Screening of antibiofilm activity and toxicity assay of methylotrophic bacteria compounds isolated from the human mouth

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Abstract: Biofilm is a community consisting of one or more species of bacteria attached to a surface using a matrix. Biofilms can cause health problems in humans, such as dental plaque. Biofilm is one form of self-defense of bacteria against antibiotics, so biofilm often has implications for the rise in resistance of bacteria. Therefore, antibiofilm compounds can be used to increase the sensitivity of bacteria to the antimicrobial agent. The purpose of this study is to determine the antibiofilm activity and the potential toxicity of the methylotrophic bacteria compound isolated from the human mouth. Based on the results of the study, 21 out of 37 isolates of methylotrophic bacteria had antibiofilm activity using two different supernatant concentrations (5% and 10%). The isolate of M28L2 showed the highest anti-biofilm inhibition activity: 47.64% against EHEC and 50.97% against Pseudomonas aeruginosa ATCC 27583. Meanwhile, M30G1 isolate showed the highest anti-biofilm destruction activity: 44.062% against EHEC and 39.17% against ETEC. Toxicity test was performed on five isolates that had the highest antibiofilm activity using a lethal dose test. Based on these test results, five isolates had LC50 values greater than 1000μg/mL, indicating that the compound was not toxic. Subsequently, PCR amplification for the DNA sequencing stage was performed on one isolate, M28L2. Based on the sequencing results, M28L2 had a 99% similarity to Pseudomonas stutzeri.

1. Introduction

Most of the bacteria are found in the environment, living in the form of biofilms. This structure is one form of adaptation for several kind of bacteria, such as Staphylococcus aureus and Pseudomonas aeruginosa. By creating a barrier using a self-synthesized matrix, it can protect them from any kind of threats such as the immune system of host and antibiotic causing chronic infections and diseases such as periodontitis, dental caries, and pulmonary infections of cystic fibrosis patients, and nosocomial infections [1,2]. The increase of resistance occurring in the biofilm structure can be caused by the density and physiological state of the cells. Sessile cells are less metabolically active than planktonic cells, thereby causing many mechanisms of antibacterial agents to become less potent [2].

Bacterial biofilm is a community of one or more species of bacteria on a solid surface that is stuck by matrix consists of polysaccharide, protein, nucleic acid, and lipids [3]. It poses a serious public health
problem because they act as an important source of the pathogen. Besides, bacteria within the biofilm structure are up to 1000 times resistance to antibacterial agents rather than planktonic cells [4]. This problem becomes a concern for researchers to look for a bioactive compound that can be used to treat biofilm-associated infections. Antibiofilm is a natural or induced process that aims to reduce bacterial biomass [2]. Antibiofilm compounds are classified into two groups based on their mechanism. The first is the antibiofilm inhibition activity; the second is the antibiofilm destruction activity. This strategy is considered promising because they specifically target bacterial behavior rather than bacterial fitness. Therefore, they are less likely to induce resistance [5].

Methylotrophic bacteria are aerobic bacteria, which utilize one-carbon compounds or reduced carbon as a source of carbon and energy, such as methane, methanol, and methylated compounds. This group of bacteria uses various enzymes. They catalyze one-carbon compounds as their substrate, including methanol dehydrogenase, methane monooxygenase, and methylamine dehydrogenase [6]. Methylotrophic bacteria can be isolated from various parts of the human body, such as mouth and feet [7,8]. Human mouth naturally produces volatile one-carbon compounds, including malodorous methylated sulfides, such as methanthiol and dimethylsulfide, as an end-product of microbial degradation of free sulfur amino acids process [8]. Therefore, methylotrophic bacteria could utilize these compounds as a source of energy.

Although there are some studies about methylotrophic bacteria, the study about their antibiofilm activity and their potential toxicity is limited. Therefore, in this study, we performed an antibiofilm assay and an acute toxicity test using the brine shrimp lethality assay (BSLA) method. This method is considered as a preliminary toxicity assay for further experiments on mammalian animal models [9]. Also, BSLA is inexpensive, fast, gives repetitive result, and accommodates a large number of nauplii for statistical validation. The result of this assay will determine the death rate of Artemia salina due to the influence of the antibiofilm compound of methylotrophic bacteria [10].

The research aims to determine the antibiofilm activity of methylotrophic bacteria isolated from human mouth against biofilm S. aureus ATCC 25923, P. aeruginosa ATCC 27583, Enterotoxigenic Escherichia coli (ETEC), and Escherichia coli O157:H7 (EHEC) and to determine their potential toxicity of the antibiofilm compound using BSLA.

2. Methods

2.1. Growth of methylotrophic bacteria
In this study, 37 isolates of methylotrophic bacteria isolated from the human mouth obtained from the previous study were used [11]. Cryopreservation isolates of methylotrophic bacteria were defrosted, inoculated on minimal medium (agar bacteriology 20 g, methanol 1% (v/v) 10 mL, and ddH2O 1,000 mL) as selective media, and incubated at 28°C for seven days. A single colony was picked and inoculated on Luria Agar (LA) (sodium chloride 10 g, tryptone 10 g, yeast extract 5 g, agar 20 g, and ddH2O 1,000 mL) as rich nutritionally medium and incubated at 28°C for 48 hours.

2.2. Bacterial isolates and culture conditions
Pathogenic bacteria used in this study were S. aureus (ATCC 25923), P. aeruginosa (ATCC 27583), ETEC (Atma Jaya Collection Culture), and EHEC (Atma Jaya Collection Culture). These bacteria were grown in LA at 37°C for overnight.

2.3. Antibacterial assay
This assay was performed using the agar diffusion method [12]. Isolates of methylotrophic bacteria were inoculated into Nutrient Broth (NB) and incubated at 28°C for 48 hours. Subsequently, the cultures were centrifuged at 12,000 x g for 20 minutes, and the supernatants were collected.
Meanwhile, isolates of pathogenic bacteria were inoculated into NB and incubated at 37°C overnight. Then, the cultures were diluted into sterile NB until the absorbance value reached 0.132 at OD = 600 nm (McFarland 0.5).

In this assay, trimethoprim (25 µg) was used as a positive control. At the same time, 10 µL of sterile NB was used as a negative control. Each plate was divided into three quadrants. Afterward, 100µL of pathogenic bacteria suspension was streaked continuously in three different directions onto Nutrient Agar (NA). Then, blank disc and the antibiotic were put in the center of each quadrant. Subsequently, 10 µL of methylotrophic bacteria suspension was dropped on the top of one blank disc, and 10 µL of sterile NB media was dropped on the top of another blank disc. Plates were incubated at 37 °C overnight. We used the Clinical and Laboratory Standards Institute (CLSI) guidelines as a standard to observe the result [13].

2.4. Antibiofilm assay
This assay was performed in various categories based on their mechanism whether the compound could inhibit biofilm formation or destruct the preformed biofilm structure. In this assay, various treatment concentrations (5% and 10%) of methylotubacteria isolates were used. Besides, the process was undertaken in round-bottom 96-well polystyrene plates under sterile conditions inside laminar. Preparation of the samples was performed by inoculating all the pathogens, and methylotubacteria isolates into Luria Broth (LB) and incubated at 37°C overnight and 28°C for 48 hours, respectively. However, in this case, S. aureus (ATCC 25923) and P. aeruginosa (ATCC 27583) were inoculated into LB + 1% glucose. Afterward, all the pathogens were diluted with sterile LB or LB + 1% glucose until reaching the absorbance value of 0.132 at OD= 600 nm (McFarland 0.5). While methylotubacteria cultures were centrifuged at 12000 x g for 20 minutes, then supernatants were collected.

For inhibition activity assay, the 96-well microplates were coated with various concentrations (10 µL and 20 µL) of methylotubacteria supernatant. Tested wells and positive-control wells were inoculated with 200 µL of pathogenic bacteria. At the same time, negative-control wells were inoculated with 200 µL of sterile LB. The plates were incubated at 37°C overnight. For destruction activity assay, tested wells and positive-control wells were inoculated with 200 µL of pathogenic bacteria. In contrast, negative-control wells were inoculated with 200 µL of sterile LB. The plates were incubated at 37°C overnight. After incubation, tested wells were inoculated with various concentrations (10 µL and 20 µL) of methylotubacteria supernatant and incubated at 37°C for 60 minutes.

After the incubation period ended, planktonic cells, along with the remaining media, were discarded, and adherent cells were rinsed with aquadest and air-dried for 30 minutes. Adherent biofilm cells were stained with 200 µL of 0.4% (w/v) crystal violet for 30 minutes. Crystal violet was discarded, rinsed five times with aquadest, and air-dried for 5 minutes. Afterward, 200 µL of absolute ethanol was resuspended to each well, and 100 µL of ethanol from each well was transferred to a new microplate. Absorbance was determined at 595 nm using a microplate reader.

2.5. Sample preparation for brine shrimp lethality assay (BSLA)
Samples were prepared by dissolving 1 mL of crude extract of methylotubacteria antibiofilm compounds to get a stock solution (10.000 ppm) using artificial seawater (38 g sodium chloride and ddH2O 1000 mL). From the stock solution, 10, 100, 500, 1000 ppm concentration were made. Five replicates were prepared for each concentration.

2.6. Hatching the shrimp
This procedure was performed using [14] methods with several modifications. Artificial seawater was prepared. Then, three mg of brine shrimp cysts were hatched in 0.75 volume of a mineral water bottle filled with artificial water. Besides, the growing media were constantly aerated with an air pump and
illuminated with tungsten bulb (40 watts). After 48 hours, the phototropic nauplii were collected by pipetted from the lighted side.

2.7. Bioassay
This assay was performed using [15] method with several modifications. Ten nauplii were transferred into a vial filled with 4.5 mL artificial seawater using a dropper. Afterward, 500 µL of the sample with different concentrations was added into each vial. Each concentration was replicated three times. All vials were maintained under illumination for 24 hours at room temperature. Then, the survivors were counted using a magnifying glass.

The percentage of mortality was determined with the formula below:

\[
\%\text{Mortality} = \frac{\text{Death nauplii}}{\text{Total nauplii}} \times 100\% \tag{1}
\]

In case, the negative control does not give 0% mortality of nauplii, and then the formula should be corrected using Abbott's formula below:

\[
\%\text{Mortality} = \frac{\text{Death nauplii in tested vial} - \text{death nauplii in negative control}}{\text{Total nauplii}} \times 100\% \tag{2}
\]

In this assay, 500 µL of K₂Cr₂O₇ was added into the vial as a positive control, while 500 µL of artificial seawater was added into the vial as a negative control.

2.8. Lethal concentration determination
Lethal concentrations of methylobacteria antibiofilm compounds resulting in 50 % of mortality of the brine shrimp (LC₅₀) were determined using probit analysis [16]. The data were processed and analyzed using Microsoft Office Excel®.

2.9. Molecular identification
One of methylobacteria isolates was identified based on 16S rDNA sequence using PCR and DNA sequencing analysis. DNA isolation was done using the Wizard® Genomic DNA Purification Kit. DNA amplification was performed using GeneAMp® PCR system 9700 using specific primers 63f (5' -CAG GCC TAA CAC ATG CAA GTC-3') and 1387r (5' GGG CGG WGT GTA CAA GGC-3') primers [17]. The result of DNA sequencing was analyzed in DNA sequencing at Macrogen, Korea.

3. Results and discussion
3.1. Bacterial isolates and cultivates
In total, 37 isolates of methylotrophic bacteria were successfully cultivated on minimal medium as selective media. Afterward, the isolates were inoculated on Luria Agar as enrichment media and showed diverse pigmentation (Table 1) [11], which describes the diversity of the oral microbiota. Morphological properties, especially the pigmentation of the isolates, were diverse. It might happen due to the carotenoid content variation. A carotenoid is a group of pigments of different tints from red to yellow. It is known for its high antioxidant activity by absorbing the excited energy of singlet oxygen onto the carotenoid chain, which degrades the carotenoids themselves, but prevents other molecules or tissue from being damaged [18].
3.2. Antibacterial assay

Antibacterial assay needs to be performed in this research because the antibacterial activity of an isolate can give a false-positive result in the antibiofilm assay. Also, non-biocidal agents target bacterial behavior rather than bacterial fitness. Therefore, they are less likely to cause resistance in bacteria [19]. Sixteen isolates of methylotrophic bacteria showed antibacterial activity against pathogens tested. For example, M7L1, M23G2, and M27L2 had antibacterial activity against EHEC, ETEC, and S. aureus ATCC 25923, respectively (data are not shown). In contrast, the other 21 isolates of methylotrophic bacteria showed no antibacterial activity (Table 1).

Table 1. Result of appearance and an antibacterial assay of several isolates.

| Isolates | Colour       | P. aeruginosa ATCC 27583 | S. aureus ATCC 25923 | ETEC | EHEC |
|----------|--------------|--------------------------|-----------------------|------|------|
| M18G1    | Orange       | -                        | -                     | -    | -    |
| M23L1    | White        | -                        | -                     | -    | -    |
| M24G1    | Yellow       | -                        | -                     | -    | -    |
| M27G3    | White        | -                        | -                     | -    | -    |
| M27L3    | White        | -                        | -                     | -    | -    |
| M28L2    | Yellow       | -                        | -                     | -    | -    |
| M2G1     | White        | -                        | -                     | -    | -    |
| M30G1    | Orange       | -                        | -                     | -    | -    |
| M32G1    | Pink-orange  | -                        | -                     | -    | -    |
| M33L1    | Yellow       | -                        | -                     | -    | -    |
| M37L1    | White        | -                        | -                     | -    | -    |
| M37L2    | White        | -                        | -                     | -    | -    |
| M39L1    | Creamy-white | -                        | -                     | -    | -    |
| M41L1    | Yellow       | -                        | -                     | -    | -    |
| M46G1    | White        | -                        | -                     | -    | -    |
| M46G2    | Orange       | -                        | -                     | -    | -    |
| M46L1    | White        | -                        | -                     | -    | -    |
| M46L2    | Orange       | -                        | -                     | -    | -    |
| M46L4    | Yellow       | -                        | -                     | -    | -    |
| M51G2    | Creamy-white | -                        | -                     | -    | -    |
| M51G3    | Creamy-white | -                        | -                     | -    | -    |

3.3. Antbiofilm assay

Tables 2 and 3 show that almost all of the isolates work most effectively at a specific concentration and mechanism against a specific pathogen. For example, M27G3 showed 57.247% inhibition antibiofilm activity against EHEC. M32G1 showed 44.02% destruction antibiofilm activity against S. aureus ATCC 25923. There are several possible mechanisms, underlying this event. One of them is due to the production of a quorum-sensing inhibitor (QSI). QSI can be achieved by inhibiting signal synthesis, direct degradation of the signal, or inhibition of signaling molecules and receptor binding. However, QSI is considered as specific antibiofilm molecules since each bacteria has different QS systems [20]. However, M28L2 showed broad antibiofilm activity (60.97% inhibition activity and 52.66% destruction activity) against P. aeruginosa ATCC 27583 (Tables 3 and 4). It had been discovered that high molecular mass of polysaccharide, EPS273 extracted from P. stutzeri 273, could effectively disperse the preformed biofilm of P. aeruginosa PA01 [21]. Several different mechanisms have been implicated in this case, including the induction of cellular motility. Motility is important in the initial attachment. However, cells need to adhere to the maturation phase. Therefore, induction of mortality in the wrong
phase of biofilm development could destabilize the biofilm formation [22]. Furthermore, this study also found that EPS273 could inhibit the production of pyocyanin and H₂O₂, playing an important role in releasing eDNA that is the major component of the biofilm structure of P. aeruginosa [21].

Extracellular polymeric substances (EPS) is one of the major components of the biofilm matrix and also play an important role in maturing the biofilm [21]. However, several recent studies found that few bacterial EPS negatively regulated biofilm formation [23]. Other studies showed several modes of action, which enable EPS to inhibit or destabilize biofilm formation. For example, studies conducted by [24] discovered that EPS A101 extracted from Vibrio sp. showed inhibition activity of biofilm formation of P. aeruginosa FRD1 and S. aureus RN6390 by disrupting the interactions of cell-surfaces and cell-cell.

Biosurfactant is an agent antibiofilm that is capable of changing the physical or chemical properties of the surfaces; hence, it might show anti-adhesion activity [25]. Also, antibiofilm components, such as polysaccharides, can inhibit intercellular adhesion by altering the properties of bacterial cell walls. Thus it can prevent the bacterial cell from binding with biotic surfaces. For example, exopolysaccharide, extracted from Bacillus licheniformis called SP1, enables to modify the physicochemical characteristics of cell surface, resulting in reduced colonization [26]. Another possible mechanism is the competitive inhibition of multivalent carbohydrate-protein interactions. Anti-adhesive by exogenous carbohydrates can inhibit the docking process of bacteria from binding physically and being stable to the host, leading to a diminished incidence of infection [27].

### Table 2. Result of inhibition antibiofilm activity of all isolates of methylotrophic bacteria tested (%).

| Isolate | S. aureus | P. aeruginosa | ETEC | EHEC |
|---------|-----------|---------------|------|------|
|         | 5% | 10% | 5% | 10% | 5% | 10% | 5% | 10% |
| M18G1   | 39.30 | 9.57 | 7.89 | 0.00 | 14.99 | 0 | 32.75 | 46.81 |
| M23L1   | 36.91 | 9.75 | 1.71 | 0.00 | 4.19 | 4.39 | 13.32 | 13.32 |
| M24G1   | 11.06 | 22.15 | 16.62 | 11.55 | 0.00 | 18.94 | 57.19 | 55.86 |
| M27G3   | 9.80 | 27.6 | 1.70 | 0.00 | 0.00 | 18.94 | 57.25 | 52.43 |
| M27L3   | 30.10 | 1.00 | 0.00 | 0.00 | 14.70 | 0.00 | 36.80 | 39.00 |
| M28L2   | 44.84 | 31.52 | 49.81 | 60.97 | 29.06 | 35.23 | 47.64 | 14.58 |
| M2G1    | 0.00 | 24.26 | 0.00 | 0.00 | 3.92 | 5.84 | 13.39 | 8.42 |
| M30G1   | 0.00 | 29.01 | 19.22 | 19.28 | 2.32 | 24.66 | 34.44 | 3.75 |
| M32G1   | 41.03 | 33.82 | 6.35 | 10.80 | 50.58 | 24.30 | 14.95 | 22.50 |
| M33L1   | 19.40 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 57.70 | 21.90 |
| M37L1   | 42.53 | 41.06 | 17.06 | 22.16 | 33.34 | 26.18 | 29.44 | 19.38 |
| M37L2   | 9.29 | 2.47 | 0.00 | 0.00 | 77.39 | 47.10 | 45.80 | 37.80 |
| M39L1   | 17.75 | 16.96 | 58.33 | 44.96 | 53.67 | 44.96 | 52.15 | 37.51 |
| M41L1   | 0.00 | 0.00 | 0.17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| M46G1   | 0.00 | 0.00 | 0.00 | 17.11 | 21.38 | 0.00 | 21.76 | 20.57 |
| M46G2   | 43.17 | 52.72 | 0.00 | 0.00 | 13.11 | 22.98 | 36.40 | 18.43 |
| M46L1   | 45.23 | 49.74 | 0.00 | 0.00 | 0.00 | 24.69 | 0.00 | 18.35 |
| M46L2   | 2.58 | 0.00 | 29.38 | 22.96 | 72.61 | 56.70 | 22.92 | 27.18 |
| M46L4   | 52.70 | 57.68 | 5.68 | 14.02 | 31.06 | 38.89 | 0.00 | 0.00 |
| M51G2   | 48.00 | 54.78 | 42.10 | 0.00 | 20.06 | 20.06 | 6.86 | 0.00 |
| M51G3   | 24.63 | 0.00 | 4.59 | 4.39 | 28.44 | 36.72 | 24.99 | 21.37 |

*the highest inhibition antibiofilm activity for each pathogenic bacteria.

### 3.4. Brine shrimp lethality assay

BSLA is a broad spectrum bioassay that aims to determine the cytotoxic effect of the specific compound using an organism called Artemia salina. BSLA is considered a useful tool for preliminary assessment of toxicity [15]. Five potential isolates (M28L2: broad antibiofilm activity against Pseudomonas aeruginosa ATCC 27583; M27G3: highest inhibition antibiofilm activity against EHEC; M30G1: highest overall
inhibition antibiofilm activity; M46G1: highest destruction antibiofilm activity against ETEC; M32G1: highest destruction antibiofilm activity against *S. aureus* ATCC 25923) were tested using brine shrimp lethality assay method.

### Table 3. Result of destruction antibiofilm activity of all isolates of methylotrophic bacteria tested (%).

| Isolate  | **S. aureus** | **P. aeruginosa** | **ETEC** | **EHEC** |
|----------|---------------|-------------------|----------|----------|
|          | 5% | 10% | 5% | 10% | 5% | 10% | 5% | 10% | 5% | 10% |
| M18G1    | 0.00 | 0.00 | 39.64 | 64.95 | 41.12 | 48.38 | 49.16 | 55.44 |
| M23L1    | 0.00 | 0.00 | 35.20 | 36.33 | 4.67  | 0.00  | 64.72 | 60.78 |
| M24G1    | 17.42 | 10.61 | 44.94 | 48.28 | 1.70  | 29.36 | 12.84 | 0.00  |
| M27G3    | 0.00 | 0.00 | 8.00  | 14.20 | 55.27 | 0.00  | 19.14 | 27.30 |
| M27L3    | 40.90 | 0.00 | 0.00  | 0.00  | 0.00  | 0.00  | 19.14 | 57.30 |
| M28L2    | 0.00 | 0.00 | 52.66 | 30.41 | 1.70  | 29.36 | 12.84 | 0.00  |
| M2G1     | 3.41 | 8.04 | 7.74  | 0.00  | 43.69 | 38.09 | 0.00  | 0.00  |
| M30G1    | 33.97 | 0.00 | 0.00  | 33.75 | 39.17 | 38.09 | 36.03 | 44.06 |
| M32G1    | 44.02 | 0.00 | 12.46 | 0.00  | 30.30 | 0.00  | 2.69  | 12.75 |
| M33L1    | 18.61 | 0.00 | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  |
| M37L1    | 31.57 | 19.54 | 24.22 | 35.73 | 2.71  | 9.24  | 27.30 | 22.83 |
| M37L2    | 61.67 | 0.00 | 3.33  | 24.50 | 32.50 | 0.00  | 0.00  | 0.00  |
| M39L1    | 0.00 | 0.00 | 11.28 | 11.28 | 14.45 | 32.20 | 27.98 | 22.83 |
| M41L1    | 0.00 | 0.00 | 0.67  | 0.58  | 0.00  | 0.00  | 0.00  | 0.00  |
| M46G1    | 0.00 | 33.07 | 0.00  | 23.49 | 51.99 | 47.83 | 45.34 | 30.34 |
| M46G2    | 8.75 | 0.00 | 4.08  | 0.00  | 29.78 | 15.84 | 35.94 | 51.70 |
| M46L1    | 8.21 | 7.84 | 27.97 | 29.37 | 41.51 | 48.09 | 0.00  | 0.00  |
| M46L2    | 17.12 | 0.92 | 0.00  | 0.00  | 25.17 | 24.29 | 55.00 | 20.61 |
| M46L3    | 20.04 | 28.29 | 0.00  | 0.00  | 48.78 | 37.44 | 0.00  | 0.00  |
| M51G2    | 13.64 | 11.12 | 10.58 | 36.80 | 9.95  | 6.26  | 0.00  | 0.00  |
| M51G3    | 3.41 | 8.04 | 0.00  | 0.00  | 43.69 | 38.09 | 0.00  | 0.00  |
|          |    |     |       |       |       |       |       |       |
|          |    |     |       |       |       |       |       |       |

Table 4 presents that LC50 of all isolates tested are more than 1000 µg/mL. Therefore, these compounds did not have a cytotoxic effect on *A. salina* larvae. Otherwise, if the LC50 value obtained is
less than 1000 µg/mL then, the compound is considered to have a cytotoxic effect against on A. salina larvae. Given the information, these compounds might be used for further research in the pharmaceutical field as antibiotics counterparts [16].

3.5. Molecular identification
Based on the result of the antibiofilm activity assay, M28L2 was selected for sequencing due to high inhibition and destruction activity of antibiofilm formation against P. aeruginosa ATCC 27583. Subsequently, the DNA sequences were inputted into the Basic Local Alignment Search Tool (BLAST) program from the National Center for Biotechnology Information (NCBI). The result of nucleotide BLAST showed that M28L2 had 99% similarity with Pseudomonas stutzeri. Besides, this isolate has been given accession number form Genbank (NCBI) KJ672386.1.

4. Conclusion
Isolates of methylotrophic bacteria isolated from previous studies were used in this study. Based on the result of the antibiofilm assay, most isolates showed antibiofilm activity against specific pathogens at specific concentrations and mechanism. Subsequently, five selected isolates tested had an LC50 value greater than 1000 μg/mL, stating that these compounds are considered non-toxic based on BSLA. Further research is needed to purify the potential bioactive compound, which has high antibiofilm activity against pathogens tested.

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