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Selective control of spoilage lactic acid bacteria during fermentation by a heat stable bacteriocin from *Pediococcus acidilactici* HW01

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Abstract

Why was the work done: Fermentation requires strict microbial control to prevent spoilage while maintaining alcoholic fermentation and sensory quality. This study evaluated a heat stable bacteriocin from *Pediococcus acidilactici* HW01 as a process compatible biopreservative for selective control of beer spoilage lactic acid bacteria (LAB) during fermentation.
How was the work done: The bacteriocin producing strain *Pediococcus acidilactici* HW01 was cultivated in unhopped wort, and antimicrobial activity against the beer spoilage bacterium *Pediococcus damnosus* assessed. Bacteriocin activity was evaluated after wort boiling and during fermentation. Selective inhibition of *P. damnosus* was examined under low and high contamination levels. Yeast growth and fermentation parameters - free amino nitrogen, reducing sugar, and specific gravity - were monitored. Sensory evaluation was performed using a sensory panel to assess overall impression and key sensory attributes.
What are the main findings: *Pediococcus acidilactici* HW01 grew in unhopped wort and produced antimicrobial activity against *P. damnosus*, persisting after wort boiling and remaining active for up to 18 days. The HW01 derived bacteriocin selectively inhibited *P. damnosus*: viable counts were significantly reduced at high contamination levels, whereas inhibition below the detection limit was achieved at low levels. Yeast growth and fermentation parameters were not adversely affected. Bacteriocin treated samples showed significantly higher overall sensory impression scores than the control, without undesirable changes in key sensory attributes.
Why is the work important: These results demonstrate that the HW01 derived bacteriocin selectively suppresses beer spoilage LAB while maintaining fermentation performance and sensory quality, highlighting bacteriocin based strategies as effective, natural, and process compatible alternatives for microbial control in industrial applications.

Keywords

bacteriocin; fermentation; beer spoilage; lactic acid bacteria; biopreservation.

Introduction

The presence of bacteria in alcoholic fermentation is undesirable in conventional beer production. However, under specific conditions, microbial interactions may arise, such as in the production of sour beer styles or with contamination, where lactic acid bacteria (LAB) can influence product stability and sensory characteristics (Bokulich and Bamforth 2013; Páez-Lerma et al. 2024; Liu et al. 2025). Although specific LAB are used in beer styles such as Berliner Weisse and Lambic to promote acidity and flavour complexity, their uncontrolled presence in conventional brewing contexts is undesirable (Chervina et al. 2019; Vermote et al. 2023). In beer, the persistence of spoilage associated LAB is linked to turbidity formation, off flavour development, and economic losses (Xu et al. 2020; Shimokawa et al. 2021). *Pediococcus damnosus* is regarded as one of the more problematic beer spoilage bacteria, owing to its tolerance to ethanol, hop derived antimicrobial compounds, and low pH, as well as its capacity to produce exopolysaccharides that contribute to turbidity and deterioration of sensory quality (Sakamoto and Konings 2003; Michel et al. 2020; Kwun et al. 2025).

The microbiological stability of beer is achieved through good hygienic practices, composition (ethanol, pH, isomerised hop compounds), filtration, and pasteurisation or sterile filtration (Michel et al. 2020; Kordialik-Bogacka 2022). Although these strategies are effective in controlling microbial contamination, thermal treatments such as pasteurisation, can be associated with undesirable effects on sensory attributes, including losses of volatile aroma compounds and reduced flavour complexity. Increasing consumer demand for minimally processed and additive free products has further driven interest in alternative strategies for microbial control in beer production (Kordialik-Bogacka 2022).

The use of chemical preservatives is restricted by limited efficacy, regulatory constraints and concerns over consumer acceptance. However, there is increasing attention to biologically derived antimicrobial compounds produced by lactic acid bacteria (LAB), particularly bacteriocins (Mokoena et al. 2021; Lahiri et al. 2022; Zeng et al. 2022).

Bacteriocins are antimicrobial peptides produced by bacteria as a form of host defence exhibiting targeted inhibitory activity against closely related microorganisms (Sugrue et al. 2024). Owing to their proteinaceous nature, specificity, and established safety in food applications, bacteriocins have been widely used as natural biopreservative agents in foods and beverages (Todorov et al. 2019; Negash and Tsehai 2020; Lahiri et al. 2022). As cell free antimicrobial compounds, bacteriocins enable selective microbial control without introducing additional metabolic activity into the system. This is advantageous in beer, where suppression of spoilage microorganisms is ideally achieved without compromising fermentation performance or sensory quality.

Pediococcus acidilactici HW01 is a bacteriocin producing lactic acid bacterium isolated from malt and its antimicrobial activity against beer spoilage LAB has been characterised (Ahn et al. 2017). Subsequent studies demonstrated its applicability as a live starter for malt and wort bioacidification, highlighting benefits during upstream brewing operations (Choi et al. 2020; Kim et al. 2021). Despite this body of work on the strain and process input levels, the fate and functional persistence of the *Pediococcus acidilactici* HW01 bacteriocin under brewing conditions remains unresolved, particularly whether the bacteriocin can withstand wort boiling and subsequently function during fermentation. Addressing this is critical, as boiling represents a process barrier that determines whether bacteriocin based interventions can be implemented without introducing additional microbial activity into fermentation.

Accordingly, the work reported here considers the potential of the cell free bacteriocin produced by *P. acidilactici* HW01 as a biopreservative agent for controlling beer spoilage lactic acid bacteria during fermentation, with particular emphasis on thermal stability and functional efficacy against *P. damnosus*. It is suggested that bacteriocin mediated suppression of spoilage LAB could be achieved without compromising fermentation performance or sensory quality. To test this, microbial stability, fermentation kinetics, and sensory attributes were compared between

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bacteriocin treated and control fermentations, thereby assessing the practical feasibility of using HW01 derived bacteriocin in beer production.

Methods and materials

Microorganisms and microbiological analysis

The bacteriocin producing lactic acid bacterium *Pediococcus acidilactici* HW01, originally isolated from malt (Ahn et al. 2017) and maintained in our laboratory, was used in this study. The strain was cultivated in MRS broth at 30°C for 24 h under static conditions. The beer spoilage lactic acid bacterium *Pediococcus damnosus* KCTC 3770 (Korean Collection for Type Cultures, Jeongeup-si, Republic of Korea) was used as the target microorganism in antimicrobial and fermentation challenge experiments. Both LAB were routinely cultivated in de Man–Rogosa–Sharpe (MRS) broth (BD Difco, Sparks, MD, USA) at 30°C under static conditions unless otherwise specified.

Preparation of bacteriocin containing cell free supernatant

Bacteriocin production was induced by cultivation of *P. acidilactici* HW01 at 30°C under static conditions. After incubation, cells were removed by centrifugation (19,461 × g, 10 min, 4°C), and the supernatant sterile filtered through a 0.22 µm membrane filter to obtain a bacteriocin containing cell free supernatant (CFS). To exclude the contribution of organic acids to antimicrobial activity, the pH of the CFS was adjusted to 6.5 using 1 N NaOH. Bacteriocin activity in the CFS was determined by an agar well diffusion assay using *P. damnosus* KCTC 3770 as the indicator strain. Antimicrobial activity was expressed as arbitrary units per millilitre (AU/mL), defined as the reciprocal of the highest twofold dilution of the CFS that produced a clearly discernible inhibition zone. Unless otherwise stated, AU values represent representative measurements obtained from three independent experiments.

Wort preparation and boiling

Unhopped wort was prepared using a stepwise mashing process. Milled Pilsner type malt (Weyermann®, Bamberg, Germany; 200 g) was mashed with 800 mL of distilled water at a grist-to-liquor ratio of 1:4 (w/v). The mash was held at 52°C for 20 min, followed by 62°C for 60 min, 72°C for 15 was filtered to separate the sweet wort from the spent grains. The collected wort was boiled at 100°C for 60 min and then cooled to fermentation temperature (14°C). Multiple wort batches prepared under identical conditions were pooled prior to fermentation. The specific gravity of the pooled wort was approximately 1.035 (extract *ca.* 9°P). For evaluation of bacteriocin thermal stability, bacteriocin containing cell free supernatant (CFS) was added to a portion of the wort prior to boiling at the designated concentration. After boiling and cooling, wort samples were analysed to confirm the absence of viable LAB and to assess residual antimicrobial activity.

Fermentation conditions and experimental design

After the wort was boiled and cooled to fermentation temperature, the pH was adjusted to 4.8 using 1 N hydrochloric acid (HCl) under aseptic conditions to standardise the initial fermentation environment across all treatments. For the pH adjusted condition, the pH of the wort was lowered further to 4.2 using 1 N HCl, corresponding to the minimum pH observed during fermentation, to evaluate the inhibitory effect of acidification independently of bacteriocin activity.

For yeast propagation, *Saccharomyces cerevisiae* WLP800 (White Labs Inc., San Diego, CA, USA) was cultivated in wort (extract *ca.* 9°P) and incubated at 20°C for 24 h under static conditions. Yeast was quantified using a haemocytometer with methylene blue staining, and the pitching rate adjusted to achieve a rate of 1×10^7 viable cells/mL. Viable cell counts determined by plate counts on YPD agar were used for monitoring of yeast populations during fermentation.

LAB and yeast populations were quantified during fermentation, so as to evaluate the selective antimicrobial effect of the HW01 derived bacteriocin and its impact on alcoholic fermentation. Viable counts of beer spoilage LAB were determined by plate counts and expressed as log CFU/mL, which together with viable yeast cell counts, were monitored throughout primary and secondary fermentation and expressed as log cells/mL. All viable cell counts were $\log_{10}$ transformed, and mean values with standard deviations (SD) were calculated. Log reductions were determined

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where applicable.

Fermentation was performed using boiled, unhopped wort in 2 L glass fermentation bottles containing 1 L of wort, with approximately 50% headspace to accommodate CO_2 evolution during static fermentation and to allow periodic sampling without disturbing the fermentation. The bottles were fitted with airlocks and maintained under static conditions throughout fermentation. No additional aeration, oxygenation, or nitrogenation was applied after yeast pitching.

The experiments were (i) control, (ii) pH adjusted, and (iii) bacteriocin treated groups. In bacteriocin treated fermentations, cell free supernatent (CFS) was added to the cooled wort immediately prior to yeast pitching, resulting in an initial bacteriocin activity of approximately 100 AU/mL; the addition of CFS slightly increased the specific gravity of the bacteriocin treated wort to approximately 1.039. Control fermentations received an equivalent volume of sterile distilled water adjusted to match the pH and volume of the bacteriocin containing CFS. *S. cerevisiae* WLP800 was pitched at a rate of 1×10^7 viable cells/mL.

To evaluate bacteriocin efficacy under different contamination levels, *P. damnosus* was inoculated into the wort at either a low initial level of approximately 1 log CFU/mL, corresponding to about 10 CFU/mL, or a high initial level of approximately 5.5 log CFU/mL, corresponding to about 3×10^5 CFU/mL, prior to fermentation. Primary fermentation was performed at 14°C for 10 days, followed by secondary fermentation (maturation) at 4°C for 21 days. All fermentations were conducted in triplicate. Samples were collected at defined intervals during primary and secondary fermentation for microbiological analysis, determination of bacteriocin activity, and assessment of fermentation related parameters.

Determination of bacteriocin activity during fermentation

Bacteriocin activity during wort boiling and subsequent fermentation was monitored to evaluate the functional persistence of the HW01 derived bacteriocin under fermentation conditions. Samples were collected from wort immediately after boiling and at defined time points during primary and secondary fermentation. Samples were centrifuged at 19,461 × g for 10 min at 4°C to remove microbial cells, and the supernatant sterile filtered through a 0.22 µm membrane filter. To exclude antimicrobial effects attributable to organic acids, the pH of each supernatant was adjusted to 6.5 using 1 N NaOH prior to analysis.

Bacteriocin activity was evaluated using an agar well diffusion assay with *P. damnosus* as the indicator strain. An overnight culture of *P. damnosus* was adjusted to approximately 10^6 CFU/mL and uniformly spread onto MRS agar plates. Wells (6 mm in diameter) were prepared in the agar, and 100 µL of serially two-fold diluted bacteriocin containing cell free supernatant was added to each well. Plates were incubated at 30°C for 24 h, and antimicrobial activity was determined based on the clear zone of inhibition. Bacteriocin activity was expressed as arbitrary units per millilitre (AU/mL), defined as the reciprocal of the highest dilution showing a detectable inhibition zone. All assays were performed in triplicate.

Fermentation parameters

Free amino nitrogen (FAN), reducing sugar, and specific gravity were monitored during primary and secondary fermentation. FAN was determined according to the European Brewery Convention method (EBC Analysis Committee 1998) based on the ninhydrin reaction, with absorbance measured at 570 nm, and expressed as mg/L. Reducing sugar was measured using the dinitrosalicylic acid (DNS) method (Miller 1959) with glucose as a standard and expressed as g/L. At each sampling point, reducing sugar was determined independently using freshly prepared DNS reagent, with absorbance measured at 540 nm after reaction. Specific gravity was measured using a hydrometer prior to yeast pitching and at the end of each fermentation stage. All parameters were measured in triplicate and reported as mean ± standard deviation.

Sensory analysis

Sensory evaluation was conducted to compare the sensory attributes of beers produced with and without the HW01 bacteriocin. A sensory panel consisting of 20 assessors (10 male and 10 female) participated in the evaluation, with all panellists

undergoing a single training session of approximately 30 minutes prior to evaluation in which they were familiarised with the sensory descriptors and corresponding reference materials (Table 1). Sensory attributes were selected based on standard beer sensory descriptors and included odour, taste, and mouthfeel. Odour attributes comprised fruity and alcoholic notes, taste attributes included bitter, sweet, and sour perceptions, and mouthfeel attributes included astringency, carbonation, and mouth coating. The intensity of each attribute was rated using a 9 point linear scale (1 = not present, 9 = very strong). Beer samples were stored at 4°C prior to evaluation and served at 20°C in coded glasses under controlled conditions. Assessors evaluated samples individually with palate cleansing with water between samples. In addition to individual attribute intensities, assessors were asked to rate their overall impression of each sample on the same 9 point scale, defined as the integrated perception of all sensory attributes including odour, taste, and mouthfeel. This was based solely on perceived intensity rather than personal preference or hedonic liking. Sensory data were collected for all attributes and subjected to statistical analysis.

Statistical analysis

All experiments were conducted using three independent fermentations, with results presented as mean ± standard deviation unless otherwise stated. Statistical significance was accepted at $p < 0.05$. Differences in viable cell counts of *P. damnosus* and yeast between control and bacteriocin treated samples across fermentation time points were evaluated using two-way ANOVA, with treatment and fermentation time as independent variables. Differences in fermentation parameters, including free amino nitrogen (FAN), reducing sugar (RS), and specific gravity (SG), between control and bacteriocin treated beers were evaluated using two tailed Student's t-tests at each sampling point. Sensory evaluation data were analysed using paired two tailed Student's t-tests applied to comparisons between matched samples. For multiple comparisons, p values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) correction to control for type I error. Statistical analyses were performed using GraphPad Prism software (GraphPad Software, CA, USA).

Results and discussion

Growth characteristics of LAB and bacteriocin production in wort

The growth and antimicrobial activity of *P. acidilactici* HW01 were evaluated in wort to assess the feasibility of bacteriocin production under brewery relevant conditions. Viable cell counts (Figure 1a) increased rapidly during the early growth phase, reaching nearly 10 log CFU/mL at approximately 14 hours, and subsequently stabilised at around 8.5–8.7 log CFU/mL by 48 h, accompanied by a marked decrease in pH, indicating active growth and metabolic adaptation of HW01 in wort.

Antimicrobial activity (Figure 1b) against the beer spoilage bacterium *P. damnosus* was evaluated using cell free supernatants adjusted to neutral pH. Inhibitory activity was first detected during exponential growth and reached a maximum level of 160 AU/mL at 16 hours. This profile is consistent with reports that bacteriocin synthesis by LAB occurs during the late exponential to early stationary growth phase (De Vuyst and Vandamme 1994; Daba and Elkhateeb 2020). This level of activity was maintained until 48 hours, suggesting sustained production of the inhibitory compound under wort conditions. All values are representative of three independent experiments.

In a previous study, HW01 bacteriocin exhibited a higher apparent activity (640 AU/mL) against *Lactiplantibacillus plantarum* NCDO 955 when produced in MRS broth (Ahn et al. 2017). However, direct quantitative comparison of bacteriocin levels here and those reported previously should be interpreted with caution, as bacteriocin yield and measured activity can vary depending on the target organism and the production matrix. Differences in medium composition and cultivation conditions can significantly influence bacteriocin production in LAB (Abbasiliasi et al. 2017). However, the maintenance of detectable antimicrobial activity in wort - a complex and industrial substrate - supports the feasibility of bacteriocin generation outside of laboratory media

Bacteriocin activity after wort boiling and during fermentation

Bacteriocin activity following wort boiling and during fermentation was evaluated to assess the process

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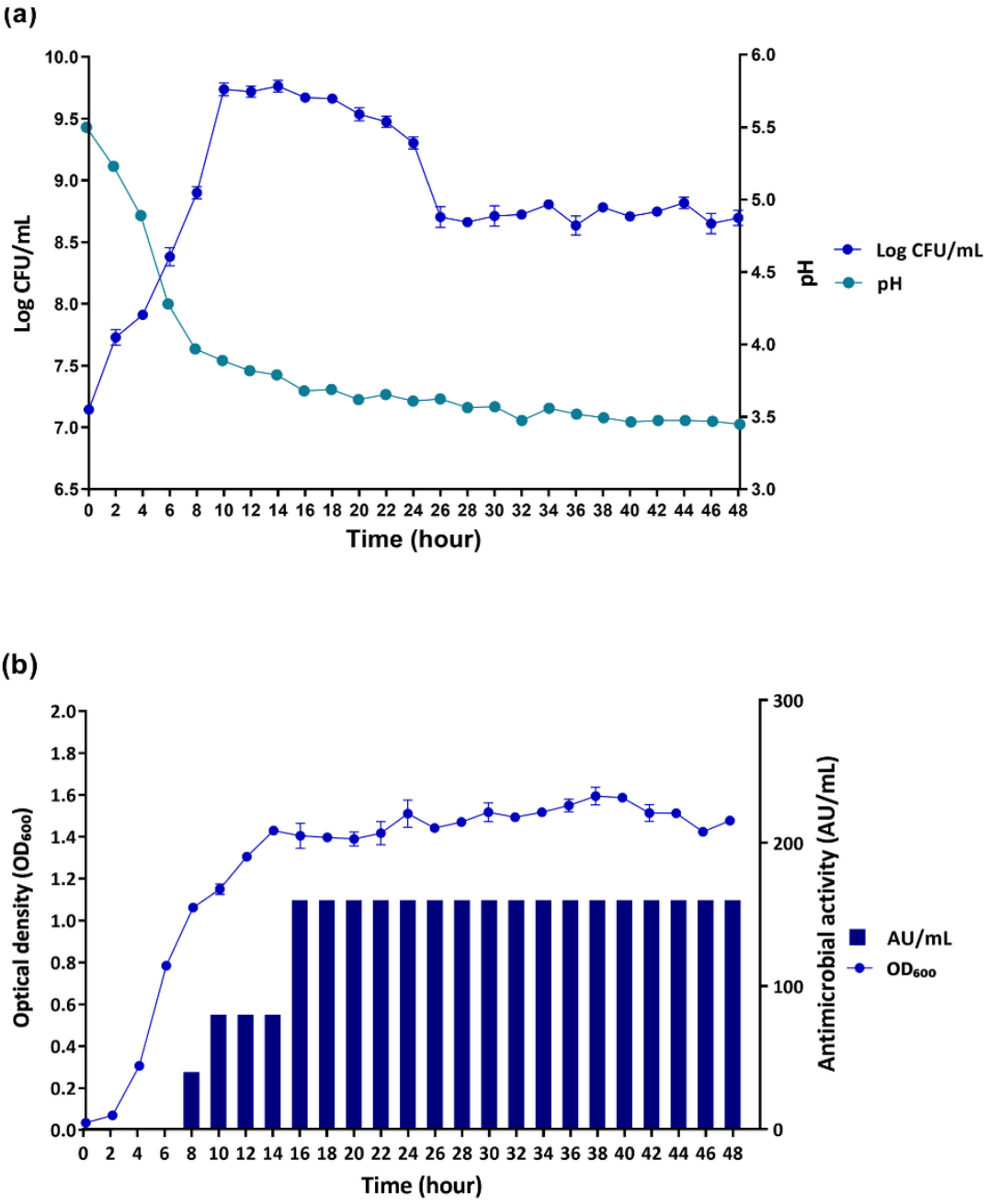
Figure 1. Growth and bacteriocin production of *Pediococcus acidilactici* HW01 during cultivation in un-hopped wort. (a) Changes in viable cell counts (log CFU/mL) and pH, (b) Changes in optical density (OD_{600}) and bacteriocin activity (AU/mL). Data from three independent experiments.



compatibility of the HW01 derived bacteriocin. Antimicrobial activity against *P. damnosus* was detected in boiled, unhopped wort (Figure 2), indicating that the bacteriocin retained functional activity after wort boiling. No viable bacterial cells were detected in the boiled wort, confirming that the observed antimicrobial activity was independent of live bacterial cells and attributable to a heat stable antimicrobial compound.

During fermentation, bacteriocin activity against *P. damnosus* remained detectable through the early and mid-stage of fermentation (Figure 2), with quantitative analysis showing a gradual decline during the later stages of fermentation (Figure 3). These results indicate that the HW01 derived bacteriocin retains inhibitory activity under dynamic fermentation conditions, with increasing ethanol concentrations and decreasing pH.

Figure 2. Retention of HW01 derived bacteriocin activity during brewing. Antimicrobial activity against *Pediococcus damnosus* was determined using an agar well diffusion assay: (a) pH-neutralised cell free supernatants after wort boiling, and (b–h) samples collected during fermentation (days 3–24). Clear inhibition zones indicate antimicrobial activity.

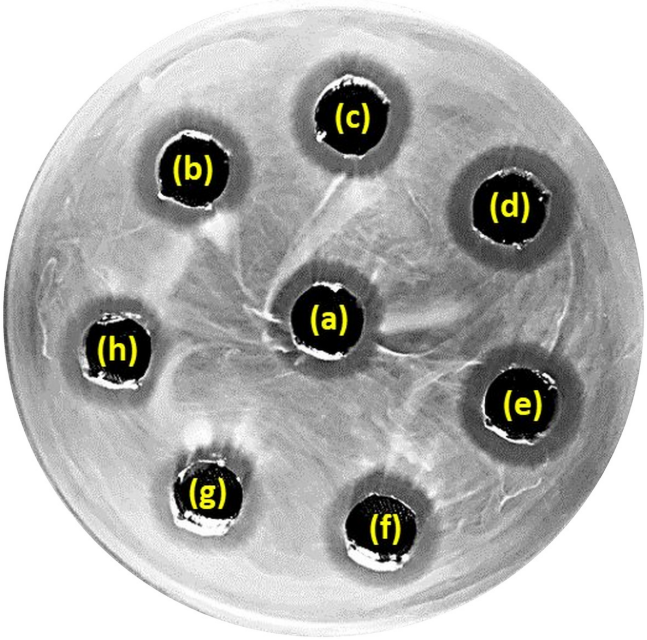
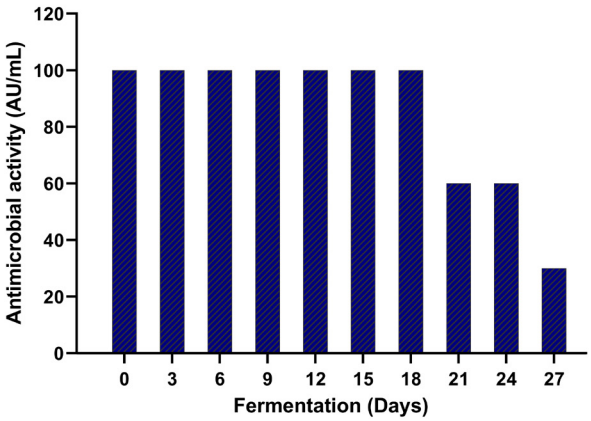


Figure 3. Changes in HW01 derived bacteriocin activity during fermentation. Bacteriocin activity against *Pediococcus damnosus* was quantified during fermentation and expressed as arbitrary units (AU/mL). The graph shows representative data from independent experiments.



These findings demonstrate that *P. acidilactici* HW01 does not survive wort boiling, whereas the bacteriocin does and remains functionally active during fermentation. The dissociation between cell survival and antimicrobial functionality highlights the potential of the HW01 derived bacteriocin as a process compatible biopreservative for selectively suppressing beer spoilage bacteria during fermentation. The retention of antimicrobial activity after pH neutralisation and wort boiling is consistent with the proteinaceous and heat stable nature of the HW01 derived bacteriocin reported by Ahn et al (2017).

Selective inhibition of spoilage bacteria during fermentation

The selective inhibitory effect of the bacteriocin on beer spoilage bacteria was evaluated during alcoholic fermentation, with parallel assessment of yeast viability (Figures 4, 5). When *P. damnosus* was inoculated at a high level into boiled wort, viable cell counts decreased during fermentation in both the pH adjusted beer and the bacteriocin treated beer (Figure 4), with *P. damnosus* declining from 5.69 ± 0.22 to 3.38 ± 0.01 log CFU/mL in the pH adjusted beer and from 5.58 ± 0.01 to 3.05 ± 0.10 log CFU/mL in the bacteriocin treated beer. This suggests that the fermentation environment contributed to growth inhibition under high inoculum conditions. Importantly, the bacteriocin treated beer exhibited significantly lower viable counts than the pH adjusted beer from day 2 of fermentation onwards ($p < 0.001$ to $p < 0.0001$). This suggests that the bacteriocin provided an additional and statistically significant antimicrobial effect beyond that of pH reduction alone.

In contrast, when *P. damnosus* was inoculated at a low initial level, distinct differences were observed. In the control and the pH adjusted beer, viable cell counts increased from 1.06 ± 0.03 to 3.01 ± 0.01 log CFU/mL suggesting that pH reduction was insufficient to suppress *P. damnosus* at low contamination levels. In contrast, in the bacteriocin treated beer, *P. damnosus* populations decreased from 1.09 ± 0.07 log CFU/mL to below the limit of detection after six days of fermentation. Similar bacteriocin mediated inhibitory effects against spoilage or closely related bacteria have been reported (Cotter et al. 2005; Silva et al. 2018). These results demonstrate that the HW01 derived bacteriocin is capable of not only providing additional suppression of *P. damnosus* beyond pH mediated inhibition at higher contamination levels but also eliminating low level spoilage contamination under fermentation conditions.

To determine whether bacteriocin mediated inhibition was selective, the viability of brewing yeast was monitored during primary and secondary fermentation (Figure 5). During primary

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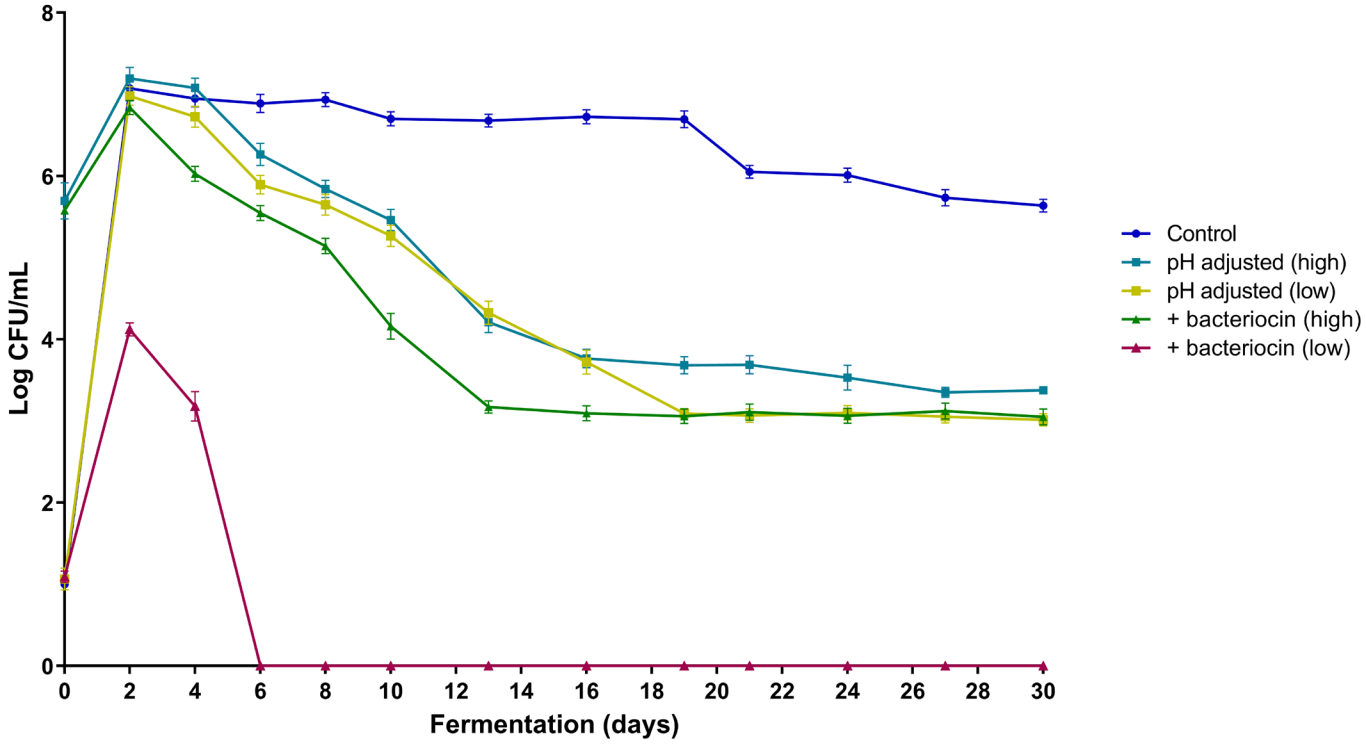
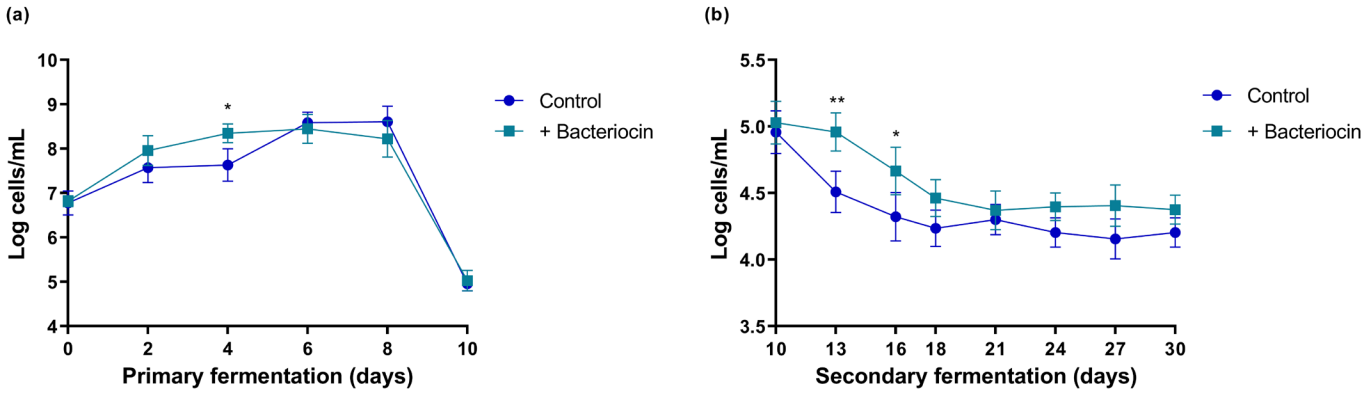
Figure 4. Inhibition of *P. damnosus* during fermentation by the HW01 derived bacteriocin. *Pediococcus damnosus* KCTC 3770 was inoculated into wort prior to fermentation at two levels, low (ca. 1 log CFU/mL) or high (ca. 5.5 log CFU/mL). Fermentations under both low and high inoculum conditions were (i) control, (ii) pH-adjusted, and (iii) bacteriocin treated groups. Viable cell counts are expressed as log CFU/mL. Error bars represent standard deviation (n = 3).



fermentation, yeast cell counts increased similarly in both the control and the bacteriocin treated beer, rising from 6.78 ± 0.27 to 8.61 ± 0.35 log cells/mL and from 6.82 ± 0.12 to 8.22 ± 0.41 log cells/mL. During secondary fermentation, yeast viability decreased in both beers, as expected under nutrient limited conditions. Yeast cell counts in the bacteriocin treated beer decreased from 5.03 ± 0.16 to 4.38 ± 0.11 log cells/mL, compared with a decrease from 4.96 ± 0.16 to 4.20 ± 0.11 log cells/mL in the control. Although statistically significant differences were observed at days 13 and 16 (p < 0.01 and p < 0.05), the bacteriocin treated beer had slightly higher yeast counts than the control indicating that these differences were not attributable to any inhibitory effect of the bacteriocin on yeast viability. Collectively, these results confirm that the HW01 derived bacteriocin exerted no inhibitory effect on brewing yeast throughout the fermentation process.

Indeed, the HW01 derived bacteriocin exerts a selective inhibitory effect during fermentation,

Figure 5. Effect of the bacteriocin on yeast viability during fermentation. (a) Changes in viable yeast cell counts during primary fermentation and (b) during secondary fermentation. Values are log cells/mL; error bars represent standard deviation (n = 3). Statistically significant differences between control and + bacteriocin beers at each time point are indicated by * and ** for FDR-adjusted p < 0.05 and p < 0.01, respectively.



suppressing *P. damnosus* under both high and low contamination conditions while maintaining the viability and fermentation performance of brewing yeast. This selective activity supports the potential application of the bacteriocin as a targeted biopreservative for controlling bacterial contamination without compromising fermentation performance.

Effects of bacteriocin treatment on fermentation performance

To evaluate whether the HW01 derived bacteriocin interferes with fermentation, changes in key parameters were monitored throughout primary and secondary fermentation, including free amino nitrogen (FAN), reducing sugar (RS), and specific gravity (SG) (Figures 6–8). These parameters are used to assess nutrient utilisation and fermentation progression in brewing (Briggs et al. 2004). Across these metrics, the profiles were comparable between the control and bacteriocin treated beers, indicating that bacteriocin treatment did not adversely affect fermentation performance.

The dynamics of FAN during fermentation are shown in Figure 6. The initial FAN level was higher in the bacteriocin treated beer (204.45 ± 7.26 mg/L) than in the control beer (158.35 ± 1.42 mg/L) (Figure 6a). This difference was attributable to the addition of HW01 derived cell free supernatant and did not affect the kinetics of FAN consumption during fermentation. Accordingly, FAN levels decreased over time with similar consumption patterns during primary and secondary fermentation (Figures 6a, b), and no noticeable divergence in FAN utilisation was observed. These results indicate that assimilable nitrogen availability and uptake by brewing yeast were not impacted by HW01 derived bacteriocin.

Consistent with FAN trends, reducing sugar levels decreased progressively as fermentation proceeded, and similar trends in RS depletion were observed between the control and bacteriocin treated beers, although differences were present at intermediate time points, while the final RS levels were comparable (Figure 7). There were no statistically significant differences in RS levels between the control and bacteriocin treated beers at the final stage of fermentation ($p > 0.05$).

Changes in specific gravity (SG) throughout fermentation are presented in Figure 8. In unhopped wort, the original SG values were 1.035 ± 0.001 for the control beer and 1.039 ± 0.001 for the bacteriocin treated beer. The SG values at the end of fermentation were not materially different between the two beers, indicating comparable attenuation and completion of fermentation. The higher initial SG of the bacteriocin treated wort, consistent with its higher initial FAN concentration, is attributable to residual medium derived solutes introduced with the added CFS rather than to differences in wort composition. As specific gravity was measured by hydrometry, which has limited resolution, these differences were not interpreted further.

Overall, the patterns observed for FAN consumption, RS depletion, and SG reduction demonstrate that treatment with the HW01 derived bacteriocin does not compromise fermentation performance.

Figure 6. Changes in free amino nitrogen (FAN) during fermentation. (a) Changes in FAN (mg/L) during primary fermentation and (b) secondary fermentation. Error bars represent standard deviation ($n = 3$). A statistically significant difference between control and + bacteriocin beers at each time point is indicated by * for FDR-adjusted $p < 0.05$.

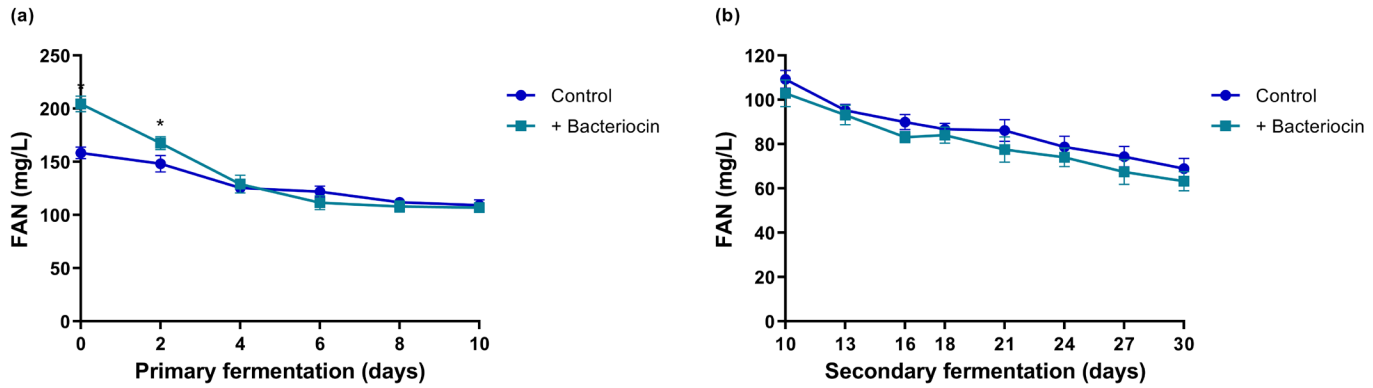
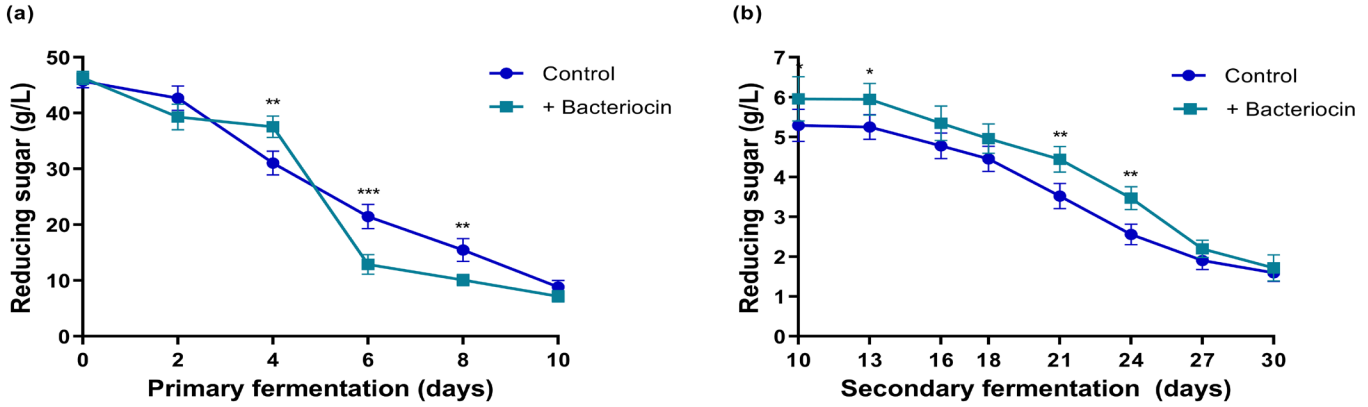


Figure 7. Changes in reducing sugar (RS) during fermentation. (a) Changes in RS (g/L) during primary fermentation and (b) secondary fermentation. Error bars represent standard deviation (n = 3). Statistically significant differences between control and + bacteriocin beers at each time point are indicated by *, **, and *** for FDR-adjusted $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.



When considered together, with the selective inhibition of *P. damnosus,* the results indicate that the bacteriocin enhances microbial stability while preserving normal fermentation. This dual functionality is critical for its practical application in brewing systems (Bokulich and Bamforth 2013; Stewart et al. 2017).

Effects of bacteriocin treatment on sensory attributes of beer

The beers produced with and without the HW01 bacteriocin (Table 1) were assessed as to whether bacteriocin addition during fermentation influenced sensory quality. The results (Table 2), and the sensory profiles are presented in Figure 9. Sensory characteristics, including aroma, taste, and mouthfeel, are key determinants of overall impression and are strongly influenced by fermentation conditions and microbial stability during brewing (Pires et al. 2014; Klimczak et al. 2024).

Sensory analysis showed that the bacteriocin treated beer exhibited significant differences in several sensory attributes compared with the control, while overall impression scores differed significantly between treatments. Among the taste related attributes, the bacteriocin treated beer showed significantly higher sour and sweet taste intensities and a significantly lower bitter taste intensity compared with the control beer. Although an increase in the perception of sourness was observed, this occurred without measurable change in fermentation parameters. Previous studies have demonstrated that fermentation management and microbial control strategies can modulate flavour profile, including perception of bitterness, without adversely affecting core fermentation processes (Schreurs et al. 2024; Ditrych et al. 2025).

Aroma attributes further supported the sensory compatibility of bacteriocin application. Fruity odour intensity was significantly higher in the bacteriocin treated beer, whereas alcoholic odour did not differ

Terms	First tier descriptors	Definitions	Reference materials
Aromatic	Fruity	Aroma of specific fruits or mixtures of fruits	Isoamyl acetate (banana like)
	Alcoholic	Aroma associated with ethanol and higher alcohols	Ethanol (5% v/v in water)
Taste	Bitter	Taste perceived as bitter	Isohumulone
	Sweet	Taste perceived as sweet	Sucrose (7.5 g/L in water)
	Sour	Acidic taste of lactic acid or sour milk	Lactic acid (0.1 g/L in water)
Mouthfeel	Mouth coating	Creamy or coating sensation in the mouth	Whole milk
	Carbonation	Tingling sensation caused by dissolved CO_2	Carbonated water
	Astringent	Mouth drying and puckering sensation, tannin like	Tannic acid (200 mg/L in water)
Fullness	Body	Fullness of flavour and mouthfeel	

Table 2. Sensory attribute scores. Values are mean ± SD (n = 20). Sensory attributes use a 9 point scale (1 = not present, 9 = very strong). Statistical significance determined using paired Student's t-tests with Benjamini–Hochberg FDR correction. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Sensory attribute	Control	+Bacteriocin
Odour - fruity	2.75 ± 0.25	6.23 ± 0.25***
Odour - alcoholic	5.08 ± 0.25	4.63 ± 0.42
Taste - bitter	5.13 ± 0.15	2.98 ± 0.19***
Taste - sweet	2.08 ± 0.03	3.65 ± 0.19**
Taste - sour	2.82 ± 0.22	5.57 ± 0.46**
Mouthfeel - mouthcoating	2.87 ± 0.45	2.95 ± 0.58
Mouthfeel - carbonation	3.55 ± 0.48	4.13 ± 0.55
Mouthfeel - astringent	4.85 ± 0.20	3.17 ± 0.38*
Mouthfeel - body	3.78 ± 0.34	3.57 ± 0.70
Overall impression	4.50 ± 0.49	6.05 ± 0.25*

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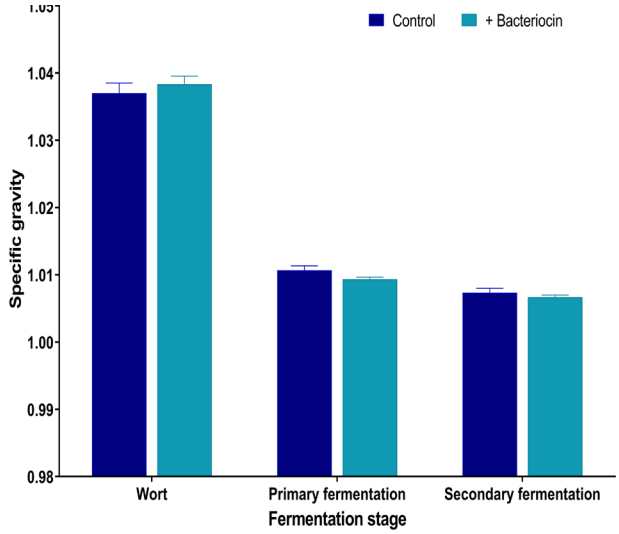
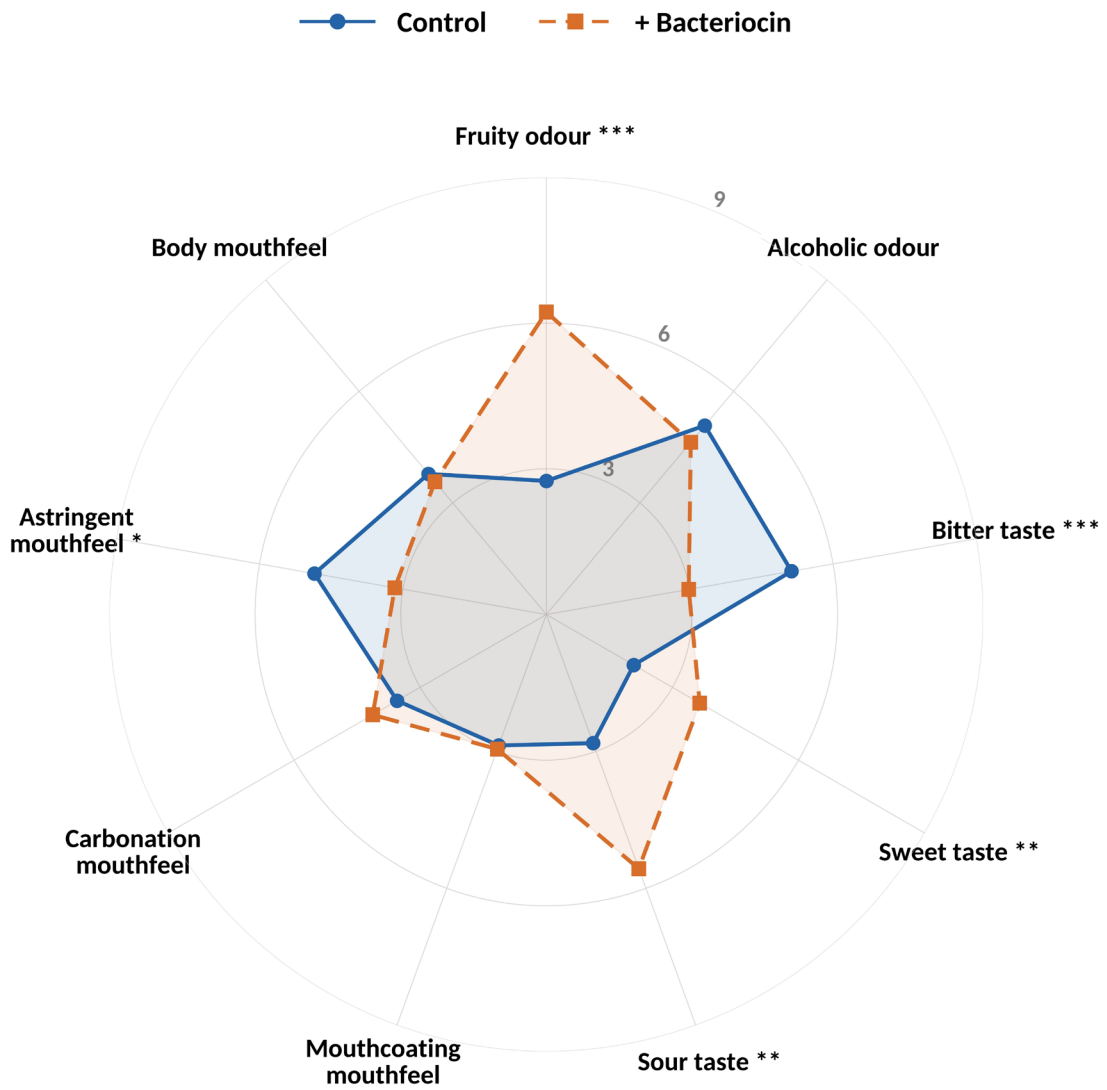
Figure 8. Changes in specific gravity (SG) during fermentation. SG in control and bacteriocin beers after primary and secondary fermentation. Error bars represent standard deviation (n = 3). No statistically significant differences were found ($p > 0.05$).



significantly between samples. These results indicate that bacteriocin treatment did not adversely affect yeast metabolic activity, as yeast derived fruity aroma compounds are associated with fermentation conditions and yeast physiological status (Pires et al. 2014), while elevated alcoholic or solvent like notes are typically linked to fermentation stress or microbial imbalance (Olaniran et al. 2017). Accordingly, bacteriocin treatment did not induce undesirable yeast metabolic responses during fermentation.

Differences in mouthfeel related attributes were also observed. The control beer showed significantly stronger astringent mouthfeel, whereas the bacteriocin treated beer was characterised by a comparatively smoother mouthfeel profile. In contrast, carbonation mouthfeel

Figure 9. Sensory profiles of control and bacteriocin-treated beers. Values represent mean scores on a 9 point scale (1 = not present, 9 = very strong) for odour, taste, and mouthfeel attributes. Statistically significant differences indicated by *, **, and *** for $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, based on paired Student's t-tests with Benjamini–Hochberg FDR correction.



and body did not differ significantly between treatments, indicating that textural properties governed by fermentation and carbonation were preserved following bacteriocin application. Studies have emphasised that effective microbial control strategies capable of suppressing spoilage microorganisms without altering yeast activity are critical for maintaining flavour and mouthfeel stability in beer (Vaughan et al. 2005; Suiker and Wösten 2022).

Consistent with these attribute level observations, the bacteriocin treated beer exhibited differences in specific sensory attributes compared with the control, while showing a significantly higher overall impression score. Taken together, the results show that application of the HW01 derived bacteriocin did not compromise sensory quality. When considered alongside the selective inhibition of beer spoilage bacteria and the preservation of fermentation parameters, the sensory data support the suitability of the bacteriocin as a process compatible biopreservative that enhances microbial stability while maintaining sensory quality during fermentation. In contrast to other bacteriocin based approaches, this study shows that a bacteriocin can remain active after wort boiling and function as a cell free antimicrobial during fermentation without compromising yeast performance or sensory quality.

Although the vast majority of commercial beers are produced from hopped wort, unhopped wort was deliberately used here to isolate the antimicrobial contribution of the HW01 derived bacteriocin from that of hop derived compounds such as iso-α acids, which inhibit Gram-positive beer spoilage bacteria including *P. damnosus* (Sakamoto and Konings 2003; Michel et al. 2020). Under hopped conditions, the effects of hop compounds and the bacteriocin may act additively, so the selective inhibition observed here may represent a conservative estimate of the efficacy achievable in conventional brewing. Nevertheless, potential interactions between hop constituents and the bacteriocin were not examined, and their combined effect under hopped wort conditions warrants further work.

It should be noted that this study did not include a sensory evaluation of beer produced without *P. damnosus* inoculation, which would have provided a more direct comparison of the impact of bacteriocin addition independent of spoilage contamination. In addition, the panel underwent only a single short training session, which may have constrained the discriminatory power and reproducibility of the ratings. These limitations suggest that the sensory findings should be regarded as indicative rather than definitive, and confirmation using an experienced sensory panel and an uninoculated control is warranted in future studies.

Conclusions

This study demonstrates that *P. acidilactici* HW01 - a bacteriocin producing lactic acid bacterium originally isolated from malt - produces a heat stable bacteriocin that remains functionally active under brewery processing conditions and during fermentation. Although *P. acidilactici* HW01 was able to grow and produce bacteriocin in unhopped wort, viable cells did not survive the wort boiling, confirming that the antimicrobial effects observed during fermentation were attributable to a cell free bacteriocin. The HW01 derived bacteriocin retained inhibitory activity against the beer spoilage bacterium *P. damnosus* following wort boiling and persisted through the early and mid-stage of fermentation. During fermentation, bacteriocin treatment selectively suppressed *P. damnosus*, eliminating low level contamination and significantly reducing bacterial populations under high inoculum conditions, without adversely affecting yeast viability. Importantly, the application of the bacteriocin did not compromise fermentation performance. Comparable profiles of free amino nitrogen consumption, reducing sugar utilisation, and decline in specific gravity were observed between bacteriocin treated and control beers, suggesting that fermentation proceeded normally in the presence of the bacteriocin. Sensory evaluation further demonstrated that the bacteriocin treated beer showed significantly higher overall impression scores than the control ($p < 0.05$), with statistically significant differences observed in fruity odour intensity, bitter, sweet, and sour taste attributes, and astringent mouthfeel, without adversely affecting the overall sensory profile. Overall, these findings demonstrate that the HW01 derived bacteriocin enables selective control of beer spoilage LAB

while preserving fermentation performance and sensory quality. This dissociation between microbial inhibition and fermentation disruption highlights the potential of the bacteriocin as a process compatible biopreservative for enhancing microbial stability during fermentation without reliance on additional thermal or chemical interventions.

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Conflict of interest

The authors declare there are no conflicts of interest.

CRediT author contributions

Ji Hyeon Kim: conceptualisation, methodology, investigation, data curation, formal analysis, visualisation, writing (original draft).
Hyun Jun Lee: conceptualisation, supervision, resources, project administration, funding acquisition, writing (review and editing).

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