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Research Article

Quality characteristics of rye sourdough fermented with a mixed culture of probiotic lactic acid bacteria and yeast exhibiting potent antioxidant properties

Eun-Seo Lim[1]*, Eun-Woo Lee[2,3]

[1]*Department of Companion Animal Industry, Tongmyong University, Busan 48520, Korea*
[2]*Department of Biomedicine and Pharmaceutics, Dong-Eui University, Busan 47340, Korea*
[3]*Research Institute for Microbiology, Dong-Eui University, Busan 47340, Korea*

Abstract This study aimed to develop probiotic-rich fermented foods by co-culturing lactic acid bacteria (LAB) and yeast strains isolated from traditional *makgeolli*, focusing on their probiotic and antioxidant activities. Three yeast strains—*Saccharomyces cerevisiae* TM15, *Pichia kudriavzevii* TM26, and *Kluyveromyces marxianus* TM39—were identified and assessed for their survival in simulated gastrointestinal conditions, bile salt hydrolase (BSH) activity, and pathogen coaggregation, confirming their potential as probiotic candidates. Safety evaluations indicated no harmful enzyme or biogenic amine production, supporting their safe use in food applications. Co-culture with the probiotic LAB *Pediococcus pentosaceus* OP91 in rye sourdough enhanced microbiological stability, antioxidant activity, and antimicrobial properties. Specifically, co-cultures with *S. cerevisiae* TM15 increased β-glucan content and DPPH scavenging activity while reducing pathogen counts, indicating the potential of these strains for health-promoting functional foods.

Keywords antioxidant activity, co-culture, pathogen inhibition, probiotic yeast, rye sourdough

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***Corresponding author**
Eun-Seo Lim
Tel: +82-51-629-1714
E-mail: limsm020@tu.ac.kr

1. Introduction

Probiotic strains, especially from the *Lactobacillus* and *Bifidobacterium* genera, are widely used to enhance nutrition, improve digestibility, and extend shelf life in fermented foods. These probiotics support digestive health, boost immune function, and enrich flavor, aligning with dietary trends focused on wellness and nutrition. Lactic acid bacteria (LAB) are particularly effective in promoting gut health by out-competing harmful pathogens and supporting beneficial microorganisms. LAB are commonly found in dairy products like yogurt and cheese, as well as fermented vegetables like kimchi and sauerkraut, providing digestive benefits and enhancing flavor (Soccol et al., 2010). Similarly, the probiotic yeast *Saccharomyces boulardii* is well-known for alleviating digestive disorders, including diarrhea, irritable bowel syndrome (IBS), and inflammatory bowel disease (IBD). Probiotic yeasts help stimulate immune responses, reduce inflammation, and resist antibiotics, allowing them to be effective alongside antibiotic treatments. Additionally, probiotic yeasts tend to have longer shelf stability, making them suitable for various food products and supplements (Kelesidis and Pothoulakis, 2012). Certain probiotics, including LAB and specific yeasts, also produce bioactive compounds like glutathione and antioxidants, neutralizing free radicals and supporting overall health. Beyond food applications, both LAB and probiotic yeasts are used in dietary supplements aimed at digestive health, immune support, and general well-being

(Adams, 2010). Probiotic yeasts contribute to fermented products like kombucha and kefir, enhancing both flavor and health benefits. This versatility positions probiotic yeasts as a promising area for future research in functional foods and probiotics, supporting digestive and immune health across the food and wellness industries (Tomicic et al., 2024).

Sourdough bread, a prominent example of fermented foods, is made through the natural fermentation of flour and water, utilizing a harmonious blend of LAB and yeasts. When sourdough is produced using LAB in monoculture, it typically develops a characteristic tangy flavor due to the production of lactic and acetic acids during fermentation. This acidic environment enhances the stability and shelf life of dough by inhibiting the growth of harmful microorganisms. Furthermore, LAB facilitate the breakdown of complex carbohydrates and gluten, resulting in improved digestibility and a softer texture in the final product. The fermentation process also increases the bioavailability of nutrients, making essential vitamins and minerals more accessible to consumers (Gobbetti et al., 2019). Meanwhile, sourdough fermented with yeasts, particularly strains like *Saccharomyces cerevisiae*, primarily focuses on the leavening process. Yeasts produce carbon dioxide during fermentation, causing the dough to rise and yield a light, airy texture. The volatile compounds generated by yeast fermentation enhance the aroma and overall flavor profile of bread (Ganzle, 2014).

When LAB and yeasts are co-cultured during sourdough fermentation, they produce a variety of bioactive compounds, including organic acids, phenolic compounds, and antioxidants such as glutathione. These components work synergistically to scavenge free radicals, reducing oxidative stress and inhibiting the formation of reactive oxygen species (ROS) in the body (Poutanen et al., 2009). Research suggests that the antioxidant properties of sourdough may lower the risk of chronic diseases linked to oxidative damage, including cardiovascular diseases and certain cancers (Gabriele et al., 2024). Additionally, LAB and yeast produce bacteriocins and phenolic compounds with antimicrobial properties, further enhancing the preservation of sourdough by inhibiting the growth of undesirable microorganisms (Perez-Alvarado et al., 2022).

For this study, we would choose rye flour over wheat flour due to its unique composition, including higher soluble fiber and bioactive compounds, which contribute to its health benefits. Rye flour also supports diverse microbial growth during fermentation, resulting in different fermentation dynamics and a denser, more flavorful loaf. Additionally, rye flour is known for its health benefits, such as improving digestive health and lowering cholesterol, and it is commonly used in traditional bread production. This makes it a valuable choice for studying fermentation effects and end-product qualities (Jonsson et al., 2018). Our research aims to develop fermented foods by co-culturing LAB and yeast strains with probiotic activity. The focus of this study was to isolate and identify yeast strains with strong probiotic and antioxidant properties from *makgeolli*. The selected yeast strains were then co-cultured with previously identified probiotic LAB to produce rye sourdough. The study investigated the microbiological and physicochemical characteristics of the rye sourdough and assessed the effects of co-culture on its antioxidant and antimicrobial activities.

2. Material and methods

2.1. Isolation and identification of yeast

Yeast was isolated from traditionally brewed makgeolli by homogenizing a 1 mL sample in phosphate buffer saline (PBS, pH 7.0) and plating it on yeast glucose chloramphenicol (YGC) agar (Difco, Detroit, MI, USA). After incubation at 25℃ for 72 h, the developed colonies were streaked onto yeast extract peptone dextrose (YEPD) agar (Difco) for pure isolation and further subcultured on the same medium. The isolated yeast was identified through molecular analysis, following the method described by Lim (2016). Briefly, the selected yeast strains were inoculated into YEPD broth and cultured. After centrifugation (7,000 ×*g*, 10 min, 4℃), cells were resuspended in TE buffer. Genomic DNA was extracted using the Promega kit. Polymerase chain reaction (PCR) was performed to amplify the D1/D2 domain of 26S rDNA with NL-1 and NL-4 primers, and the ITS1/5.8S rDNA/ITS2 domain with ITS-1 and ITS-4 primers. The reaction conditions were as follows: an initial denaturation at 94℃ for 3 min, followed by 36 cycles of denaturation at 94℃ for 2 min, annealing at 42℃ for 1 min, and elongation at 72℃ for 2 min, with a final extension step at 72℃ for 7 min. Purified PCR products (Qiagen, Hilden, Germany) were sequenced and analyzed for gene sequence homology using BLAST from NCBI. The strains were then preserved in liquid media with 10% dimethyl sulfoxide (DMSO, Merck, Kenilworth, NJ, USA) at -70℃ for future experimental use.

2.2. Evaluation of potential probiotic yeast

2.2.1. Resistance to artificial digestive juice

Yeast strains were cultured overnight in YEPD broth at 37°C and then centrifuged at 5,500 ×*g* for 10 min at 4°C. The resulting pellets (5.0×10^8 CFU/mL) were resuspended in artificial gastric juice [125 mM NaCl, 7 mM KCl, 45 mM $NaHCO_3$, 0.3% pepsin (Sigma-Aldrich, St. Louis, MO, USA)] to achieve pH of 2.5. The strains were then inoculated into YEPD broth containing artificial intestinal fluid with pancreatin (0.1 g/L), bile salts (3 g/L), KCl (0.835 g/L), NaCl (6.5 g/L), $CaCl_2$ (0.22 g/L), and $NaHCO_3$ (1.386 g/L). Cultures were incubated at 37°C for 2 h in gastric juice and 4 h in intestinal fluid. After incubation, the remaining viable cell count was measured on YEPD agar using the standard agar plate method.

2.2.2. Bile salt hydrolase (BSH) activity

BSH activity was assessed following the method outlined by Wang et al. (2024).

2.2.3. Co-aggregation

The co-aggregation assay of the isolates with pathogenic bacteria was conducted following the methods of Wang et al. (2024), with modifications. Probiotic yeast strains were cultured overnight in YEPD broth, and pathogenic bacteria (*Bacillus cereus* ATCC 11778, *Escherichia coli* O157 ATCC 43889, *Salmonella typhimurium* KCTC 3514, and *Staphylococcus aureus* ATCC 6538) were cultured in BHI broth (Difco). Both yeast and bacterial cells were harvested by centrifugation, washed with 0.9% NaCl, and resuspended to a concentration of 10^8 CFU/mL. Equal volumes (2 mL) of the isolates and bacterial suspensions were mixed, vortexed, and incubated at 25°C for 4 h. Absorbance at 600 nm was measured for the mixture (Amix), as well as for individual yeast (Apro) and bacterial (Apath) controls. Co-aggregation (%) was calculated using the formula: Co-aggregation (%) was calculated using the formula:

$$\text{Co-aggregation (\%)} = \frac{(\text{Apro} + \text{Apth}) / 2 - \text{Amix}}{(\text{Apro} + \text{Apath}) / 2} \times 100$$

2.2.4. Adhesion to Caco-2 cells

The adhesion ability of the isolated strain to Caco-2 cells was evaluated using the method described by Kil et al. (2023).

2.2.5. Cell surface hydrophobicity

The cell surface hydrophobicity of the isolated strain was assessed using the method outlined by Wang et al. (2024).

2.3. Safety assessment of isolates

2.3.1. Hemolytic, proteolytic, and gelatinase activities

The hemolytic, proteolytic, and gelatinase activities of the isolated strain were measured following the method of Wang et al. (2024).

2.3.2. Coagulase and deoxyribonuclease (DNase) activities

The coagulase and DNase activities of the isolated strain were evaluated using the method described by Fernandez-Pacheco et al. (2021).

2.3.3. Biogenic amines (BA) production

The isolated strains were inoculated into decarboxylase broth (Difco) supplemented with precursor amino acids (l-arginine monohydrochloride, l-histidine monohydrochloride monohydrate, l-lysine monohydrochloride, l-ornithine monohydrochloride, l-phenylalanine, and l-tyrosine hydrochloride, Sigma-Aldrich, 1 g/L) and pyridoxal 5-phosphate (1 mg/L). This mixture was incubated at 37°C for 24 h over five cycles to induce enzyme activity. Following this, 0.5 mL of the precultured broth was transferred to 1 mL of decarboxylase broth containing 2% (w/v) precursor amino acids in microtiter plates (Falcon, Franklin Lakes, NJ, USA) and incubated anaerobically at 37°C for 72 h. Strains exhibiting a purple coloration were identified as positive.

2.4. Antioxidant activity of isolates

The DPPH, hydroxyl, and superoxide radical scavenging activities of intact cells (IC) and intracellular cell-free extracts (ICFE) were measured according to the method of Abduxukur et al. (2023), using ascorbic acid and BHA (100 μg/mL) as positive controls. Antioxidant activity was evaluated by categorizing the isolated strain into IC and ICFE. To prepare IC, the isolated strain was inoculated in YEPD broth and cultured at 28°C for 24 h. The culture was subsequently centrifuged at 10,000 ×*g* for 10 min at 4°C to collect the pellet, which was then washed three times with PBS (pH 7.0) and resuspended in the same buffer. For ICFE preparation, IC

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cells were washed twice with deionized water and disrupted in an ice bath using sonication (Q700, Qsonica, Newtown, CT, USA) for 10 min. The disrupted sample was then centrifuged (10,000 ×*g*, 10 min) to remove cell debris, and the supernatant was collected and filtered through a 0.45 μm membrane filter (Millipore, Billerica, MA, USA).

2.5. Preparation of rye sourdough

Pediococcus pentosaceus OP91, identified for its probiotic and antioxidant properties (Lim, 2023), was utilized as the LAB strain in rye sourdough fermentation, demonstrating its potential as a functional starter culture. LAB were cultured in MRS broth (Difco) at 37℃ for 24 h, while the isolated yeast from this study was cultured in YEPD broth at 30℃ for 24 h. After optimal cultivation, cells were harvested by centrifugation at 7,000 ×*g* for 10 min at 4℃ and washed twice with PBS (pH 7.0) for further analysis. Rye sourdough was prepared following the method by Kim and Chun (2009), with minor modifications. A mixture of 600 g rye flour and 600 g water was inoculated with 10 g each of LAB (1.0×10^3 CFU/g) and yeast (1.0×10^3 CFU/g), both suspended in PBS (pH 7.0), and blended using a mixer (CFM-E201XB, CUCKOO, Yangsan, Korea) for approximately 5 min. The dough was allowed to ferment at 30℃ for 20 h in a stationary fermenter (GWF-1020, Grand Woosung, Kimpo, Korea) (Day 1). Subsequently, 900 g of the 1,200 g fermented rye sourdough was retained, and 900 g each of rye flour and water were added. The dough was fermented again at 30℃ for 20 h (Day 2). For the final step, 2,400 g of the 2,700 g second-fermented sourdough was taken, mixed with an additional 1,200 g each of rye flour and water, and left for a final 20 h fermentation at 30℃ (Day 3).

2.6. Microbiological and physicochemical characteristics

The measurements of LAB count, yeast count, pH, total titratable acidity (TTA), ethanol, and exopolysaccharide (EPS) content in the fermented sourdough were conducted according to the method described by Lim et al. (2017).

2.7. Preparation of water/salt-soluble extract of rye sourdough

The antioxidant activity of sourdough was measured using a modified method based on Coda et al. (2012). First, 10 g of sourdough was mixed with 30 mL of 50 mM Tris-HCl buffer (pH 8.8) and stirred at 4℃ for 1 h. The mixture was then centrifuged at 20,000 ×*g* for 20 min. The resulting supernatant, referred to as the water/salt-soluble extract (WSE), was freeze-dried. The concentration of peptides in the sample was determined using the o-phthaldialdehyde (OPA) method as described by Church et al. (1983).

2.8. Antioxidant capacity of rye sourdough

The antioxidant properties of rye sourdough were evaluated using several methods: total polyphenol content (TPC), DPPH radical scavenging ability, lipid peroxidation inhibitory activity, and β-glucan content. TPC was measured according to the method outlined by Ivanisova et al. (2023), while the β-glucan content was determined based on the protocol established by McCleary and Codd (1991). The DPPH radical scavenging activity and lipid peroxidation inhibitory activity were assessed following the methodology described by Lim et al. (2017).

2.9. Antimicrobial activity against foodborne pathogens

B. cereus ATCC 11778 and *S. aureus* ATCC 6538 were inoculated into BHI broth and incubated at 37℃ for 24 h. The resulting cultures were then centrifuged at 7,000 ×*g* for 10 min at 4℃ to harvest the cells, which were subsequently washed twice with PBS (pH 7.0) and adjusted to a concentration of 1.0×10^3 CFU/mL. A 10 g suspension of these foodborne pathogens was artificially inoculated into the sourdough ingredients and starter mixture, followed by fermentation at 30℃ for 60 h. The fermented sourdough was stored at 25℃ for 5 days, after which the remaining number of foodborne pathogens was measured. For analysis, a 10 g sample of sourdough was homogenized with 90 mL of PBS (pH 7.0), and the homogenate was serially diluted. *B. cereus* was enumerated on Mannitol Egg Yolk Polymyxin (MYP) agar (Difco), while *S. aureus* was quantified on *Staphylococcus* 110 medium (Difco) after incubation at 37℃ for 48 h.

2.10. Statistical analysis

All experiments were conducted in triplicate, with results expressed as the mean±standard deviation (SD). Statistical analyses were performed using SPSS Statistics (version 19.0, SPSS Inc., Chicago, IL, USA), applying one-way ANOVA followed by Duncan's multiple range test to assess differences between group means. Statistical significance was set at $p<0.05$.

3. Results and Discussion

3.1. Isolation and identification of the yeast

Traditionally brewed *makgeolli* hosts a diverse microbial community of yeasts and bacteria, shaping its unique fermentation profile. Sequence analysis identified the yeast strains as *S. cerevisiae* TM15 (100%), *Pichia kudriavzevii* TM26 (99.4%), and *Kluyveromyces marxianus* TM39 (99.8%), confirmed through NCBI BLAST homology searches (Table 1).

The isolated yeast strains—*S. cerevisiae* TM15, *P. kudriavzevii* TM26, and *K. marxianus* TM39—were identified through sequence analysis, aligning with findings from previous research. Similarly, studies by Kwon et al. (2012) and Jung et al. (2017) recognized these yeasts as dominant species, highlighting their vital roles in the fermentation process and quality improvement of *makgeolli*. Their exceptional adaptability to diverse environmental conditions ensures a stable and dependable fermentation process.

3.2. Probiotic activity of the yeast

As summarized in Table 2, the probiotic activities of the yeast strains *S. cerevisiae* TM15, *P. kudriavzevii* TM26, and *K. marxianus* TM39 from *makgeolli* were evaluated. *P. kudriavzevii* TM26 showed exceptional gastric acid resistance (93.05±3.01%) and significant BSH activity, supporting its role in lipid digestion and cholesterol metabolism. *K. marxianus* TM39 exhibited strong adhesion to Caco-2 cells (22.42±3.84%), high surface hydrophobicity (69.39±0.59%), and notable coaggregation with foodborne pathogens, highlighting its potential for gastrointestinal colonization and gut health promotion. *S. cerevisiae* TM15 demonstrated superior adhesion, hydrophobicity, and stronger BSH activity compared to the other strains, confirming its robust probiotic properties.

Previous studies have established the strain-specific nature of probiotic activities. *S. cerevisiae* C41 from Tibicos, for example, demonstrated resilience in acidic environments (pH 2.0), strong bile salt resistance, effective cellular aggregation, adherence to gastrointestinal cells, and notable antioxidant activity (Romero-Luna et al., 2019). Similarly, *P. kudriavzevii* Y33 exhibited robust acid tolerance, antimicrobial activity, high resilience to bile concentrations, cholesterol assimilation, and excellent autoaggregation (Lata et al., 2022). Strains of *K. marxianus* (JYC2614, JYC2610, and KU140723-02) showed tolerance to bile salts and acidic environments, strong autoaggregation, cell surface hydrophobicity, and enzymatic activities that promote gut health (Cho et al., 2018; Hsiung et al., 2021). Fernandez-Pacheco et al. (2021) observed that four *Pichia* strains (1,003, 1,200, 1,082, and 1,090) were BSH-positive, while *Hanseniaspora* strains (1,056 and 1,094)

Table 1. Identification of yeast with antioxidant and probiotic activity from *makgeolli* through molecular analysis

Strain	26S rRNA sequencing	Accession No.	Similarity (%)	Identification	Length (bp)
	Related strain in NCBI				
S. cerevisiae TM15	*Saccharomyces cerevisiae* CHY1011	EU649672	100	*Saccharomyces cerevisiae* TM15	1,458
P. kudriavzevii TM26	*Pichia kudriavzevii* Atz-EN-01	KC886644	99.4	*Pichia kudriavzevii* TM26	1,621
K. marxianus TM39	*Kluyveromyces marxianus* UniMAP 1-1	KX538800	99.8	*Kluyveromyces marxianus* TM39	1,170

Table 2. Probiotic properties of yeast isolated from *makgeolli*

Strain	Survival (%)		BSH activity (mm)	Adhesion (%)	Hydrophobicity (%)	Co-aggregation (%)			
	Gastric juice	Intestinal juice				*Bacillus cereus*	*Escherichia coli O157*	*Salmonella typhimurium*	*Staphylococcus aureus*
S. cerevisiae TM15	88.40±2.43[1)b]	96.73±2.89[b]	7.50±0.50[b]	12.83±0.41[b]	60.42±1.30[b]	55.07±2.85[b]	40.81±2.91[b]	78.62±5.59[a]	53.69±3.06[b]
P. kudriavzevii TM26	93.05±3.01[a]	100.52±1.05[a]	15.33±0.58[a]	5.99±1.50[c]	53.77±5.77[c]	49.38±1.55[c]	29.43±4.20[c]	33.87±2.73[b]	50.06±3.19[b]
K. marxianus TM39	86.34±0.64[b]	98.13±1.78[ab]	5.67±0.29[c]	22.42±3.84[a]	69.39±0.59[a]	61.26±4.03[a]	66.24±1.10[a]	75.13±7.80[a]	80.40±6.01[a]

[1)]All values are means±SD (n=3). Different superscript letters ([a-c]) in a column indicate significant differences at $p<0.05$ by Duncan's multiple range test.

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exhibited no activity. Certain *S. cerevisiae* strains also displayed BSH activity, indicating its strain-specific nature and potential for cholesterol reduction.

Probiotics offer several key benefits for gut health. Their resistance to digestive acids and bile salts enables them to survive harsh stomach conditions and reach the intestines, where they support colonization and help maintain a balanced microbiome. Probiotics inhibit pathogen growth, enhancing overall gut health (Ganzle and Evers, 2012). BSH activity aids in deconjugating bile salts, influencing cholesterol metabolism, and promoting a healthier microbiome. Their ability to adhere to intestinal cells facilitates effective colonization, prevents pathogen attachment, and stimulates immune responses (Agolino et al., 2024). Hydrophobicity enhances adhesion, contributing to biofilm formation and strengthening the gut barrier. Co-aggregation with pathogens helps prevent their attachment and supports microbiome balance while modulating immune responses (Collado et al., 2007). In conclusion, the selection of resilient yeast strains with strong survival and functional characteristics is crucial for developing effective probiotic supplements and functional foods. This emphasizes the critical need for strain-specific evaluation of probiotic activities, highlighting the potential health benefits and therapeutic applications of probiotics in enhancing gut health and preventing gastrointestinal disorders.

3.3. Safety profile of the yeast

The safety profiles of the three strains were assessed through key indicators, including hemolytic activity, enzyme production, and BA production, as summarized in Table 3. *S. cerevisiae* TM15 showed α-hemolytic activity, while *P. kudriavzevii* TM26 and *K. marxianus* TM39 exhibited γ-hemolytic activity, indicating the TM39 strain may be particularly safe for consumption. All strains produced proteolytic enzymes but showed no activity for plasminogen activase, gelatinase, or DNase. Furthermore, none of the strains produced the tested BA, confirming a low risk of amine-related toxicity. These results validate the safety of the strains and their suitability for functional food applications promoting gut health.

Evaluating the safety of probiotic yeasts is essential to ensure they pose no risks when incorporated into food products (Alkalbani et al., 2022). Safety assessments encompass tests for antibiotic and antifungal resistance, BA production, bile salt deconjugation activity, and various enzymatic activities that may indicate pathogenicity (Fernandez-Pacheco et al., 2021). These evaluations ensure that probiotic yeasts retain their beneficial properties while minimizing potential hazards. The findings of this study align with previous research. Fernandez-Pacheco et al. (2021) demonstrated that probiotic yeasts, including *Candida vini*, *Hanseniaspora osmophila*, *Lachancea thermotolerans*, *Pichia anomala*, *P. kudriavzevii*, *S. boulardii*, *S. cerevisiae*, and *Zygosaccharomyces bailii*, produced minimal BA (<1 mg/L) and exhibited no pathogenic traits, such as DNase activity, hemolysin activity, or plasma coagulation, confirming their suitability for food applications. Romero-Luna et al. (2019) found that *S. cerevisiae* C41 from Tibicos displayed no hemolytic activity and was sensitive to nystatin, an antifungal agent, further supporting its safety as a probiotic. Lata et al. (2022) reported that *P. kudriavzevii* Y33 demonstrated antibiotic resistance, produced EPS, and showed no hemolytic activity, highlighting its probiotic potential. Youn et al. (2023) assessed *K. marxianus* strains A4 and A5 from Korean kefir and found no gelatinase activity or hemolysis *in vitro*. Comprehensive safety evaluations are crucial to confirm that probiotic strains can be safely used in the food industry. These assessments ensure the absence of pathogenic traits, safeguard consumer health, and enhance product integrity. Identifying potential risks early facilitates regulatory compliance

Table 3. Safety assessment of yeast isolated from *makgeolli*

Strain	Hemolytic activity	Proteolytic activity	Coagulase activity	Gelatinase activity	DNase activity	Biogenic amine production			
						Cadaverine	Histamine	Putrescine	Tyramine
S. cerevisiae TM15	α	+	-	-	-	-	-	-	-
P. kudriavzevii TM26	γ	+	-	-	-	-	-	-	-
K. marxianus TM39	γ	+	-	-	-	-	-	-	-

+, positive result; -, negative result.

and fosters consumer confidence, paving the way for the reliable application of probiotics in food products.

3.4. Antioxidant activity of the yeast

As shown in Table 4, the DPPH, hydroxyl, and superoxide anion radical scavenging activities of IC and ICFE from the isolated strains were evaluated. *K. marxianus* TM39 exhibited the highest DPPH scavenging activity (51.95±3.86% in IC, 33.96±1.97% in ICFE), though lower than the positive controls BHA (91.63±5.24%) and ascorbic acid (96.87±2.99%). It also showed strong hydroxyl radical scavenging (93.03±3.85% in IC), closely followed by *S. cerevisiae* TM15 ICFE (90.62±4.21%), both significantly outperforming BHA (24.22±2.75%) and ascorbic acid (67.03±4.05%) ($p<0.05$). For superoxide scavenging, K. marxianus TM39 IC (40.23±3.55%) and *S. cerevisiae* TM15 ICFE (55.13±3.04%) displayed promising activity, albeit lower than BHA (91.52±1.68%) and ascorbic acid (98.70±0.72%).

The differences between IC and ICFE in terms of antioxidant activity can be attributed to the distinct mechanisms by which yeast cells exert their antioxidant effects. The antioxidant activity of IC is primarily associated with cell surface components, such as polysaccharides, proteins, and cell wall-bound antioxidants. Yeast cell walls contain β-glucans and mannoproteins, which have been reported to scavenge free radicals and exhibit antioxidant properties. Additionally, intact yeast cells may contribute to antioxidant activity through metal ion chelation, reducing oxidative stress by limiting the availability of pro-oxidant metals (Machova and Bystricky, 2013). Whereas, the antioxidant activity observed in ICFE is largely attributed to intracellular compounds, such as glutathione, superoxide dismutase (SOD), catalase, and other enzymatic and non-enzymatic antioxidants. These intracellular components actively neutralize ROS and contribute to cellular defense against oxidative stress (Liu et al., 2021).

The antioxidant activity of the yeast strains, as demonstrated by their radical scavenging abilities, suggests multiple underlying mechanisms. *K. marxianus* TM39 exhibited the highest DPPH and hydroxyl radical scavenging activities, indicating its strong ability to donate electrons or hydrogen atoms, which is characteristic of non-enzymatic antioxidants such as polyphenols, glutathione, or carotenoids. The superior hydroxyl radical scavenging activity, especially in *S. cerevisiae* TM15 ICFE, suggests the presence of intracellular antioxidants like glutathione and phenolic compounds, which neutralize highly reactive hydroxyl radicals. Additionally, the significant superoxide anion scavenging activities observed in *K. marxianus* TM39 IC and *S. cerevisiae* TM15 ICFE point to the role of enzymatic antioxidants such as SOD, which catalyzes the conversion of superoxide anions into less harmful molecules. The differences between IC and ICFE activities imply that both cell wall components (e.g., β-glucans and mannoproteins) and intracellular metabolites contribute to antioxidant defense of the yeast. These findings highlight the potential of these yeast strains as functional fermentation starters with robust antioxidant properties.

Previous studies have highlighted the significant antioxidant activities of various yeast strains. Wang et al. (2024) reported that strains such as *Debaryomyces prosopidis*, *Zygosaccharomyces lentus*, *Dekkera bruxellensis*, *Schizosaccharomyces pombe*, *Hanseniaspora valbyensis*, *Brettanomyces anomalus*, *P. kudriavzevii*, and *S. cerevisiae* exhibited remarkable antioxidant properties, with DPPH free radical reductions exceeding 80%. Notably, *P. kudriavzevii* GBY1 and *S. cerevisiae* GBY2 demonstrated DPPH scavenging rates above 90%. Chen et al. (2010) evaluated the antioxidant potential of 12 yeast strains, finding that both IC and ICFE from these strains exhibited strong antioxidant activity. The enhanced activity in ICFE, compared to IC, was attributed to the presence of complex

Table 4. Antioxidant properties of yeast isolated from *makgeolli*

Strain	DPPH radical scavenging (%)		Hydroxyl radical scavenging (%)		O^2- radical scavenging (%)	
	IC	ICFE	IC	ICFE	IC	ICFE
S. cerevisiae TM15	44.47±4.21[1)b]	24.11±4.32[b]	84.52±5.01[b]	90.62±4.21[a]	35.76±4.43[b]	55.13±3.04[a]
P. kudriavzevii TM26	36.09±0.66[c]	13.02±5.00[c]	72.46±5.23[c]	88.13±4.70[b]	19.63±1.97[c]	42.19±0.92[b]
K. marxianus TM39	51.95±3.86[a]	33.96±1.97[a]	93.03±3.85[a]	80.34±5.15[c]	40.23±3.55[a]	30.84±1.43[c]

[1)]All values are means±SD (n=3). Different superscript letters ([a-c]) in a column indicate significant differences at $p<0.05$ by Duncan's multiple range test. IC, intact cells; ICFE, intracellular cell-free extracts.

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polymers such as β-glucans, α-mannans, mannoproteins, chitin, and cell wall proteins. Additional factors contributing to antioxidant effects included ascorbate, erythroascorbate, and enzymes like superoxide dismutase, catalase, and glutathione peroxidase. Romero-Luna et al. (2019) reported that *S. cerevisiae* C41 from Tibicos exhibited strong antioxidant activity, particularly in neutralizing DPPH radicals, enhancing its probiotic potential. Cho et al. (2018) found that *K. marxianus* KM2 exhibited the highest antioxidant activity, which was further enhanced when combined with polyphenol-rich grape seed flour or extract, suggesting a synergistic effect and its potential as a functional food ingredient. Abduxukur et al. (2023) demonstrated that *P. fermentans* QY-4 exhibited significant antioxidant enzyme activities, making it a promising candidate for the food and fermentation industries.

The antioxidant capabilities of yeast are highly valuable in both food and health applications, helping to reduce oxidative damage and potentially prevent chronic diseases related to oxidative stress, such as cardiovascular diseases and cancer. Their ability to preserve the quality and shelf-life of food products by preventing lipid peroxidation further enhances their value in the food industry. Additionally, their antioxidant activity supports their role as probiotics, modulating oxidative stress in the gastrointestinal tract and contributing to gut health (Alkalbani et al., 2022).

In conclusion, the yeast strains examined in this study exhibited strong antioxidant potential, reinforcing their role in promoting health and preventing disease. The selection of robust yeast strains with potent antioxidant properties is essential for the development of functional foods and health supplements. Notably, the yeast strains isolated from *makgeolli* demonstrated probiotic activity comparable to the well-established *S. boulardii*, while also ensuring safety and exhibiting remarkable antioxidant properties. These characteristics highlight their significant potential as fermentation starters for sourdough production, contributing to both its nutritional and functional enhancement.

3.5. Microbiological and physicochemical properties of rye sourdough

The microbiological and physicochemical characteristics of rye sourdough fermented with *P. pentosaceus* OP91 and selected yeast strains are summarized in Table 5. The LAB count in naturally fermented sourdough ($2.5\pm0.8\times10^7$ CFU/g) was similar to yeast-only samples, while LAB fermentation alone resulted in a significantly higher LAB count compared to the control. Co-culture with LAB and yeast further increased LAB counts, and yeast numbers were significantly higher in yeast-only and co-cultured samples than in the control and LAB-only samples ($p<0.05$). The LAB-yeast co-culture also led to significantly lower pH, higher TTA, and increased alcohol and EPS content compared to single-

Table 5. Microbiological and physicochemical properties of rye sourdough fermented with selected probiotic yeast

Starters		Viable cell counts (CFU/g)		pH	TTA (%)	Alcohol content (g/kg)	EPS content (g/kg)
LAB	Yeast	LAB	Yeast				
Control (spontaneous fermentation)[1)]		$2.5\pm0.8\times10^{72)e}$	$2.7\pm0.1\times10^{6f}$	5.40 ± 0.11^{a}	5.87 ± 0.06^{f}	0.87 ± 0.10^{d}	6.71 ± 1.12^{g}
P. pentosaceus OP91		$9.0\pm1.5\times10^{8c}$	$6.5\pm1.7\times10^{6e}$	4.03 ± 0.04^{d}	11.46 ± 0.10^{b}	0.99 ± 0.13^{d}	17.42 ± 0.87^{c}
	S. cerevisiae TM15	$5.4\pm0.3\times10^{7d}$	$7.1\pm2.2\times10^{8c}$	4.95 ± 0.16^{b}	7.98 ± 0.14^{e}	1.39 ± 0.08^{c}	8.57 ± 0.58^{f}
	P. kudriavzevii TM26	$6.8\pm2.4\times10^{7d}$	$5.9\pm3.0\times10^{8c}$	4.70 ± 0.13^{c}	8.63 ± 0.33^{c}	1.30 ± 0.05^{c}	14.52 ± 4.05^{d}
	K. marxianus TM39	$3.2\pm1.1\times10^{7de}$	$4.5\pm0.3\times10^{8d}$	5.01 ± 0.02^{b}	8.37 ± 0.09^{d}	1.33 ± 0.09^{c}	10.19 ± 0.18^{e}
P. pentosaceus OP91	*S. cerevisiae* TM15	$8.5\pm0.9\times10^{9ab}$	$7.0\pm1.4\times10^{9a}$	3.98 ± 0.05^{de}	13.82 ± 0.40^{ab}	1.86 ± 0.41^{a}	27.53 ± 1.72^{b}
P. pentosaceus OP91	*P. kudriavzevii* TM26	$1.9\pm0.2\times10^{10a}$	$3.8\pm0.2\times10^{9b}$	3.62 ± 0.05^{f}	14.96 ± 0.04^{a}	1.80 ± 0.28^{ab}	34.66 ± 5.08^{a}
P. pentosaceus OP91	*K. marxianus* TM39	$6.2\pm2.0\times10^{9b}$	$4.9\pm0.5\times10^{9ab}$	3.85 ± 0.01^{e}	12.14 ± 0.37^{b}	1.71 ± 0.14^{b}	30.10 ± 3.34^{ab}

[1)]The control sourdough is prepared through spontaneous fermentation by allowing wild yeasts and LAB to naturally inoculate the dough without the addition of specific starter cultures.
[2)]All values are means±SD (n=3). Different superscript letters ($^{a-g}$) in a column indicate significant differences at $p<0.05$ by Duncan's multiple range test.
LAB, lactic acid bacteria; TTA, total titratable acidity; EPS, exopolysaccharide.

strain fermentations ($p<0.05$). Notably, sourdough co-cultured with *P. kudriavzevii* TM26 and *P. pentosaceus* OP91 showed the highest LAB count and TTA, outperforming those with *S. cerevisiae* TM15 and *K. marxianus* TM39. These results demonstrate the synergistic benefits of probiotic *P. pentosaceus* OP91 and yeast strains in enhancing rye sourdough quality.

Previous studies provide strong support for these findings. Banu et al. (2010) reported that sourdoughs fermented with a starter culture exhibited lower pH and higher TTA, indicative of intense fermentation driven by lactic and acetic acids produced by LAB. Our results align with those of Lim et al. (2017), who observed higher counts of LAB and yeast in co-cultured sourdoughs compared to controls, with LAB strains contributing to lower pH and increased acidity. Fang et al. (2023) also noted that co-culture of LAB and yeast accelerated growth and fermentation activity, leading to reduced pH and increased TTA. Furthermore, Tieking et al. (2003) found that LAB strains producing EPS could replace traditional bread additives, enhancing dough properties such as texture and moisture retention. Ketabi et al. (2008) suggested that EPS addition reduces resistance to extension, benefiting dough processing. Probiotic LAB, such as those studied here, produce EPS like levan, improving sourdough and bread texture, moisture, and volume, further supporting the potential of LAB to enhance dough properties.

Sourdough fermentation benefits from the symbiotic relationship between LAB and yeast. LAB lower the pH, promoting preservation by inhibiting pathogen growth and improving gluten digestibility. They also generate organic acids and volatile compounds that enhance flavor (Ganzle, 2014). Yeast contributes to leavening, produces alcohol that enhances aroma, and stimulates EPS production, improving texture and potentially extending shelf life. Co-culturing stabilizes LAB and yeast populations, resulting in sourdough with lower pH, higher TTA, and superior texture compared to LAB-only fermentation. This integrated process yields sourdough with enriched flavor, aroma, texture, and health benefits (Fang et al., 2023; Gobbetti et al., 2019).

A study by Lim (2023) demonstrated that the survival rate of LAB, along with probiotic and antioxidant activities, was significantly higher in sweet pumpkin yogurt made with *P. pentosaceus* OP91 compared to the control. Similarly, this study confirmed the superior microbiological and physicochemical characteristics of rye sourdough co-cultured with highly antioxidant yeasts and LAB. Therefore, the functionality of fermented foods can be enhanced by selecting strains with probiotic activity from diverse environments and using them as fermentation starters.

3.6. Antioxidant activity of rye sourdough

As shown in Table 6, the antioxidant activity of rye sourdough was evaluated. The control sample showed the lowest TPC, β-glucan, and DPPH radical scavenging activity,

Table 6. Antioxidant activity of rye sourdough fermented with selected probiotic yeast

Starter		TPC (mg GAE/g)	β-Glucan content (%)	DPPH radical scavenging activity (%)	Lipid peroxidation inhibitory activity (OD_{500} nm)
LAB	Yeast				
Control (spontaneous fermentation)[1)]		0.21±0.06[2)f]	0.19±0.01[e]	21.78±4.05[f]	0.96±0.03[a]
P. pentosaceus OP91		0.50±0.02[c]	0.32±0.02[c]	35.89±5.14[d]	0.74±0.02[d]
	S. cerevisiae TM15	0.47±0.05[cd]	0.29±0.04[cd]	32.46±1.28[de]	0.80±0.02[c]
	P. kudriavzevii TM26	0.41±0.01[e]	0.26±0.01[d]	29.65±3.66[e]	0.85±0.04[b]
	K. marxianus TM39	0.46±0.03[d]	0.25±0.02[d]	26.40±6.71[e]	0.82±0.04[bc]
P. pentosaceus OP91	*S. cerevisiae* TM15	0.68±0.07[a]	0.50±0.06[a]	55.13±2.38[a]	0.36±0.05[g]
P. pentosaceus OP91	*P. kudriavzevii* TM26	0.60±0.01[b]	0.43±0.03[b]	48.63±0.59[b]	0.43±0.03[f]
P. pentosaceus OP91	*K. marxianus* TM39	0.69±0.03[a]	0.41±0.04[b]	44.92±2.99[c]	0.49±0.04[e]

[1)]The control sourdough is prepared through spontaneous fermentation by allowing wild yeasts and LAB to naturally inoculate the dough without the addition of specific starter cultures.
[2)]All values are means±SD (n=3). Different superscript letters ([a-g]) in a column indicate significant differences at $p<0.05$ by Duncan's multiple range test.
TPC, total polyphenol content.

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but the highest lipid peroxidation absorbance. Sourdough fermented with LAB alone had higher TPC than yeast-only samples ($p<0.05$), while LAB-yeast co-cultured samples exhibited the highest TPC, particularly with *S. cerevisiae* TM15 or *K. marxianus* TM39 ($p<0.05$). The combination of *P. pentosaceus* OP91 and *S. cerevisiae* TM15 yielded the highest β-glucan content, DPPH activity, and lowest lipid peroxidation, highlighting its strong antioxidant potential.

Banu et al. (2010) found that organic acids produced by LAB, such as lactic and acetic acids, play a pivotal role in lowering pH, preserving antioxidant compounds, and preventing oxidation. Similarly, our results demonstrate that co-culture with LAB and yeast enhances antioxidant properties, likely due to the synergistic production of bioactive compounds and EPS. Coda et al. (2012) highlighted that LAB and yeast enzymes help release bound phenolic compounds, thus increasing their bioavailability and contributing to antioxidant capacity. Our study supports these findings, showing that co-cultured samples exhibited higher TPC compared to single-strain fermentations. Gabriele et al. (2024) further emphasized that LAB and yeast co-culture fosters a metabolic environment that mitigates oxidation, thereby enhancing the overall antioxidant capacity of sourdough. Lim et al. (2016) demonstrated that LAB-fermented sourdough had significantly higher antioxidant capacity than controls, consistent with our findings. They reported DPPH radical scavenging abilities ranging from 25% to 40% for specific LAB strains, aligning with our observation that co-culture with *P. pentosaceus* OP91 and *S. cerevisiae* TM15 enhanced antioxidant properties. Banu et al. (2010) and Michalska-Ciechanowska et al. (2007) highlighted the impact of flour type and fermentation methods on antioxidant properties, noting that controlled fermentation with specific starter cultures significantly increased TPC and DPPH radical scavenging activity. Our study similarly observed higher TPC and enhanced antioxidant activity in rye sourdough co-cultured with LAB and yeast, reinforcing the importance of starter cultures in optimizing antioxidant properties.

Ivanisova et al. (2023) found that adding oat flour to wheat bread significantly increased β-glucan content, a finding mirrored in our study, where rye sourdough co-cultured with *P. pentosaceus* OP91 and *S. cerevisiae* TM15 exhibited enhanced β-glucan content. This suggests that incorporating functional ingredients like oats can further boost the health benefits of sourdough. Coda et al. (2012) observed higher antioxidant activity and peptide content in sourdoughs made with various grains, supporting our finding that fermentation enhances antioxidant properties. The formation of antioxidant peptides during fermentation plays a key role in the enhanced antioxidant capacity of sourdough. In conclusion, our results reinforce and expand upon previous research, demonstrating that sourdough fermentation with LAB and yeast boosts antioxidant activity through the production of phenolic compounds, β-glucan, and antioxidant peptides. These findings elevate the functional properties of sourdough, positioning it as a promising health-promoting ingredient with significant potential in food and health applications.

3.7. Antimicrobial activity against foodborne pathogens

The pathogen counts in rye sourdough inoculated with foodborne pathogens, measured immediately after fermentation and after 5 days of storage at 25℃, are presented in Table 7. In the control sample, both *B. cereus* and *S. aureus* counts increased significantly during storage. Sourdough fermented with *P. pentosaceus* OP91 alone showed significantly lower *B. cereus* counts than the control. Yeast-only samples had higher *B. cereus* counts, with the highest observed in *K. marxianus* TM39. In contrast, co-cultured sourdough with both LAB and yeast had significantly lower *B. cereus* counts than the control and LAB- or yeast-only cultures ($p<0.05$), a reduction that persisted during storage. For *S. aureus*, counts were lower than *B. cereus* in both the control and *P. pentosaceus* OP91 alone, with *S. cerevisiae* TM15 exhibiting the strongest inhibitory effect compared to *P. kudriavzevii* TM26 and *K. marxianus* TM39.

Our findings align with those of Lim et al. (2017), who demonstrated that fermentation with LAB strains, including *Lactobacillus dextranicum* SRK03, *Lactobacillus acidophilus* SRK30, *Lactobacillus plantarum* SRK38, and *Lactobacillus delbrueckii* SRK60, in combination with *S. cerevisiae* KCTC 7246, led to a significant reduction in *B. cereus* and *S. aureus* populations in sourdough compared to controls. This antimicrobial effect was attributed to the production of organic acids and bacteriocins, particularly by *L. acidophilus* SRK30. Their study also noted a sustained reduction in bacterial counts after storage, further confirming the antimicrobial benefits of LAB fermentation. Similarly, Katina et al. (2002) reported that LAB strains such as *L. plantarum*,

Table 7. Antibacterial activity against foodborne pathogens of rye sourdough fermented by selected probiotic yeast

Starter		*Bacillus cereus* (CFU/g)		*Staphylococcus aureus* (CFU/g)	
LAB	Yeast	Immediately after fermentation	Storage for 5 days at 25℃	Immediately after fermentation	Storage for 5 days at 25℃
Control (spontaneous fermentation)[1)]		$4.0±0.7×10^{72)a}$	$6.7±3.3×10^{9a}$	$6.3±1.2×10^{6a}$	$7.6±1.1×10^{7a}$
P. pentosaceus OP91		$5.4±1.8×10^{5d}$	$4.8±0.9×10^{6e}$	$8.0±0.9×10^{3d}$	$9.9±0.9×10^{3f}$
	S. cerevisiae TM15	$9.4±0.5×10^{5c}$	$9.8±2.6×10^{6d}$	$4.2±2.0×10^{5b}$	$2.4±0.4×10^{6c}$
	P. kudriavzevii TM26	$2.2±1.1×10^{6b}$	$3.5±1.1×10^{7c}$	$2.5±0.1×10^{6a}$	$6.0±3.3×10^{6c}$
	K. marxianus TM39	$1.1±2.4×10^{7a}$	$2.9±0.8×10^{8b}$	$5.3±3.5×10^{6a}$	$1.8±2.2×10^{7b}$
P. pentosaceus OP91	*S. cerevisiae* TM15	$6.5±3.6×10^{4f}$	$9.0±1.5×10^{4g}$	$9.9±4.3×10^{3d}$	$5.1±1.8×10^{4e}$
P. pentosaceus OP91	*P. kudriavzevii* TM26	$2.8±0.1×10^{5e}$	$8.7±4.0×10^{5f}$	$4.1±1.7×10^{4c}$	$8.8±3.0×10^{4e}$
P. pentosaceus OP91	*K. marxianus* TM39	$5.9±2.2×10^{5d}$	$1.6±0.7×10^{6ef}$	$8.2±3.9×10^{4c}$	$3.6±0.5×10^{5d}$

[1)]The control sourdough is prepared through spontaneous fermentation by allowing wild yeasts and LAB to naturally inoculate the dough without the addition of specific starter cultures.
[2)]All values are means±SD (n=3). Different superscript letters ($^{a-g}$) in a column indicate significant differences at $p<0.05$ by Duncan's multiple range test.
LAB, lactic acid bacteria.

P. pentosaceus, and *L. brevis* inhibited the growth and spore germination of rope-forming *Bacillus* species, thereby enhancing bread shelf life. Mentes et al. (2007) found that sourdough fermented with *L. plantarum* and *Lactobacillus alimentarius* at a lower pH (3.5-4.0) significantly reduced *Bacillus*-induced rope formation, underscoring the role of organic acids in suppressing *Bacillus* growth. Choi et al. (2012) observed that the antimicrobial activity of LAB strains like *Leuconostoc citreum* HO12 and *Weissella koreensis* HO20 was primarily due to organic acids, which were effective against spoilage molds and *Bacillus subtilis*, and remained stable under heat or protease treatments. *Lactobacillus reuteri* LTH3584 produced reutericyclin, an antimicrobial compound that effectively inhibited spore germination of *S. aureus* and *B. subtilis*, with *Bacillus* species showing susceptibility to bacteriocins such as BLIS C57, reutericyclin, and plantaricin ST31 (Messens and De Vuyst, 2002). These findings support the potential of incorporating bacteriocin- or acetic acid-producing strains in sourdough fermentation as a viable method to prevent spoilage.

The reduction of harmful bacteria in sourdough fermented with LAB and yeast can be attributed to several antimicrobial mechanisms. LAB produce organic acids (lactic and acetic acids) that lower the pH of the dough, creating an inhospitable environment for pathogenic bacteria such as *Bacillus* and *Staphylococcus* species (Lim et al., 2017). As the pH drops below 4.8, these bacteria are unable to proliferate due to membrane destabilization, leading to cell damage and death. Additionally, LAB produce bacteriocins, antimicrobial peptides that disrupt bacterial cell membranes and interfere with protein synthesis, further enhancing the antimicrobial properties of sourdough (Messens and De Vuyst, 2002). *S. cerevisiae* contributes to preservation by producing ethanol, which has mild antimicrobial effects, and carbon dioxide for leavening. Both LAB and yeast also compete with pathogenic bacteria for nutrients, limiting their growth.

The enhanced antimicrobial activity observed in co-cultured LAB and yeast can be attributed to synergistic mechanisms, such as increased organic acid production, enhanced exopolysaccharide synthesis, the production of antimicrobial compounds, competitive exclusion of pathogens, and modulation of oxidative stress (Nenciarini et al., 2023). These combined factors—pH reduction, organic acid and bacteriocin production, ethanol presence, and nutrient competition—create a protective environment in sourdough, inhibiting spoilage and pathogenic microorganisms while extending shelf life of the product (Perez-Alvarado et al., 2022). In conclusion, our study confirms that co-culturing LAB and yeast in rye sourdough fermentation significantly reduces pathogen counts, consistent with previous research. This reduction is primarily driven by the production of organic acids, bacteriocins, and competitive inhibition, providing a promising strategy to

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enhance the safety and shelf life of sourdough products.

The *makgeolli*-derived yeast strains possess several advantages over conventional probiotic yeasts. They exhibit strong probiotic potential, including high gastrointestinal survival, adhesion to intestinal cells, and competitive exclusion of pathogens. Their potent antioxidant activity surpasses that of many well-known yeast strains, offering enhanced cellular protection and health benefits. Additionally, these strains display notable antimicrobial properties, effectively inhibiting harmful foodborne pathogens. Their ability to co-culture with LAB further enhances the functional and physicochemical characteristics of fermented products such as rye sourdough. These unique attributes underscore the value of *makgeolli*-derived yeast strains in the development of functional foods with both probiotic and bioactive properties (Ferreira et al., 2022).

In conclusion, this study demonstrates the potential of co-culturing probiotic LAB and yeast strains with antioxidant properties to enhance the nutritional quality and safety of rye sourdough. By isolating and characterizing yeasts from *makgeolli* with probiotic, safety, and antioxidant properties, and co-culturing them with probiotic LAB, we observed a significant increase in both LAB and yeast populations. This mixed fermentation not only improved the physicochemical properties of the sourdough but also enhanced its antioxidant activity and inhibited harmful foodborne pathogens, such as *Bacillus* and *Staphylococcus* species. The findings underscore the effectiveness of combining LAB and yeast strains to create functional foods that offer health benefits, including improved gut health and food safety.

This study emphasizes the synergistic effects of LAB and yeast co-culture, further enhancing the potential applications of probiotics. The incorporation of rye flour further sets this research apart, demonstrating its potential to enhance health benefits through its high fiber content and bioactive compounds. By integrating traditional fermentation practices with modern probiotic science, this study provides valuable insights into how co-cultured LAB and yeast strains can improve the functional properties of fermented foods. These findings present a promising strategy for developing probiotic-enriched products that promote health and help prevent foodborne illnesses.

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The authors declare no potential conflicts of interest.

Author contributions

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Ethics approval

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ORCID

Eun-Seo Lim (First & Corresponding author)
https://orcid.org/0000-0001-9645-7408
Eun-Woo Lee
https://orcid.org/0000-0003-4091-6606

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