting cell pellets were dried at 45 °C until a constant weight was achieved. Biomass yield was expressed as dry cell weight (g/L), and the effects of SC concentration on bacterial growth performance and cell production were evaluated relative to the control medium (Iraporda et al., 2019). Conventional MRS broth and MRS supplemented with 5% and 10% IN were used as negative and positive control media, respectively.

Preparation of cell-free culture supernatants

Cell-free culture supernatants (CFSs) were prepared from the synbiotic fermentation cultures following bacterial growth in the respective media. Briefly, *L. brevis*, *L. rhamnosus*, and *L. paracasei* were cultivated in control MRS broth, IN and SC-supplemented MRS media (5% and 10%, w/v) at 37 °C for 48 hr. At the end of the incubation period, cultures were centrifuged at 10,000 rpm for 10 min at 4 °C to separate bacterial cells from the fermentation broth. The resulting supernatants were carefully collected and filtered through sterile 0.22 μm membrane filters (Millipore, USA) to completely remove residual bacterial cells. The obtained sterile filtrates were designated as CFSs and were used for subsequent antimicrobial activity, antioxidant activity, enzyme activity, and organic acid analyses. All CFS samples were stored at 4 °C and analysed within 24 hr of preparation.

Determination of biological activities

Antimicrobial activity

The antimicrobial activity of CFSs obtained from fermentation cultures was evaluated using the agar well diffusion method (Solak et al., 2025). The test microorganisms included *E. coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923. Fresh cultures of the test microorganisms were adjusted to 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU/ml). Sterile MHA plates were inoculated with 100 μl of each standardised pathogen suspension. Wells with a diameter of 6 mm were aseptically punched into the agar, and 80 μl of each sterile-filtered CFS was added to the wells. Following incubation at 37 °C for 24 hr, inhibition zone diameters were measured in millimetres. CFSs obtained from *L. brevis*, *L. rhamnosus*, and *L. paracasei* cultivated in standard MRS broth were used as the fermentation control, while CFSs obtained from cultures grown in MRS supplemented with IN (5% and 10%, w/v) served as the established prebiotic reference controls. These groups were compared with CFSs obtained from cultures grown in MRS supplemented with 5% and 10% (w/v) *S. cerevisiae* yeast cell wall (SC) to determine the effect of SC supplementation on antimicrobial activity. All experiments were performed in triplicate.

Antioxidant activity

The antioxidant activity of CFSs was determined using the DPPH free radical scavenging assay according to Hoşer et al., 2026, with minor modifications. The 100 μl of each CFS sample was mixed with 100 μl of freshly prepared DPPH solution (0.1 mM in methanol) in a 96-well microplate and incubated in the dark for 30 min at room temperature. Absorbance was measured at 517 nm using a microplate reader (BioTek Synergy HTX, USA). The tested CFSs were obtained from probiotic cultures grown in conventional MRS broth, MRS supplemented with 5% and 10% (w/v) IN, and MRS supplemented with 5% and 10% (w/v) *S. cerevisiae* yeast cell wall. Ascorbic acid (2 μg/ml) was used as a positive control. Radical scavenging activity was calculated according to the following equation:

$$\text{DPPH scavenging activity } (\%) = \left[(\text{Acontrol} - \text{Asample}) / \text{Acontrol}\right] \times 100$$

All measurements were performed in triplicate, and the antioxidant activities of SC-supplemented cultures were compared with those of both the control and IN-supplemented cultures.

Enzyme activity assays

The enzymatic activities of probiotic strains cultivated in control and synbiotic fermentation media were evaluated to determine the effect of *S. cerevisiae* yeast cell wall supplementation on bacterial metabolic functionality. Fermentation liquids obtained after 48 hr of incubation were centrifuged (10,000 rpm, 10 min, 4 °C), and the resulting CFSs

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were used for all enzymatic analyses. For each strain, the fermentation liquid exhibiting the highest biomass production among the modified media was selected for enzymatic characterisation. Fermentation liquids obtained from cultures grown in standard MRS broth were included as controls. Lipase activity was determined using a Lipase Assay Kit (MAK046, Sigma-Aldrich, St. Louis, MO, USA), protease activity was determined using a Protease Assay Kit (539125, Sigma-Aldrich, St. Louis, MO, USA), and phytase activity was measured using a Phytase Assay Kit (K-Phytase, Megazyme International, Bray, Ireland), all according to the manufacturers' instructions. Enzyme activities were quantified spectrophotometrically using a microplate reader at the wavelengths specified in each kit protocol. Activities were calculated using the calibration curves supplied with the respective assay kits and expressed as enzyme activity units per millilitre of fermentation liquid (U/ml). The enzyme activities of cultures grown in MRS broth supplemented with 10% (w/v) *S. cerevisiae* yeast cell wall were compared to evaluate the influence of prebiotic supplementation on the metabolic activity of the probiotic strains. All measurements were performed in triplicate ($n = 3$), and the results are presented as $M \pm SD$.

Organic acid determination

The production of organic acids by the probiotic strains was evaluated using CFSs obtained after 48 hr of fermentation in conventional MRS broth supplemented with 10% (w/v) *S. cerevisiae* yeast cell wall. Lactic acid, acetic acid, and butyric acid concentrations were quantified using commercial assay kits according to the manufacturers' protocols. Lactic acid was determined using the L-Lactic Acid Assay Kit (Megazyme, Ireland; K-LATE), acetic acid was quantified using the Acetic Acid Assay Kit (Megazyme, Ireland; K-ACETRM), and butyric acid was determined using the Butyric Acid Assay Kit (Megazyme, Ireland; K-HDBA). Absorbance measurements were performed using a microplate reader (BioTek Synergy HTX, USA), and organic acid concentrations were calculated according to the equations provided by the kit manufacturers. Results were expressed as g/L.

Statistical analysis

All experiments were performed in triplicate ($n = 3$), and the results are presented as $M \pm SD$. Statistical analyses were performed using GraphPad Prism version 10.0 (GraphPad Software Inc., San Diego, CA, USA). Differences among experimental groups were evaluated using one-way analysis of variance, followed by Tukey's multiple-comparison test. Statistical significance was accepted at $p < .05$. In Figures 3–7, different lowercase letters indicate statistically significant differences among the compared groups, whereas groups sharing the same letter are not significantly different ($p \geq .05$).

Results and discussion

FTIR characterisation of *Saccharomyces cerevisiae* yeast cell wall

The chemical composition of the *S. cerevisiae* yeast cell wall preparation was characterised by FTIR spectroscopy, and the obtained spectrum is presented in Figure 1. The FTIR profile revealed characteristic absorption bands associated with the major structural components of yeast cell walls, including β-glucans, mannans, and mannoproteins. A broad absorption band centred at 3,278 cm^{-1} was observed and attributed to O–H and N–H stretching vibrations, indicating the presence of hydroxyl-rich polysaccharides and protein-associated amino groups. This band is commonly reported in yeast cell wall preparations due to the abundance of β-glucans and mannans containing numerous hydroxyl groups (Kogan & Kocher, 2007; Lipke & Ovalle, 1998). The absorption bands detected at 2,929 and 2,850 cm^{-1} corresponded to asymmetric and symmetric C–H stretching vibrations of aliphatic methylene groups, respectively. These peaks may be associated with residual lipid structures and hydrocarbon chains present in cell wall-associated proteins and membrane-derived components (Liu et al., 2021). The prominent bands observed at 1,619 and 1,555 cm^{-1} were assigned to amide I and amide II vibrations, respectively. These signals are characteristic of proteinaceous materials and indicate the presence of mannoproteins, which constitute an important fraction of the outer layer of the yeast cell wall. The amide I band originates primarily from C=O stretching vibrations, whereas the amide II band results from N–H bending and C–N stretching vibrations (Borchani et al., 2016). Additional absorption peaks at 1,458 and 1,370 cm^{-1} were attributed to C–H bending and C–O–H deformation vibrations, respectively, reflecting the carbohydrate-rich nature of the sample. These bands have previously been reported in β-glucan- and mannan-containing polysaccharide structures isolated from *Saccharomyces* species. The most significant region of the spectrum was located within the fingerprint region (1,200–900 cm^{-1}), where polysaccharide-specific vibrations are generally observed. The intense absorption band at 1,041 cm^{-1} was assigned to C–O–C glycosidic stretching vibrations and is characteristic of β-(1→3)- and β-(1→6)-glucans as well as mannan structures. This band confirms the predominance of carbohydrate-based polymers within the SC preparation. The absorption bands at 787 and 768 cm^{-1} were detected in the lower wavenumber region. These peaks are associated with anomeric vibrations of glycosidic linkages and may indicate the presence of mannan-related structures within the yeast cell wall matrix. Similar absorption bands have been reported for *S. cerevisiae* cell wall preparations rich in mannoproteins and mannan polysaccharides. The FTIR spectrum confirmed that the obtained SC preparation was primarily composed of polysaccharide-rich cell wall components, particularly β-glucans, mannans, and mannoproteins. The presence of these bioactive constituents supports the potential use of the prepared yeast cell wall as a microbial-derived prebiotic substrate capable of promoting probiotic growth and metabolic activity.

Growth performance and biomass production of probiotic strains in *Saccharomyces cerevisiae* yeast cell wall-supplemented media

The growth kinetics of *L. paracasei*, *L. brevis*, and *L. rhamnosus* cultivated in MRS broth supplemented with IN and *S. cerevisiae* yeast cell wall are presented in Figure 2, while the corresponding biomass yields are shown in Figure 3. In all strains, conventional MRS medium supported the most rapid growth and the highest final OD values, confirming its suitability as a nutritionally rich medium for lactic acid bacteria. Similar observations have been widely reported, as MRS contains readily metabolisable carbohydrates, peptides, vitamins, and growth factors specifically formulated to support LAB proliferation (De Man et al., 1960).

Growth curve analysis showed that IN supported faster bacterial growth than *S. cerevisiae* yeast cell wall (SC), with cultures reaching the stationary phase after approximately 20–24 hr and exhibiting growth profiles similar to those in MRS broth. This finding is consistent with the well-established ability of lactobacilli to readily

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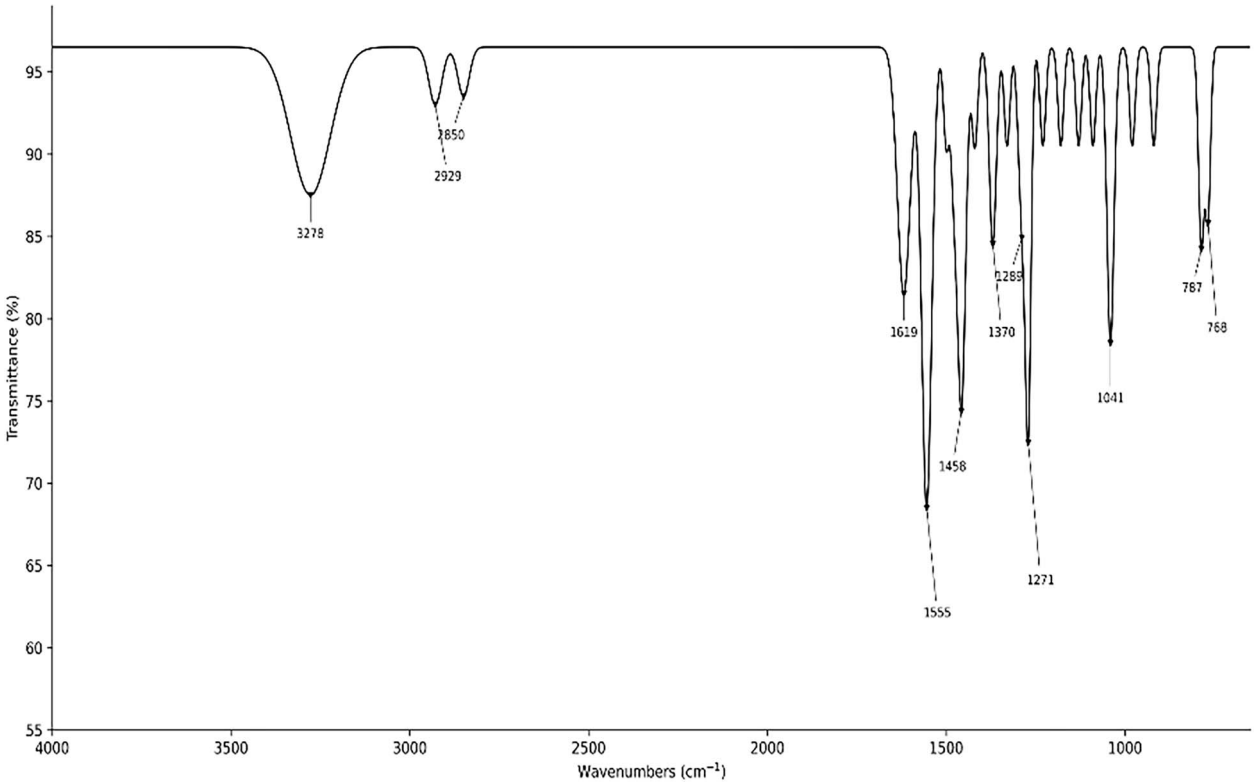

Figure 1 FTIR spectrum of the prepared *Saccharomyces cerevisiae* yeast cell wall (SC). The broad absorption band at approximately 3,278 cm^{-1} was attributed to O–H stretching of polysaccharides, while the band at 2,929 cm^{-1} corresponded to aliphatic C–H stretching. Bands observed at approximately 1,619 and 1,555 cm^{-1} were associated with protein-related amide vibrations, supporting the presence of mannoprotein components. Absorption bands in the 1,458–1,370 cm^{-1} region were related to C–H bending vibrations, whereas the prominent carbohydrate fingerprint region at approximately 1,271–1,041 cm^{-1} was consistent with C–O and C–O–C vibrations of yeast cell wall polysaccharides. Collectively, the FTIR profile supports the presence of polysaccharide- and protein-associated structural components characteristic of *Saccharomyces cerevisiae* yeast cell wall.

metabolise IN through fructan-degrading enzymes (Gibson et al., 2017; Roberfroid, 2007). In contrast, SC-containing media resulted in slower growth kinetics and lower final OD_{600} values, suggesting a more gradual utilisation of yeast-derived polysaccharides. The biomass production revealed a different trend. The highest biomass of the study was obtained for *L. paracasei* cultivated in 10% SC (5.85 g), exceeding both 10% IN (3.24 g) and MRS (3.75 g). Likewise, *L. rhamnosus* produced greater biomass in SC-supplemented media (3.68–4.00 g) than in IN-containing media (2.70–2.84 g), whereas *L. brevis* showed only a modest increase. These findings indicated that SC promotes biomass accumulation despite slower optical growth. The discrepancy between OD_{600} and biomass may be attributed to the complex composition of the yeast cell wall, which contains β-glucans, mannoproteins, and chitin. The discrepancy between OD_{600} and biomass may be related to the structurally complex nature of yeast cell wall components, particularly β-glucans and mannoproteins, which can influence microbial interactions, aggregation, and substrate utilisation. Recent studies have further highlighted the functional role of yeast cell wall biopolymers in supporting probiotic viability and metabolic performance (Baek, Rani Ramakrishnan et al., 2024; Utama et al., 2023).The response to SC was clearly strain dependent. *Lacticaseibacillus paracasei* exhibited the greatest adaptation, suggesting an efficient utilisation of yeast-derived carbohydrates, consistent with previous reports describing its ability to metabolise mannan- and β-glucan-derived oligosaccharides (Markowiak-Kopeć & Śliżewska, 2020). *Lacticaseibacillus rhamnosus* also responded positively, with biomass increasing as SC concentration increased, supporting earlier findings that yeast β-glucans and mannan oligosaccharides enhanced the growth and metabolic activity of this species (Slizewska & Chlebicz-Wójcik, 2020). In contrast, the weaker response of *L. brevis* likely indicated strain-specific differences in the utilisation of complex polysaccharides (Gibson et al., 2017). The increasing SC concentration from 5% to 10% improved biomass production in all strains, particularly in *L. paracasei*. These findings revealed that *S. cerevisiae* yeast cell wall can be served as an effective microbial-derived prebiotic capable of supporting probiotic biomass production in a strain-dependent manner while offering a sustainable strategy for the valorisation of industrial yeast by-products within circular bioeconomy and zero-waste frameworks.

Antimicrobial activity of fermentation supernatants

The antimicrobial activities of cell-free fermentation supernatants obtained from *L. paracasei*, *L. rhamnosus*, and *L. brevis* cultivated in modified MRS media supplemented with IN or *S. cerevisiae* yeast cell wall (SC) were evaluated using the agar well diffusion method against *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 25923 (Figure 4).

The inhibition zone diameters ranged from 0 to 5 mm, with differences observed among probiotic strains, prebiotic substrates, and target microorganisms. Compared with the standard MRS

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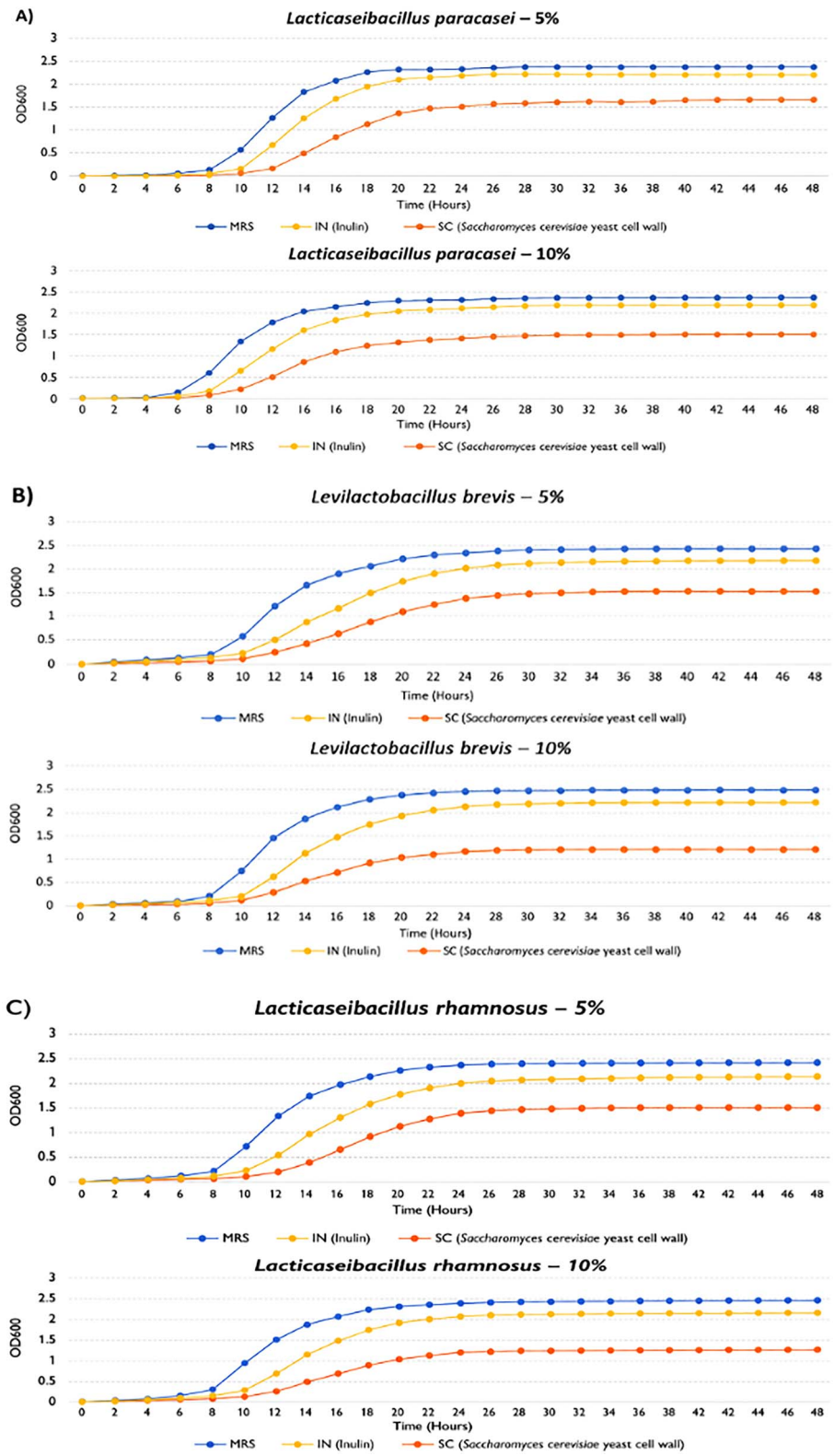

Figure 2 Growth kinetics of (A) *Lacticaseibacillus paracasei*, (B) *Levilactobacillus brevis*, and (C) *Lacticaseibacillus rhamnosus* grown in media supplemented with 5% and 10% (w/v) prebiotic substrates.

fermentation control, SC supplementation generally maintained antimicrobial activity, although the magnitude of the response varied depending on the probiotic strain, SC concentration, and target microorganism. *Lacticaseibacillus rhamnosus* exhibited the strongest antimicrobial performance. Fermentation supernatants obtained from both IN- and SC-supplemented media produced inhibition zones of up to 4 mm against *E. coli* and *S. aureus*, demonstrating effective inhibition of both Gram-negative and Gram-positive bacteria.

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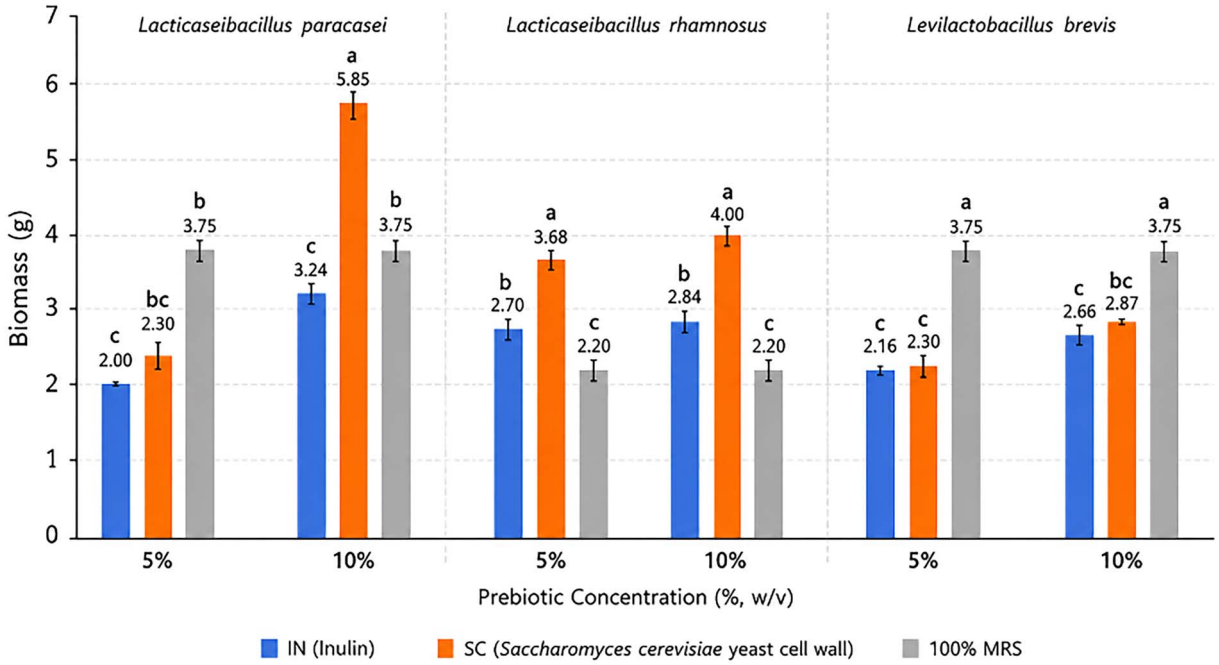

Figure 3 Biomass production of *Lacticaseibacillus paracasei*, *Lacticaseibacillus rhamnosus*, and *Levilactobacillus brevis* cultivated in MRS supplemented with IN or *Saccharomyces cerevisiae* yeast cell wall (SC) at 5% and 10% (w/v). Standard MRS (100% MRS) was used as the control. Data are presented as $M \pm SD$ ($n = 3$). Different lowercase letters indicate statistically significant differences among treatments within each probiotic strain, as determined by one-way ANOVA followed by Tukey's multiple comparison test ($p < .05$); bars sharing the same letter are not significantly different. ANOVA = analysis of variance; IN = inulin; MRS = de Man, Rogosa, and Sharpe.

Lacticaseibacillus paracasei displayed a more selective antimicrobial profile. Inhibition against *E. coli* remained constant at approximately 3 mm, whereas no activity was detected against *P. aeruginosa*, and only limited inhibition (2–3 mm) was observed against *S. aureus*. *Lacticaseibacillus brevis* showed intermediate activity, with the greatest inhibition against *E. coli* (3.5 mm) in 10% IN- and SC-supplemented media, while activity against *P. aeruginosa* was weak or absent. The influence of prebiotic supplementation was strain dependent. Increasing the substrate concentration from 5% to 10% generally enhanced antimicrobial activity in *L. rhamnosus* and *L. brevis*, particularly against *E. coli*, whereas no improvement was observed for *L. paracasei*. The higher inhibitory activity may be associated with increased production of antimicrobial metabolites, including organic acids, hydrogen peroxide, and bacteriocin-like compounds. Among the test organisms, *P. aeruginosa* was consistently the least susceptible test microorganism, which may be associated with its well-established intrinsic resistance mechanisms, including restricted outer-membrane permeability and multidrug efflux systems. Recent evidence further indicates that the antimicrobial activity of LAB-derived cell-free supernatants is highly dependent on both the producing strain and metabolite composition, with organic acids and proteinaceous compounds representing major contributors to pathogen inhibition. Recent evidence further indicates that the antimicrobial activity of LAB-derived cell-free supernatants is highly dependent on both the producing strain and metabolite composition, with organic acids and proteinaceous compounds representing major contributors to pathogen inhibition (Mani-López et al., 2022; Poole, 2011). Accordingly, the differences observed among *L. paracasei*, *L. rhamnosus*, and *L. brevis* may reflect strain-dependent variations in the antimicrobial metabolites generated during fermentation. In contrast, *E. coli* and *S. aureus* were more susceptible, especially to fermentation supernatants produced by *L. rhamnosus*. Similar antimicrobial effects have been attributed to metabolites produced by lactic acid bacteria, including lactic acid, acetic acid, hydrogen peroxide, diacetyl, reuterin, and bacteriocins (Markowiak-Kopeć & Śliżewska, 2020). Although the antimicrobial activity of SC-supplemented cultures was generally comparable to or slightly lower than that of the MRS control, SC successfully maintained probiotic antimicrobial functionality. These findings indicated that *S. cerevisiae* yeast cell wall can serve as a sustainable microbial-derived prebiotic capable of supporting the production of antimicrobial metabolites while simultaneously valorising industrial yeast by-products.

Antioxidant activity of cell-free fermentation supernatants

The antioxidant activities of cell-free fermentation supernatants obtained from *L. paracasei*, *L. rhamnosus*, and *L. brevis* cultivated in media supplemented with IN or *S. cerevisiae* yeast cell wall (SC) were evaluated using the DPPH radical scavenging assay (Figure 5).

Antioxidant activity varied markedly among the probiotic strains and culture media, indicating that antioxidant metabolite production was highly strain dependent. *Lacticaseibacillus paracasei* exhibited the highest antioxidant activity when cultivated in 10% IN (81.60%), followed by 10% SC (76.06%), suggesting efficient utilisation of both substrates for the production of antioxidant compounds. Similar improvements have been associated with the production of EPS and other bioactive metabolites during LAB fermentation. Recent evidence demonstrates that LAB-derived EPS can exhibit substantial radical-scavenging and antioxidant activities, although these properties vary considerably according to the producing strain and structural characteristics of the metabolites (Amaretti et al.,

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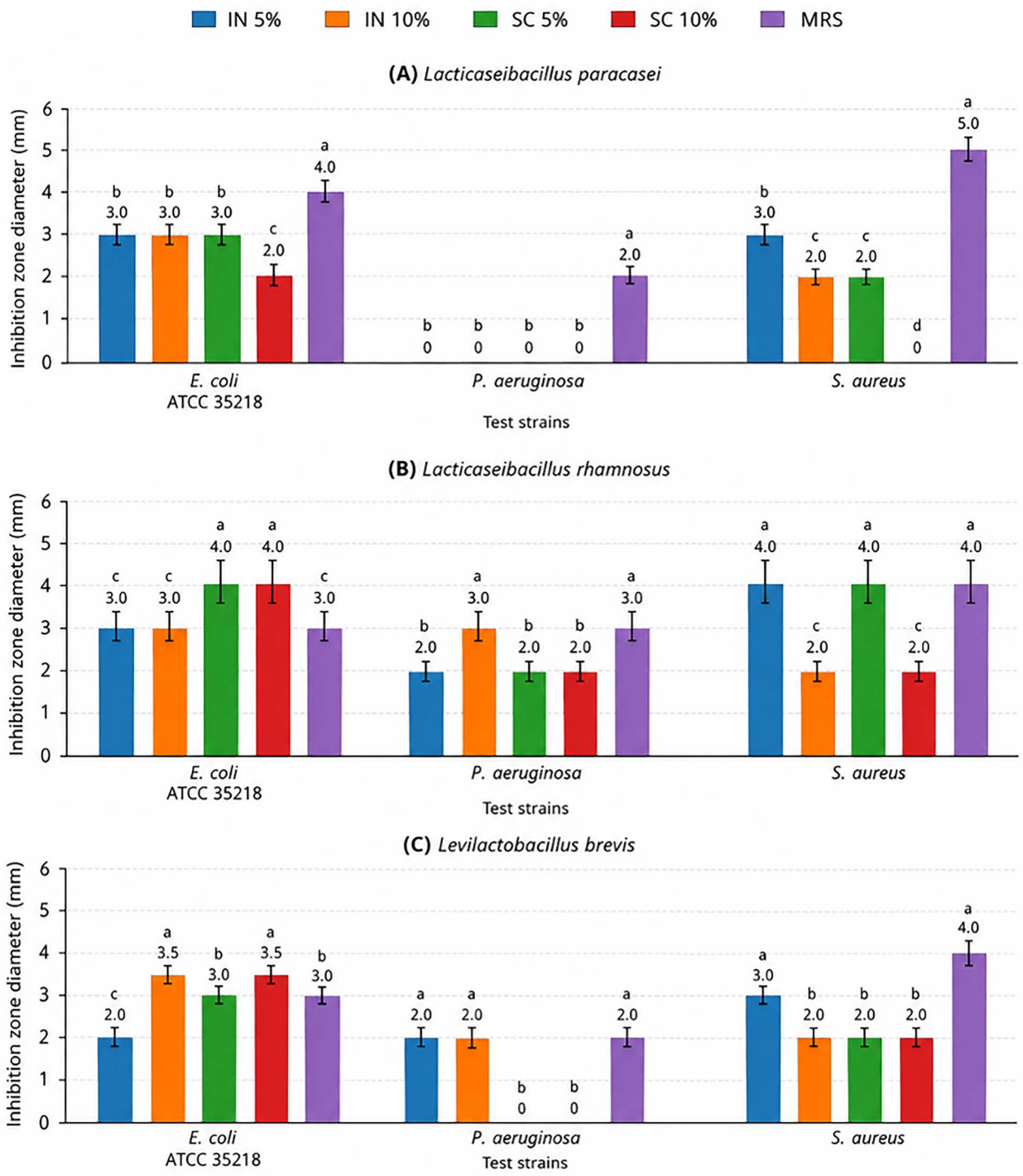

Figure 4 Antimicrobial activity of CFSs obtained from *Lacticaseibacillus paracasei* (A), *Lacticaseibacillus rhamnosus* (B), and *Levilactobacillus brevis* (C) cultivated in standard MRS broth (fermentation control), MRS supplemented with 5% or 10% (w/v) IN (established prebiotic reference), or MRS supplemented with 5% or 10% (w/v) *Saccharomyces cerevisiae* yeast cell wall (SC). Antimicrobial activity against *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923 was determined by agar well diffusion. Data are presented as $M \pm SD$ ($n = 3$). Different lowercase letters indicate statistically significant differences among treatments within each test microorganism (one-way ANOVA followed by Tukey's multiple-comparison test, $p < .05$); bars sharing the same letter are not significantly different. ANOVA = analysis of variance; CFSs = cell-free culture supernatants; IN = inulin; MRS = de Man, Rogosa, and Sharpe.

2012; Kaplan & Hutkins, 2000; Sharma et al., 2024; Zhang et al., 2024). In contrast, *L. rhamnosus* showed the highest antioxidant activity in MRS medium (78.96%), whereas supplementation with 10% IN and 10% SC reduced DPPH scavenging activity to 55.24% and 62.02%, respectively. This finding indicates that increasing prebiotic concentration does not necessarily enhance antioxidant production and supports previous reports describing strain-specific metabolic responses to different prebiotic substrates. Compared with the other

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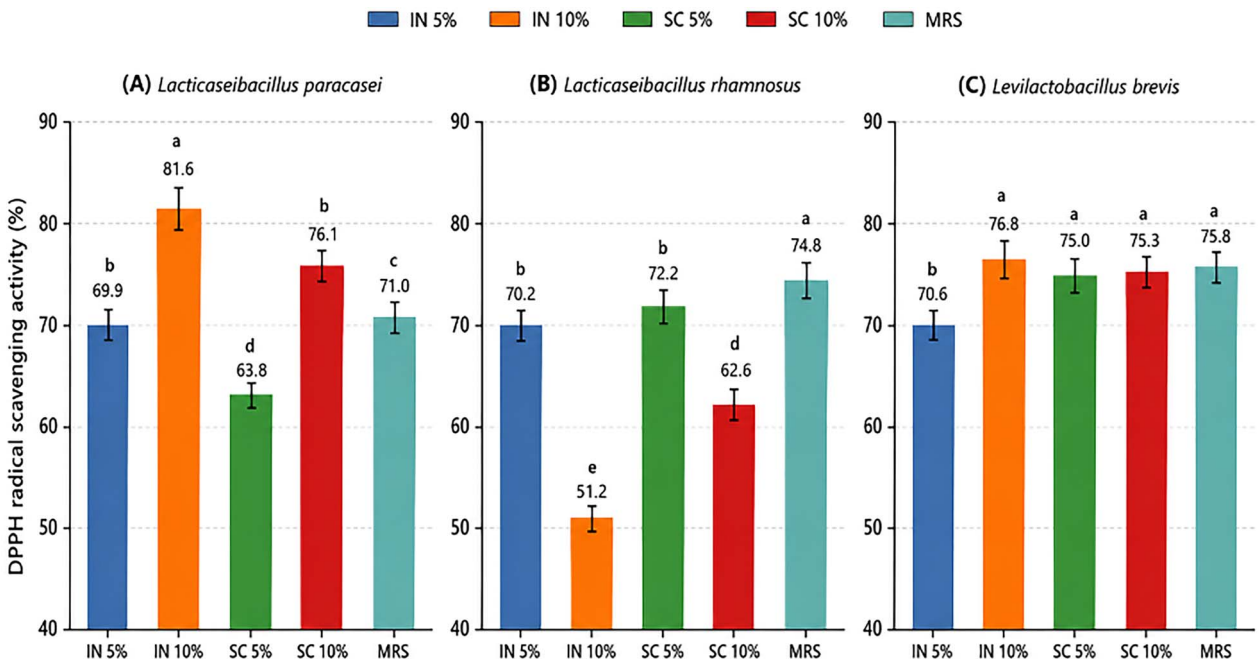

Figure 5 DPPH radical-scavenging activity of cell-free fermentation supernatants obtained from *Lacticaseibacillus paracasei* (A), *Lacticaseibacillus rhamnosus* (B), and *Levilactobacillus brevis* (C) cultivated in MRS supplemented with IN or *Saccharomyces cerevisiae* yeast cell wall (SC) at 5% and 10% (w/v), compared with standard MRS. Data are presented as $M \pm SD$ ($n = 3$). Different lowercase letters indicate statistically significant differences among treatments within each probiotic strain, as determined by one-way ANOVA followed by Tukey's multiple-comparison test ($p < .05$); bars sharing the same letter are not significantly different. ANOVA = analysis of variance; DPPH = 2,2-diphenyl-1-picrylhydrazyl; IN = inulin; MRS = de Man, Rogosa, and Sharpe.

strains, *L. brevis* maintained relatively stable antioxidant activity across all cultivation conditions (73.00%–76.97%), suggesting that its antioxidant metabolism is less influenced by substrate composition. This agrees with previous studies demonstrating the strong intrinsic antioxidant defence systems of *L. brevis*, including glutathione production and efficient reactive oxygen species scavenging (Mishra et al., 2015; Wang et al., 2017). The relatively high antioxidant activities observed in SC-supplemented media, particularly for *L. paracasei* and *L. brevis*, support the prebiotic potential of yeast-derived β-glucans and mannan oligosaccharides, which have been associated with enhanced antioxidant metabolite production during fermentation (Patel et al., 2010). The results demonstrated that *S. cerevisiae* yeast cell wall effectively maintains antioxidant functionality, although its effects are strongly strain dependent.

Enzyme activity assays

The enzymatic activities of the selected probiotic strains cultivated in MRS broth supplemented with 10% (w/v) *S. cerevisiae* yeast cell wall (SC) were evaluated by measuring lipase, phytase, and protease activities (Figure 6). The 10% SC condition was selected for enzymatic analysis because it resulted in the highest biomass production among the SC-supplemented conditions and was therefore considered the most appropriate condition for further functional characterisation. Considerable strain-dependent differences were observed, indicating that the utilisation of yeast cell wall components affected bacterial metabolism differently depending on the probiotic strain.

Distinct strain-dependent differences were observed in extracellular enzyme production following supplementation with 10% *S. cerevisiae* yeast cell wall (SC). *Lacticaseibacillus rhamnosus* exhibited the highest lipase activity (5.80 ± 0.42 U/ml), followed by *L. paracasei* (5.20 ± 0.36 U/ml) and *L. brevis* (4.90 ± 0.31 U/ml). This finding suggests that *L. rhamnosus* utilised yeast-derived nutrients more efficiently, promoting lipase synthesis. Lipases produced by lactic acid bacteria contribute to lipid hydrolysis and the formation of aroma compounds, thereby improving the nutritional and technological properties of fermented foods. Moreover, yeast-derived β-glucans and mannoproteins have been reported to stimulate extracellular enzyme production by supporting bacterial metabolism (De Vuyst & Leroy, 2011). In contrast, phytase activity was highest in *L. paracasei* (0.21 ± 0.02 U/ml), followed by *L. brevis* (0.15 ± 0.02 U/ml) and *L. rhamnosus* (0.12 ± 0.01 U/ml). Since phytases improve mineral bioavailability through phytic acid hydrolysis, the superior activity of *L. paracasei* highlights its potential for functional fermented foods (Lei et al., 2013). Protease activity remained comparatively low in all strains (0.05–0.06 U/ml), with *L. brevis* showing the highest value. Although differences were small, this result suggests a greater potential for generating bioactive peptides, which are associated with antioxidant, antimicrobial, and antihypertensive properties. Proteolytic activity of LAB contributes to the hydrolysis of food proteins and the release of peptides and amino acids, including bioactive peptides with potential antioxidant, antimicrobial, antihypertensive, and immunomodulatory properties. Recent reviews have further emphasised that proteolytic capacity varies considerably among LAB strains and can substantially influence the nutritional and functional characteristics of fermented products (Pessione & Cirrincione, 2016; Ter et al., 2024; Zou et al., 2024). Each probiotic strain exhibited a distinct enzymatic profile: *L. rhamnosus* showed superior lipase production, *L. paracasei* demonstrated the highest phytase activity, and *L. brevis* exhibited the greatest protease activity. These findings indicated that supplementation with 10% *S. cerevisiae* yeast cell wall effectively modulates probiotic metabolism in a strain-dependent manner, supporting its potential as a sustainable

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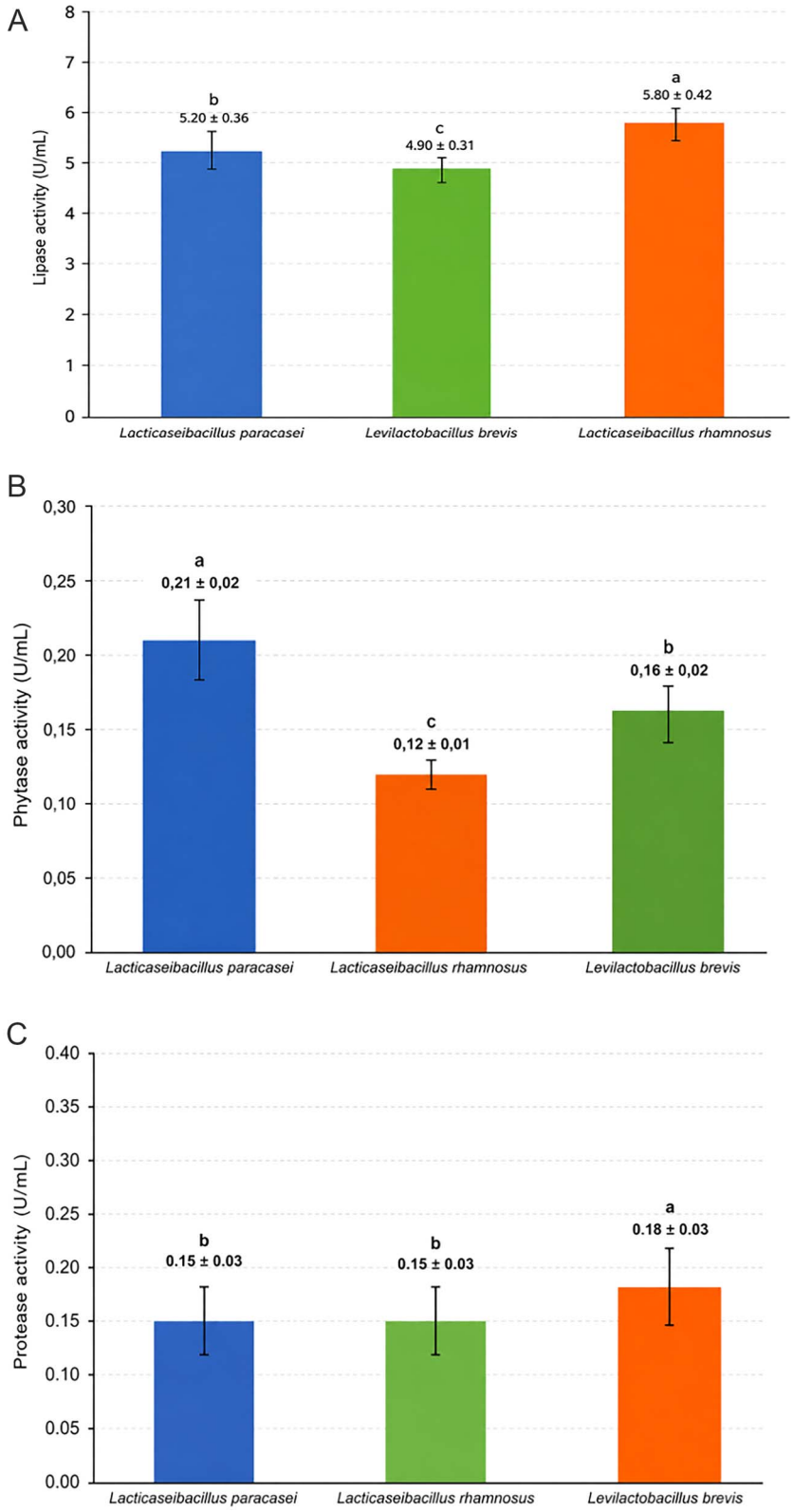

Figure 6 Activities of lipase (A), phytase (B), and protease (C) produced by *Lacticaseibacillus paracasei*, *Lacticaseibacillus rhamnosus*, and *Levilactobacillus brevis* cultivated in MRS broth supplemented with 10% (w/v) *Saccharomyces cerevisiae* yeast cell wall (SC). Data are presented as $M \pm SD$ ($n = 3$). Different lowercase letters indicate statistically significant differences among probiotic strains within each enzyme assay, as determined by one-way ANOVA followed by Tukey's multiple-comparison test ($p < .05$); bars sharing the same letter are not significantly different. ANOVA = analysis of variance.

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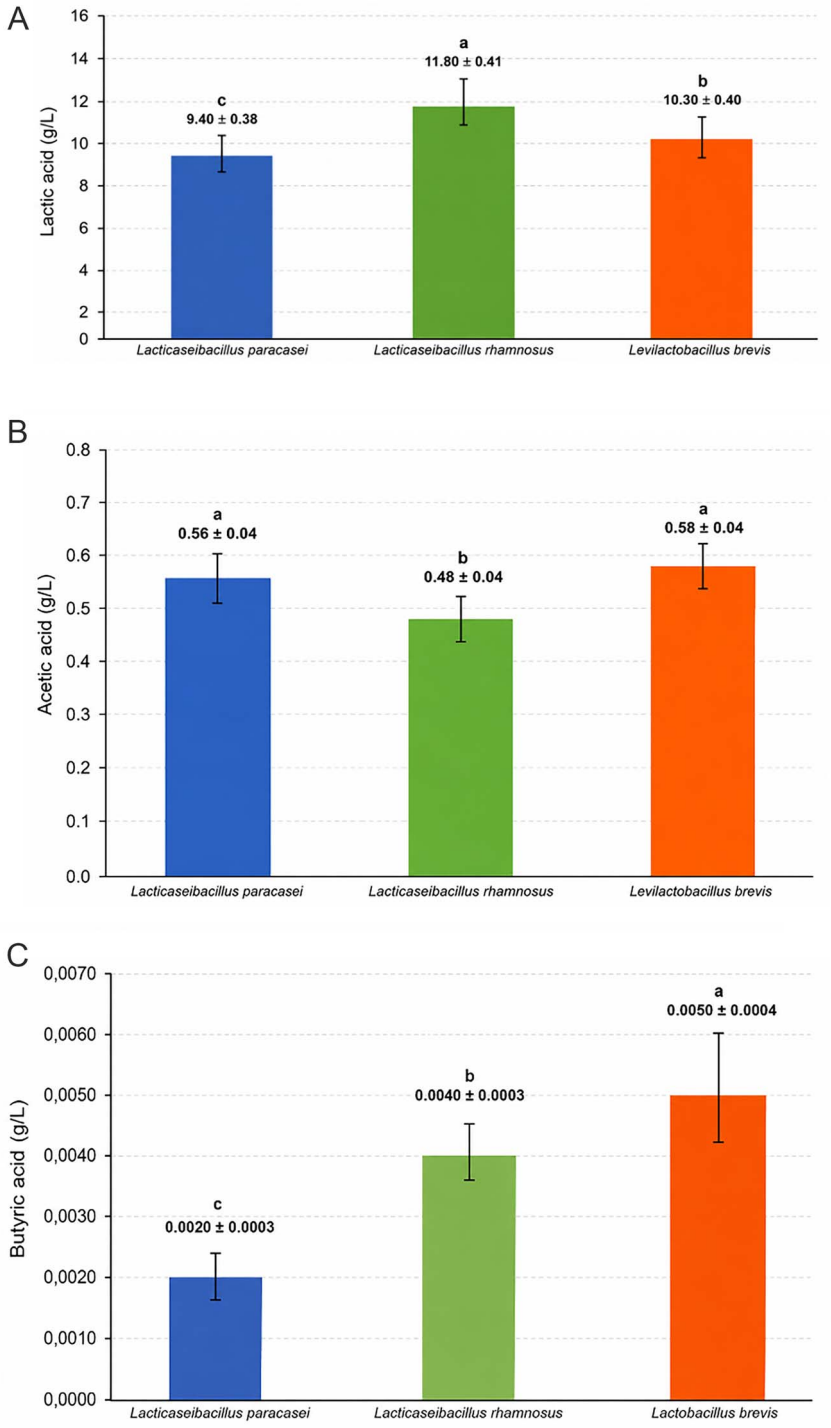

Figure 7 Organic acid production by *Lacticaseibacillus paracasei*, *Lacticaseibacillus rhamnosus*, and *Levilactobacillus brevis* cultivated in MRS broth supplemented with 10% (w/v) *Saccharomyces cerevisiae* yeast cell wall (SC): (A) lactic acid, (B) acetic acid, and (C) butyric acid. Data are presented as $M \pm SD$ ($n = 3$). Different lowercase letters indicate statistically significant differences among probiotic strains within each organic acid assay, as determined by one-way ANOVA followed by Tukey's multiple-comparison test ($p < .05$); bars sharing the same letter are not significantly different. ANOVA = analysis of variance; MRS = de Man, Rogosa, and Sharpe.

microbial-derived prebiotic for enhancing the functional properties of fermented products. More broadly, recent evidence indicates that LAB can produce a diverse range of fermentation-associated bioactive compounds and enzymes, including proteases and lipases, with their production strongly influenced by the bacterial strain and fermentation conditions (Abdul Hakim et al., 2023).

Organic acid production

The production of organic acids by the selected probiotic strains cultivated in MRS medium supplemented with 10% (w/v) *S. cerevisiae* yeast cell wall (SC) is presented in Figure 7. Lactic acid was the predominant metabolite produced by all strains, whereas acetic and

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butyric acids were detected at substantially lower concentrations. This metabolic profile is consistent with the characteristic carbohydrate fermentation pathways of lactic acid bacteria, in which pyruvate is primarily reduced to lactic acid to regenerate NAD^+ (Nicotinamide adenine dinucleotide) and sustain glycolytic activity (Gänzle, 2015).

Among the tested strains, *L. rhamnosus* produced the highest lactic acid concentration (11.80 ± 0.41 g/L), followed by *L. brevis* (10.30 ± 0.40 g/L) and *L. paracasei* (9.40 ± 0.38 g/L). The superior lactic acid production by *L. rhamnosus* suggested a more efficient utilisation of nutrients released from the *S. cerevisiae* yeast cell wall, which is rich in β-glucans, mannoproteins, and oligosaccharides known to stimulate probiotic metabolism and fermentation efficiency (Markowiak-Kopeć & Śliżewska, 2020). The enhanced lactic acid production was also consistent with the higher biomass observed for this strain. Although *L. brevis* produced less lactic acid, it generated the highest concentrations of acetic acid (0.56 ± 0.04 g/L) and butyric acid (0.0050 ± 0.0004 g/L), reflecting its heterofermentative metabolism through the phosphoketolase pathway (Gänzle, 2015). The increased acetic acid production may contribute to the antimicrobial properties of fermentation products. Recent evidence confirms that undissociated organic acids can permeate bacterial membranes and disturb intracellular proton and anion homeostasis, thereby interfering with essential cellular functions (Alakomi et al., 2000; Yoon et al., 2024). In contrast, *L. paracasei* produced moderate acetic acid and the lowest butyric acid concentrations but exhibited superior biomass formation and phytase activity, suggesting that carbon metabolism was preferentially directed toward cell growth and functional metabolite production rather than secondary organic acid synthesis. Similar strain-dependent metabolic differences have been reported previously for probiotic lactobacilli (Papadimitriou et al., 2016). The supplementation with 10% *S. cerevisiae* yeast cell wall supported active fermentation while producing distinct strain-specific organic acid profiles. *Lacticaseibacillus rhamnosus* favoured lactic acid production, *L. brevis* generated a broader spectrum of organic acids, and *L. paracasei* exhibited a balanced metabolic profile associated with enhanced biomass and functional activity. These findings demonstrated that yeast cell wall supplementation modulates probiotic metabolism according to the physiological characteristics of individual strains.

Conclusion

This study demonstrated that *S. cerevisiae* yeast cell wall (SC) can support the growth and metabolic activity of *L. paracasei*, *L. rhamnosus*, and *L. brevis*. FTIR analysis confirmed characteristic β-glucans, mannans, and mannoproteins in the prepared SC material. SC supplementation produced distinct strain-dependent functional responses: *L. paracasei* exhibited the highest biomass production and phytase activity, *L. rhamnosus* showed the strongest antimicrobial activity together with the highest lipase and lactic acid production, and *L. brevis* produced the highest concentrations of acetic and butyric acids while maintaining stable antioxidant activity. These findings indicated that SC can influence probiotic functionality beyond growth promotion by modulating antimicrobial, antioxidant, enzymatic, and organic acid profiles, emphasising the importance of strain selection when designing synbiotic fermentation systems. The use of *S. cerevisiae* yeast cell wall as a microbial-derived prebiotic provides a promising strategy for developing functional fermentation systems while valorising an abundant fermentation by-product within circular bioeconomy approaches. Future studies should investigate the molecular mechanisms underlying probiotic–yeast cell wall interactions and validate SC-based synbiotic systems in food matrices and in vivo models.

Data availability

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

Author contributions

Pervin Soyer (Conceptualization [equal], Methodology [equal], Investigation [equal], Formal analysis [equal], Data curation [equal], Visualization [equal], Writing—original draft [equal]), Melisa Ayhan (Investigation [equal], Data curation [equal], Validation [equal]), Emine İrdem (Investigation [equal], Methodology [equal], Validation [equal]), Miriş Dikmen (Investigation [equal], Formal analysis [equal]), and Zerrin Cantürk (Supervision [equal], Conceptualization [equal], Funding acquisition [equal], Project administration [equal], Writing—review & editing [equal]).

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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Ethical approval statement

Ethical approval was not required for this study because it did not involve human participants or animals.

Patient consent statement

Not applicable.

Clinical trial registration

Not applicable.

Permission to reproduce material from other sources

Not applicable.

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