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The Antagonistic Potential of Kimchi-Associated LAB Against Histamine-Producing Pathogens in Seafood

Potensi Antagonistik Bakteri Asam Laktat (LAB) dari Kimchi Terhadap Patogen Penghasil Histamin pada Makanan Laut

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ABSTRACT

Lactic acid bacteria (LAB) play a crucial role in food fermentation, for example in kimchi, a traditional Korean dish. These bacteria aid in food preservation and probiotics, with antimicrobial compounds like bacteriocins directly responsible for extending shelf life. This study introduces an approach in using LAB from kimchi for inhibiting the growth of *Morganella morganii*, a major histamine-producing pathogen in fish products. Histamine accumulation poses severe food safety risks, necessitating an innovative natural solution. LAB isolates from both solid and liquid fractions of kimchi were screened MRS Agar and characterized biochemically for traits such as fermentation type and acid production. Antimicrobial efficacy, assessed via the Kirby-Bauer disk diffusion method, revealed inhibition zones averaging 6.98 ± 0.04 mm for liquid and 6.40 ± 1.34 mm for solid fractions. Despite their lower efficacy compared to chloramphenicol (30.80 ± 1.92 mm), the natural attributes of LAB highlights their unique potential as a sustainable alternative for histamine control. This study is among the first to explore kimchi-derived LAB as antagonists against *M. morganii*, providing a novel perspective for developing green technologies in food safety and quality management.

Keywords: Lactic acid bacteria, kimchi, histamine control, *Morganella morganii*, sustainable food safety

INTISARI

Bakteri asam laktat (BAL) memiliki peran penting dalam fermentasi makanan, seperti pada proses pembuatan kimchi, hidangan tradisional Korea. Bakteri ini tidak hanya berfungsi dalam pengawetan makanan dan probiotik, tetapi juga menunjukkan aktivitas antimikroba melalui produksi senyawa seperti bakteriosin. Penelitian ini memperkenalkan pendekatan baru dalam penggunaan BAL dari kimchi untuk menghambat pertumbuhan *Morganella morganii*, patogen utama penghasil histamin pada produk perikanan. Akumulasi histamin menimbulkan risiko serius terhadap keamanan pangan, sehingga diperlukan solusi inovatif dan alami. Isolat BAL dari fraksi padatan dan cairan kimchi diseleksi menggunakan media selektif dan dikarakterisasi secara biokimia untuk sifat-sifat seperti tipe fermentasi dan produksi asam. Efikasi antimikroba, yang diuji menggunakan metode difusi cakram Kirby-Bauer, menunjukkan zona hambat rata-rata $6,98 \pm 0,04$ mm untuk fraksi cairan dan $6,40 \pm 1,34$ mm untuk fraksi padatan. Meskipun efektivitasnya lebih rendah dibandingkan

dengan kloramfenikol ($30,80 \pm 1,92$ mm), sifat ramah lingkungan BAL mengunggulkan potensinya sebagai alternatif berkelanjutan untuk pengendalian histamin. Penelitian ini merupakan salah satu penelitian terbaru yang mengeksplorasi BAL dari kimchi sebagai antagonis terhadap *M. morganii*, dan memberikan perspektif baru dalam pengembangan teknologi hijau untuk keamanan dan kualitas pangan.

Kata kunci: Bakteri asam laktat, kimchi, pengendalian histamin, *Morganella morganii*, keamanan pangan ramah lingkungan

INTRODUCTION

The global rise of foodborne illnesses linked to histamine contamination presents a critical challenge in food safety (Oktariani et al., 2022). Histamine, a biogenic amine, forms through the decarboxylation of histidine in protein-rich foods like fish, facilitated by bacteria such as *Morganella morganii*. Increasing seafood consumption, coupled with improper handling and storage, amplifies the risks of histamine-related food poisoning (Visciano et al., 2020). Regulatory agencies, including the FDA and EFSA, set histamine limits at 50 ppm and 400 ppm, respectively, to prevent poisoning (DeBeeR et al., 2021). Despite these regulations, histamine poisoning remains prevalent, especially in regions with inadequate cold chain infrastructure. Symptoms, including flushing, headaches, and respiratory distress, often resemble allergic reactions, leading to underreporting (Visciano et al., 2020; Oktariani et al., 2022).

Temperature control is a key strategy to prevent histamine formation. Refrigeration below 4°C slows bacterial activity but cannot eliminate pre-formed histamine (Oktariani et al., 2022). Chemical preservatives, such as sulfites and benzoates, are effective but face scrutiny due to health risks and consumer preference for natural products (Nnaji et al., 2025). Irradiation, though efficient, has limited acceptance due to high costs and consumer resistance (Nishihira, 2020). Natural antimicrobials like plant extracts offer sustainable alternatives but are influenced by food composition and storage conditions (Yiasmin et al., 2021). Essential oils, for example, can control histamine but may affect food flavor (Teshome et al., 2022).

Probiotics, particularly lactic acid bacteria (LAB), have emerged as promising agents to mitigate histamine contamination (Zapaśnik et al., 2022). LAB produce antimicrobial compounds such as organic acids, hydrogen peroxide, and bacteriocins, creating hostile conditions for histamine-producing bacteria (Vieco-Saiz et al., 2019). Their Generally Recognized as Safe (GRAS) status makes them suitable for food applications (Christianah & Oyewumi, 2024). Kimchi, a traditional Korean fermented vegetable dish, is a rich source of LAB, including *Lactobacillus*, *Leuconostoc*, and *Weissella* (Park et al., 2014). The fermentation process in kimchi enhances LAB growth and antimicrobial properties (Cha et al., 2024). While LAB from kimchi have demonstrated efficacy against pathogens like *Escherichia coli* and *Staphylococcus aureus*, their potential against histamine producers like *M. morganii* is less explored.

This study aims to fill this gap by evaluating LAB derived from kimchi as antagonists against *M. morganii*. Key objectives include isolating and characterizing LAB from kimchi's solid and liquid fractions, assessing their antimicrobial activity via the Kirby-Bauer disk diffusion method, and comparing their efficacy to conventional antibiotics, in hope to examine the feasibility of using LAB in seafood preservation as a natural, eco-friendly solution to histamine contamination. The novelty of this research lies in its focus on kimchi-derived LAB for histamine control, targeting *M. morganii*, a key histamine producer. By emphasizing LAB's practical applications in seafood preservation, this study aligns with global efforts to enhance food safety and sustainability.

MATERIALS AND METHOD

Isolation of lactic acid bacteria

Lactic acid bacteria (LAB) were isolated from commercial kimchi's solid and liquid (sauce) fractions using serial dilution and spread plating on MRS Agar (Oxoid). Ten grams or 10 mL of kimchi were diluted in 0.9% saline solution to a final volume of 100 mL (10^{-1} dilution). The solution was further diluted to 10^{-5} , and

100 μ L from dilutions 10^{-2} to 10^{-5} were spread on MRS Agar plates. Plates were incubated anaerobically at 37°C for 24 hours until bacterial colonies were visible. Distinct colonies were picked using a sterile loop and purified using the streak plate method to obtain single colonies. These purified colonies were screened by spot inoculation onto MRS agar (Merck) containing Bromocresol Purple (BCP, Merck) as a pH indicator. Colonies that changed BCP from purple to yellow were identified as potential LAB candidates. These LAB isolates were then preserved as stock cultures for further research.

Subculturing of *Morganella morganii*

The *M. morganii* used in this study was obtained from the official culture collection of the Center for Food and Nutrition Studies, Universitas Gadjah Mada. To reactivate the bacteria, the stock culture was transferred onto Nutrient Agar (NA, Oxoid) using a sterile inoculating loop and streaked using the quadrant method. The plates were incubated at 37°C for 24 to 72 hours to promote growth.

Biochemical tests for identification of LAB and *M. morganii*

We conducted several tests to confirm the identity of LAB and *M. morganii*. Various biochemical tests were performed on LAB, including Gram staining, catalase activity, fermentation type, acid production, and motility, following the method reported by Teriyani (2023). For *M. morganii*, the biochemical tests included motility, H₂S production, and catalase activity, as outlined previously (Sintyadewi et al., 2015).

In vitro* dual culture assay of LAB against *M. morganii

The antagonistic activity of LAB against *M. morganii* was conducted using a modified Kirby-Bauer disk diffusion method based on an established method (Ramona, 2021). This method aimed to evaluate the ability of LAB to inhibit *Morganella morganii* growth. A sterile cotton swab was used to spread *M. morganii*, prepared at 0.5 McFarland standard, across the surface of Nutrient Agar (NA, Merck) plates. A volume of 10 μ L of LAB suspension with potential antimicrobial activity was then spot inoculated on this *M. morganii* lawn, incubated at 37°C, and the inhibition zone around the LAB spot was observed. Each 10 μ L of sterile MRS broth medium and 0.01 g/mL chloramphenicol served as negative and positive controls, respectively. The experiment was repeated five times to obtain representative data.

The diameter of the inhibition zones was measured using a vernier calipers by determining the vertical, horizontal, diagonal I, and diagonal II lengths of the clear zone. Careful measurements were taken to ensure data accuracy. The result was averaged and categorized based on the inhibition zone classification defined by Kerr (2025).

Data analysis

The data collected in this study consisted of both qualitative and quantitative information. Qualitative data included the biochemical characteristics of LAB & *Morganella morganii*, and the ability of LAB to create inhibition zones in the screening tests. Quantitative data comprised the average diameters of inhibition zones, categorized based on Kerr (2025) classification. These quantitative data were analyzed using ANOVA (Analysis of Variance) with a significance level of 5% ($p < 0.05$) through IBM SPSS version 26.0. If the analysis indicated $p < 0.05$, further evaluation was conducted using the Duncan Multiple Range Test.

RESULTS

Isolation, Characterization, and Screening of LAB Isolates

A total of 10 isolates were successfully obtained in this study from the solid and liquid parts of kimchi. The candidates of LAB isolates were subsequently tested for their antagonistic activity against *Morganella morganii* before undergoing characterization to confirm that they belong to the lactic acid bacteria group. The results are presented in Figure 1.

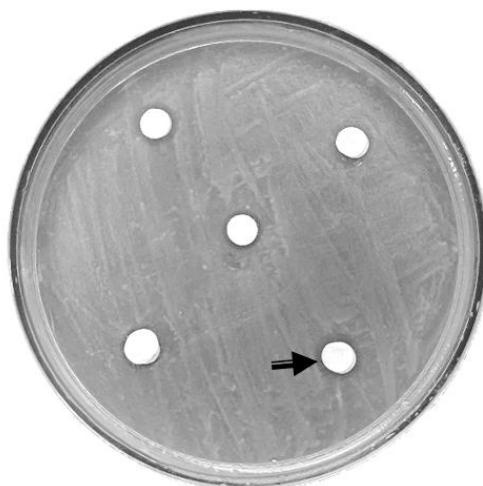


Figure 1. Inhibition zones formed on *M. morganii* lawn by lactic acid bacteria in a dual culture assay. Inhibition zone is pointed by an arrowhead.

As shown in Figure 1, although the inhibition zones around the tested LAB isolates are small, they remain observable (indicated by black arrow). This provided preliminary evidence of antagonistic activity of LAB against *M. morganii*.

Two LAB isolates (one from the solid fraction and one from the liquid fraction of kimchi) that exhibited the best inhibition zones during the preliminary screening were subjected to biochemical characterizations, with the results presented in Table 1. Both isolates exhibited similar characteristics, except for their fermentation types. Despite this minor variation, both isolates can be classified as LAB based on the results presented in Table 1. Specific traits of LAB include their ability to produce acid, coagulate milk, their Gram-positive nature, and their catalase-negative property.

Table 1. Some important characteristics of LAB isolates

Types of tests	Isolate A (solid kimchi)	Isolate B (liquid part of kimchi)
Gram stain	Gram +	Gram +
Catalase	Negative	Negative
Acid production	+	+
Fermentation types	Homofermentative	Heterofermentative
Motility tests	Non motile	Non motile
Ability to clot milk	+	+
Change of BCP indicator	Purple to yellow	Purple to yellow

Dual culture assays of LAB isolate from kimchi against *M. morganii*

The two previously obtained isolates consistently demonstrated antagonistic activity against *M. morganii* in inhibition tests using the dual culture assay, as shown in Figure 2 and Table 2. Overall, the diameters of the inhibition zones formed by these isolates did not differ significantly ($p>0.05$) but were significantly different from the positive and negative controls (Table 2). Although the inhibition zones were categorized as weak, their formation offers potential for applying these isolates in preserving seafood, which is highly susceptible to contamination by histamine-producing bacteria, such as *M. morganii*.

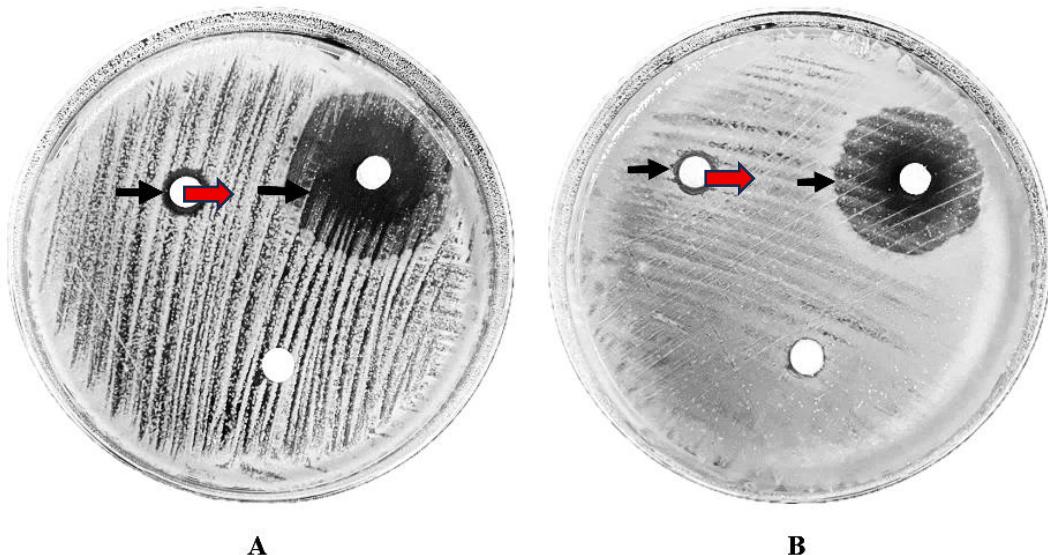


Figure 2. Antagonistic activity of LAB isolates from kimchi, as indicated by clear zone formation. (A) LAB isolate from solid part of kimchi and (B) LAB isolate from liquid (sauce) of kimchi. Black arrowheads point the inhibition zones produced by LAB isolates and red arrowheads point the inhibition zones of positive control on *M. morganii* lawn.

The absence of inhibition zones in the negative control confirms that the zones formed by LAB isolates on the *M. morganii* lawn were solely due to compounds released by the LAB isolates. These inhibitory compounds diffused through the agar, creating clear zones on the *M. morganii* lawn, as shown in Figure 2.

Table 2. Diameter of inhibition zones formed by LAB isolate on *M. morganii* in dual culture assays

Treatments	Diameter of inhibition (mm)*	Category of inhibition
LAB from solid kimchi	6.98 ± 0.04 ^b	Weak
LAB from kimchi sauce	6.40 ± 1.34 ^b	Weak
Positive control	30.80 ± 1.92 ^c	Strong
Negative control	0.00 ± 0.00 ^a	Weak

*The values in Table 2 represent the mean ± standard deviation from five replicates. Values followed by the same letter indicate no statistically significant differences, as determined by Duncan's test at $p < 0.05$ following ANOVA analysis.

DISCUSSION

Kimchi is a fermented food product naturally containing various microorganisms, particularly lactic acid bacteria (LAB), which develop during its fermentation and storage. The presence of LAB in kimchi plays a significant role in forming its distinct flavor, enhancing nutritional value, and providing probiotic benefits to health (Cha et al., 2024). The characteristics of LAB colonies on MRS Agar and their biochemical characteristics found in our study (Table 1) align with findings of Gopal (2022), describing LAB colonies as milky white, round, with smooth edges, and a convex surface.

Lactic acid bacteria (LAB) are generally classified into two groups based on their hexose sugar fermentation pathways: homofermentative and heterofermentative, as shown in Table 1. Homofermentative LAB utilize the Embden-Meyerhof-Parnas (EMP) pathway to convert hexose and pentose sugars into lactic acid through glycolysis, facilitated by the enzyme aldolase (Salvetti et al., 2013). This pathway does not produce secondary byproducts such as carbon dioxide (CO_2), meaning no gas bubbles are observed during fermentation tests (Bustos et al., 2008).

In contrast, heterofermentative LAB found in kimchi sauce fermentation use the phosphoketolase pathway to metabolize glucose into lactic acid, CO₂, and ethanol (Stephen & Saleh, 2023). Ethanol is produced through the reduction of acetyl-CoA by excess NAD(P)H, where CO₂ is released during the initial decarboxylation step of the pentose phosphate pathway (Lee et al., 2020). The presence of CO₂ leads to detectable gas bubbles in the hot loop dip test. According to (Gunkova et al., 2021), homofermentative LAB produce lactic acid in higher proportions, approximately 90–95%, compared to heterofermentative LAB, which yield about 50% (Blajman et al., 2020). Due to their efficiency, homofermentative LAB are preferred for industrial applications in lactic acid production.

The two LAB isolates obtained in this study demonstrated acid production, as indicated by a color change in the MRSA medium containing BCP (Table 1). This result was further supported by reduction in pH and the coagulation of skim milk inoculated with the isolates (Table 1). The color change in the acid production test medium is due to the use of bromocresol purple (BCP), a standardized pH indicator containing approximately 90% active dye components (Hemdan et al., 2023). This indicator is highly sensitive to pH changes and provides a rapid response. At a pH below 5.2, the indicator turns yellow, while at a pH above 6.8, it changes to violet (Funnekotter et al., 2023). Lactic acid bacteria (LAB) produce lactic acids during metabolism, lowering the surrounding pH and causing the medium to change from purple to yellow, signaling fermentation activity.

In the acidity test using in skim milk solution, after 24 hours of LAB inoculation, the milk separates into solid (curd) and liquid (whey) components. Curd formation resulted from the coagulation of milk proteins, primarily casein, while whey remains as the liquid fraction. Acid fermentation by LAB destabilizes the casein structure through dissociation mechanisms, leading to casein aggregation and separation from the whey (Wang & Zhao, 2023).

In the motility test, none of the LAB isolates exhibited motility (Table 1), indicating that our isolates lack flagella for movement and are non-motile, as LAB supposed to be. In contrast, *Morganella morganii* is motile due to the presence of peritrichous flagella distributed over its surface. These flagella enable *M. morganii* to adapt to its environment by seeking nutrients or avoiding unfavorable conditions, which explains its movement away from the inoculation point (J. Chen et al., 2024).

The inhibition screening results revealed that LAB possess the potential to inhibit the growth of *Morganella morganii*, demonstrating their antibacterial capability (Figure 1). According to Kusharyati et al. (2021), LAB can produce bacteriocins, compounds effective against both Gram-positive and Gram-negative pathogens. Bacteriocins perform optimally in neutral pH media, increasing pathogen membrane permeability and damaging pore structures. Lactic acid produced by LAB may also contribute to inhibit the growth of *M. morganii*. These findings strengthen the potential of LAB as antibacterial agents for controlling *M. morganii*. However, the inhibition tests showed that LAB isolates from kimchi solids and sauce produced weak inhibition zones compared to antibiotics, which exhibited strong inhibition. This indicates the need for further efforts to enhance LAB efficacy in controlling *M. morganii*.

Although specific studies on the effects of chloramphenicol on *M. morganii* are lacking, research by Sood (2016) demonstrated that chloramphenicol has strong antimicrobial activity against Gram-negative bacteria. *M. morganii* is known to be lacking of intrinsic resistance to chloramphenicol and is highly sensitive to this antibiotic (Zaric et al., 2021). Chloramphenicol is a broad-spectrum antibiotic that inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit. This mechanism blocks the activity of peptidyl transferase, disrupting protein translation and ultimately halting bacterial growth or causing cell death. However, the strong bactericidal effects of chloramphenicol also pose potential long-term side effects if used continuously (Madavi and Sonwane 2024).

In the main experiment of inhibition tests for LAB, it was observed that LAB could inhibit *M. morganii*, although only in the weak category. According to Darbandi et al. (2022), LAB, particularly from the

Lactobacillus genus, exhibit limited antagonistic activity due to low production of bacteriocins and organic acids, making them less effective against *M. morganii*. The high acid tolerance of *M. morganii* is another limiting factor, as the bacteria can survive at pH levels as low as 3.5 (Chen et al., 1989), allowing growth to continue even in acidified environments created by LAB.

In the case of bacteriocins produced by LAB, they are also reported to be less effective against Gram-negative bacteria. As explained by Nissen-Meyer et al. (2009) and Miller (2016), bacteriocins can more easily bind and penetrate to the peptidoglycan layer of Gram-positive bacteria. In contrast, Gram-negative bacteria like *M. morganii* possess an outer membrane composed of lipopolysaccharides (LPS), phospholipids, and proteins, forming a physical barrier that prevents bacteriocin entry (Gong et al., 2021). This outer membrane reduces permeability, which is crucial for bactericidal activity, thereby limiting the antibacterial effectiveness of LAB against *M. morganii*.

Further research is needed to enhance LAB efficacy, such as incorporating natural additives to boost acid and bacteriocin production. Additionally, exploring genetically engineered LAB strains could help develop variants capable of producing bacteriocins and acids with stronger inhibitory effects.

CONCLUSION

This study demonstrated the potential of lactic acid bacteria (LAB) from kimchi as natural inhibitors of *Morganella morganii*, a histamine-producing pathogen in seafood. Although their inhibitory effect was weak, LABs show promise as eco-friendly alternatives to chemical preservatives. Their GRAS status and safety make LAB a viable option, but enhancing their antimicrobial activity through improved bacteriocin and acid production or genetic modifications is necessary. Future research should focus on optimizing LAB applications for seafood preservation and overcoming challenges posed by Gram-negative bacterial membranes to develop sustainable and effective food safety solutions.

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