Characteristics of antibacterial activity stability of crude bacteriocin *Pediococcus acidilactici* BK01

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**Abstract.** One of the lactic acid bacteria that has the potential to produce crude bacteriocin is *Pediococcus acidilactici* BK01, which is the result of lactic acid bacteria isolated from Bekasam. Crude Bacteriocin *Pediococcus acidilactici* BK01 has a high-temperature resistance of 121°C, which is indicated by the antibacterial activity of *E. coli* O15: H7 and *S. aureus* ATCC25923 and has extensive pH stability against *Escherichia coli* O15: H7 and is sensitive to proteolytic enzymes. Crude Bacteriocin *Pediococcus acidilactici* BK01 still shows antibacterial activity during 30 days of storage at room, refrigerator, and freezer temperatures. So Crude Bacteriocin *Pediococcus acidilactici* BK01 can be recommended as a bio-preservative in processed food products involving the heating process.

1. **Introduction**

Lactic acid bacteria produce different antibacterial compounds that can inhibit the growth of gram-positive and gram-negative bacteria. One of the lactic acid bacteria is *Lactobacillus fermentum* L23, which can inhibit *Escherichia coli* and *Staphylococcus aureus* and *Listeria monocytogenes* [1-4]. Various types of antibacterial substances produced by pro-biotic bacteria are organic acids, hydrogen peroxide, diacetyl, and bacteriocin [5].

Bacteriocin produced by lactic acid bacteria is increasingly paying attention as food additives that can inhibit the growth of pathogenic bacteria as food contamination. Bacteriocin is a peptide synthesized inside ribosomes by bacteria to inhibit the growth of other bacteria. Bacteriocin has antibiotic-like properties, but several properties differ from antibiotics, i.e., bacteriocin synthesized in ribosomes, and host cells are immune to bacteriocin [6]. Bacteriocin can be produced from gram-positive bacteria, such as *Lactobacillus bulgaricus*, *Lactobacillus lactis*, *Lactobacillus acidophilus*, *Lactococcus lactis*, *Streptococcus cremoris*, *Pediococcus halophilus* and *Pediococcus cerevisiae* isolated from yogurt, cheese and fermented milk [7].

Bacteriocin can be extracted from bacteria through a propagation process in the media under environmental conditions that can induce it to produce such peptide compounds. Bacteriocin produced of various types depends on the producing strain. Bacteriocin produced by *Lactobacilli* and *Bifidobacteria* received more attention given the positive role of this bacteria in the human intestines [8].

One of the lactic acid bacteria that has the potential to produce bacteriocin is *Pediococcus acidilactici* BK01. This bacteria is the result of isolation from bekasam, a fermented fish product typical of Banyuasin area in South Sumatra, Indonesia [3]. *Pediococcus acidilactici* is widely applied to dairy processing products because it is a probiotic bacteria beneficial for health. It has an anti-
microbe activity that can inhibit the growth of pathogenic bacteria and is isolated from faeces [9]. 
*Pediococcus acidilactici* produces pediocin (class II bacteriocin) [10]. Pediocin belongs to a type of thermostable protein that can prevent food decay and kill pathogenic bacteria such as *L. monocytogenes, Enterococcus faecalis, S. aureus, Clostridium perfringens* [11, 12]. According to [13] pediocin can not only inhibit the growth of gram-positive bacteria, but also effectively inhibit gram-negative bacteria. Bacteriocin produced by *P. acidilactici* is AcH, PA-1, JD and 5 [14]. Pediocin PA-1 is an antibacterial peptide that is utilized as a bio preservative in the food processing industry [15]. Pediocin PA-1 is a bacteriocin of lactic acid bacteria with a broad spectrum and effectively inhibits *Listeria monocytogenes*, a pathogenic bacteria often found in food products [16]. [17] states that *P. acidilactici* AK effectively inhibits *S. aureus*.

However, the antibacterial characteristics of crude bacteriocin *Pediococcus acidilactici* BK01 have not been further studied. So, this study aims to find out the characteristics of antibacterial stability crude bacteriocin *Pediococcus acidilactici* BK01, i.e., the stability to temperature, pH, enzymes, and stability during storage.

2. Materials and methods

2.1. Materials

*Pediococcus acidilactici* BK01 obtained from the isolation of lactic acid bacteria from bekasam [3], can be seen in Figure 1. The media used were Man Rogossa Sharpe Agar (MRSA) (Merck), MRS Broth (Merck), Nutrient Agar (NA) (Merck), and the pathogenic bacteria tested are *Escherichia coli* O157: H7 and *Staphylococcus aureus* ATCC25923.

The equipment used were a cool box, anaerobic jar, thermometer, pH meter, petri dish, ose needle, incubator (Infors HT-ecotron), measuring glass, analytical scales, erlemeyer; bunsen lamp, test tube, measuring glass, cotton buds, beaker glass, drip pipette, hockey stick, autoclave, centrifuge 5417R, vortex, and micropipette.

![Phylogenetic tree of 16S rRNA gene of Pediococcus acidilactici BK01](image)

*Figure 1*. Phylogenetic tree of 16S rRNA gene of *Pediococcus acidilactici* BK01 [3]

2.2. Methods
This research was a descriptive research and experimental, which tested the stability of crude bacteriocin against pH, temperature, enzymes, and stability during storage.
2.2.1. Preparation of crude bacteriocin [18]. One ml of culture was incubated for 24 hours inside 9 ml of MRS broth for 24 hours at a temperature of 37°C. Then, the culture was centrifuged at 14,000 rpm for 5 minutes, and the supernatant was filtered with a filter membrane of 0.22 μl. Cell-free supernatants were set to pH 6.5 with NaOH 1 N, to remove the barrier effect due to the absence of organic acids [18]. Pathogenic bacteria were grown aerobically at 37°C for 24 hours. Then, a 0.2% pathogenic bacterial culture was put into 20 ml of MHA at a temperature of 50°C. After the MHA was solid, a well with 4 mm was made, using a cork borer.

Furthermore, as much as a 50 μl supernatant was taken, put in each well, and set aside for 15-20 minutes. It was then incubated for 24 hours at 37°C in aerobic conditions. Then measured the zone using a long-term shove. If an obstacle zone was found in the well, it could be said that lactic acid bacteria isolates contained bacteriocin compounds.

2.2.2. Crude bacteriocin stability testing to temperature [19]. The method used in this study was descriptive. Temperature treatment was: control (room temperature), 40°C, 60°C, 80°C, 100°C for 30 minutes and 121°C for 15 minutes. Resistance activity for all treatments was determined by diffusion method using *E.coli* O15:H7 and *S. aureus* ATCC25923 as bacterial indicators. The measurements were done for three replications with the standard deviation.

2.2.3. Crude bacteriocin stability testing against pH [19]. Ten ml of MRS broth was prepared to reach a pH of 2, 4, 6, 8, and 10 using 1 N HCl or 1 N NaOH and autoclaved. After that, it was incubated for 2 hours at a temperature of 37°C, and each pH was set back to pH 6.0. Resistance activity for all treatments was determined by diffusion method using *E.coli* O15 : H7 and *S. aureus* ATCC25923 as bacterial indicators. The measurements were done for three replications with the standard deviation.

2.2.4. Crude bacteriocin stability against enzymes [20]. Supernatant was dissolved in a 20 μl solution of protease-K enzymes (pH 7), and 20 μl trypsin (pH 7) (Sigma-Aldrich), 20 μl enzyme solution in NaOH or pH phosphate buffer 7) and incubated for 2 hours at 37°C. Then, the mixtures were boiled for 5 minutes. Enzyme-sensitive supernatant was defined as those that did not produce clear zones.

2.2.5. Crude stability of bacteriocin during storage. The crude bacteriocin was stored at 37°C, refrigerator temperature (4-10°C), and freezer temperature (-20°C) for 30 days. Inhibition activity was measured by all treatment determined by clear zone created against Indicator strains (*E.coli* O15:H7 and *S. Aureus* ATCC 25923). Each treatment was repeated three times followed by a standard deviation

3. Results and discussion

3.1. Stability of crude bacteriocin on temperature

| Temperature (°C) | *E. coli* O15: H7 | *S. aureus* ATCC25923 |
|------------------|-------------------|-----------------------|
| Room Temperature | 20.76 ± 0.23      | 7.12 ± 0.34           |
| 40               | 20.25 ± 0.89      | 7.12 ± 0.34           |
| 60               | 20.25 ± 0.89      | 7.63 ± 0.67           |
| 80               | 20.25 ± 0.89      | 6.11 ± 0.45           |
| 100              | 18.74 ± 0.47      | 6.62 ± 0.78           |
| 121              | 20.25 ± 0.89      | 6.62 ± 0.78           |

Values are mean of triplicate and presented as means ± S.E.
The stability of crude bacteriocin from *Pediococcus acidilactici* BK01 at some room temperature (control), 40, 60, 80, 100 for 30 minutes, and 121°C for 15 minutes can be seen in Table 1. Crude bacteriocin stability against temperature using pathogenic bacteria *E. coli* O15: H7 and *S. aureus* ATCC25923. *E. coli* O15: H7 has a pretty good resistance at various temperatures. The rise of temperature up to 121°C still showed resistance activity with a diameter of inhibition zone 20.25 ± 0.89. This inhibition is higher than *S. aureus* ATCC25923.

The results show that crude bacteriocin was resistant to high temperatures to be later utilized as a bio-preservative in the processing of food products. Similar to [21] research, *Lactobacillus coryformis* subsp. *Torquens* JCM 1166 has a temperature resistance of 121°C for 30 minutes. The resistance demonstrated its ability to inhibit the growth of *Escherichia coli* ATCC25922 and *Staphylococcus aureus* ATCC2913. Moreover, CV7 bacteriocin produced by *Enterococcus faecalis* CV7, has antibacterial activity at temperatures of 30, 50, 70, and 90°C for 20 minutes and still has antibacterial activity at 121°C for 10 minutes [21].

### 3.2. Stability of crude bacteriocin on pH

| pH | *E. coli* O15: H7 | *S. aureus* ATCC25923 |
|----|------------------|----------------------|
| 2  | 26.65 ± 0.12     | 10.49 ± 0.56         |
| 4  | 24.96 ± 0.28     | 8.80 ± 0.25          |
| 6  | 22.61 ± 0.28     | 0                    |
| 8  | 18.23 ± 0.47     | 0                    |
| 10 | 0                | 0                    |

Values are mean of triplicate and presented as means ± S.E

Table 2 presents, it can be seen the results of crude bacteriocin *Pediococcus acidilactici* BK01 stability test against pH 2, 4, 6, 8 and 10 by using test bacteria *E. coli* O15: H7 and *S. aureus* ATCC25923. Crude bacteriocin still has resistance activity to *E. coli* O15: H7 to pH 8 (18.23 ± 0.47) and decreased compared to pH 2 (26.65 ± 0.12). The decrease in inhibition activity in pH eight and the absence of activity in pH 10, caused by the denaturation of proteins in the alkaline pH condition. This was in accordance with the opinion of [21], the presence of strong intramolecular electrostatic interactions arising from dissociation between amino acids and carboxyl groups resulting in an extreme pH (alkaline), resulting in the denaturation of proteins. Meanwhile, crude bacteriocin stability against *S. aureus* ATCC25923 was only shown at pH 2, and 4. [22] reported that Bacteriocin CV7 was produced from *Enterococcus faecalis* CV7 was active at pH 2, 4, 6, 8, 10, and 12. [23] explained Pentocin MQ1 was stable at pH 2-5, and activity decreased at pH 8-10.

### 3.3. Stability of crude bacteriocin on enzymes

| Treatment | *E. Coli* O15: H7 Inhibition Zone (mm) | *S. aureus* ATCC25923 Inhibition Zone (mm) |
|-----------|--------------------------------------|--------------------------------------------|
| Control   | 20.76 ± 0.23                         | 6.54 ± 0.23                               |
| Protease-K| 0                                    | 0                                          |
| Trypsin   | 0                                    | 0                                          |

Values are mean of triplicate and presented as means ± S.E
Antibacterial activity of crude bacteriocin *Pediococcus acidilactici* BK01 can be partially or overall activated with proteolytic enzymes. Sensitivity to proteolytic enzymes (Protease-K and Trypsin), where examined to see how enzymes affect the antibacterial activity of crude bacteriocin. Table 3 shows the crude bacteriocin with the addition of protease-K enzymes and trypsin had no inhibition activity. This indicated that bacteriocin was a protein inactive by protease enzymes so as not to show its action. In contrast, at the controls (without the addition of enzymes), crude bacteriocin exhibited its activity against *E. Coli* O15: H7 (20.76 ± 0.23) and *S.aureus* ATCC25923 (6.54 ± 0.23). [24], stated that the antibacteriocin CV7 antibacteriocin activity, effective against proteolytic enzymes such as proteinase-k, trypsin, chymotrypsin, and bacteriocin from *E. faecalis* DU10, was sensitive to proteinase k and pepsin and ineffective against lipase [22].

3.4. Stability of crude bacteriocin during storage
Crude bacteriocin stability during 30 days storage at various temperatures (room temperature refrigerator temperature, and freezer temperature) against *E. Coli* O15 : H7 and *S. aureus* ATCC25923 can be seen in this table 4 below.

| Storage Temperatures | E. coli O15: H7 | S. aureus ATCC25923 |
|-----------------------|----------------|---------------------|
| Room temperature      | 18.33 ± 0.11   | 5.24 ± 0.22         |
| Refrigerator          | 22.33 ± 0.27   | 6.15 ± 0.47         |
| Freezer               | 32.33 ± 0.49   | 6.20 ± 0.47         |

Values are mean of triplicate and presented as means ± S.E

The results show that crude bacteriocin had inhibition activity against tested bacteria, *E. coli* O15: H7 and *S. aureus* ATCC25923 during 30 days of storage at different temperatures. Activity resistance to *E. coli* O15:H7 at room temperature, refrigerator temperature, and freezer temperature were 18.33, 22.33, and 32.33, respectively. In general, the resistance activity of *S. aureus* ATCC25923, in-room temperature, refrigerator temperature, and freezer temperature was lower than that of *E. coli* O15 : H7 (Table 4). The result indicates that bacteriocin, which was a protein, was stable at low temperatures.

4. Conclusions
Crude bacteriocin *Pediococcus acidilactici* BK01 is stable on sterilization temperature (121 °C for 15 minutes and has a wide range pH. However, the bacteriocin had inactivated by proteolytic enzyme (protease-K and trypsin). Crude Bacteriocin. *Pediococcus acidilactici* BK01 still leads to antibacterial activity during 30 days of storage at the room, refrigerator, and freezer temperatures. Crude Bacteriocin *Pediococcus acidilactici* BK01 can be recommended as bio preservative in processed food products involving the heating process.

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