Artikel indah 3

by Artikel Indah 3 Artikel Indah 3

Submission date: 12-Jun-2023 06:32AM (UTC+0200)
Submission ID: 2114189659
File name: 03._Artikel_IJLERA.pdf (185.49K)
Word count: 3340
Character count: 17972

International Journal of Latest Engineering Research and Applications (IJLERA) ISSN: 2455-7137

Volume – 08, Issue – 01, January 2023, PP – 70-75

Optimization of Fermentation Condition of Lansano Endophyte Bacteria (*Pterocarpus Indicus* Willd) Isolate BA 1 to Produce Antibiotic Compounds

Nurhasnah[1], Dwi Hilda Putri[2], Media Roza[1], Milya Sari[1], Indah Kencanawati[3]

[1]*Department of Science Education , Faculty of Tarbiyah and Teacher Training, Universitas Islam Negeri Imam Bonjol Padang, Indonesia*

[2]*Department of Biology, Faculty of Mathematics and Sciences, Universitas Negeri Padang, Indonesia*

[3]*Department of Biology Education, Faculty of Tarbiyah and Teacher Training, State Islamic Institute of Kerinci, Indonesia*

Abstract: Increasing cases of antibiotic resistance encourage researchers to find new bioactive compounds. The source of the newly developed antibiotics is utilizing endophytic bacteria. BA1 isolate is an endophytic bacterium from Lansano plant (Pterocarpus indicus Willd) which has high antimicrobial activity. Antibiotic compounds are produced through a fermentation process. The purpose of this study was to optimize the fermentation conditions of endophytic bacteria Lansano isolate BA1 in producing antibiotic compounds. Optimization of the fermentation time was carried out for 72 hours. Optimization of starter fermentation using concentrations (1%, 5%, 10%, and 15%). While the optimization of the fermentation medium used the types of medium MH, NB, LB, and LBB. Antibiotic activity test (antifungal and antibacterial) was carried out using the paper disc diffusion method. The results showed that the endophytic bacteria Lansano isolate BA1 had a growth curve consisting of: a lag phase at 0 to 4 hours of fermentation, a log phase at 8 to 12 hours of fermentation, and a stationary phase starting after fermentation. 12 o'clock. The best fermentation medium for producing antibacterial compounds by endophytic bacteria Lansano isolate BA1 is MH medium. There was an effect of the duration of fermentation of the endophytic bacteria Lansano isolate BA1 on different types of medium to produce antibiotic compounds. The best concentration of starter fermentation for producing antibiotic compounds by endophytic bacteria Lansano isolate BA1 was 10% for antifungal and 5% for antibacterial.

Keywords: Optimization, Endophyte Lansano bacteria (Pterocarpus indicus Willd), BA 1 isolate, Antibiotic

I. Introduction

One of the new sources of antibiotics developed is by utilizing endophyte bacteria. Endophyte bacteria are bacteria that live in plant tissues that are able to grow colonization without disturbing their host. Some types of endophyte bacteria are already known to produce active compounds that are antibiotic, antimalarial and antibacterial[1], [2]. Research conducted in 2018 successfully isolated 7 endophytic isolates from Lansano roots, stems, and leaves (*Pterocarpus indicus* Willd) that have antibacterial activity [3]. BA 1 isolate is one of the isolates that has high antibiotic activity. Antibiotic activity test conducted by [4], showed that this isolate was able to inhibit the growth of *S. aureus* with a bland zone of 1.50 cm and inhibit the growth of *E. coli* with a bland zone of 1.25 cm.

To produce antibiotic compounds produced by Lansano endophyte bacteria ba 1 isolate needs to be done fermentation process. The fermentation process must take place in optimal conditions in order to get the maximum product. The optimum conditions of fermentation are influenced by external and internal factors. Environmental conditions in the form of temperature, pH, *starter* concentration, medium composition and fermentation time are external factors that also play a role in determining the optimum conditions of the fermentation process [5], [6]. Internal factors that affect the fermeation process are types of bacteria. Different types of bacteria will affect metabolic pathways and the types of active compounds produced [7].

The optimum time of fermentation is also influenced by external factors of bacteria, including the type of medium [8]. According to [9], the medium is a nutrient used for bacterial growth. Each bacterium needs a different nutrient content. Based on the study [10], *Streptomyces gulbargensis* DAS 131 bacteria showed high antibiotic activity when cultured in media with glucose (2%), pepton (1%), NaCl (5%), and K2HPO4 (0.05%).

Another external factor that also affects the optimum conditions of fermentation is *strarter* concentration. According to [11], starters are a number of microbes that have been grown at their best, after previously the microbes were dormant. The concentration of starters calculated in the fermentation medium exerts an influence

on the optimum process of fermentation. Low starter concentration will cause the resulting product is also low, conversely very high *starter* concentration will inhibit bacterial growth due to nutritional limitations [12].

Based on the description above, it is necessary to know the optimum fermentation conditions of Lansano endophyte bacteria isolated BA 1, which is already known for its ability to produce antibiotic compounds. This study will optimize the fermentation conditions of BA 1's endophyte bacteria to produce antibiotic compounds. Optimized fermentation conditions are limited to fermentation time, medium type and starter concentration.

II. Experimental Section

1. Optimization of Fermentation Medium

Starter culture is made by inserting 1 ose isolate BA 1^{-6} into the *Erlenmeyer* containing 10 mL of sterile medium. *Starters* are made on different mediums, according to the medium used. The *starter* of $BA1^{-6}$ endophyte bacteria isolates that have been grown, then diluted with NaCl 0.9% until the turbidity is equivalent to *Mc Farland's* 1. *Starters* areculated as much as 10% into each fermentation medium (NB, LBB, MH, LB). In this study, the volume of the fermentation medium used was 10 mL. Furthermore, the culture is incedated in the incubator shaker at room temperature at a speed of 150 rpm for 48 hours. Footage of the fermentation medium is taken every 24 hours (as much as 300 µL each) using a micropipet and inserted into an eppendorf tube. Fermentation is concentrated at 4000 rpm at 4^{o}C for 30 minutes. Supernatan is stored at a temperature of 4^{o}C which will be used for antibacterial activity tests.

2. Optimization of Fermentation *Starter* Concentration

Starter culture is made by inserting 1 ose isolate BA 1^{-6} into erlenmeyer which contains 10 mL of fermentation medium (best medium according to medium optimization) that has been sterile. The *starter* culture is incessed in the *incubator shaker* at room temperature at a speed of 150 rpm for 12 hours. Furthermore, a *starter* of BA 1^{-6} isolate bacteria that has been grown on a fermentation medium is diluted with 0.9% NaCl until its turbidity is equivalent to *Mc Farland's* 1. The *starter* is incorporated into a 10 mL fermentation medium according to the variation in the concentration of the *starter*. In this study used variations in concentration in the form of 5%, 10% and 15%. The culture is incessed in the *incubator shaker* at room temperature at a speed of 150 rpm for 48 hours. Footage of the fermentation medium is taken every 24 hours (as much as 300 µL each) using a micropipet and inserted into an *eppendorf* tube. Fermentation is concentrated at a speed of 4000 rpm at 4^{o}C for 15 minutes. Supernatan is stored at 4^{o}C, which will be used for antibiotic activity tests.

3. Observation

3.1 *Optical Density* Calculation

OD calculations aim to determine the density of bacterial cells in the fermentation culture medium. A total of 200 µL of fermented culture is incorporated into the kuvet, then diluted by adding with 1800 µL of sterile *aquadest*. OD values are measured based on absorbance values using spectofotometers at wavelengths of 625 nm [13]. Calculations are done on each replay.

3.2 Antibiotic Activity Test

The method used for antibacterial activity tests is diffusion of disc paper [14]. The test microbes used at this stage are *C. albicans* and *E.coli*, which are grown on PDA mediums. Before use, the *E.coli* isolate is rejuvenated first. The *E. coli* suspension is made with a 0.9% NaCl solvent, with turbidity equivalent to *Mc Farland's* 0.5. The suspension is then calculated into the PDA medium, by applying it using a sterile *cotton bud*. The roughly fermented extract of BA 1 isolates to be tested, taken as much as 20 µL, is then dripped on sterile disc paper. Next the disc paper is placed on a PDA medium that has been inoculated with the test microbes *C. albicans*. Petri dishes are inchabited in reverse at room temperature. The bland zone around the disc paper is measured using a funnel period after 24-48 hours of incubation.

4. Data and Data Analysis

This research data is primary data. The bacterial growth curve is analyzed based on OD measurement results. Antibiotic activity is analyzed based on the diameter of the hambat zone. Both of these data are shown in one graph, which will show the relationship of growth curve and antibacterial activity produced by Lansano endophyte bacteria isolated BA 1. The fermentation medium optimization data and the concentration of fermentation *starters* are statistically processed using the ANOVA test. If there is a significant difference, it will be continued with a further test of 5% BNJ. Statistical analysis data is displayed in the form of a table.

III. Result and Discussion

1. Optimization of Isolated Fermentation Time BA 1

Fermentation time optimization is analyzed by creating a graph that illustrates the relationship between the bacterial growth curve and the activity of the resulting antibiotic active compound. The bacterial growth curve is based on *optical dencity* (OD) values the results of OD measurements produced by Lansano BA1 endophyte bacteria for 72 hours can be seen in Fig 1.

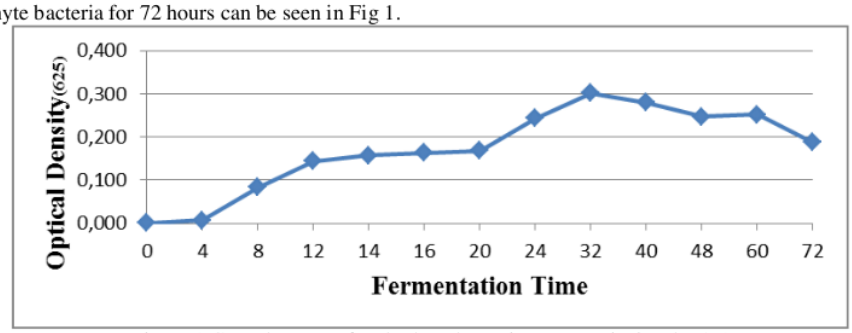

Figure 1 Growth curve of endophyte bacteria Lansano isolated BA 1

Based on the growth curve in Fig.1 it can be seen that the lag phase (adaptation) occurs in the fermentation of the 0th hour until the fermentation of the 4th hour. The exponential phase (log) occurs at the fermentation of the 4th hour until the fermentation of the 32nd hour. After fermentation at the 32nd hour, the growth of the bacteria further leads to a stationary phase.

However the resulting growth curve is not constant during the stationary phase. So that the activity of antibiotic active compounds again increases at the 20th hour fermentation and leads to the death phase in the fermentation of the 40th hour.

2. Optimized Medium Fermentation Isolated BA 1

The mediums used in the optimization of fermentation mediums are MH, NB, LB and LBB. Fermentation is only done for 3 days, because based on data on the bacterial growth curve, antibiotic activity is no longer present on the fermentation of the 4th day and so on. The results of the statistical analysis of medium optimization showed that there was a real difference (5%) in the ability to produce antibiotic compounds by Lansano BA1 endophyte bacteria grown in 4 different types of medium and fermentation times. The results of the BNJ advanced test level of 5% optimization of the fermentation medium can be seen in Table 1.

Table 1 Optimization Profile of The Endophytic Bacteria Lansano Isolate BA1 in Producing Antibiotic Compounds Against *C.albicans*

Medium Type	Average Inhibitory Zone Diameter (cm) Fermentation Hour to-			Main Influence Medium
	24 Hours	48 Hours	72 Hours	
MH	4.02^{d}	2.81^{b}	2.42^{b}	3.08^{B}
NB	3.09^{bc}	3.34^{cd}	2.75^{b}	3.05^{B}
LB	2.65^{b}	3.03^{b}	0.00^{a}	1.89^{A}
LB-B	3.23^{c}	1.99^{b}	1.92^{b}	2.38^{AB}
Main Effects of Fermentatio Time	3.24^{B}	2.79^{B}	1.77^{A}	

Description: The number followed by the same notation indicates a treatment that is not real different (5% level). Notation is sorted from the highest. It is the notation "a". Capital letter notation states the main influence. Lowercase notation states interaction

Based on Table 1 it can be seen that the treatment of this type of fermentation medium exerts a noticeable influence (5% level) on the ability of Lansano endophyte bacteria to isolate BA1 in producing antifungal

compounds. The best type of medium in producing antifungal compounds is medium MH. In the treatment of long fermentation times there is also a noticeable influence in producing antifungal compounds. The best fermentation time in producing antifungal compounds is on 24-hour fermentation.

Table 2 Fermentation optimization profile of Lansano endophyte bacteria isolates BA 1 in producing antibiotic compounds against *E.coli*

Medium Type	Average Inhibitory Zone Diameter (cm)			Main Influence Medium
	Fermentation Hour to-			
	24 Hours	48 Hours	72 Hours	
MH	2.13^{c}	2.02^{b}	0.00^{a}	1.38^{B}
NB	2.23^{c}	2.28^{c}	0.00^{a}	1.50^{B}
LB	1.46^{b}	1.05^{b}	0.00^{a}	0.83^{B}
LB-B	2.05^{b}	1.14^{b}	0.00^{a}	1.06^{B}
Main Effects of Fermentatio Time	1.97^{B}	1.62^{B}	0.00^{A}	

Description: The number followed by the same notation indicates a treatment that is not real different (5% level). Notation is sorted from the highest. It is the notation "a". Capital letter notation states the main influence. Lowercase notation states interaction.

Based on Table 2 it can be seen that the treatment of this type of fermentation medium exerts a noticeable influence (5% level) on the ability of Lansano endophyte bacteria to isolate BA1 in producing antibacterial compounds. The best type of medium in producing antibacterial compounds is medium MH. In the long treatment of fermentation time there is also a noticeable influence in producing antibacterial compounds.The best fermentation time in producing antibacterial compounds is on 24-hour fermentation.

From the two data above it can be seen that the treatment of the type of fermentation medium to the ability of Lansano endophyte bacteria to isolate BA 1 in producing the best antibiotic compound is medium MH.

3. Optimizing Starter Fermentation Isolated BA 1

Table 3 Fermentation Optimization Profile of Lansano Endophyte Bacteria Isolates BA1 in Producing Antibiotic Compounds Against *C.albicans*

Starter Concentration (%)	Average Inhibitory Zone Diameter (cm)			Main Influence of *Starter* Concentration
	Fermentation Hour to-			
	24 Hours	48 Hours	72 Hours	
1	2.97^{a}	2.68^{a}	2.57^{a}	2.74^{A}
5	3.05^{a}	2.79^{a}	2.68^{a}	2.84^{A}
10	3.89^{b}	2.81^{a}	2.74^{a}	3.14^{B}
15	2.93^{a}	3.02^{b}	2.19^{a}	2.71^{A}
Main Effects of Fermentation Time	3.20^{C}	2.82^{B}	2.54^{A}	

Description: The number followed by the same notation indicates a treatment that is not real different (5% level). Notation is sorted from the highest. It is the notation "a". Capital letter notation states the main influence. Lowercase notation states interaction.

Based on Table 3 it can be seen that the treatment of the concentration of fermented *starters* exerts a noticeable influence (5% level) on the ability of Lansano endophyte bacteria to isolate BA1 in producing antifungal compounds. The best *starter* concentration in producing antifungal compounds is a concentration of 10%. In the treatment of long fermentation times there is also a noticeable influence in producing antifungal compounds. The best fermentation time in producing antifungal compounds is on 24-hour fermentation.

Table 4 Fermentation Optimization Profile of Lansano Endophyte Bacteria Isolates BA1 in Producing Antibiotic Compounds Against *E.coli*

Starter Concentration (%)	Average Inhibitory Zone Diameter (cm)			Main Influence of *Starter* Concentration
	Fermentation Hour to-			
	24 Jam	48 Jam	72 Jam	
1	1.73^{a}	1.88^{b}	2.64^{d}	2.08^{B}
5	2.38^{cd}	2.05^{bc}	1.85^{b}	2.09^{B}
10	1.76^{a}	1.77^{ab}	1.91^{bc}	1.81^{A}
15	1.74^{a}	1.65^{a}	2.15^{c}	1.84^{A}
Main Effect of Fermentation Time	2.13^{B}	1.90^{A}	1.83^{A}	

Description: The number followed by the same notation indicates a treatment that is not real different (5% level). Notation is sorted from the highest. It is the notation "a". Capital letter notation states the main influence. Lowercase notation states interaction

3

Based on Table 4 it can be seen that the treatment of the concentration of fermented *starters* exerts a noticeable influence (5% degree) on the ability of Lansano endophyte bacteria to isolate BA1 in producing antibacterial compounds. The best *starter* concentration in producing antibacterial compounds is the concentration of 5%. In the long treatment of fermentation time there is also a noticeable influence in producing antibacterial compounds. The best fermentation time in producing antibacterial compounds is on 24-hour fermentation.

From the two data above it can be seen that the treatment of the type of fermentation *starter* concentration against the ability of Lansano endophyte bacteria to isolate BA 1 in producing different antibiotic compounds in both types of test microbes. In testing using *C. albicans* the best starter concentration is 10% concentration. While testing using *E. coli* test microbes the best starter concentration is a concentration of 5%.

IV. Conclusion

The stationary fermentation phase of the BA 1 isolate of the Lansano endophyte bacterium was initiated after 32 hours of fermentation. The optimal fermentation condition for producing antibiotic compounds was to use a MH medium with 10% starter fermentation (for antifungals) and 5% starter fermentation (for antibacterial).

Acknowledgements

This study was supported by Penelitian Dasar Interdisipliner (Penelitian Terapan) BOPTN 2021 UIN Imam Bonjol Padang.

References

[1]. M. F. Putri, M. Fifendy, and D. H. Putri, "Diversitas Bakteri Endofit Pada Daun Muda dan Tua Tumbuhan Andaleh (Morus macroura miq.)," *EKSAKTA*, vol. 19, no. 1, pp. 125–130, 2018.

[2]. D. H. Putri, R. Rahayu, D. Sahara, N. Nurhelmi, and V. Violita, "Antimicrobial Activities of Extract of Andalas Endophytic Bacterial Fermentation Products in Overcoming Oral Cavity Infection," *EKSAKTA Berk. Ilm. Bid. MIPA*, vol. 20, no. 2, pp. 1–5, 2019.

[3]. F. R. Dharmawan, "Pengujian aktivitas hipoglikemik ekstrak air daun angsana (pterocarpus indicus willd) terhadap histopatologi sel hati tikus diabetes aloksan.[CD-ROM]." Widya Mandala Catholic University Surabaya, 2013.

[4]. N. Nurhasnah, "Potensi Antimikroba Dari Bakteri Endofit Pada Tumbuhan Angsana (Pterocarpus indicus Willd).," *Penelit. Dasar Pengemb. Progr. Stud. (PDPS). Padang*, 2018.

[5]. N. Nofion, D. H. Putri, and Irdawati, "Optimization of Medium Fermentation for Production of Antimicrobial Compounds by Endofit Bacteria Andalas Plant (Morus macroura Miq.) B.J.T.A-6 Isolate," vol. 2, no. 1, pp. 79–84, 2018.

[6]. T. Y. Yun, R. J. Feng, D. B. Zhou, Y. Y. Pan, Y. F. Chen, F. Wang, L. Y. Yin, Y. D. Zhang, and J. H. Xie, "Optimization of fermentation conditions through response surface methodology for enhanced antibacterial metabolite production by Streptomyces sp. 1-14 from cassava rhizosphere," *PLoS One*, vol. 13, no. 11, 2018.

[7]. L. S. Sulistyaningrum, "Optimasi fermentasi asam kojat oleh galur mutan Aspergillus flavus NTGA7A4UVE10," *Skripsi. Fak. Mat. dan Ilmu Pengetah. Alam. Dep. Farm. Univ. Indones.*, 2008.

[8]. D. H. Putri, V. Violita, Irdawati, M. Fifendy, and N. Nurhasnah, "Production of Antifungal Compounds by Andalas Endophytic Bacteria (Morus macroura Miq.) Isolate ATB 10-6 at Fermentation Medium with Optimum Carbon and Organic Nitrogen Source," in *Journal of Physics: Conference Series*, 2021, vol. 1940, no. 1.

[9]. M. J. Pelczar and E. C. S. Chan, "Dasar-Dasar Mikrobiologi Jilid I. Jakarta." Ui Press, 2008.

[10]. U. Mangamuri, "Optimization of the Cultural Parameters for Improved Production of Antimicrobial Metabolites by Streptomyces gulbargensis DAS 131.," *Br. J. Pharm. Res.*, vol. 4, no. 9, pp. 1130–1145, 2014.

[11]. A. Prabowo, "Pengawetan Dedak Padi dengan cara fermentasi," *URL http//sumsl. litbang. deptan. go. id/index. php/component/content/article/53-it-1/206-dedak-padi*, vol. 6, 2011.

[12]. W. Wijanarka, E. Kusdiyantini, and H. Hermin, "Optimasi Starter dalam Memproduksi Inulinase dan Identifikasi Khamir Inulinolitik BAN-1 dari Umbi Dahlia (Dahlia variabilis Willd)," *Maj. Ilm. Biol. Biosf. A Sci. J.*, vol. 27, no. 3, pp. 147–152, 2010.

[13]. M. B. Aji, "Aktivitas senyawa antimikroba dari bakteri biokontrol D2. 2 terhadap bakteri patogen pada udang dan ikan secara in vitro," *Skripsi. Univ. Lampung. Lampung*, 2014.

[14]. S. T. Pratiwi, "Mikrobiologi Farmasi. Jakarta: Penerbit Erlangga. Hal," 2008.

Artikel indah 3

ORIGINALITY REPORT

11%	9%	6%	5%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to University of Kufa Student Paper	4%
2	D H Putri, V Violita, Irdawati, M Fifendy, N Nurhasnah. "Production of Antifungal Compounds by Andalas Endophytic Bacteria (Morus macroura Miq.) Isolate ATB 10-6 at Fermentation Medium with Optimum Carbon and Organic Nitrogen Source", Journal of Physics: Conference Series, 2021 Publication	3%
3	jurnalpeternakan.unisla.ac.id Internet Source	1%
4	repository.unp.ac.id Internet Source	1%
5	www.ijsht-journals.org Internet Source	1%
6	garuda.kemdikbud.go.id Internet Source	<1%
7	123dok.com Internet Source	<1%

8	ejurnal.ung.ac.id Internet Source	<1%
9	download.atlantis-press.com Internet Source	<1%

Exclude quotes	On	Exclude matches	< 10 words
Exclude bibliography	On		