ANTITUBERCULOSIS EFFECTIVENESS OF ENDOPHYTIC ANDALAS MUTANT BACTERIA PRODUCS INDUCED BY UV LIGHT WILL BE CONDUCTED

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ABSTRACT

Indonesia is the 3rd largest country in the world in cases of tuberculosis (TB) infection. Infections caused by the bacteria Mycobacterium Tuberculosis have claimed 100,000 lives per year. This death rate is expected to continue to increase due to resistance to Antituberculosis Drugs (OAT). One strategy to overcome the increasing number of resistance of M. tuberculosis bacteria to OAT is to find new and better antituberculosis active compounds. This study aims to determine the potential of Andalas Endophytic Bacteria mutated by UV light on antituberculosis activity. This research is descriptive research. The research was conducted at the Research Laboratory of the Faculty of Mathematics and Natural Sciences, Padang State University. The mutation process is carried out as a form of increasing the production of active compounds. Natural anti microbial compounds can be obtained through the fermentation process. The active compounds from the fermentation products were extracted using methanol and aquadest as solvents. Testing against M. tuberculosis using the Mycobacterium Growth Indicator Tube (MGIT) 960 System as a medium for sensitivity testing. The fermented supernatant with absolute concentration and extract of each solvent with a concentration of 75% were mixed into the MGIT 960 tube. The results showed that the length of time UV irradiation could reduce the number of endophytic bacterial cells of Andalas isolate ATB 10-6. UV light also causes changes in the morphology of mutant bacterial colonies. UV irradiation can produce mutant isolates that have better antifungal activity, where of the 8 mutant bacteria produced, 7 mutants have better antifungal activity than the wild type, and the growth of mutant bacteria is faster than the wild type.

Keywords: Mycobacterium tuberculosis; Antibacterial; Andalas Endophytic Bacteria Isolate; UV Light.

1. INTRODUCTION

1.1.Background

The increasing number of resistance to Antituberculosis Drugs (OAT) is the biggest problem in overcoming cases of tuberculosis (TB) infection. In 2011 cases of tuberculosis in the world is estimated to reach 8.7 million cases. WHO data (2019) states that the estimated number of TB cases in Indonesia is 843,000 people and according to Indonesia's TB data in 2020, the number of TB cases has increased to 845,000 and the number of deaths is more than 13,000 people. Director of Prevention and Control of Directly Infectious Diseases at the Ministry of Health, Wiendra Waworuntu, said that the handling of Drug Resistance TB (RO) is also problematic in Indonesia. The number increases every year and it is predicted that there will be around 24,000 in 2020. This is in line with the WHO estimate that there are 23,000 cases of Multi Drug Resistance (MDR) / Rifampicin Resistance (RR) in Indonesia. Treatment using rifampin and isoniazid (INH) does not provide high effectiveness against new strains of Mycobacterium tuberculosis MDR bacteria, so a combination therapy with other anti tuberculosis drugs is needed (Zhang, 2005). Researchers at the UGM Center for Tropical Medicine in 2021 revealed that this drug-resistant TB condition requires more complex and long-term treatment, as well as greater side effects. This encourages efforts to overcome tuberculosis resistance, one of which is by exploring active compounds from natural ingredients that have the potential as alternative TB treatment. Researchers at the UGM Center for Tropical Medicine in 2021 revealed that this drug-resistant TB condition requires more complex and long-term treatment, as well as greater side effects. This encourages efforts to overcome tuberculosis resistance, one of which is by exploring active compounds from natural ingredients that have the potential as alternative TB treatment. Researchers at the UGM Center for Tropical Medicine in 2021 revealed that this drug-resistant TB condition requires more complex and long-term treatment, as well as greater side effects. This encourages efforts to overcome tuberculosis resistance, one of which is by exploring active compounds from natural ingredients that have the potential as alternative TB treatment.

Andalas plant (Morus macroura) is an endemic plant of West Sumatra which contains active compounds that have the potential as antituberculosis. Based on the research of Soekamto et al (2003), several compounds contained in this plant are stilbene derivatives, 2-arylbenzofuran, morasin M, Andalasin A, Andalasin B and other active compounds. These compounds can be obtained from endophytic bacteria that live on Andalas plants. Many efforts can be made to increase the activity of compounds in endophytic bacteria that have antituberculosis potential, one of which is by mutation.

According to Hollander (1995) mutation is a change in the nucleotide sequence of a nucleic acid. One of the ways to induce mutations is by means of ultraviolet (UV) irradiation. Ultraviolet light is known to penetrate strongly enough in the cell walls of microorganisms that are able to change the composition of their nucleic acids. Research by Alfiky (2019) using Trichorderma spp. irradiated with ultraviolet light increased antagonistic activity against soil-borne fungal pathogens.

Mycobacterium tuberculosis is a bacterium that has a more complex cell structure and function than other bacteria. So, we need active compounds that have better antimicrobial activity. Exploration of the ability of Andalas endophytic bacteria to produce antibacterial compounds (gram positive and gram negative) has been carried out by Putri (2018), Afifah (2018) and Yandila (2018). The optimum conditions for fermentation of Andalas endophytic bacteria in maximizing the production of antibacterial compounds have also been successfully carried out by Rifa (2019), Anggiastanti (2019) and Mahjani (2019). However, each antimicrobial active compound has a different mechanism of action against the target microbe. Likewise, each type of microbe has a different response to the active compound.

There has been no scientific report on the antituberculosis activity of the Andalas endophytic bacteria which have been mutated so that they are able to produce antituberculosis active compounds. For this reason, a study entitled "Antituberculosis Effectiveness of Endophytic Andalas Mutant Bacteria Products Induced by UV Light will be conducted"

1.2.Research Specific Purpose

To determine the effect of UV light induction to produce mutant Andalas endophytic bacteria. To determine the effect of UV light induction on the growth of Andalas mutant endophytic bacteria. To determine the effect of UV light induction on the antituberculosis activity produced by Andalas mutant endophytic bacteria.

2. LITERATURE REVIEW

A. Tuberculosis Resistance.

Indonesia is the 3rd largest country in the world in cases of tuberculosis (TB) infection. Infections caused by Mycobacterium tuberculosis have claimed 100,000 lives per year (Intan, 2019). According to WHO data (2020) an estimated 1.2 million (range, 1.1 - 1.3 million) deaths are caused by TB. This death rate is expected to continue to increase due to resistance to Anti tuberculosis Drugs (OAT). Based on the WHO statement in 2018 there were half a million people experiencing resistance to OAT W, only 1 in 3 people received treatment and only 56% of treatment was successful. One strategy to overcome the increasing number of resistance of M. tuberculosis bacteria to OAT is to find new, better anti tuberculosis active compounds (Gurib-Fakim, 2006).

B. Andalas Endophytic Bacteria as Producing Antimicrobial Compounds

Andalas plant with the scientific name Morus macroura Miq. (Family Moraceae) is an endemic plant of West Sumatra which the local community calls Andaleh. (Sosef et al., 1998). Chinese medicine has long used several types of Morus including; M. Alba, M. Bombycis, M. Multicaulis, used for cough medicine, asthma, hypertension, influenza and rheumatism. Based on the results of research conducted (Hakim et al., 2008) Andalas plant contains chemical compounds in the form of betulinic acid and other similar chemical compounds. These compounds can inhibit viral replication, antitumor, melanoma and inflammation in humans. Andalas plants are also known to contain phenolic compounds derived from stilbenes and coumarin derivatives. The stilbene-derived phenolic compounds found in the Andalas plant are; oxyresveratrol and stilbene dimers, namely Andalasin A and Andalasin B mulberofuran K, and one coumarin derivative compound, namely 7 hydroxycoumarin or umbeliferone (Sukanto et al., 2001). Stilbene derivatives exhibit antimicrobial and antioxidant activity (Ferdiaz et al, 2003). Andalas plants are also difficult to cultivate (Syamsuardi et al., 2015). Therefore, the direct production of bioactive compounds from Andalas plants is not effective, because it can damage existing biological resources. Bioactive compounds can be obtained efficiently by using endophytic bacteria. Andalas plants are also difficult to cultivate (Syamsuardi et al., 2015). Therefore, the direct production of bioactive compounds from Andalas plants is not effective, because it can damage existing biological resources. Bioactive compounds can be obtained efficiently by using endophytic bacteria. Andalas plants are also difficult to cultivate (Syamsuardi et al., 2015). Therefore, the direct production of bioactive compounds from Andalas plants is not effective, because it can damage existing biological resources. Bioactive compounds can be obtained efficiently by using endophytic bacteria.

Endophytic bacteria are bacteria that have a mutualistic symbiosis with their host, these bacteria live in plant tissues (Kumala et al., 2008). Andalas endophytic bacteria (Morus macroura) is one of the candidates for producing antituberculosis compounds. Exploration of the ability of Andalas endophytic bacteria to produce antibacterial compounds (gram positive and gram negative) has been carried out by Putri (2018), Afifah (2018) and Yandila (2018). The optimum conditions for fermentation of Andalas endophytic bacteria in producing antibacterial compounds have also been successfully carried out by Rifa (2019), Anggiastanti (2019) and Mahjani (2019).

C. UV irradiation

Mutation is a process of changes that occur in the nucleotide sequence in nucleic acids (Hollaender, 1995). Mutations can be non-lethal, sub-lethal, or lethal. Mutations can also cause a gene to lose its function or have a new function. Mutations can occur due to the presence of mutagens (Nasir, 2002). Ultraviolet light is one of the most effective mutagens. As a mutagen resulting from physical treatment, ultraviolet light has a lower penetration power than gamma rays and X rays (Hardianto et al., 2015). Mutagenesis induced using UV light is known to be effective for selecting microorganisms to produce biologically active substances and increasing their activity (Goodarzi, 2016). The use of UV light as a mutagen has been widely reported. Research conducted by Widowati et al., (2018) mutations using nitric acid for 30-120 minutes and UV light at 20 minutes of irradiation on TC isolate 4.3.1.2 were able to increase the production of IAA (Indole Acetic Acid). IAA production by UV mutants was higher than pure strains.

D. Extraction and Activity Test of Antimicrobial Compounds

The extraction method can be used to separate the antimicrobial compounds produced in the fermentation process with the fermentation medium. The extraction process will produce concentrated and pure antimicrobial compounds (Susanty and Bachmid, 2016). The solvents commonly used in the extraction process are ethanol, n-hexane, isopropanol, ethyl acetate, acetone, and methanol. Methanol is the most widely used solvent in the process of extracting organic compounds from natural ingredients (Susanti, 2012). The resulting methanol extract has a high total content of phenolic compounds. The activity of active compounds extracted from natural ingredients needs to be tested. The antimicrobial activity test method can be carried out by diffusion or dilution (Pratiwi, 2008). The activity of extracts of ATB 10-6 isolates worked to inhibit the growth of their respective microbes on S. mutans and C. albicans with the best concentration of 12.5%. The mouthwash formula of methanol extract of Andalas endophytic bacterial fermentation product has the potential to inhibit the growth of pathogenic microbes in the oral cavity. (Rahayu, 2020).

Research methods

This type of research method is carried out through empirical experiments carried out through laboratory research.

Research Stages

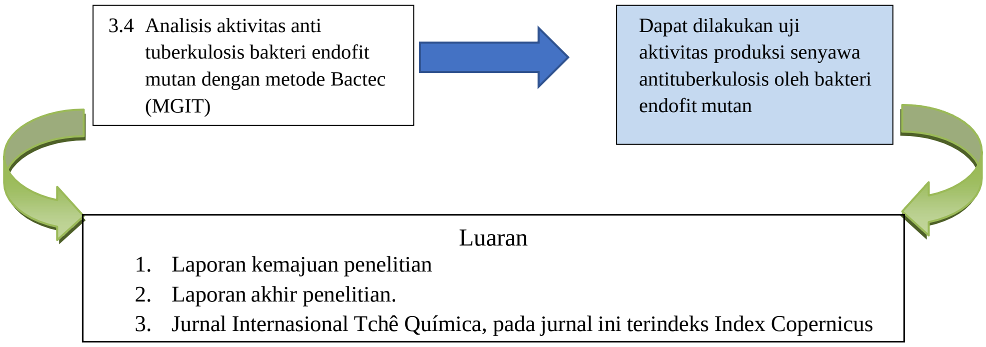

Figure 1. Research Stages
(source : Courtesy of Annisa Mitri)

Figure 2. Research procedure
(source : Courtesy of Annisa Mitri)

3. EXPERIMENTAL

Table 1. Number of Endophytic Bacterial Cells Growing After UV Radiation

Exposure Time	Amount Bacterial Colony	Number of Bacterial Cells (Cells/mL)
wild type	3	3.107
20 minutes	Infinity	Infinity
30 minutes	29	290
40 Minutes	6	60
50 Minutes	0	0
60 Minutes	2	20

Table 2. Results of UV Radiation on Colony Morphology of Endophytic Bacteria Isolate ATB 10-6.

Isolate code	**Colony Morphology**			
	Form	**Edge**	**Elevation**	**Color**
wild type	**Irregular**	**Undulate**	**Convex**	**White**
Mu ATB 10-6 40-K1(A)	circular	Undulate	Raised	White
Mu ATB 10-6 40-K2 (B)	circular	Undulate	Raised	White
Mu ATB 10-6 40-K3 (C)	circular	Undulate	Raised	White
Mu ATB 10-6 40-K4 (D)	circular	Undulate	Raised	White
Mu ATB 10-6 40-K5 (E)	circular	Undulate	Raised	White
Mu ATB 10-6 40-K6 (F)	circular	Undulate	Raised	White
Mu ATB 10-6 60-K1 (G)	circular	Undulate	Flat	White
Mu ATB 10-6 60-K2 (H)	circular	Undulate	Raised	White

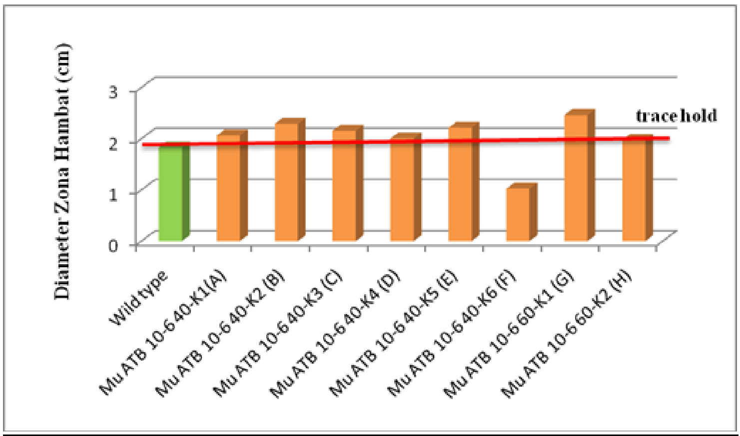

Figure 3. Test Results of Endophytic Bacteria Isolate ATB 10-6 after UV irradiation. (source : Courtesy of Annisa Mitri)

4. RESULTS AND DISCUSSION

4.1 Effect of UV Radiation on Microscopic Character

Based on the data in Table 1, it is known that the bacterial irradiation with UV for 20 minutes was still able to maintain the colonies that grew in large enough numbers. The number of bacterial colonies that were able to grow after irradiation of more than 30 minutes was reduced and less when compared to the wild type. On UV irradiation for 30 minutes the colonies grew 29 colonies, irradiation for 40 minutes there were 6 colonies, irradiating for 50 minutes no colonies grew, and irradiation for 60 minutes there were 2 colonies that grew.

4.2 Increase Production of Antifungal Compounds Isolate ATB 10-6 by Fermentation

Mutant isolates which had better activity than the wild type were fermented. Fermentation is carried out for 24 hours. Based on the graph in Figure 5, it is known that the fermentation of mutant and wild type ATB 10-6 isolates had the best activity during 24-hour fermentation. The highest activity was produced by Mu ATB 10-6 60-K2 (H) with an inhibition zone diameter of 3.73 cm. Based on these data, the fermented Mu ATB 10-6 60-K2 (H) was continued to the maceration process and made in the form of an extract.

4.3 Extraction of Endophytic Bacterial Fermentation Products Andalas Isolate Mutant ATB.10-6

Mu ATB 10-6 60-(K2) isolate which had the best activity after fermentation, was macerated using aquadest and methanol as solvents. The results obtained were 1000 mL brownish yellow filtrate. Evaporation results using*rotary vacuum evaporator*obtained a thick brown extract weighing 1.38 g for aquadest solvent and 2.55 g for methanol solvent. The comparison of the antimicrobial activity test of the fermented extract can be seen in Figure 6. The results of the observation of the inhibition zone of the endophytic bacterial fermentation product extract using aquadest solvent were better than using methanol as solvent.

In this study, two mutant isolates of Andalas endophytic bacteria were tested, namely Mu BJTA Isolate 2.1 (the mutation process until extraction was carried out by other researchers) and Mu ATB isolate 10-6. The two isolates had different activities. Mu isolate BJTA 2.1 had antibacterial activity, while Mu isolate ATB 10-6 had antifungal activity. In theory*M. tuberculosis*has characteristics that distinguish it from bacteria in general. The cell wall of this bacterium is very complex, consisting of a layer of fat with a percentage of up to 60%, mycolic acid, complex wax, trehalose dimycolate, and mycobacterial sulfolipids that play a role in virulence (Karina, 2013). Thus, it is necessary to do a comparison to see the most potential compounds in inhibiting the growth of M. tuberculosis.

The potential results of this study are pthe discovery of antituberculosis compounds as a substitute for OAT. This is also an effort to preserve the endemic plants of West Sumatra. z If the active compound works well against M. tuberculosis as a substitute for OAT, then the Andalas mutant endophytic bacteria and the new antituberculosis active compound are patented.

5. CONCLUSION

Tuberculosis is the first infectious cause of death in the world. Tuberculosis cases continue to increase, one of the causes is resistance to OAT. Bacteria have the potential to produce tuberculosis compounds. UV irradiation can increase the active compound of tuberculosis. After UV irradiation, the results showed that the number of colonies and the morphology of wild type and mutants had significant differences. Mutant isolate activity was better than wild type. After fermentation, the best activity was obtained by isolate code Mu ATB 10-6 60-K2. The aquadest solvent fermentation product extract has better activity than methanol solvent.

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