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Antibacterial Activity of Bacteriocin of *Bifidobacterium longum* against *Salmonella typhimurium*

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Abstract **Original Research Article**

Salmonella typhimurium is a food-borne pathogen that causes gastroenteritis. Until now, antibiotics have been used to treat gastroenteritis. Nowadays, some antibiotics have become resistant to a variety of pathogens. Therefore, alternative treatments based on natural ingredients need to be explored. Lactic acid bacteria, or so-called probiotics, that are naturally found in human digestion have been studied for their antibacterial properties, such as bacteriocins. The purpose of this study is to see if bacteriocin from *Bifidobacterium longum* FNCC 0210 has any inhibitory activity against *Salmonella typhimurium* ATCC 14028 in vitro. Bacteriocin was extracted from *B. longum* as cell-free culture supernatant (CFCS) in MRS broth, which was neutralized at pH 6.5 with 1 N NaOH and heated at 100°C for 10 minutes. The antibacterial assay of *B. longum* bacteriocin against *S. typhimurium* was performed with the agar-well diffusion method with concentrations of 100, 50, 25, 12.5, 6.25% (v/v), and determined based on the diameter of the zone of inhibition (mm). The results of the tests revealed that *B. longum* CFCS 100% (v/v) had the highest antibacterial activity against *S. typhimurium* with intermediate category. This study provides information on the ability of *B. longum* bacteriocin that has antibacterial activity against food-borne pathogens.

Keywords: Antibacterial, antibiotic resistance, *Bifidobacterium longum*, Bacteriocin, *Salmonella typhimurium*.

INTRODUCTION

Salmonella typhimurium also known as Non-typhoidal Salmonella (NTS), is a pathogenic bacterium that can cause gastroenteritis or stomach flu [1]. Gastroenteritis can occur because of eating pathogen-contaminated food (food-borne disease) and has been treated with ORS fluids, antidiarrheal drugs (zinc), antibiotics, and other medications. However, using chemicals as medicine carries the risk of causing side effects such as disrupting normal microflora in the digestive tract and causing antibiotic resistance [2]. Therefore, research into alternative medicine that is less risky, such as the use of natural ingredients, is critical.

Lactic Acid Bacteria (LAB), also known as probiotics, are non-pathogenic bacteria that naturally exist in the digestive tracts of humans and animals. The presence of LAB is essential for digestive health because it regulates the balance of intestinal microflora, boosts the immune system, and inhibits the growth of harmful bacteria [3]. The antibacterial compounds it produces, one of them is bacteriocin, give it this inhibitory activity. Hence, LAB can be used as an antibacterial agent.

The genus Bifidobacterium was found to dominate the human digestive tract in the entire age range (infants to the elderly) [4]. *Bifidobacterium longum*, a species of Bifidobacterium, has been shown to adhere and survive in human intestinal mucus, which supports its potential use when consumed later [5]. Furthermore, it has been discovered that *B. longum* bacteriocin was able to inhibit Gram-positive and Gram-negative bacteria, specifically *E. coli*, *P. aeruginosa*, *Enterococcus faecalis*, and *S. aureus* in vitro [6,7]. Thus, probiotics as natural ingredients should be investigated for their inhibitory potential against pathogens. The goal of this study is to know the inhibitory activity of bacteriocin from the probiotic *B. longum* against *S. typhimurium* with in-vitro experiments.

MATERIALS AND METHODS

Pathogen and Probiotic Strain

The pathogen used in this study is *Salmonella typhimurium* ATCC 14028 which was obtained from American Type Culture Collection, Yogyakarta Health and Calibration Laboratory. The probiotic,

Bifidobacterium longum FNCC 0210 was obtained from Food and Nutrition Culture Collection (FNCC), Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta.

Preparation of bacteriocin-like substance

B. longum stock culture was grown in MRS broth which was neutralized at pH 6.5 with 1 N NaOH solution, then incubated at 37 °C for 36 hours. The growth-culture centrifuges at 6.000-rpm for 15 minutes to obtain cell-free culture supernatant (CFCS). The supernatant was then heated at 100° C for 10 minutes, collected, and kept in the refrigerator until it would be used [8].

Determination of Antibacterial Activity

The agar-well diffusion assay was conducted modifying the Kirby-Bauer method. Before the assay, pathogen strain was pre-cultured overnight on Brain Heart Infusion Broth (BHI, Merck^{TM}) at 37ºC for 24 hours. The growth culture was centrifuged at 6,000 rpm for 15 minutes. Individual colonies were suspended in sterile distilled water until it was reaching a turbidity corresponding to McFarland 0.5 standard or equivalent as 1, 5 x 108 CFU/ml. [9-11].

The pathogen suspense was spread on the Mueller Hinton Agar (MHA, Merck^{TM}) by sterile cotton swab. Ciprofloxacin lactate (2 mg/ml, Kalbe) was used as a positive control and sterile distilled water as a negative control. *B. longum* CFCS in 100, 50, 25, 12.5, 6.25% (v/v) was added to each well and incubated at 37 °C under aerobic conditions for 24 h. The assays were carried out three times in duplicate [9, 10].

DATA ANALYSIS

The antibacterial activity was detected by measuring the zone of inhibition (clear zone) around the well containing samples. The diameter zone of inhibition was analyzed descriptively based on the categories: Susceptible (S) (≥ 20 mm), Intermediate (I) (15-19 mm), and Resistant (R) (≤ 14 mm) [9, 10].

RESULT AND DISCUSSION

Bacteriocins are nanomolar-sized proteins or protein complexes produced by LAB to inhibit the growth of other bacteria and are not toxic to the bacteria themselves [12]. Bacteriocin is a unique component of each LAB that is secreted outside the cell and has bacteriostatic and bactericidal activity. LAB produces bacteriocins during the exponential growth phase. Bacteriocin activity decreases when the incubation time reaches the stationary phase due to the release of protease enzymes from cells that enter the death stage [13].

The supernatant was harvested during the exponential phase, at 36 hours, as cell-free culture supernatant (CFCS) containing bacteriocin in this study. The neutralization of the media at pH 6.5 aims to neutralize the organic acids produced by *B. longum*, whereas heating at 100 °C for 10 minutes deactivates the existing protease enzymes, hydrogen peroxide, and other antibacterial components. This treatment is also based on the properties of heat-resistant bacteriocins [12].

Ciprofloxacin (2 mg/mL) was used as a positive control, with an inhibition zone diameter of 17 mm (intermediate category). Because of its sensitivity to *Salmonella sp* bacteria, Ciprofloxacin was used as a positive control [10]. Ciprofloxacin is an antibiotic in the fluoroquinolone class. This antibiotic works by interfering with enzymes involved in DNA repair, transcription, recombination, and replication, thereby inhibiting the growth of pathogenic bacteria [14].

The presence/absence of an inhibition zone was determined using the modified Kirby-Bauer diffusion method to determine the effect of *B. longum* bacteriocin on *S. typhimurium* growth. The diameters of the inhibition zone are shown in Table 1.

Table-1: Diameter Inhibition Zone of *B. longum* bacteriocin against *S. typhimurium*

***B. longum* CFCS**	**Diameter Inhibition Zone (mm) ± STD1**	**Category**
100%	18,50±1,76	Intermediate
50%	14,00±0,57	Resistant
25%	0,00±0,00	ND
12,5%	0,00±0,00	ND
6,25%	0,00±0,00	ND
Positive control*	46,67±2,88	Susceptible
Negative control**	0,00±0,00	ND

Diameter inhibition zone (DIZ, mm) includes the diameter of well (6 mm); values are expressed as the means±SD (n=3).
ND: Not detected if the DIZ value is ≤ 6 mm.
*Positive control: Ciprofloxacin lactate (2 mg/ml)
**Negative control: Distilled water

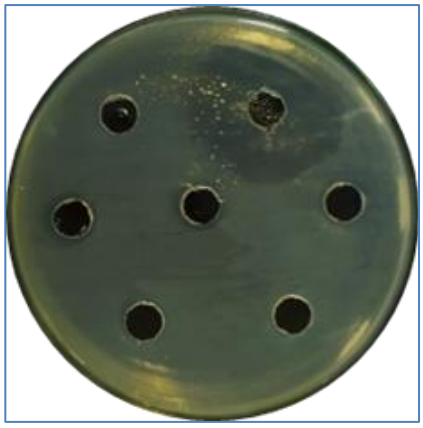

Fig-1: Antibacterial activity of *B. longum* bacteriocin against *S. typhimurium* by agar-well diffusion method

According to (Table 1), *B. longum* bacteriocin inhibited the growth of *S. typhimurium* only at 100% concentration with intermediate category which is shown in Figure 1. The bactericidal mechanism of bacteriocin involves disrupting pathogenic bacterial cells' cytoplasmic membrane potential. Bacteriocins that meet the pathogen cell membrane cause membrane permeability to be reduced and the Proton Motive Force (PMF) to be eliminated, resulting in cytoplasmic membrane potential instability. This causes cell leakage or whole formation, which results in cell death [15].

A resistant category was detected at a concentration of 50%, and no clear zone was detected at a lower concentration below that (Table 1 & Figure 1). Because of the less-than-ideal diffusion process between the sample, well, and media, the absence of the inhibition zone by the sample in the media was possible. Furthermore, the strain or species of LAB that produces bacteriocins with distinct properties influences bacteriocin production [16].

CONCLUSIONS

Bacteriocin from *Bifidobacterium longum* has antibacterial activity against *Salmonella typhimurium* at 100% concentration with an intermediate inhibition category. Thus, it can be used as an alternative treatment for gastroenteritis and other food-borne diseases. Further research still needs to be done to explore the potential probiotics against food pathogens.

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