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Original Article

In vitro antimalarial activity of the biosurfactant produced by *Serratia marcescens* MBC1

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Abstract

Serratia is a bacterium with a distinctive red-pigment prodigiosin, known as a plasmodium growth inhibitor. This species is also known for its reliability as a producer of biosurfactants. Furthermore, the ability of these bacteria to reduce the interface tension in antimalarial activity has not been reported. Therefore, this study aimed to develop biosurfactants as antimalarial drugs candidate. Additionally, tryptone Soy Broth is used as a fermented media to produce biosurfactants with the addition of *Serratia marcescens* MBC1. Biosurfactant activity was evaluated on a hydrocarbon substrate consisting of used motor lubricants, used-cooking oil, and diesel. Emulsifying activity, oil spread test, blue agar, and infrared spectroscopy were methods used for evaluating biosurfactants. Used motor lubricants produced the highest emulsification index at 41.40%. Spectroscopic results using Fourier-transform infrared spectroscopy (FTIR) revealed that the compounds contain glycolipids and lipopeptides. The antimalarial test using *Plasmodium falciparum* d37 obtained an inhibitory concentration 50 of 3.66 µg/mL. There was limited information on the toxicity of biosurfactants in cells of Plasmodium parasites. The use of biosurfactants from *Serratia marcescens* MBC1 to control plasmodium infection needs to be improved to provide an alternative to malaria control from natural ingredients.

Keywords: Antimalarial, Biosurfactant, Glycolipid, Lipopeptide, *Serratia* sp.

1. Introduction

Malaria is one of the deadliest diseases in tropical countries, caused by *Plasmodium* sp., infection [1]. The use of secondary metabolites of biological agents has been used extensively in attempts to control malaria [2]. Meanwhile, it is believed that bacteria have a shorter life cycle for obtaining their secondary metabolites. It has been reported that *Serratia* sp., plays a role in inhibiting the growth of *Plasmodium* sp., responsible for malaria infection [3].

Serratia sp., a member of the Enterobacteriaceae family, is known as a producer of biosurfactants, which are active compounds synthesized on the surface of microbial cells to reduce surface and interface tension [4]. Some biosurfactants are classified

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into lipoproteins, fatty acids, glycolipids, phospholipids, lipopeptides, and other particulate compounds. Furthermore, microbes that produce biosurfactants are widespread in several species, one of which is bacteria [5]. *Serratia marcescens* produced serrawettins, (non-ionic biosurfactants), while *Serratia rubidaea* produced rubiwettin [6].

The bacteria from *Drosophila melanogaster* larvae were isolated and identified as *Serratia marcescens* MBC1. These bacteria were able to produce antioxidant activity in heavy metal stress, larvicide, and high lipase [7–9]. The pigment prodigiosin from *Serratia* sp. has long been known for its use as antimalarial [10]. However, information on the potential of biosurfactants from these bacteria to inhibit the growth of Plasmodium parasites is not widely reported. Therefore, this study aimed at producing biosurfactants from some substrates, and the highest biosurfactant activity was used for the in vitro antimalarial test.

2. Materials and Methods

2.1. Media Culture and Fermentation

Serratia marcescens MBC1 was cultured on tryptone soy agar (TSA) media and adjusted to a pH of 7.3, and the sloping culture was prepared for stock. A single loop inoculum was removed and inoculated into a 10 mL test tube containing tryptone soy broth (tryptone water/TW) media. A total of 10 ml of 24 h ripened sourdough was taken and placed in 90 ml of TW media. Bacteria were incubated for 7 days at room temperature. The supernatant was harvested by centrifuging the culture at 6000 rpm for 30 minutes. The crude extract was filtered through filter paper and dissolved in a 1:1 mixture of methanol and ethyl acetate solvent [12].

2.2 Biosurfactant screening activity

2.2.1 Emulsification activity

The performance of biosurfactants was tested using the emulsification index method [13]. Used motor lubricants, used cooking oil, and diesel oil were the hydrocarbons that were applied. The crude extract was homogenized with the hydrocarbons through a vortex machine at high speed for 2 min. The stability was measured after an hour compared to 48 h, with 3 replications. The following formula was used to measure the emulsification operation (E24 or EI):

$$EI = \frac{Total\ height\ of\ the\ emulsion\ layer}{Total\ height\ of\ the\ system}\ x\ 100 \quad (1)$$

2.2.2 Displacement of oil

Oil dispersibility was evaluated using a process devised by Morikawa [14]. In the center of a Petri dish containing 10 mL of distilled water, 1 mL of hydrocarbons (used motor lubricant, used cooking oil, and diesel fuel) was added. 20 µL of culture supernatant was dropped on top of the oil layer. The diameter of the displacement zone in oil was observed and measured using 3 replications.

2.2.3 Glycolipid detection

The blue agar test was used to detect biosurfactants from the glycolipid group [15]. 0.2g cetyltrimethylammonium bromide (CTAB), 0.005g methylene powder, and a minimally saline medium are dissolved in 1000 ml sterile water. 50 microliters of crude biosurfactant was added to the blue agar medium in the Petri dish. The plates were left at room temperature for 2 days. The blue hazes formation was indicated as a positive result. The treatment was carried out in 3 replications.

2.2.4 Infrared Spectroscopy

An Agilent Cary 630 spectrometer was used to detect infrared (IR) spectroscopy of the crude extract. Infrared spectra ranging from 400 to 4000 wave numbers per centimeter were obtained (cm^{-1}). In the resolution, there were two sizes per wave number.

2.3 In vitro antimalarial testing procedure

The chloroquine-sensitive *Plasmodium falciparum* strain d37 was selected and used in an in vitro antimalarial activity test [16]. About 1 mg of the sample extract was dissolved in 100 µL of dimethyl sulfoxide (DMSO) (stock solution, concentration 10.000 µg / mL). Furthermore, a serial dilution was carried out from the stock solution and synchronous parasites (ring stage) with about 1% parasitemia were used in this test. Parasites were added to a 96-well plate using a high-flow liquid handler, and then 2 µl of the test solution at different concentrations (100, 10, 1, 0.1, and 0.01 µg/ mL) dripped in. The gas mixture (5% O2, 5% CO2, and 90% N2) was flowed on a test plate placed in the chamber, then incubated for 48 hours at 37 °C. The culture was harvested and a thin blood layer was prepared with 20% Giemsa staining. The blood smear was observed under a microscope, and the number of infected erythrocytes was then counted per 1000 normal erythrocytes, in Duplo measurement. To determine the percentage of growth, the following formula was used:

$$\% \text{ growth} = \% \text{ parasitemia} - \text{D0} \quad (2)$$

While D0 informed as the initial hour of growth (%)

The inhibition ability was expressed as a percentage calculated using the formula below:

$$\text{The percentage of inhibition} = 100\ \% - ((\text{Xu} - \text{Xk}) \times 100\%) \quad (3)$$

Description:

Xu = % growth on the test solution

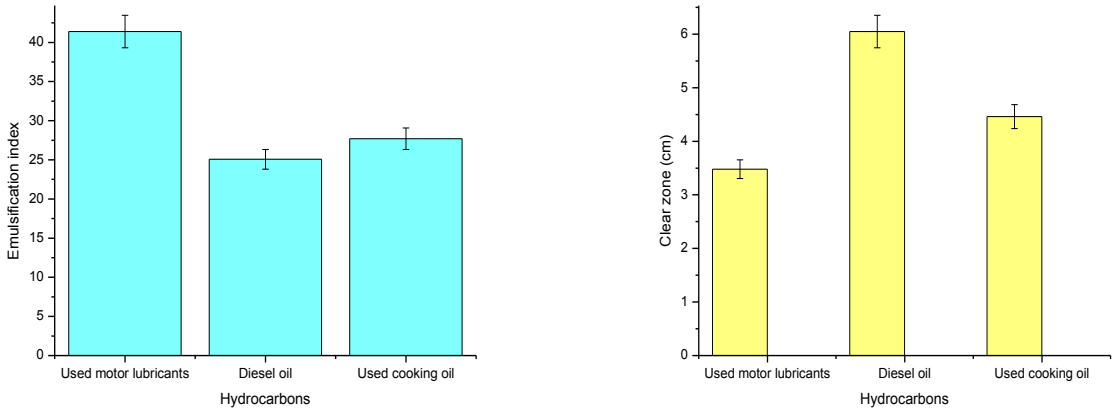
Xk = % growth on the negative control

The two formulas above were used to determine the concentration of the test material that can inhibit the growth of parasites to about 50%. The inhibitory concentration (IC-50) was estimated, through probit analysis according to the inhibition percentage data.

3. Results and Discussion

3.1. Biosurfactant screening activity

The members of the *Serratia* genera mainly produced two classes of biosurfactants, namely



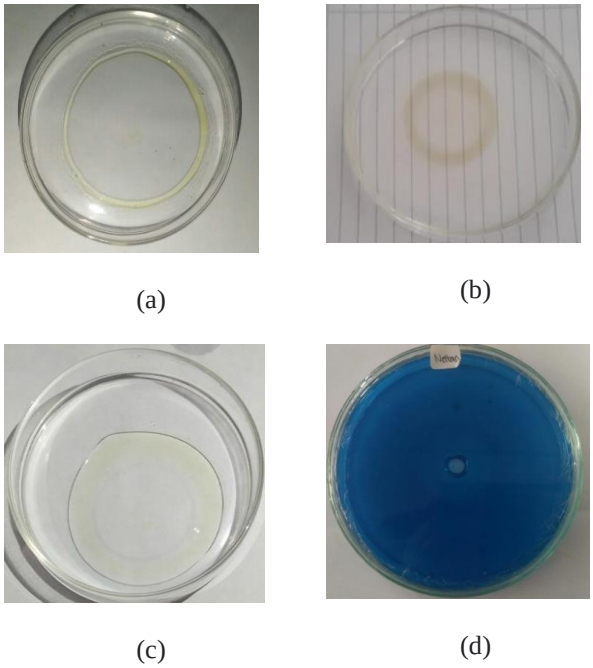
(a) (b)

Figure 1. Emulsification activity (a) and spreading oil diameter (b) of free cell supernatant of *Serratia marcescens* MBC1.

lipopeptides, and glycolipids. Subsequently, lipopeptides produced by *Serratia* species included serrawettins and Stephens slides. In addition, the glycolipids identified included rubiwettins and rhamnolipids [17].

Serratia marcescens strain MBC1 has shown the ability to emulsify hydrocarbons. These results were identified from the formation of emulsions in the addition of free cell biosurfactants to used motor lubricants, used cooking oil, and diesel oil. The highest emulsification index was achieved with the addition of used motor lubricants at 41.40%, followed by used cooking oil at 27.70% and diesel fuel at 25.07% (**Figure 1**). The emulsification test was an indirect method that examined the presence of biosurfactants. The reason was that if the supernatant contained a biosurfactant, it would be able to emulsify the hydrocarbons, which act as hydrophobic substrates [18]. One of the roles of biosurfactants was to increase the contact between the hydrophobic substrate and water as well as to figure out the role of the emulsification index [19].

Oil displacement was used as a direct test for the presence of biosurfactants in forming clear zones in the presence of hydrocarbons [20]. The results of the oil dispersion observations showed that the crude extract biosurfactant formed the largest clear zone in the motor lubricant used (**Figure 2**). The anionic surfactants were



(a) (b)

(c) (d)

Figure 2. Oil spreading test in (a) used cooking oil, (b) used motor lubricants, (c) diesel oil, and (d) the blue agar assay result.

specifically tested using the CTAB agar test. Anionic surfactants in aqueous solutions control the formation of insoluble ion pairs with certain cationic chemicals [21]. The blue agar was considered a semi-qualitative method for determining the presence of glycolipid-type biosurfactants [22].

This study showed that biosurfactants produced by *Serratia marcescens* MBC1 can emulsify various hydrocarbons, thereby increasing the availability of recalcitrant hydrocarbons. These properties explain the applicability of this strain to multiple hydrocarbon contaminants.

Our study showed that biosurfactants produced by *Serratia marcescens* MBC1 could emulsify various hydrocarbons, thereby increasing the availability of stubborn hydrocarbons. These properties explained the applicability of this strain to several hydrocarbon contaminants.

FTIR analysis (**Figure 3**) detected bandwidth at 3373.99 cm^{-1} and it is believed to be related to O-H stretching and its width has been attributed to hydrogen bonding [23]. The bands at 3267.92 cm^{-1} showed the presence of NH groups, and the absorption at 400 to 1250 cm-1 indicated the deformation of the C-O bond [24]. The spectrum at positions 1577 and 1406 cm^{-1} was related to the

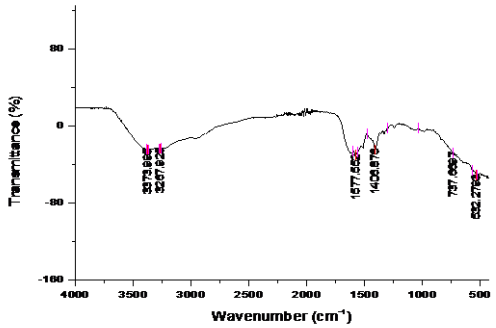


Figure 3. Infrared spectroscopy using FTIR at biosurfactant produced by *Serratia marcescens* MBC1.

vibrational strain of the asymmetric and symmetrical free carboxylate groups [25]. Meanwhile, two classes of biosurfactants, classified into lipopeptides and glycolipids, have been discovered from *Serratia* species. Strains that have produced biosurfactants include *Serratia surfactantfaciens*, *Serratia marcescens*, and *Serratia rubidaea* [17]. Previous research found a high level of lipolytic activity in the fermented extract of TSA medium using *Serratia marcescens* MBC1 [7, 26]. These results demonstrated the lipopeptide-type biosurfactant character. Then, blue agar (**Figure 2**) confirmed the presence of a glycolipid group.

3.2 Antiplasmodium Activity

Plasmodium showed greater growth after administration of the lower concentration of biosurfactant, while growth was inhibited with increasing doses (**Figure 4**). The level of

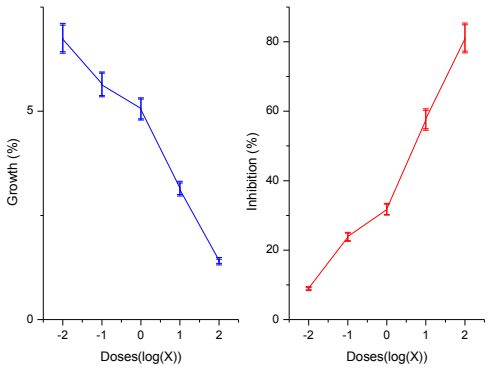


Figure 4. The effect of biosurfactant treatment at *Plasmodium falciparum* d37 after 48 h in the growth and inhibition.

concentration that inhibited half of the population was called IC50. The biosurfactant extracted from *Serratia marcescens* MBC1 was able to prohibit *Plasmodium falciparum* d37 at a level of 3.66 µg/mL (Fig 5). This biosurfactant was not separated

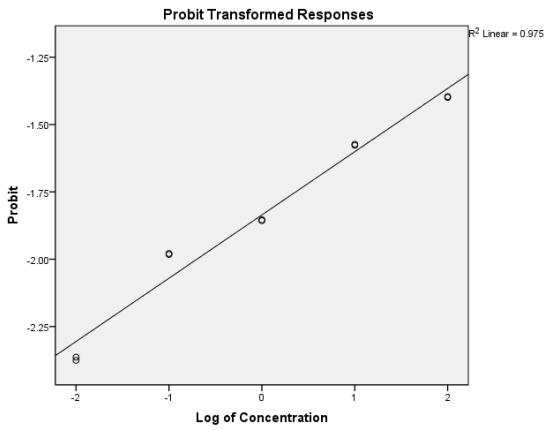


Figure 5. Probit analysis to determine the inhibitory concentration of *Plasmodium falciparum* d37 after 48 h treated using biosurfactants. IC50=3.66 µg/ mL.

into a purified compound. The biosurfactant extract from *Serratia marcescens* was identified as a glycolipid and a lipopeptide. Purified glycolipid produced by marine sponges has been confirmed to inhibit the growth of chloroquine-resistant plasmodium to about 0.53 µM. Furthermore, *Okeania* sp. marine cyanobacteria were reported to produce a type of lipopeptide identified as an ikoamide. This compound exhibited plasmodium inhibitory activity with an IC50 value of 0.14 µM [27].

Lipopeptide and glycolipid showed no significant cytotoxicity for different human and cell lines [27, 28]. It was also reported that glycolipids can activate immunocompetent cells. This became the reason for the adjuvant effect of the vaccine [29, 30]. Ceramide-analog sphingolipids and other glycolipids were reported to inhibit *P. falciparum* growth by altering internal ceramide content. The prospective result was required in detail for further research on glycolipid and lipopeptide. The prospective result was needed in detail for further research on glycolipids and lipopeptides. If the glycolipid was found to be able to modulate the immune system and kill plasmodium cells, the biosurfactant MBC1 from *Serratia marcescens* has the potential as a vaccine adjuvant and drug candidate. Moreover, the lipopeptide or glycolipid was harmless to human cells.

The mechanisms by which biosurfactants can be toxic to plasmodium cells is limited. Oxygen stress treatment showed no parasitic activity [31]. While the ability to lower the surface tension of water reduces the availability of oxygen, leading to cell death. However, biosurfactants synthesized by *Bacillus subtilis* A1 and *Pseudomonas stutzeri* NA3 were also able to eradicate *Anopheles* mosquitoes (plasmodium host) [32]. The notion that biosurfactants affect the conformation of sulphide bonds in DNA, cause denaturation of enzymes, disrupt membrane permeability, and disruption of proton driving forces, resulting in cell damage, was still questionable. Especially in the case of the physiological effect on the plasmodium parasite [33].

4. Conclusion

The crude extract produced by *Serratia marcescens* MBC1 exhibited biosurfactant properties. This was evidenced by hydrocarbon emulsifying activity, oil displacement, glycolipid properties, and spectroscopic-based group identification. This compound was able to inhibit the growth of the Plasmodium parasite. Further research is needed to develop better results, especially in vivo and the effects of toxicity on non-target cells.

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Conflict of interest

The authors declare to have no conflict of interest.

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