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Original Research

The Bacteriocin Potential of Lactic Acid Bacteria from Cincalok as an Antibacterial Against *Shigella* and *Salmonella*

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ABSTRACT

Background: Lactic Acid Bacteria (LAB) are a group of bacteria that produce antimicrobial compounds called bacteriocins. The antibacterial properties of LAB have been proven effective in inhibiting pathogenic bacteria that cause infection in humans, including *Shigella* sp. and *Salmonella* sp. LAB can be found in fermented foods, one of which is cincalok, a traditional fermented food from West Kalimantan, Indonesia. **Aim:** This study **aim**ed to determine the antibacterial potential of bacteriocins from LAB isolated from cincalok against *Shigella* sp. and *Salmonella* sp. **Methods:** This study used a true experimental design with a post-test-only control group arrangement. Data were analysed using SPSS to evaluate inhibition zone differences among three treatment groups: bacteriocin from *Weissella* sp. at 24 hours, *Weissella* sp. at 48 hours, and antibiotics (positive control). **Results:** Bacteriocin from *Weissella* sp. produced measurable inhibition zones against both target pathogens. For *Shigella* sp., mean inhibition zone diameters were 24.4 mm (W24), 13.2 mm (W48), and 42.2 mm (cotrimoxazole). For *Salmonella* sp., mean values were 24.2 mm (W24), 12.8 mm (W48), and 45.0 mm (chloramphenicol). One-Way ANOVA and post-hoc LSD tests confirmed statistically significant differences among all three groups ($p < 0.05$). **Conclusion:** Bacteriocins from LAB cincalok (*Weissella* sp.) have a significant antibacterial effect against *Shigella* sp. and *Salmonella* sp., with the 24-hour preparation demonstrating superior activity compared to the 48-hour preparation.

Keywords: Bacteriocin; Cincalok; *Salmonella* sp.; *Shigella* sp.; *Weissella*

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1. INTRODUCTION

Lactic Acid Bacteria (LAB) are a group of Gram-positive bacteria that do not produce spores and have either homofermentative or heterofermentative metabolism, producing lactic acid from carbohydrates (Mokoena, 2017). Fermentation is one of the oldest forms of food processing and is still widely used to extend the shelf life of food (Juodeikiene et al., 2011). During fermentation, carbohydrates and other macromolecules are converted into organic acids by bacteria under anaerobic conditions. Fermented foods have been demonstrated to function as functional foods that provide health benefits, helping to minimise the risk of conditions such as irritable bowel syndrome, inflammatory bowel disease, and colorectal cancer (Juodeikiene et al., 2011; Getahun et al., 2017; Sanlier et al., 2019; Marco et al., 2017).

Fermented foods produced using LAB are widely available in Indonesia, including sayur asin, bekasam, tempoyak, and cincalok (Masdarini, 2011). Cincalok is a traditional fermented food from West Kalimantan made from shrimp of the Acetes species. It can also be found in Malaysia, Thailand, and the Philippines (Syahmurdiandi, 2011; Hajep & Jinap, 2012). LAB are known to produce antimicrobial compounds called bacteriocins, which are utilised in probiotic applications, as food preservatives, and as alternatives to conventional antibiotics (Sunaryanto, 2015). Bacteriocins exert bactericidal or bacteriostatic effects against pathogenic bacteria.

The antibacterial activity of LAB has been demonstrated against pathogenic organisms responsible for human infections. According to the World Health Organization (2013), diarrhoea accounts for 29% of child mortality worldwide, exceeding two million deaths annually. Key causative agents include *Salmonella* typhi and *Shigella* dysenteriae (Zein et al., 2004). In tropical countries, diarrhoea caused by *Salmonella* sp. ranks second in incidence at 15%, followed by *Shigella* sp. at 10% (Agtini et al., 2005).

Shigellosis is characterised by bloody, mucus-filled diarrhoea with fever and abdominal distension, caused by *Shigella* sp. (Nur et al., 2014). Salmonellosis presents as gastroenteritis with enteric fever and localised infections caused by *Salmonella* sp. (Apriliana, 2020). Both pathogens contaminate food sources including raw eggs, raw meat, fresh vegetables, and water (Moekti, 2020), and are transmitted via the fecal-oral route (Okafor, 2021). *Salmonella* sp. is a Gram-negative, rod-shaped, non-spore-forming organism that grows at pH 4-9 (Suryandari et al., 2018). *Shigella* sp. is a Gram-negative, non-motile rod (0.5 \times 1-3 \mu m) that grows optimally at 37^\circ C and pH 7.4 (Sari, 2018a). Both are selectively detected on *Salmonella-Shigella* Agar (SSA) (Apriani et al., 2019).

Previous studies have demonstrated the inhibitory activity of LAB against these pathogens. Fitrianarni et al. (2014) confirmed the ability of LAB to inhibit the growth of *Salmonella* typhii, while Mira et al. (2015) showed that a probiotic drink derived from red guava juice reduced the colony count of both Escherichia coli and *Shigella* dysenteriae. Based on these findings, this study investigated the bacteriocin potential of LAB from cincalok (*Weissella* sp.) as an antibacterial agent against *Shigella* sp. and *Salmonella* sp.

2. METHODS

2.1 Study Design

This study employed a true experimental design with a post-test-only control group arrangement. The study was conducted at the Biomedical Laboratory, Faculty of Medicine, Wahid Hasyim University, Semarang, over five consecutive working days.

2.2 Biological Materials and Bacterial Strains

A pure culture of *Weissella* sp. was obtained from the Microbiology Laboratory collection of Diponegoro University, originally isolated from cincalok. The test pathogens, *Shigella* sp. and *Salmonella* sp., were obtained from the same laboratory collection. All strains were rejuvenated in BHIB at 37^\circ C for 24 hours and standardised to a 0.5 McFarland turbidity standard before use (Singh et al., 2025).

2.3 Sample Size

The minimum number of repetitions was determined using the Federer formula: (t-1) (n-1) ≥15 (Federer, 1955). With four groups, the formula yielded n = 6, corrected to n' = 8 after

20% dropout adjustment (n' = 6/0.8 = 7.5 \approx 8). Five repetitions per group were performed, as sample dropout is not applicable in vitro. This minor deviation is acknowledged as a study limitation; statistical assumptions of normality and homogeneity were nonetheless met.

2.4 Experimental Variables

Independent variable: bacteriocin from *Weissella* sp. (24-hour and 48-hour incubation). Dependent variables: inhibition zone diameters (mm) against *Shigella* sp. and *Salmonella* sp.

2.5 Bacteriocin Preparation and Antibacterial Activity Testing

Crude bacteriocin preparations were obtained by immersing sterile blank paper discs (6 mm) in 24-hour or 48-hour *Weissella* sp. culture for 15 minutes (Isaac et al., 2024). Positive controls: cotrimoxazole 30 ug (Oxoid, UK) for *Shigella* sp. and chloramphenicol 30 ug (Oxoid, UK) for *Salmonella* sp. Negative control: blank discs in sterile distilled water.

Antibacterial activity was assessed by disc diffusion on MHA (CLSI M02-Ed14; CLSI, 2024a). Standardised suspensions were inoculated by the streak method; discs were placed on inoculated MHA and incubated at 37^\circ C for 24 hours. Inhibition zone diameters were measured with a digital calliper and interpreted as: >20 mm (very strong), 10-20 mm (strong), 5-10 mm (moderate), <5 mm (weak) (Wayah et al., 2024).

2.6 Statistical Analysis

Data were analysed using IBM SPSS Statistics (Version 25). Normality was assessed by Shapiro-Wilk test; homogeneity by Levene's test. One-Way ANOVA was performed, followed by post-hoc LSD analysis; significance threshold $p < 0.05$ (CLSI, 2024b).

3. RESULTS

3.1 Inhibition Zone Measurements

Inhibition zones were observed in all treatment groups; no inhibition was observed in the negative control.

Table 1. Inhibition zone diameters against *Shigella* sp. (mm)

Rep.	*Weissella* 24 h	*Weissella* 48 h	Cotrimoxazole
1	21	15	40
2	27	15	40
3	30	13	43
4	22	13	43
5	22	10	45
Mean	24.4	13.2	42.2

Weissella 24 h (24.4 mm) and cotrimoxazole (42.2 mm): very strong (>20 mm). *Weissella* 48 h (13.2 mm): strong (10-20 mm).

Table 2. Inhibition zone diameters against *Salmonella* sp. (mm)

Rep.	*Weissella* 24 h	*Weissella* 48 h	Chloramphenicol
1	23	15	46
2	25	15	46
3	23	12	44
4	20	12	44
5	30	10	45
Mean	24.2	12.8	45.0

Weissella 24 h (24.2 mm) and chloramphenicol (45.0 mm): very strong (>20 mm). *Weissella* 48 h (12.8 mm): strong (10-20 mm).

3.2 Statistical Analysis

3.2.1 Normality and Homogeneity Shapiro-Wilk

All groups were normally distributed ($p > 0.05$). Levene's test: *Shigella* sp. $p = 0.068$; *Salmonella* sp. $p = 0.164$. One-Way ANOVA was therefore applied.

3.2.2 One-Way ANOVA

Table 3. One-Way ANOVA -- *Shigella* sp.

Source	SS	df	MS	F	Sig.
Between Groups	2138.800	2	1069.400	132.561	0.000
Within Groups	96.800	12	8.067		
Total	2235.600	14			

Table 4. One-Way ANOVA -- *Salmonella* sp.

Source	SS	df	MS	F	Sig.
Between Groups	2665.734	2	1332.867	206.108	0.000
Within Groups	77.600	12	6.467		
Total	2743.334	14			

3.2.3 Post-Hoc LSD Test

Table 5. Post-hoc LSD -- *Shigella* sp.

Group I	Group II	Mean Diff (I-II)	p
Weissella 24 h	*Weissella* 48 h	11.200*	0.000*
Weissella 24 h	Antibiotic	-17.800*	0.000*
Weissella 48 h	*Weissella* 24 h	-11.200*	0.000*
Weissella 48 h	Antibiotic	-29.000*	0.000*
Antibiotic	*Weissella* 24 h	17.800*	0.000*
Antibiotic	*Weissella* 48 h	29.000*	0.000*

*Significant ($p < 0.05$)

Table 6. Post-hoc LSD -- *Salmonella* sp.

Group I	Group II	Mean Diff (I-II)	p
Weissella 24 h	*Weissella* 48 h	11.400*	0.000*
Weissella 24 h	Antibiotic	-20.800*	0.000*
Weissella 48 h	*Weissella* 24 h	-11.400*	0.000*
Weissella 48 h	Antibiotic	-32.200*	0.000*
Antibiotic	*Weissella* 24 h	20.800*	0.000*
Antibiotic	*Weissella* 48 h	32.200*	0.000*

*Significant ($p < 0.05$)

In both pathogens, the 24-hour *Weissella* preparation produced significantly larger inhibition zones than the 48-hour preparation, and both were significantly inferior to the antibiotic controls.

4. DISCUSSION

This study demonstrated that bacteriocins producaed by *Weissella* sp. -- a LAB isolated from cincalok -- inhibited the growth of both *Shigella* sp. and *Salmonella* sp. in vitro, with the 24-hour preparation showing superior antibacterial activity compared to the 48-hour preparation.

The 24-hour *Weissella* preparation yielded mean inhibition zone diameters of 24.4 mm against *Shigella* sp. and 24.2 mm against *Salmonella* sp., both classified as very strong (>20 mm). The 48-hour preparation produced zones of 13.2 mm and 12.8 mm, classified as strong (10-20 mm). These findings are consistent with Fitrianarni et al. (2014), who demonstrated that LAB isolates significantly inhibited *Salmonella typhii* and *Shigella dysenteriae* in vitro, and with Sari et al. (2016), who reported bacteriocin-mediated inhibition of *Salmonella* typhi by Lactobacillus plantarum with a zone of 9.0 mm. The inhibitory activity observed here is higher than values previously reported from yogurt-based systems, suggesting that *Weissella* sp. from cincalok may represent a potent source of LAB-derived bacteriocins.

The statistically significant decline in activity from 24 to 48 hours ($p < 0.05$) can be attributed to several mechanisms. Accumulation of endogenous proteases can degrade proteinaceous bacteriocin molecules (Wayah et al., 2024). Excessive acidification during extended incubation may induce conformational changes or peptide aggregation, reducing efficacy against target pathogens (Wayah et al., 2024). Nutrient depletion further compromises bacteriocin biosynthesis (Sari, 2018a). These results accord with Fitrianarni et al. (2014), in which One-Way ANOVA ($p < 0.05$) confirmed significant between-group differences.

The antibiotic controls -- cotrimoxazole (42.2 mm) and chloramphenicol (45.0 mm) -- produced larger inhibition zones than the

bacteriocin preparations, as expected given the higher concentration and specificity of standardised antibiotic discs. Nevertheless, the 24-hour *Weissella* bacteriocin achieved zones exceeding 20 mm, supporting its potential as an alternative or adjunct antimicrobial agent.

Limitations: (1) Crude preparations were used; protease sensitivity and pH-neutralisation tests are recommended to confirm bacteriocin specificity. (2) Five rather than the Federer-recommended six repetitions were conducted. (3) MIC values were not determined; MIC assessment is recommended for future work.

5. CONCLUSION

Bacteriocins from *Weissella* sp. isolated from cincalok LAB demonstrated significant antibacterial activity against *Shigella* sp. and *Salmonella* sp. in vitro. The 24-hour preparation was more effective than the 48-hour preparation ($p < 0.05$). Statistically significant differences were confirmed among all treatment groups by One-Way ANOVA and post-hoc LSD analysis. These findings support the potential of cincalok-derived *Weissella* sp. bacteriocins as candidates for further development as natural antimicrobial agents.

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