Molecular Identification, GC-MS Analysis of Bioactive Compounds and Antimicrobial Activity of Thermophilic Bacteria Derived from West Sumatra Hot-Spring Indonesia

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1. Introduction

Antibiotics have been used a lot to treat diseases caused by pathogenic microbial infections. Antibiotics are compounds that can inhibit the growth of pathogenic microorganisms, including bacteria and fungi (Gould 2016). The ability of microorganisms to modify themselves due to antibiotics has led to an increase in the resistance of pathogenic microorganisms to currently used antibiotics (Peterson and Kaur 2018). MRSA (methicillin-resistant Staphylococcus aureus) and VRSA (vancomycin-resistant Staphylococcus aureus) are examples of infection with antibiotic-resistant pathogenic bacteria. Cases of antibiotic resistance continue to increase every year (Scheffler et al. 2013). The increasing antimicrobial resistance of isolates found in dairy products due to contamination S. aureus and MRSA on these food products (Alghizzi and Shami 2021). According to the World Health Organization (WHO), antibiotic resistance has posed a severe threat to human health worldwide (Romandini et al. 2021). Based on data from WHO, at least 700,000 people worldwide die each year due to antibiotic resistance, of which around 200,000 are newborns (WHO 2021). This case is expected to increase in 2050 to 10 million (Mancuso et al. 2021). Data from the Indonesian Ministry of Health in 2019 shows an increase in antibiotic resistance to antibiotics Carbapenems, Fluoroquinolones, and third-generation cephalosporins for some bacteria such as E.coli and K. Pneumoniae (R.I. Ministry of Health 2020). Antibiotic resistance in Indonesia...
can also be increased by bacterial coinfection in COVID-19 patients due to the widespread use of broad-spectrum antibiotics (Prasetyoputri 2021). Search for new natural biologically active compounds and their characterization is one of the urgent tasks in modern biotechnology. One of the sources of bioactive compounds is microorganisms. Thus, they are an important source of antimicrobial compounds. Secondary metabolite compounds produced by bacteria can be antimicrobial, antitumor agents, immunosuppressant agents, herbicides, pesticides, antiparasitic agents, and enzymes. In addition, thermophilic bacteria can be used to produce bioethanol and other industrial chemicals (Zeldes et al. 2015; Panda et al. 2018; Gurumurthy et al. 2020). Thermophilic bacteria live optimally at 45°C between 80°C and can be isolated from various environments such as deep sea, soil, hot springs, and compost (Pandey et al. 2015). The provinces on the Indonesian island of Sumatra are traversed by mountainous trails and have many hot springs. These natural conditions are a source of diversity of thermophilic microorganisms. Previously, was reported the potential of thermophilic bacteria in Sumatra as a source of amylase (Ardhi et al. 2020), protease, and inulinase enzymes (Fachrial et al. 2019). Thermophilic bacteria can be used to produce bioethanol and other industrial chemicals (Zeldes et al. 2015; Panda et al. 2018; Gurumurthy et al. 2020). Thermophilic bacteria also have the potential to produce antibiotic compounds, such as cyclohexyl acrylate, imiloxan, tabtoxinine-lactam, and filberton (Alrumman et al. 2018; Gurumurthy et al. 2020). Thermophilic bacteria in Sumatra can be antimicrobial, antitumor agents, immunosuppressant agents, herbicides, pesticides, antiparasitic agents, and enzymes. In addition, thermophilic bacteria can be used to produce bioethanol and other industrial chemicals (Zeldes et al. 2015; Panda et al. 2018; Gurumurthy et al. 2020). Thermophilic bacteria live optimally at 45°C between 80°C and can be isolated from various environments such as deep sea, soil, hot springs, and compost (Pandey et al. 2015). The provinces on the Indonesian island of Sumatra are traversed by mountainous trails and have many hot springs. These natural conditions are a source of diversity of thermophilic microorganisms. Previously, was reported the potential of thermophilic bacteria in Sumatra as a source of amylase (Ardhi et al. 2020), protease, and inulinase enzymes (Fachrial et al. 2019). Thermophilic bacteria can be used to produce bioethanol and other industrial chemicals (Zeldes et al. 2015; Panda et al. 2018; Gurumurthy et al. 2020). Thermophilic bacteria also have the potential to produce antibiotic compounds, such as cyclohexyl acrylate, imiloxan, tabtoxinine-lactam, and filberton (Alrumman et al. 2018; Gurumurthy et al. 2020). Thermophilic bacteria in Sumatra can be antimicrobial, antitumor agents, immunosuppressant agents, herbicides, pesticides, antiparasitic agents, and enzymes. In addition, thermophilic bacteria can be used to produce bioethanol and other industrial chemicals (Zeldes et al. 2015; Panda et al. 2018; Gurumurthy et al. 2020).

2. Materials and Methods

2.1. Screening for Thermophilic Bacteria that have Antimicrobial Activity from Laboratory Collections

Table 1 is the data of thermophilic bacteria from the collection of the Biochemistry Laboratory of the University of Riau. The total number of samples in this study was 50 isolates of thermophilic bacteria and coded LBKURCC (Laboratorium BioKimia Universitas Riau Culture Collection). These thermophilic bacteria were tested for antimicrobial ability against Staphylococcus aureus ATCC 29213, Escherichia coli ATCC 35218, and the fungus Candida albicans ATCC 10231. Pathogenic bacteria are purchased from a collection of microorganism cultures which are standard bacteria and are pathogenic for humans. E. coli ATCC 35218 is an Enteropathogenic Escherichia coli (EPEC) strain that can cause diarrhea. S. aureus ATCC 29213 and C. albicans ATCC 10231 are clinical isolates used as a standard quality control strain in laboratory testing. Bacteria were cultured in Nutrient Broth for 48 hours at 45°C. The supernatant was obtained by centrifugation for ten minutes at 10,000 rpm. The antimicrobial activity of thermophilic bacteria against microbial pathogens was tested by the disc diffusion method. Disc with a diameter of 6 mm was immersed in the bacterial culture supernatant. After 1 hour, the discs were placed on solid media containing pathogenic microbes and

| Origin               | Number of isolates | LBKURCC code                  |
|----------------------|--------------------|--------------------------------|
| Sungai Pinang, Riau Province | 17                | 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 |
| Rimbo Panti, West Sumatera Province | 10               | 214, 215, 216, 217, 218, 219, 220, 221, 222, 223 |
| Padang Gantiang, West Sumatera Province | 5                | 224, 225, 226, 227, 228 |
| Pawan, Riau Province | 6                 | 229, 230, 231, 232, 233, 234 |
| Bukik Gadang, West Sumatera Province | 6               | 235, 236, 237, 238, 239, 240 |
| Bukik Kili, West Sumatera Province | 6                | 241, 242, 243, 244, 245, 246 |
then incubated for 24 hours at 45°C. The zone of inhibition of pathogen growth in mm was used as antimicrobial activity. The following scoring system was used: Diameter of inhibition zone >15mm (++), 13-15 mm (+), <13 mm ((+)/-) and absent (-) (Esikova et al. 2002).

2.2. Morphological Characterization of Selected Thermophilic Bacteria

The pure isolate of the most potential bacteria was incubated at 45°C for 24 hours on a Nutrient Agar medium. Single bacteria colonies were taken to observe cell shape and Gram stain test. Gram stain test was carried out referring to Al-Dhabi et al. 2016. Bacterial growth temperature is determined by growing bacteria in a temperature range of 35-60°C. Bacterial growth was then observed on Nutrient Agar (Merck) for 2 days.

2.3. Molecular Identification of the Promising Thermophilic Bacteria

Bacteria were grown in a liquid medium for 24 hours at 45°C. Bacterial DNA was isolated using a modified GES method (Pitcher et al. 1989). The mixture of PCR reaction consisted of 39.5 µL of ddH2O, 0.5 µL of primers 27F: (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492 R (5'--GGT TAC CTT GTT ACG ACT T-3'), 1 µL of 10 mM dNTPs, 0.5 µL of Taq-polymerase, and 5 µL of reaction buffer. PCR was performed using either a Perkin-Elmer Cetus Thermal Cycler with a cycle profile of pre-run at 96°C for 2 minutes, denaturing at 96°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1.5 minutes and a total of 35 cycles was followed by a post-run at 72°C for 2 minutes (White et al. 1990; O'Donnell 1996). The PCR products were purified by the PEG precipitation method (Hiraishi et al. 1995). An automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) sequence bacterial ribosomal DNA. Sequencing data were processed using the BioEdit program. The length of the DNA fragment obtained in sequencing was 1,411 bp. The results were then analyzed for sequence homology using the mega BLAST program, accessed on the NCBI website. Sequence alignment of thermophilic bacterial strains with sequences already stored in NCBI based on the similarity of the 16S rRNA gene was carried out using Clustal W software. The resulting data were processed to obtain bacterial kinship relationships (Kumar et al. 2016).

2.4. Liquid Culture Fermentation and Crude Extract Extraction

Bacterial isolates that showed the highest antimicrobial activity in the previous test were selected for fermentation. Bacterial liquid culture fermentation was carried out in a Nutrient Broth (Merck) liquid medium (2 x 250 ml) at pH 7. The culture was incubated for 2 days at 45°C with a stirring speed of 150 rpm. The cultures were centrifuged at 300 rpm for 30 min to obtain cell-free extracts. Extraction with ethyl acetate was carried out by adding ethyl acetate in a ratio of 1:1 with the volume of the supernatant. Extraction was carried out by adding the volume of ethyl acetate 3 times in a separating funnel. The solvent layer was separated and evaporated at 40°C temperature by Rotavapor (Buchi R-100) under a vacuum. The extract was used as a stock for GC-MS analysis and performed antimicrobial activity tests.

2.5. Analysis by Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis of the obtained ethyl acetate extract was carried out with the SHIMADZU GCMS-QP2010S. The column used was (fused silica) with 30 m x 250 µm x 0.25 µm. Three µl of the sample were injected at 300°C and the GC run times were 80 minutes. Helium gas velocity was 0.5 ml/min with a pressure of 13.7 kPa. The electron energy was 70 Ev and the measured mass was 28-600 amu. The chromatogram results were compared with the compounds in the database. Then identify the name of the components, chemical structure, weight, and molecular formula.

2.6. Antimicrobial Activity

Twenty microliters of crude extract of ethyl acetate (200 ppm) were dropped on a paper disc and then tested for antimicrobial activity. The pathogenic microbes used are bacteria and fungi. Gram-negative bacteria *E. Coli*, gram-positive *S. aureus*, and fungi *C. Albicans*. The concentration of the suspension of pathogenic microbes was 1.5 x 10⁸ CFU/ml. It conforms to the 0.5 McFarland standard. The paper disc containing the extract was placed on the solid media inoculated with the test microbes. Positive control for an antibacterial was 5µL Ampicillin (100 µg/ml), antifungal was nystatin (100 µg/ml), and negative control ethyl acetate. Incubation was carried out for 24 hours at 37°C. (Alrumman et al. 2019). The zone of resistance formed around the disc
is measured with a ruler. The experiment was done in three replications.

3. Results

3.1. Screening for Thermophilic Bacteria that have Antimicrobial Activity

The result of the primary screening showed that the isolate LBKURCC218 displayed antimicrobial activity against *E. coli*, *S. aureus*, and *C. albicans* (Table 2). Among a total of 50 isolates showing antimicrobial activity, isolates which were code as LBKURCC198, LBKURCC200, LBKURCC201, LBKURCC202, LBKURCC204, LBKURCC207, LBKURCC212, LBKURCC216, LBKURCC217, LBKURCC218, LBKURCC220, LBKURCC223, LBKURCC224, LBKURCC227, LBKURCC231, LBKURCC232, LBKURCC235, LBKURCC243, and LBKURCC244, displayed antimicrobial activity against *C. albicans*. In this study, three (LBKURCC206, LBKURCC218, and LBKURCC222) isolates were active against Gram-negative and Gram-positive bacteria.

One of the most potential bacterial isolates is LBKURCC218 which has the antimicrobial activity against pathogenic fungi and bacteria. The inhibition zone around the paper disc indicates the presence of secondary metabolites produced by thermophilic bacteria that function as antimicrobials. One isolate (LBKURCC218) from Rimbo Panti hot spring, West Sumatera (Figure 1) was selected for identification according to their antimicrobial activity. We further characterized the selected isolates through analyses of the 16S rRNA region, gram staining, antimicrobial activities of crude extract, and metabolic profiling by GC-MS.

3.2. Morphological Characterization of Selected Thermophilic Bacteria

LBKURCC218 thermophilic bacterial colonies were circular, convex, and yellowish-white (Figure 2A). From the gram staining test, LBKURCC218 isolate was identified as a Gram-positive bacterium. The inhibition zone around the paper disc indicates the presence of secondary metabolites produced by thermophilic bacteria that function as antimicrobials. One isolate (LBKURCC218) from Rimbo Panti hot spring, West Sumatera (Figure 1) was selected for identification according to their antimicrobial activity. We further characterized the selected isolates through analyses of the 16S rRNA region, gram staining, antimicrobial activities of crude extract, and metabolic profiling by GC-MS.

### Table 2. Preliminary screening of LBKURCC thermophilic bacteria

| Isolate code | Inhibition zone category | Isolate code | Inhibition zone category |
|--------------|--------------------------|--------------|--------------------------|
| E. coli      | S. aureus    | C. albicans  | E. coli      | S. aureus    | C. albicans  |
| Sungai Pinang | +/−          | −            | Padang Gantiang | +/−          | −            | +/−          |
| LBKURCC197  | +/−          | −            | LBKURCC224  | +/−          | −            | +/−          |
| LBKURCC198  | +/−          | +/−          | LBKURCC225  | +/−          | −            | −            |
| LBKURCC199  | +/−          | −            | LBKURCC226  | −            | −            | −            |
| LBKURCC200  | +/−          | −            | LBKURCC227  | −            | −            | −            |
| LBKURCC201  | +/−          | −            | LBKURCC228  | −            | +/−          | −            |
| LBKURCC202  | +/−          | +/−          | LBKURCC229  | −            | −            | −            |
| LBKURCC203  | −            | −            | LBKURCC230  | −            | −            | −            |
| LBKURCC204  | +/−          | −            | LBKURCC231  | +/−          | −            | +/−          |
| LBKURCC205  | +/−          | −            | LBKURCC232  | −            | +/−          | +            |
| LBKURCC206  | +/−          | −            | LBKURCC233  | −            | +/−          | −            |
| LBKURCC207  | +/−          | −            | LBKURCC234  | +/−          | −            | −            |
| LBKURCC208  | +/−          | −            | Bukit Gadang | +/−          | −            | +/−          |
| LBKURCC209  | −            | −            | LBKURCC235  | −            | −            | +/−          |
| LBKURCC210  | −            | −            | LBKURCC236  | +/−          | −            | −            |
| LBKURCC211  | +/−          | −            | LBKURCC237  | −            | −            | −            |
| LBKURCC212  | +/−          | −            | LBKURCC238  | −            | −            | −            |
| LBKURCC213  | +/−          | −            | LBKURCC239  | −            | −            | −            |
| Rimbo Panti | −            | −            | LBKURCC240  | −            | −            | −            |
| LBKURCC214  | +/−          | −            | Bukit Kili   | +/−          | −            | −            |
| LBKURCC215  | +/−          | −            | LBKURCC241  | −            | +/−          | −            |
| LBKURCC216  | +/−          | −            | LBKURCC242  | −            | −            | −            |
| LBKURCC217  | +/−          | −            | LBKURCC243  | +/−          | −            | +/−          |
| LBKURCC218  | +/−          | +/−          | LBKURCC244  | −            | +/−          | +/−          |
| LBKURCC219  | −            | +/−          | LBKURCC245  | −            | +/−          | +/−          |
| LBKURCC220  | −            | +/−          | LBKURCC246  | −            | −            | −            |
| LBKURCC221  | −            | +/−          | −            | −            | −            | −            |
| LBKURCC222  | −            | +/−          | −            | −            | −            | −            |
| LBKURCC223  | −            | +/−          | −            | −            | −            | −            |

Inhibition zone category: diameter of inhibition zone >15 mm (++), 13-15 mm (+), <13 mm (+/−), and absent (−)
was gram-positive and rod-shaped (Figure 2B). The LBKURCC218 strain can grow in a temperature range of 35 to 55°C. This strain grows optimally at 50°C (Table 3). Bacillus sp. LBKURCC218 was fermented at 45°C to produce secondary metabolites. Antimicrobial activity was indicated by a clear zone around the paper disc (Figure 2C).

3.3. Molecular Identification of the Selected Thermophilic Bacteria

DNA sequences of selected thermophilic bacterial with 1411 bp were used for identification based on the 16S rRNA gene. The sequence was submitted to GenBank with accession number OM802613. Analysis of thermophilic bacteria LBKURCC218 16S
rRNA gene sequences with existing sequences in GenBank showed similarities to the genus *Bacillus* sp. This thermophilic bacterium showed 99.93% similarity with *Bacillus paramycoides* with accession number MW065486 (Figure 3).

Table 3. The growing temperature of bacterial isolate LBKURCC218

| Temperature (°C) | Growth |
|------------------|--------|
| 35               | +      |
| 40               | +      |
| 45               | ++     |
| 50               | +++    |
| 55               | +      |
| 60               | -      |

- not grow, +: moderate growth, ++: good growth and +++: prolific growth

3.4. Analysis by Gas Chromatography-Mass Spectrometry

The GC-MS chromatogram of the ethyl acetate extract of *Bacillus paramycoides* LBKURCC218 produced 33 peaks (Supplementary Figure 1). Table 4 shows the names of the compounds extracted with ethyl acetate. Dodecanoic acid, which represented 23.62% of the total compound, was the main compound, followed by 11-Dodecanoic acid at 17.84%. The compound detected at moderate concentration was eicosane (5.08%) followed by 4.15% of phenol 2, 6-bis(1,1 dimethyl ethyl)-4-methyl. The chemical structure of major compounds of *Bacillus paramycoides* LBKURCC218 crude extract is shown in Figure 5. Other compounds were detected in the little amount such as 1-tetradecene.
Table 4. GC-MS analysis of ethyl acetate extract *Bacillus Paramycoides* LBKURCC218

| Peak | Compounds | Chemicals formula | Molecular weight | RT (min) | Area (%) | Similarity index (%) |
|------|-----------|-------------------|------------------|----------|----------|----------------------|
| 1    | Isoxazolidine | C₃H₇NO | 73 | 8,817 | 1.75 | 70 |
| 2    | Butanoic acid 2-methyl | C₅H₁₀O | 102 | 8,896 | 0.89 | 92 |
| 3    | Phenol 2, 6-bis(1,1 dimethyl ethyl)-4-methyl | C₂₅H₂₄O | 220 | 27,329 | 4.15 | 75 |
| 4    | Undecane, 2-methyl | C₁₁H₂₂ | 170 | 29,281 | 1.04 | 96 |
| 5    | 1-Dodecane | C₁₂H₂₆ | 168 | 33,820 | 1.72 | 93 |
| 6    | Dodecane | C₁₂H₂₄ | 170 | 33,970 | 1.44 | 96 |
| 7    | Dodecanolic acid | C₁₂H₂₄O₂ | 214 | 36,893 | 23.62 | 94 |
| 8    | Decane | C₁₀H₂₂ | 198 | 37,045 | 2.40 | 83 |
| 9    | 1-Iodo-2-methylnonane | C₁₉H₃₁O | 268 | 37,178 | 1.42 | 89 |
| 10   | 1-Tetradecene | C₁₄H₂₈ | 196 | 38,096 | 3.26 | 92 |
| 11   | Pentadecene | C₁₅H₃₀ | 212 | 38,199 | 2.33 | 96 |
| 12   | 11-octadecanoic acid | C₂₀H₄₀O₂ | 296 | 40,380 | 17.84 | 92 |
| 13   | Octadecanoic acid | C₂₀H₄₀O₂ | 298 | 40,722 | 1.92 | 94 |
| 14   | Octadecane | C₂₀H₄₀ | 254 | 40,856 | 1.02 | 94 |
| 15   | 1-Hexadecene | C₁₆H₃₂ | 224 | 41,952 | 2.50 | 92 |
| 16   | Hexadecane | C₁₆H₃₂ | 226 | 42,034 | 1.67 | 95 |
| 17   | Naphthalene | C₁₀H₈ | 268 | 42,245 | 1.22 | 69 |
| 18   | Hexadecane | C₁₆H₃₂ | 226 | 43,790 | 1.41 | 95 |
| 19   | Methyl ester of ricinoleic acid | C₁₃H₂₄O₃ | 312 | 43,974 | 1.29 | 86 |
| 20   | Cyclotetradecane | C₁₄H₂₈ | 196 | 45,483 | 2.17 | 89 |
| 21   | Hexadecane | C₁₆H₃₂ | 226 | 45,565 | 2.53 | 96 |
| 22   | Hexadecane | C₁₆H₃₂ | 226 | 47,194 | 1.88 | 96 |
| 23   | 2-Undecene | C₁₁H₂₀ | 182 | 47,284 | 0.90 | 75 |
| 24   | Nonadecane 2-methyl | C₁₉H₃₂ | 282 | 47,747 | 1.12 | 89 |
| 25   | 1,2 Benzenedicarboxylic acid | C₁₈H₄₀O₄ | 390 | 48,151 | 2.09 | 83 |
| 26   | Eicosane | C₂₀H₄₀ | 282 | 48,349 | 1.31 | 92 |
| 27   | Eicosane | C₂₀H₄₀ | 282 | 48,802 | 5.08 | 96 |
| 28   | Tetradecane | C₁₄H₂₀ | 226 | 49,732 | 0.87 | 87 |
| 29   | Eicosane | C₂₀H₄₀ | 282 | 50,310 | 2.30 | 95 |
| 30   | Eicosane | C₂₀H₄₀ | 282 | 50,785 | 1.47 | 93 |
| 31   | Hexatriacontane | C₃₆H₇₂ | 507 | 51,371 | 1.14 | 91 |
| 32   | Hexacosane | C₂₄H₄₈ | 366 | 51,771 | 2.48 | 95 |
| 33   | Propane 2-(1,1 dimethyl ethyl) sulfonyl 12-methyl | C₁₈H₃₄O₂S | 178 | 69,301 | 1.76 | 81 |

(3.26%), hexadecane (2.53%), 1-hexadecene (2.50%), hexacosane (2.48%), decane (2.40%), pentadecene (2.33%), cyclotetradecane (2.17%), Propane 2-(1,1 dimethyl ethyl) sulfonyl 12-methyl (1.76%), and isoxazolidine (1.75%).

3.5. Antimicrobial Activity

Our findings showed that ethyl acetate extract of *Bacillus paramycoides* LBKURCC218 inhibited the growth of *E. coli*, *S. aureus*, and *C. Albicans* (Figure 4). The inhibition diameter zone value of *Bacillus paramycoides* LBKURCC218 against *E. coli*, *S. aureus*, and *C. albicans* was 10.67 mm, 11.67 mm, and 23 mm (Table 5).

4. Discussion

Thermophilic bacteria from the collection of the Biochemistry Laboratory of the University of Riau have been tested for their antimicrobial activity. The thermophilic bacteria that was able to inhibit both bacterial and fungal pathogens was the LBKURCC218 isolate. The area of origin of LBKURCC218 is the hot springs of Rimbo Panti, West Sumatra Province, Indonesia. When viewed from the earth’s surface, the Rimbo Panti hot spring is a dense forest. Forest areas have typical environmental conditions for hot spring bacteria to live. Existing environmental conditions are also influenced by temperature, acidity (pH), and the diversity of isolates in specific locations can affect the types of secondary metabolites produced by bacteria (Poli *et al.* 2017). The presence of microbial interactions in competition for food causes microorganisms to produce secondary metabolites that act as antibiotics. Hot springs are a habitat for thermophilic bacteria that can be used as a source of antibiotics (Panda *et al.* 2018). Thermophilic bacteria have been isolated from hot springs with antimicrobial activity in southern Saudi Arabia. There were 50 bacteria from 84 bacterial isolates with antimicrobial activity (Alrumman *et al.* 2019). Aldhabi has also isolated bacteria from sediments...
from hot springs at Tharban. This bacterium is a type of *Streptomyces* sp. Al-Dhabi. This strain has also been investigated, which has antimicrobial activity (Al-Dhabi et al. 2016).

The type of *Bacillus* isolated from hot springs was also reported by Arzu et al. (2012) and Oztas Gulmus and Gormez (2020). Another study reported the ability of *Bacillus* thermophilic bacteria to produce antimicrobials isolated from hot water in Jordan (Fandi *et al.* 2014) and *Bacillus subtilis* KFSB5 isolated from the Kinwat teak forest Kanse *et al.* (2014). This has increased the interest of researchers and industry to explore the potential of *Bacillus* class bacteria. Coupled with this research explores the potential of the *Bacillus* class from hot springs in Indonesia.

Microorganisms that live in hot springs have diversity and produce various types of secondary metabolites that function as antimicrobials. Bacillus-type bacteria can produce metabolites or enzymes (Kumar and Raja 2019), including antifungal and antimicrobial (Khan *et al.* 2018), (Caulier *et al.* 2019). This study also reported the ability of the thermophilic bacteria *B. paramycoides* LBKURCC218 to produce antibacterial and antifungal compounds. The inhibition zone (Figure 4) of *Bacillus paramycoides* LBKURCC218 against *Candida albicans* was 23 mm. The antifungal ability of *B. paramycoides* LBKURCC218 was higher than *Bacillus sonorenensis* KJU-KS2 (15 mm) (Alrumman *et al.* 2019) and *Streptomyces* sp. Al-Dhabi (14 mm) (Al-Dhabi *et al.* 2016). While the antimicrobial activity *Bacillus paramycoides* LBKURCC218 against pathogenic bacteria *E. coli* and *S. aureus* (Table 4) had similar inhibition zones to *Bacillus sonorenensis* KJU-KS2 and *Streptomyces* sp. Al-Dhabi. The antibacterial activity of *B. paramycoides* LBKURCC218 against *E. coli* was smaller than the thermophilic lactic acid bacteria *Pediococcus pentosaceus* N6 isolated from Rimbo Panti hot springs, which had an inhibition zone diameter of 20 mm (Yah *et al.* 2014).

Our study also showed that the thermophilic bacteria *Bacillus paramycoides* LBKURCC218 can produce compounds that have antimicrobial activity.

**Table 5. Antimicrobial activity of *B. paramycoides* LBKURCC218 ethyl acetate extract using disc diffusion method**

| Test sample                        | Mean of zone inhibition against pathogenic microbes (mm) | E. coli | S. aureus | C. albicans |
|------------------------------------|---------------------------------------------------------|---------|-----------|-------------|
| Ethyl acetate extract              | 23.00±0.47                                              | 11.67±0.47 | 10.67±0.47 |
| Nystatin                           | 17.33±0.47                                              | -        | -         |
| Ampicillin                         | 14.67±0.47                                              | 18.67±0.47 |
| Negative control (ethyl acetate)   | -                                                       | -        | -         |

**Figure 4. Inhibition zone of ethyl acetate extract strain *B. paramycoides* LBKURCC218 against microbial tested. (A) *E. coli*, (B) *S. aureus*, and (C) *C. albicans* (PC: positive control, NC: negative control, and 218: ethyl acetate extract of *Bacillus paramycoides* LBKURCC218)**
The main compound of the ethyl acetate extract analyzed by GC-MS was dodecanoic acid (Figure 5). Dodecanoic acid is a fatty acid methyl ester. Previous studies reported that this compound has antiviral, antifungal, and antibacterial properties (Özçelik et al. 2005; Chandrasekaran et al. 2008). Dodecanoic acid or lauric acid has been tested in vitro and in vivo to inhibit the growth of Propionibacterium acnes. This compound has a minimal inhibitory concentration (MIC) 15 times lower than benzoyl peroxide (BPO) in inhibiting P. acnes, S. aureus, and S. epidermidis (Nakatsuji et al. 2009).

Long-chain fatty acid compounds can function as antibacterial. This is because the outer membrane of bacteria is very sensitive to fatty acid compounds. The difference in the sensitivity of the outer membrane of Gram-positive and Gram-negative bacteria may be due to the impermeability of the outer membrane, which serves as an effective barrier to hydrophobic substances (Heesterbeek et al. 2019). This study found that the antimicrobial compound B. paramycoides LBKURCC218 could inhibit S. aureus and E. coli with inhibition zones of 11.67 mm and 10.67 mm, respectively. This shows that gram-negative bacteria are more resistant to fatty acids than gram-positive bacteria as reported by Klobucar et al. (2021). Agoramoorthy et al. (2007) investigated the leaves of Excoecariaagallocha and found the excellent antimicrobial activity of dodecanoic acid (lauric acid) against S. aureus and E. coli. This compound was also active against several pathogenic microbes such as Micrococcus luteus, Salmonella typhimurium, Pseudomonas aeruginosa, Bacillus subtilis, and Klebsiella pneumonia.

The compound 11-octadecanoic acid was the second most abundant component produced by Bacillus paramycoides LBKURCC218. This long-chain fatty acid is synthesized from linoleic acid by Lactobacillus Plantarum. Miyamoto showed that 11-octadecanoic acid has anti-inflammatory activity in the gut (Miyamoto et al. 2015). Extract of Streptomyces strain KX852460 has the main components eicosane (C20H42) and dibutyl phthalate (C16H22O4), where the extract can inhibit the fungus R. solani AG-3. This indicates that the two compounds above have antifungal activity (Ahsan et al. 2017). Aissaoui investigated the potential of thermophilic Bacillus isolated from hot springs in Algeria. These thermophilic bacteria are good producers of phenolic compounds that can be used against clinical strains (Aissaoui et al. 2018). The crude bioactive extracts produced in our study contain phenol 2, 6-bis(1,1 dimethyl ethyl)-4-methyl. These phenolic compounds showed antibacterial, antifungal, and antioxidant activity (Zhao et al. 2020).

![Dodecanoic acid](image1.png)

![11-octadecanoic acid](image2.png)

![Eicosane](image3.png)

![4-Phenol 2,6-bis(1,1dimethyl ethyl)-4-methyl](image4.png)

Figure 5. Chemical structure of major compounds of B. paramycoides LBKURCC218 crude extract.
The first peak of GC-MS ethyl acetate extract of *Bacillus paramycoides* LBKURCC218 (Table 4) in this study was a compound similar to isoxazolidine (compound similarity index 70%). Previously, new isoxazolidine synthetic compounds have been found and have antifungal activity against plant pathogens (Ra et al. 2003). Isoxazolidine and γ-lactam analogs have antiviral activities because they can damage DNA or RNA from viruses. In addition, isoxazolidine is more active as an antitumor drug than antiviral (Piotrowska et al. 2019).

In conclusions, this study provides new information about the thermophilic bacteria *B. paramycoides* LBKURCC218 isolated from the Rimbo hot springs in West Sumatra, Indonesia, and its potential as an antifungal and antibacterial agents. The antimicrobial ability has been tested against the fungus *C. albicans* and *S. aureus* and *E. coli*. The diversity of compounds produced by this hot spring isolate was analyzed by GC-MS. The GC-MS chromatogram showed that this isolate produced general metabolites that could function as antibacterial and antifungal compounds. This study succeeded in exploring the potential of thermophilic isolates that have the potential as a source of antimicrobial compounds.

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Supplementary Figure 1. Chromatogram of ethyl acetate extract of Bacillus paramycoides LBKURCC218 analyzed by GC-MS