# *Antibacterial Activity of Bacillus sp. HSFI Isolated from Holothuria scabra*

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**ABSTRACT**

*Staphylococcus aureus* and *Escherichia coli* are important bacteria that cause various infectious diseases. Clinically, the incidence of antibiotic resistance against these two bacteria needs serious attention. Therefore, the search for new antibiotics is important. Microorganisms including bacteria can be a source of discovery of new antibiotics. This study carried out the extraction of secondary metabolites from nine *Bacillus sp.* HSFI isolated from intestinal fermented sea cucumber (*Holothuria scabra*). Secondary metabolites of *Bacillus sp.* HSFI was produced using media with Starch, Yeast, Peptone, and then extracted using ethyl acetate. As much 20 µg of extract of each *Bacillus sp.* HSFI was tested for antibacterial activity using the disc diffusion method. The results showed that the ethyl acetate extract of *Bacillus sp.* HSFI-2 and HSFI-9 can produce *S. aureus* growth inhibition zone with diameters of 6.7 and 17.0 mm, respectively. Meanwhile, *E. coli* can be inhibited by ethyl acetate extracts of *Bacillus sp.* HSFI-2, HSFI-4, HSFI-6, and HSFI-9 with inhibition zone diameters of 3.0; 2.0; 2.67, and 11.3 mm, respectively. Ethyl acetate extract of *Bacillus sp.* HSFI-2 and HSFI-9 potential to be developed into antibiotics because they have moderate to strong inhibitory activity against *S. aureus* and *E. coli*.

**Keywords:** Antibacterial agent; *Bacillus sp.*; drug resistance; *Escherichia coli*; *Staphylococcus aureus*

## Introduction

*Staphylococcus aureus* and *Escherichia coli* are pathogenic bacteria that can cause various infectious diseases. Both bacteria can enter the bloodstream or internal tissues, and have the potential to cause serious infections such as blood stream or systemic infections (1)(2). For example, *S. aureus* can cause *Staphylococcus aureus* Bacteremia (SAB) as well as endocarditis. *Escherichia coli* which is also a normal flora of the human gut can be pathogenic. Some strains of *E. coli* can cause various diseases such as diarrhea, urinary tract infections, meningitis, and even sepsis which can lead to death (3).

Clinically, *S. aureus* and *E. coli* need attention, especially because of the development of antimicrobial resistance (AMR) (4). In the last two decades the incidence of SAB has been controlled, but recently there has been a fluctuation in this number due to the presence of Methicillin-Resistant *S. aureus* (MRSA) (5). Meanwhile, because *E. coli* is also found in the intestines, it can often be exposed to antibiotics and put it at antibiotic resistance risk (6).

The problem of resistance is one of the reasons for the discovery and development of new antibiotics. There are many ways to find new antibiotics, including synthesizing chemical compounds and searching for natural sources (7). Known sources of natural products include secondary metabolites produced by plants, fungi, microbial endophytes, and also marine microorganisms (8).

Secondary metabolites are metabolic products that do not play an important role in the growth, development and reproduction of a microorganism. The formation of secondary metabolites depends on the physical and chemical

conditions around the producing microorganisms, such as the amount of water, pH, the amount of oxygen, and nutrients (9). Secondary metabolites produced by microorganisms usually function to support the life of these microorganisms in the wild. In addition, these secondary metabolites are also known to have various kinds of bioactivity such as antimicrobial, enzyme inhibitor, antitumor, immunosuppressant, antiparasitic, plant growth stimulant, herbicide, insecticide, or antihelmintic (10).

One of the microorganism sources for the discovery of new antibiotics is *Bacillus sp*. An example, *Bacillus pumilus* can produce secondary metabolites that can inhibit the growth of *S. aureus, E. coli, Aspergillus niger,* and *Aspergillus flavus* (11). Other species that can also produce antibacterial compounds include *Bacillus subtilis* (12), *Bacillus sp*. strain JS04 (13), *Bacillus amyloliquefaciens* (14)(15), and *Bacillus tequilensis* (16).

Therefore, this study will explore antimicrobials from the extraction of secondary metabolites of *Bacillus sp*. Nine *Bacillus sp*. HSFI (*Holothuria scabra* Fermented Intestine) has been isolated from intestinal fermented sea cucumber (*Holothuria scabra*). The results of previous studies showed that the nine isolates could produce proteolytic and thrombolytic enzymes (17), However, studies showing the antibacterial potential of its secondary metabolites have never been carried out.

## METHOD

### *Research Design and Object*

This research is an exploratory experimental study to determine the antibacterial activity of the secondary metabolites produced by nine isolates of Bacillus sp. The nine isolates were named *Bacillus sp*. HSFI-2, HSFI-4, HSFI-5, HSFI-6, HSFI-8, HSFI-9, HSFI-10, HSFI-11, and HSFI-12. Activity tests were carried out on *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922).

### *Culture of Bacillus sp. HSFI to Produce Secondary Metabolites and the Extraction Process*

Each *Bacillus sp*. HSFI was pre-cultured using 2 mL of Mueller-Hinton Broth (MHB) for 24 hours at room temperature. Furthermore, bacteria growth in pre culture process continued to the culture process using SYP NaCl media (1.0% soluble starch, 0.4% yeast extract, 0.2% bacto peptone, and 0.5% NaCl in 1 L sterile water). Each *Bacillus sp*. HSFI was then cultured at an incubation temperature of 28-30 °C, using an orbital shaker at a speed of 120 rpm for 72 hours(18).

The supernatant from the culture of each *Bacillus sp*. HSFI was separated from the cells using a centrifuge at 6000 rpm for 15 minutes. The separated supernatant was extracted with ethyl acetate (1:1) v/v. After that, the ethyl acetate fraction was separated using separator funnel, and then dried using a ceramic dish for 24 hours. The dried extract was put into a sterile microtube and dissolved with 5% DMSO until the test concentration was obtained(18).

### *Antibacterial activity testing of secondary metabolites of Bacillus sp. HSFI*

Antibacterial activity test of ethyl acetate extract of *Bacillus sp*. HSFI was performed by disc diffusion method using Mueller-Hinton Agar (MHA) media. *Staphylococcus aureus* and *E. coli* were prepared by suspension in 0.9% NaCl until the turbidity was equivalent to 0.5 McFarland. Bacterial suspension was grown in MHA using streak plate method.

The concentration of each ethyl acetate extract of *Bacillus sp*. HSFI was 1000 µg/mL and was dripped onto a paper disc as much as 20 µL. Furthermore, a paper disc containing ampicillin 5 µg was used as a control antibiotic for the *S. aureus* susceptibility test and meropenem 10 µg was used as a control antibiotic for the *E. coli* susceptibility test. The activity test was carried out by triplication, and incubation was carried out for 24 hours at 37°C (19). The inhibition zone formed was measured with the calculation results are categorized into weak, moderate, strong, and very strong (20).

## RESULT

Based on the test results of the ethyl acetate extract of *Bacillus sp*. HSFI against *S. aureus*, it was found that the ethyl acetate extract of *Bacillus sp*. HSFI-2 and HSFI-9 could inhibit the growth of *S. aureus*. Meanwhile, ampicillin strongly inhibit the growth of *S aureus* (Table 1 and Figure 1).

*Table 1. Mean ± standard deviation (SD) of inhibition zone of ethyl acetate extract of Bacillus sp. HSFI, ampicillin, and 5% DMSO against S. aureus*

| Treatment Group | Mean ± SD | Inhibition Category |
|---|---|---|
| 5% DMSO | 0 ± 0.0 | No inhibition |
| Ampicillin 5 µg | 44.0 ± 0.0 | Very Strong |
| *Bacillus sp*. HSFI-2 | 6.7 ± 0.57 | Moderate |
| *Bacillus sp*. HSFI-9 | 17.0 ± 1.89 | Strong |

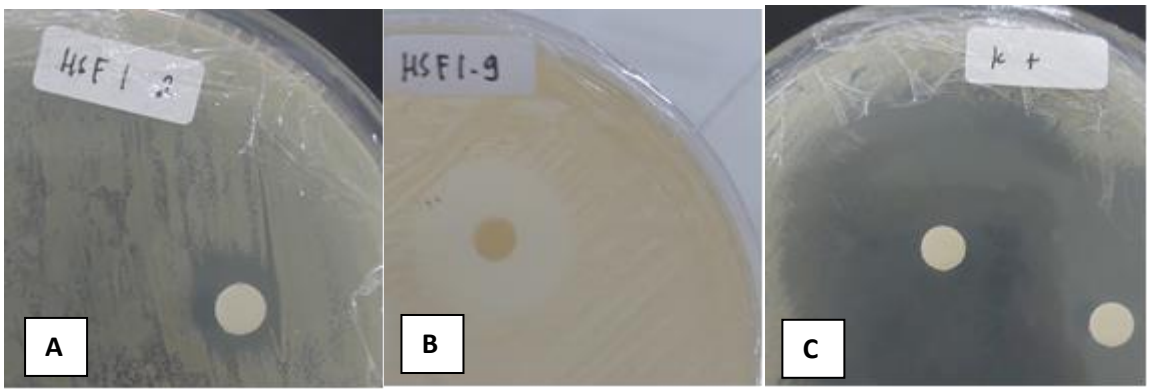


Figure 1. Photograph of growth inhibition zone of *S. aureus* due to administration of a) 20 µg of *Bacillus sp*. HSFI-2 ethyl acetate extract, b) 20 µg of *Bacillus sp*. HSFI-9 ethyl acetate extract, and c) positive control (5 µg ampicillin).

For the disc diffusion test results of ethyl acetate extract of *Bacillus sp*. activity against *E. coli*, it was found that ethyl acetate extract of *Bacillus sp*. HSFI-2, HSFI-4, HSFI-6, HSFI-9 have the potential to inhibit the growth of *E. coli*. The inhibition ability of ethyl acetate extracts of *Bacillus sp*. HSFI-2, HSFI-4, and HSFI-6 was in the weak category, while the ethyl acetate extract of *Bacillus sp*. HSFI-9 could inhibit in the strong category. The positive control (10 µg meropenem) could very strongly inhibit the growth of *E. coli* with the inhibition zone diameter reaching 32 mm (Table 2 and Figure 2).

*Table 1. Mean ± standard deviation (SD) of inhibition zone of ethyl acetate extract of Bacillus sp. HSFI, meropenem, and 5% DMSO against E. coli*

| Treatment Group | Mean ± SD | Inhibition Category |
|---|---|---|
| 5% DMSO | 0 ± 0.0 | No inhibition |
| Meropenem 10 µg | 32.0 ± 0.0 | Very strong |
| *Bacillus sp.* HSFI-2 | 3.0 ± 1.0 | Weak |
| HSFI-4 | 2.0 ± 0.0 | Weak |
| HSFI-6 | 2,67 ± 2,31 | Weak |
| HSFI-9 | 11,3 ± 0,57 | Strong |

## DISCUSSION

In this study, both *S. aureus* and *E. coli* were still sensitive to control antibiotics. *Staphylococcus aureus* is still sensitive and its growth can be inhibited by ampicillin, while *E. coli* is still sensitive to meropenem. *Staphylococcus aureus* was declared sensitive to ampicillin if the diameter of the inhibition zone was ≥ 29 mm. Meanwhile, *E. coli* is still sensitive to meropenem if an inhibition zone of ≥ 16 mm is formed (21).

Ampicillin is a penicillin class of antibiotics, while meropenem is a carbapenem antibiotic. Both are antibiotics with a β-lactam structure. Both antibiotics can bind to the Penicillin Binding Protein (PBP), then inhibit the transpeptidation reaction during the formation of the bacterial cell wall, so that the bacterial cell wall can be lysed (22).

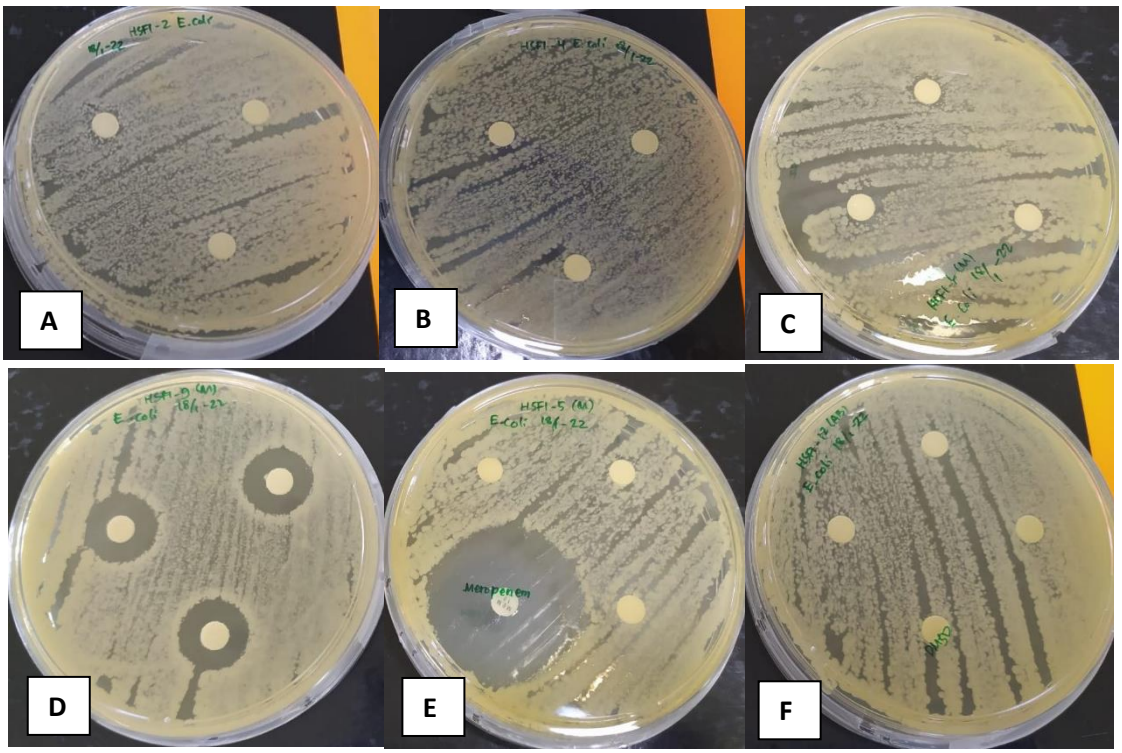


Figure 2. Photograph of growth inhibition zone of *E. coli* due to administration of a) 20 µg of *Bacillus sp*. HSFI-2 ethyl acetate extract, b) 20 µg of *Bacillus sp*. HSFI-4 ethyl acetate extract, c) 20 µg of *Bacillus sp*. HSFI-6 ethyl acetate extract, d) 20 µg of *Bacillus sp*. HSFI-9 ethyl acetate extract, e) positive control (10 µg meropenem), and f) negative control (5% DMSO)

For negative control, five percent Dimethyl Sulfoxide (DMSO) was used because it is a solvent for *Bacillus sp*. HSFI extract. Dimethyl Sulfoxide is a colorless liquid which is generally used as a polar aprotic solvent which is miscible with water and is capable of dissolving various kinds of polar and non-polar molecules (23).

Meanwhile, for the test extract from *Bacillus sp*. HSFI, it was found that there were four *Bacillus sp*. HSFI which have the potential to produce antibacterial compounds, including *Bacillus sp*. HSFI-2, HSFI-4, HSFI-6, and HSFI-9. However, based on the inhibition produced, the ethyl acetate extract of *Bacillus sp*. HSFI-2 and HSFI-9 was the most potent extract because it could inhibit the growth of both *S. aureus* and *E. coli*, and had moderate to strong inhibitory capacity.

Antibacterial compounds contained in the ethyl acetate extract of *Bacillus sp*. HSFI-2 and HSFI-9 have not been identified. Species of *Bacillus sp*. HSFI-2 and HSFI-9 have also not been identified molecularly using 16S rRNA gene sequencing, so it is not possible to estimate the content of compounds that may be present in the two extracts. *Bacillus sp*. itself is known to produce antibacterial compounds. For example, in *Bacillus subtilis*, it is known to produce compounds that have an antibacterial activity, namely benzaldehyde and acetophenone (12). Benzaldehyde can work by causing bacterial lysis (24), while acetophenone works by inhibiting the bacterial nutrition, so that these bacterial cells cannot grow (25). The types of compounds that can be produced by *Bacillus sp*. were varies depending on the source of nutrients in the culture media used (16).

In this study, there were differences in the activity of *Bacillus sp*. HSFI ethyl acetate extract on the growth of *S. aureus* and *E. coli*. In tests using *S. aureus*, only *Bacillus sp*. HSFI-2 and HSFI-9 extracts were able to inhibit the growth of *S. aureus*. Meanwhile, for the test using *E. coli*, the extracts that were able to inhibit the growth of these bacteria were the ethyl acetate extract of *Bacillus sp*. HSFI-2, HSFI-4, HSFI-6, and HSFI-9. Apart from the different compounds in each ethyl acetate extract of *Bacillus sp*. HSFI, the difference in activity could also be due to differences in the nature of the two types of bacteria tested. *Staphylococcus aureus* is a Gram positive bacteria, while E. coli is a Gram negative. If the assumption of the mechanism of action of antibacterial compounds contained in the secondary metabolite extract is bacteriolytic, it is necessary to consider the differences in the structure of the bacterial cell walls of *S. aureus* and *E. coli*. Gram positive bacteria have a thicker layer of peptidoglycan, while Gram negative bacteria have a double membrane system with the outer membrane covered by a series of lipopolysaccharides (26).

## CONCLUSION

*Bacillus sp*. HSFI -2 and HSFI-9 have the potential to produce antibacterial compounds that inhibit the growth of *S. aureus* and *E. coli*. The inhibitory capacity of ethyl acetate extract of *Bacillus sp*. HSFI-2 was categorized as moderate, while *Bacillus sp*. HSFI-9 was classified as strong inhibitor.

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