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Evaluation of Plantaricin Gene Expression in *Lactiplantibacillus plantarum Strain SU-KC1a*

[*Evaluasi Ekspresi Gen Plantarisin pada Lactiplantibacillus plantarum Strain SU-KC1a*]

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ABSTRACT

Lactobacillus species are normal gastrointestinal flora and are commonly used in the food industry for fermented milk products. Due to their probiotic properties, *Lactobacillus* strains are considered safe and effective for human consumption, with antimicrobial activity often attributed to bacteriocins. *Lactobacillus plantarum* (known as *Lactiplantibacillus plantarum*) produces plantaricin, a ribosomally synthesized bacteriocin with activity against a range of pathogens and potential as a bio-preservative. In this study, *L. plantarum* SU-KC1a, isolated from human breast milk, was assessed for its antimicrobial activity through the production of plantaricin. Since plantaricin is typically produced at low concentrations, reverse transcription polymerase chain reaction (RT-PCR) was employed. PCR amplification of the *plnJ* and *plnK* genes resulted in amplicons of approximately 168 bp and 173 bp, respectively. These findings suggest that *L. plantarum* SU-KC1a produces plantaricin JK. However, further sequence analysis is required to confirm the homology of the amplified genes with known plantaricin J and K genes in GenBank.

Keywords: antimicrobial; bacteriocin; lactic acid bacteria; probiotic; RT-PCR

ABSTRAK

Spesies *Lactobacillus* adalah flora gastrointestinal normal dan sering digunakan dalam industri makanan untuk produk susu fermentasi. Karena sifat probiotiknya, strain *Lactobacillus* dianggap aman dan efektif untuk konsumsi manusia, dengan aktivitas antimikroba yang sering dikaitkan dengan bakteriosin. *Lactobacillus plantarum* (dikenal sebagai *Lactiplantibacillus plantarum*) menghasilkan plantarisin, sebuah bakteriosin yang disintesis ribosom dengan aktivitas melawan berbagai patogen dan berpotensi sebagai bio-preservatif. Dalam penelitian ini, *L. plantarum* SU-KC1a yang diisolasi dari ASI manusia dievaluasi aktivitas antimikrobanya melalui produksi plantarisin. Karena plantarisin biasanya diproduksi dalam konsentrasi rendah, dilakukan *reverse transcription polymerase chain reaction* (RT-PCR). Amplifikasi PCR dari gen *plnJ* dan *plnK* menghasilkan produk amplifikasi sekitar masing-masing 168 bp dan 173 bp. Hasil tersebut mengindikasikan bahwa *L. plantarum* SU-KC1a menghasilkan plantarisin JK. Namun, analisis urutan lebih lanjut diperlukan untuk mengonfirmasi kesamaan gen yang diamplifikasi dengan gen plantarisin J dan K yang diketahui di GenBank.

Kata kunci: *antimikroba; bakteriosin;bakteri asam laktat; probiotik, RT-PCR*

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INTRODUCTION

Lactobacillus species are normal flora of the human gastrointestinal tract. They have been widely used in the food industry as starters for various fermented products. The growing interest in using *Lactobacillus* as a probiotic has led to the development of many probiotic products on the market, which are considered safe and effective for human consumption (Maldonado *et al*., 2004). The World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) define probiotics as live organisms which, when administered in adequate amounts, confer a health benefit on the host (Hill *et al*., 2014). One of the most important properties of a probiotic is its antimicrobial activity (Fijan, 2014).

Lactobacillus plantarum, now preferably known as *Lactiplantibacillus plantarum*, is a lactic acid bacterium (LAB) that is considered a probiotic. Various strains of *L. plantarum* are known for their ability to inhibit the growth of pathogens through several mechanisms, one of which is the production of bacteriocins. The bacteriocin produced by *L. plantarum* is known as plantaricin. Plantaricin consists of ribosomally synthesized peptides that effectively target a range of harmful bacteria through various bactericidal and bacteriostatic mechanisms. Moreover, the use of plantaricin as a bio-preservative has been widely studied due to its safety and its ability to extend the shelf life of food without altering its nutritional properties or antimicrobial activity (Todorov, 2009).

Various types of plantaricin have been identified, including plantaricin A, C, D, E, F, J, K, S, T, Y, 423, 163, 149, 35D, BN, SA6, LC74, KW30, ZJ008, LD1, UG1, NC8, C11, and NA (Meng *et al*., 2015). Additionally, several *pln* loci have been sequenced from different *L. plantarum* strains (NC8, WCFS1, J23). These loci contain approximately 22–26 genes spanning a 19 kb DNA region, which are organized into 4–6 operons. Two of these operons include transport and regulatory functions, while the remaining operons encode bacteriocins and other cognate immune proteins (Diep *et al*., 2009).

An indigenous *L. plantarum* strain isolated from human breast milk, *L. plantarum* SU-KC1a, was first reported by Sugata *et al.* (2024). This strain can inhibit pathogenic bacteria such as *E. coli* and *S. aureus*, suggesting their potential as probiotic. The antibacterial effect is likely attributed to plantaricin activity**.** However, plantaricin is usually produced in low concentrations, which makes the purification process challenging and

complicates the confirmation that the observed antibacterial activity is due to the presence of plantaricin. Previous research reported that plantaricin LR14 production reached only 59.21 µg/L, while the highest recorded plantaricin production was in *L. plantarum* A-1, at 3.5 mg/L (Tiwari & Srivastava, 2008; Hata *et al.*, 2010). These factors present a significant obstacle for further research and the application of plantaricin. In this study, the expression of the plantaricin genes in *L. plantarum* SU-KC1a under normal conditions was evaluated using RT-PCR to determine whether the gene is naturally expressed without the need for external signals or specific conditions.

MATERIALS AND METHOD

Lactobacillus plantarum strain SU-KC1a was isolated from human breast milk, as previously reported by Sugata *et al.* (2024). The culture stock was revived by growing the strain in de Man, Rogosa, and Sharpe (MRS) medium for 48 h at 37°C under microaerophilic conditions. The culture was then transferred into sterile MRS broth and incubated at 37°C until it reached the mid-exponential phase (± 4 h). Cells were harvested by centrifuging the culture at 5,000 g for 10 min and washed twice with TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]). Genomic RNA was extracted using the PathoGene-spin™ DNA/RNA Extraction Kit (iNtRON Biotechnology). Subsequently, RNA concentration was quantified using a spectrophotometer (Nanodrop).

Plantaricin genes were amplified by reverse transcription polymerase chain reaction (RT-PCR) using specific primers (Table 1). The RT-PCR was performed using the QIAGEN OneStep RT-PCR Kit. The PCR mixture composition was as follows: 10 µL QIAGEN OneStep RT-PCR Buffer (5x), 2 µL dNTPs (10 mM), 2 µL Enzyme Mix, 18 µL RNA template, 3 µL of each primer, and 12 µL RNase-free water. The final primer concentration was 10 µM. The PCR conditions are outlined in Table 2.

Table 1. Primer sequence used for plantaricin gene amplification

Genes		Sequence (5' – 3')	Annealing temp (°C)	Reference
PlnJ	F	TAA CGA CGG ATT GCT CTG		
	R	AAT CAA GGA ATT ATC ACA TTA GTC	51	Chaalel *et al.,* (2015)
PlnK	F	CTG TAA GCA TTG CTA ACC AAT C		
	R	ACT GCT GAC GCT GAA AAG	53	

Table 2. PCR conditions for plantaricin gene amplification

Stage	Cycle (times)	Temp (°C)	Duration (min)

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Reverse transcription	1	50	30
Initial PCR activation step	1	95	15
Denaturation		94	0.50
Annealing	35	49	0.50
Extension		72	1
Final Extension	1	72	10

The migration, visualization, and quantification of the migrating products were performed by electrophoresis in 2% (w/v) agarose gels. The results were visualized by ethidium bromide staining. HyperLadder™ 50 bp (Meridian Life Science Inc., USA) was used as the molecular size standard.

RESULTS AND DISCUSSION

Bacteriocins and antibiotics share similarities as bactericidal compounds; however, bacteriocins are synthesized by ribosomes, while antibiotics are typically secondary metabolites. Bacteriocins can be encoded either on the chromosome or on plasmids. Most bactericidal bacteriocins act primarily against closely related bacterial species or competing microbial species. Additionally, the genes involved in bacteriocin production and immunity are often organized into operon clusters—such as *plnABCD, plnEFI, plnJKLR, plnGHSTUVW,* and *plnMNOP*. As a result, the biosynthesis of plantaricin is considered a key factor in the development of probiotics (Basa *et al*., 2020).

Plantaricin, a type of bacteriocin, is generally recognized as safe for human consumption due to its degradation by gastric and pancreatic enzymes during digestion (Simons *et al.*, 2020). While it exhibits strong antibacterial activity and low toxicity, its limited production remains a significant barrier to industrial-scale applications. As a result, gaining insight into the regulatory mechanisms governing plantaricin synthesis is essential (Bu *et al.,* 2021).

According to Bu *et al*. (2021), the plataricin synthesis by *L. plantarum* Q7 can be divided into four stages. In the first stage (0–2 h), the bacteria were still adjusting to the environment, and plantaricin production was low. During the second stage (2–6 h), the bacteria began growing quickly, and plantaricin was produced rapidly. In the third stage (6–12 h), growth slowed down, and the production rate of plantaricin decreased, reaching its highest level at 12 h. In the final stage (12–24 h), both bacterial growth and plantaricin production stabilized, with a slight drop in production at 16 h, after which it remained steady.

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Key time points were identified: the start of production at 2 h, the fastest production at 6 h, the highest amount at 12 h and a decrease in production at 16 hours. A notable decline in yield during the later stages of fermentation is often attributed to proteolytic processes.

In this study, to determine whether *L. plantarum* SU-KC1a produces plantaricin J and K, RT-PCR analysis was performed using specific primers targeting the respective bacteriocin genes. The presence of plantaricin JK was investigated after 4 h of incubation, which is the stage when plantaricin is expected to be produced rapidly. The PCR results revealed two amplicons of approximately 168 bp and 173 bp, obtained using primers specific to *plnJ* and *plnK*, respectively (Figure 1).

This result was further confirmed by comparing the fragment sizes obtained with the plantaricin sequences found in the *L. plantarum* SU-KC1a genome. Plantaricin JK has also been identified in other *L. plantarum* strains, such as C11, WCFS1, and LbM2a (Moll *et al.*, 1999; Chanel *et al.*, 2015). *plnJK* encodes complementary peptides that must function together to achieve optimal antimicrobial activity. These two peptide bacteriocins belong to the large group of heat-stable, small, non-lantibiotic bacteriocins classified as class II bacteriocins. Both are cationic peptides, consisting of 25 and 32 amino acids, with molecular weights of 2,929 and 3,503 Da, respectively. Plantaricin JK plays a crucial role in pore formation in target cell membranes (Chanel *et al*., 2015).

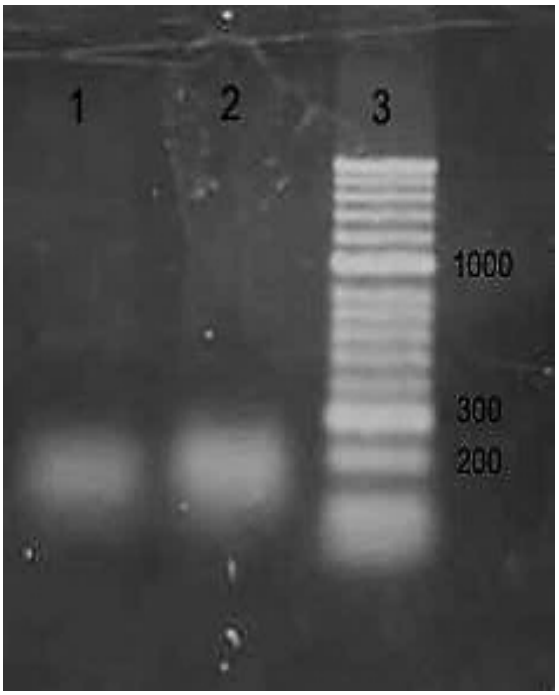

Figure 1. PCR amplification of genomic RNA from SU-KC 1a using a specific primer pair of plantaricin J (Lane 1) and K (Lane 2).
The Hyperladder 50 bp (Meridian Life Science) was used as nucleic acid molecular size marker (Lane 3).

Since no external signal was added and no specific conditions were applied to the culture, Figure 1 also suggests that plantaricin JK was naturally produced by *L. plantarum* SU-KC1. Based on the transcriptomics analysis, Bu *et* al. (2021) reported that the synthesis of plantaricin Q7 was related to ABC transport system, quorum sensing system and proteolysis

system. In accordance, a study by Diep *et al.* (2009) found that plantaricin biosynthesis in monoculture is regulated in a cell density-dependent manner through an interspecies quorum-sensing (QS) system mediated by an autoinducing peptide (AIP).

Multiple strategies have been explored to enhance plantaricin biosynthesis. Among these, co-cultivation with selected bacterial strains has emerged as a potential method to stimulate plantaricin production, likely through environmental signaling. This approach may offer advantages in the food industry by enabling the synergistic utilization of the metabolic pathways of all interacting microbial species.

CONCLUSION

The results indicated that the expression of *plnJ* (168 bp) and *plnK* (173 bp) genes was detected in *L. plantarum* SU-KC1a after 4 h of incubation under standard conditions. However, further analyses, including sequence analysis, are necessary to determine whether the two amplicons share sequence homology with the structural genes of plantaricin J and K in the GenBank database.

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