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Microbial Growth as Determinant of Antibiotic Production with Biotic Elicitors Stimulation

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Abstract

The emergence of antibiotic-resistant and multidrug-resistant (MDR) bacteria stress the importance of researchers to continue research for natural potential as a source of new antimicrobials from the environment. One of the potential fungi isolates that can produce antimicrobial active compounds from Indonesian tropical peat soils is *Penicillium sp.* LBKURCC34. In this study, the production of antimicrobial compounds from local isolates were carried out by batch fermentation method in liquid media with the addition of biotic elicitors to increase the extracted antibiotic activity and yield. This study aims to optimize the results based on the time the elicitor was added. *Staphylococcus aureus* was used as a biotic elicitor, which was added on days 2, 3, and 4 in the fermentation antibiotic production media, with a total fermentation time of 6-14 days. After fermentation, the antibiotic production media was extracted with ethyl acetate and evaporated. The antimicrobial test was carried out by the disk diffusion method against the pathogenic bacteria *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Staphylococcus epidermidis* using three crude extract contents (19; 38; and 57 µg/disc). Amoxicillin® was used as a positive control (10 µL/disc). The results showed that the addition of *S. aureus* biotic elicitor extended the log phase growth of the fungus *Penicillium sp.* LBKURCC34. The optimum condition of production was obtained by adding initiator treatment on the 3rd day for 14 days incubation, giving the highest yield that could inhibit the growth of all pathogenic microbes.

1. Introduction

Human resistance to pathogenic microbes is a big challenge in the pharmaceutical and biomedical fields. The increasing number of pathogenic microbes that are resistant to antibiotics on the market, especially multidrug-resistant (MDR) bacteria, encourages researchers to continue to explore the potential of nature as a new source of antimicrobials [1]. One of the potential microorganisms to be explored is the fungus *Penicillium sp.* from peat soils. The presence of humic acid in peat soil provides an environment for the growth of microbe that producing secondary metabolites that have complex structures as antimicrobials [2]. Antimicrobial compounds from the metabolism of microorganisms originating from extreme conditions such as peat soils with low pH and dissolved oxygen content in the temperature range of 24-32°C have the possibility as potential sources of metabolites [3]. Laboratory of Enzyme, Fermentation and Biomolecular, Department of Chemistry, FMIPA, University of Riau has isolated several types of microbes from the peat soils of the Biosphere Reserve Primary Forest, Giam Siak Kecil Bukit Batu (GSKBB), Riau Province, one of which is the fungus *Penicillium sp.* LBKURCC34 with high biological activity as a producer of the chitinase enzyme isolated by Utamy [4].

Antibiotics are one of the secondary metabolites secreted by the fungus *Penicillium sp.* in the stationary phase to defend themselves. Antibiotic compounds can be obtained by extraction of the fermentation media from the batch fungus fermentation process [5]. Fitri [6] produced an antibiotic extract with antimicrobial activity, which was only able to inhibit the growth of *E. coli* by 53% compared to the positive control of Amoxsan®. Optimization of the production media still needs to be done because of the low yield and unsatisfactory antimicrobial activity.

Elicitor is a substance that can induce plant resistance processes against pathogenic microbial infections [7]. Several studies have shown that addition of a biotic elicitor, also known as co-culture, on the production of antibiotic compounds from microbes to be able to increase the antimicrobial activity of secondary metabolite extracts. According to Todar [8], penicillin compounds produced by the fungus *Penicillium sp.* are active against Gram-positive bacteria and less active against Gram-negative bacteria. Khairullinas et al. [9] has produced bioactive compounds from the fermentation media of *Penicillium sp.* LBKURCC34 with and without the *Staphylococcus aureus* (*S. aureus*) as elicitor, which showed that the addition of elicitor increased the extract activity. Therefore, in this research, optimization of the production of *Penicillium sp.* LBKURCC34 antibacterial compounds with the addition of S. aureus pathogenic bacterial elicitors was done by varying the time of elicitor addition based on the microbial growth phases involved. First, the growth curve of the microbes involved were analyzed so that the variation time to add an elicitor in order to obtain an optimal ratio of bioactive compound extracts could be determined.

2. Methodology

This research was preceded by determining the growth curve of the fungus *Penicillium sp.* LBKURCC34, *S. aureus*, and a consortium of *Penicillium sp.* LBKURCC34, and *S. aureus*. Determination of growth curves using the determination of biomass gravimetric method (for fungi and consortium) and bacterial growth with optical density (OD) values (for bacteria). The second stage was the production of antibiotic compounds by *S. aureus* stimulation which was carried out by the addition of pathogenic bacteria at the beginning of the acceleration phase and the beginning of the deceleration phase in accordance with the growth curve of the fungus *Penicillium sp.* taking into account the growth curve of *S. aureus* bacteria and their consortium. The ratio of fungi and pathogenic bacteria was 10: 1, and the incubation time variation of production was 6-14 days. The process was continued with the extraction of production media using ethyl acetate, antimicrobial activity test of several pathogenic bacteria, and analysis of the number of compounds in the extract using HPLC. Higher production was determined by comparing extraction yields and activity of antimicrobial compounds.

2.1. Equipment and Materials

The equipment used in this study were Autoclave All America model 1925/KY-23D, UV-Vis spectrophotometer (Genesys 10S UV-VIS v4.002 2L9N175013, Madison, USA), Genie 2TM vortex mixer, rotary shaker, water bath, Heidolph WB 2000 vacuum rotary evaporator, UV hand lamp, GF/C Whatman filter paper (Catalog No. 1882055), Durchmesser disk paper (Catalog No. 52355 Duren, Germany), calipers (Vernier Caliper, 150x0.05mm, China), HPLC (Shimadzu LC solution UFLC series, Kyoto, Japan), analytical scales and other laboratory standard tools according to work procedures.

The materials used in this study were Potato Dextrose Agar (PDA) (Merck Cat. No. 1.10130), Potato Dextrose Broth (PDB) (Merck Cat. No. 1.08339), Nutrient Agar (NA) (Merck Cat. No. 1.05450), Nutrient Broth (NB) (Merck Cat. No. 1.06649), distilled technical grade ethyl acetate ($CH_3COOC_2H_5$), distilled technical grade methanol (CH_3OH), citric acid (Merck Cat No. 1.00244), sodium chloride (Merck Cat No. 1.06404), sterile distilled water, and Amoxsan® (Sigma AB523-5G).

The microorganism used was *Penicillium sp.* Culture. (Collection Number: LBKURCC34) collection of Enzyme, Fermentation and Biomolecular Laboratory, Department of Chemistry, FMIPA, University of Riau, pathogenic microbes: *Escherichia coli* ATCC35218 (*E. coli*), *Staphylococcus aureus* ATCC 29213 (*S. aureus*), *Bacillus subtilis* ATCC11774 (*B. subtilis*), *Staphylococcus epidermidis* ATCC12228 (*S. epidermidis*).

2.2. Determination of Microbial Growth Curves

In 3 treatment groups consisting of *Penicillium sp.* LBKURCC34, *S. aureus* inoculum, and *Penicillium sp.* LBKURCC34 consortium inoculum added *S. aureus* in Potato dextrose broth (PDB) media then shaken for 1 to 10 days. Every day, an inoculum erlenmayer was harvested to determine the bacterial mass or bacterial optical density (OD) in 3 replications. Determination of fungal mass was conducted by filtering fungus, then dried in an oven and weighed, using gravimetric methods in determining the mass of this fungus. For *S. aureus* bacteria, optical density was measured using a spectrophotometer. Next, a growth curve was created by plotting the incubation time (X) with the value of the fungal mass and bacterial optical density (Y).

2.3. Secondary Metabolite Production of Candidate Antibiotic Compounds from *Penicillium sp.* LBKURCC34

Production of antimicrobial compounds by stimulating pathogenic bacteria *S. aureus* was performed following the method of Lee *et al.* [10] with some modifications. The colonies of *Penicillium sp.* LBKURCC34 which had been rejuvenated, were inoculated into potato dextrose broth (PDB) media with the number of spores that ± 7 x 10^{12} spores into 50 mL PDB media. The inoculum was incubated at room temperature for seven days on a rotary shaker at a speed of 150 rpm. After seven days, the entire inoculum in PDB media was transferred to 1000 mL of antimicrobial production liquid media. Pathogenic bacterial elicitor for antibacterial stimulation, with a ratio

of fungus : bacteria (10:1), were added to the antimicrobial production media containing the inoculum of *Penicillium sp.* fungus. For that, the addition of the pathogenic bacteria, *S. aureus* was conducted on days, which were 1/2 and 2/3 of the log phases, according to the results of the growth curve data obtained.

2.4. Extraction of antimicrobial compounds

After 14 days of fermentation incubation, mycelium and media were separated by filtering on Whatman GF/C filter paper with a Buchner funnel. For every 200 mL of filtered antimicrobial production media, extraction was carried out three times with 100 mL ethyl acetate. The collected ethyl acetate extract was evaporated with a rotary evaporator at room temperature until a crude extract was obtained. This crude extract was stored at -20°C before further use.

2.5. Testing the antimicrobial activity of some pathogenic bacteria by disk diffusion method

The extract was tested for its activity against pathogenic microbes (*E. coli*, *S. aureus*, S. epidermidis, and *B. subtilis*). Dilution of the bacterial and fungus suspensions with a 0.85% NaCl solution was carried out if the OD_{600nm} after incubation for 24 hours was higher than 0.1. For antibacterial testing, 1 mL bacteria were inoculated into a test tube containing 15 mL liquid nutrient agar (NA) media at 45°C and homogenized with a vortex. This media was then poured into a Petri dish and allowed to condense. Metabolic extract of secondary metabolite *Penicillium sp.* LBKURCC34 obtained was then tested for its antibacterial activity by the agar diffusion method. The antibacterial activity test of the secondary metabolite methanol extract was carried out by placing 10 µL secondary metabolites on 6 mm disc paper and allowed to stand for 1-2 minutes until the solvent evaporated. In this test, the positive control was 10 µL Amoxsan® with a content of 38 µg/disc, and the negative control was 10 µL methanol. The solvent-vaporized disc paper was placed on a petri dish that had been inoculated with test bacteria. The cup was then incubated for one day at 37°C in an incubator by turning the petri dish upside down. Clear zone diameters were measured after incubation carried out for 24 hours to 3 days.

2.6. HPLC analysis to determine the ratio of the number of compounds in the extract

Extracts of secondary metabolites from the fungus *Penicillium sp.* LBKURCC34 was analyzed using reverse-phase HPLC. The extracts in methanol were filtered using a 0.45 µm Grace filter. The 20 µL filtrate was injected into the HPP Shim-pack VP-ODS column (250 x 4.6 mm). Samples were analyzed for 20 minutes using acetonitrile-water with a ratio of 70:30 with a gradient elution system. UV light detector was used at wavelengths of 210 nm and 254 nm. This wavelength selection was based on preliminary measurements using a UV spectrophotometer. HPLC analysis was carried out at the Department of Chemistry, FMIPA, University of Riau.

2.7. Data analysis.

Data obtained from antimicrobial activity tests were presented in tables and figures. To compare the average value of the results of the antibacterial activity of the extracts with the positive control of Amoxsan®, the Duncan New Multi Range Test (DNMRT) was used.

3. Results and Discussion

3.1. Microbial growth curve

Growth curves describe changes in the number of cells of an organism over time. The curve can be determined by graphing the relationship between time and cell count. In this study, the observation time of the number of cells was chosen from day 0 to day 10, because in that time range, generally the microbial growth phase can be observed as a whole. The initial growth phase is the lag phase, which is the adaptation stage of the fungus to the maintenance environment [7, 11]. In this phase, cell number multiplication does not occur, but the increase in cell size, DNA synthesis, enzymes, and ribosomes takes place [12]. Multiplication of cell numbers and active division occurs in the logarithmic phase, where primary metabolites are used as a source of nutrition. The exponential phase lasts as long as the cell gets enough nutrition and supporting environmental conditions [12]. Furthermore, due to the depletion of nutrients in the medium occupied, microbes enter a stationary phase in which the addition and reduction of the number of microbes are almost the same. The growth curve will be seen as a straight line [13]. In this condition, the number of primary metabolites decreases, so the microbes will excrete secondary metabolites to defend themselves because of the accumulation of organic acids and biochemical compounds that are toxic to cells [12]. The number of living microbes is less than the number of dead microbes because there are no more sources of nutrients, and this is called the death phase. The death phase becomes the last phase of the microbial growth curve.

From Figure 1, it is observed that there are differences in each growth curve, where each microbe has a time trend of its growth phases. The end of the log phase for the fungus *Penicillium sp.* and bacteria *S. aureus* is the 4^{th} and the 3^{rd} day, respectively. These results are different from Prihanto [14] report. Growth curve of *Penicillium sp.* R1M and *Penicillium notatum* ATCC 28089 in that study had longer lag and log phases, which were 2-7 days and 2-8 days, respectively. These results indicate that the adaptability of each fungus to the growth media is different, where *Penicillium sp.* LBKURCC34 is more adaptable.

While in the form of a consortium with the bacterium *S. aureus*, the log phase of *Penicillium sp.* LBKURCC34 extended to the 6^{th} day. The presence of pathogenic bacteria affects the log phase of the fungus. The stationary phase of *Penicillium sp.* with and without elicitor bacteria is the same as two days, then proceed to the death phase. It is thought that the presence of a pathogenic elicitor does not affect the duration of the stationary phase.

Based on this growth curve, the production of *Penicillium sp.* antibacterial by stimulation with the bacterial pathogenic elicitor *S. aureus*, i.e., were done with the addition of elicitor on the 2nd day in a 14 day fermentation period (H2P14); the addition of elicitor on the 4th day in a 6 days ferementation period (H4P6), and the addition of elicitor on the 4th day in a 14 day incubation (H4P14). Saputra *et al.* [5] incubated for 14 days for the production of antibacterial compounds against the plant pathogenic bacteria Erwinia corotovora subs carotovora. Incubation on the 6th day was conducted by considering the phase of fungal death starting on the 6th day (Figure 1).

3.2. Production, Extraction, and Antibacterial Activity of the Extract

Several studies produced antimicrobial compounds and antibiotics using microorganisms. Supartono *et al.* [15] produced antibiotics using *Bacillus subtilis* M10 in urea-sorbitol media, Prihanto [14] produced antibacterial mycelia and *Penicillium notatum* ATCC28089 and *Penicillium sp.* R1M with the fermentation method, while Tanuwijaya [16] produced penicillin from *Penicillium chrysogenum* with the addition of phenylalanine. In this study, the production of secondary metabolites from the fungus *Penicillium sp.* LBKURCC34 was carried out by a batch fermentation method in which nutrients were given at the beginning of the fermentation process. The effect of adding elicitor *S. aureus* were observed at different addition times based on the growth curve that had been analyzed in advance with the fermentation incubation time for six days and 14 days. After fermentation were carried out, secondary metabolite compounds were extracted using ethyl acetate.

According to Nugroho *et al.* [17], direct extraction with ethyl acetate obtains secondary metabolites and antibiotic peptaibol compounds without requiring additional steps to remove substrate salts from the media. Ethyl acetate extract that had been obtained was given anhydrous Na_2SO_4 to bind the water contained in the extract, so that evaporation could take place more rapidly. Evaporation was carried out at temperatures between 30°C and 50°C to prevent damaging of the compound bioactivity.

Antimicrobial test of the fungal *Penicillium sp.* LBKURCC34 extract were performed by the disk diffusion method, which were carried out on five types of pathogenic microbes, i.e., *S. epidermidis*, *S. aureus*, *B. subtilis*, *E. coli*, and *C. albicans*. The antimicrobial extract were prepared with three concentration variations, 57, 38, and 19 µg/disc. To each disc was applied 10 µL extract. The positive control used was Amoxsan®, and the negative control was methanol. The positive control was used as a comparison of the extracts tested, while negative control served to ensure that the solvent used did not provide activity against the pathogenic microbes tested.

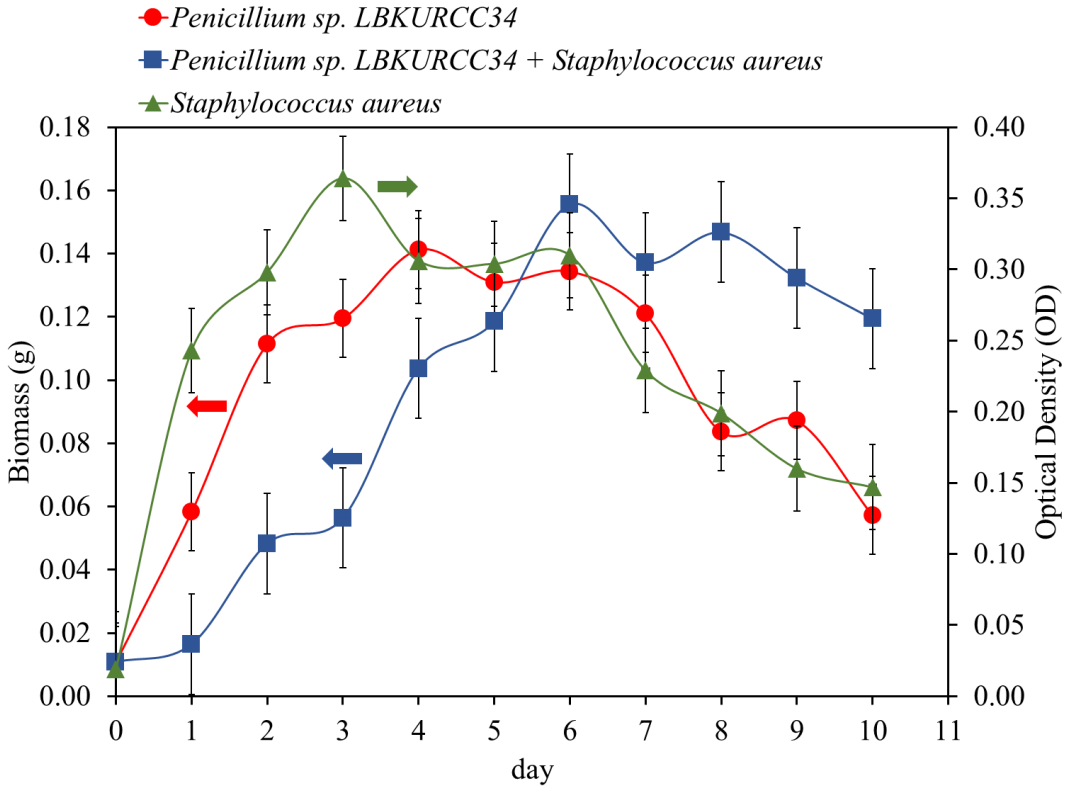


Figure 1. Growth curves of microbes involved in the production of antimicrobial bioactive compounds from *Penicillium sp.* LBKURCC34 with the elicitor of *Staphylococcus aureus*

Based on the data obtained, H2P14 extract has antimicrobial activity against pathogenic bacteria (Table 1). The highest activity of the extract to inhibit the growth of *S. epidermidis* is at a concentration of 57 µg/disc. The percentage of ability to inhibit it is still small compared to the positive control. H4P6 extract did not have antimicrobial activity against pathogenic bacteria. This can be suspected because of the harvest time of the

fungus *Penicillium sp.* LBKURCC34, which is too fast so that the microbes have not appropriately divided, and the number of cells produced has not been maximized, so the secretion of bioactive compounds has not yet occurred. Based on this data, it can be assumed that the fermentation time of secondary metabolite production media has a significant influence on the activity of the extract produced.

H4P14 extract has antimicrobial activity against the five pathogenic microbes tested. The highest activity was found in the H4P14 extract against *B. subtilis* and *S. epidermidis* bacteria with an activity percentage of approximately 59-60% compared to the positive control, namely Amoxsan® at the same concentration (Table 2). The addition of elicitor and the right time of fermentation affect the antimicrobial activity of the extract produced. Khairullinas *et al.* [9] produced fungal bioactive compounds sampled with the same elicitor but added the stimulator on the 3rd day and incubated for 14 days, where the results showed higher activity than all the treatments of this study. The highest inhibition against *B. subtilis* was 178% higher than the positive control of Amoxsan® (Table 2).

Table 1. Antibacterial activity of ethyl acetate extracts of antibiotic production media *Penicillium sp.* LBKURCC34 with the elicitor *S. aureus* on various elicitor addition day

Pathogenic Microbes	Concentration	Inhibition (clear zone, mm)			
		H2P14	H4P6	H4P14	Amoxsan®
E. coli ATCC35218	19 µg/disc	0.25 ± 0.35	0.00 ± 0.00	1.07 ± 0.09	-
	38 µg/disc	0.15 ± 0.21	0.00 ± 0.00	2.99 ± 3.20	6.43 ± 0.52
	57 µg/disc	0.00 ± 0.00	0.00 ± 0.00	2.86 ± 1.32	-
B. subtilis ATCC11774	19 µg/disc	0.29 ± 0.19	0.00 ± 0.00	2.26 ± 0.33	-
	38 µg/disc	0.20 ± 0.28	0.00 ± 0.00	4.39 ± 0.66	7.28 ± 0.83
	57 µg/disc	0. 00 ± 0.00	0.00 ± 0.00	5.56 ± 1.13	-
S. aureus ATCC29213	19 µg/disc	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	-
	38 µg/disc	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	7.05 ± 1.20
	57 µg/disc	0.00 ± 0.00	0.00 ± 0.00	1.66 ± 0.09	-
S. epidermidis ATCC12228	19 µg/disc	0.00 ± 0.00	0.00 ± 0.00	2.19 ± 0.62	-
	38 µg/disc	0.20 ± 0.28	0.00 ± 0.00	3.64 ± 0.87	6.14 ± 1.99
	57 µg/disc	0.53 ± 0.10	0.00 ± 0.00	5.23 ± 1.70	-

Note: H2P14 is ethyl acetate extract from production media with the addition of an elicitor on the second day in 14 days incubation. H4P6 is ethyl acetate extract from production media with the addition of an elicitor on the fourth day in 6 days incubation. H4P14 is ethyl acetate extract from production media with the addition of an elicitor on the fourth day in 14 days of incubation.

Table 2. Percentage of antibacterial activity of ethyl acetate extracts of antibiotic production media *Penicillium sp.* LBKURCC34 with the elicitor *S. aureus* on various elicitor addition day on the positive control of Amoxsan® at a concentration of 38 µg/disc

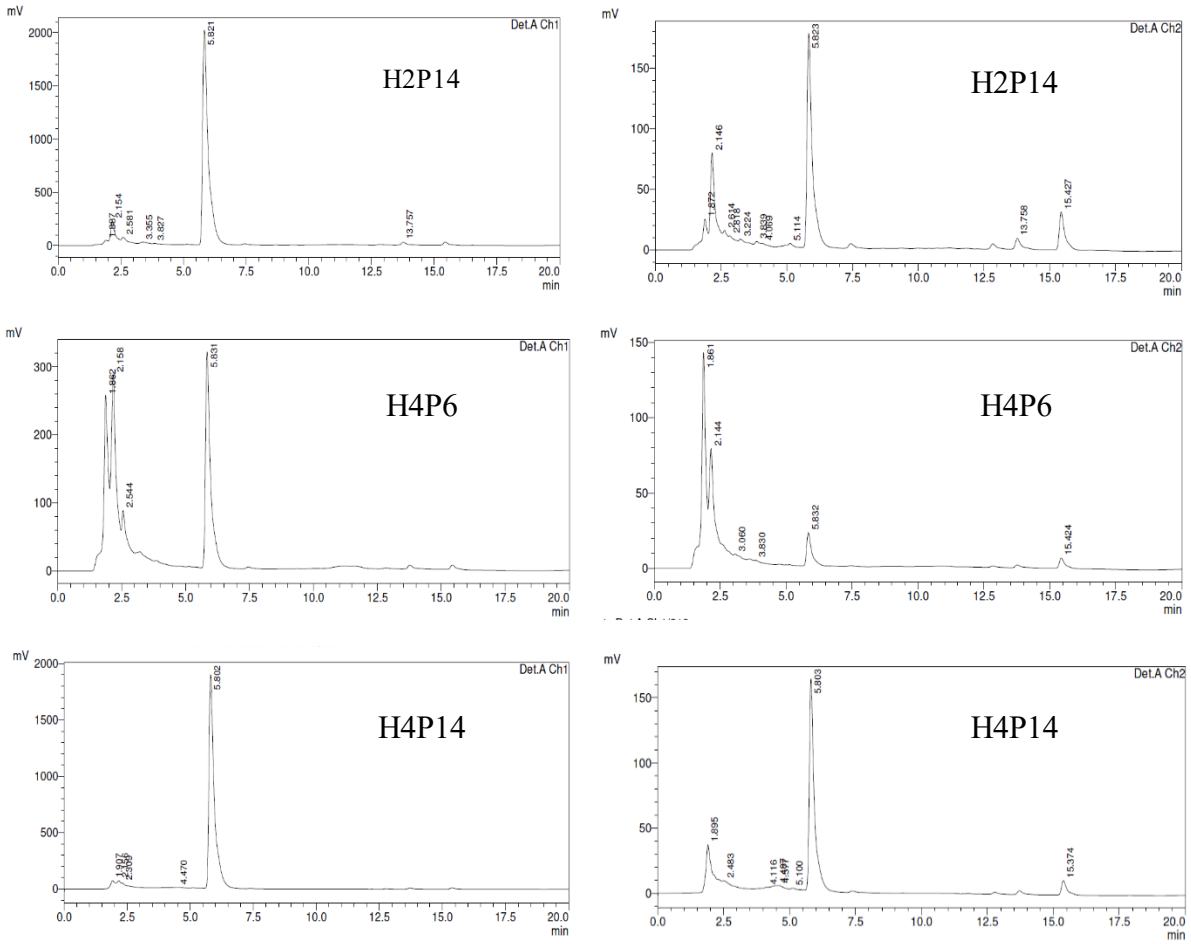
Pathogenic microbes	Inhibition percentage (%)			
	H2P14	H4P6	H4P14	H3P14 [9]
E. coli ATCC35218	2.3	0	46.5	115.3
B. subtilis ATCC11774	2.7	0	60.3	178.2
S. aureus ATCC29213	0	0	0	73.3
S. epidermidis ATCC12228	3.2	0	59.3	61.5

Note: H2P14 is an ethyl acetate extract from the production media with the addition of an elicitor on the second day in 14 days incubation. H4P6 is ethyl acetate extract from production media with the addition of an elicitor on the fourth day in 6 days incubation. H4P14 is ethyl acetate extract from production media with the addition of an elicitor on the fourth day in 14 days of incubation.

3.3. HPLC Analysis

According to Thakur and Sohal [18], elicitors specifically work to induce the production of compounds for defense against pathogenic attack. H2P14, H4P6, and H4P14 extract analysis used HPLC to predict the number of compounds contained in the extract produced. The reverse phase HPLC method with an elution gradient system causes polar compounds to come out first due to the HPLC principle of "like dissolve like". The results of HPLC at wavelengths of 210 nm and 254 nm show that generally, the compounds in the extracts are polar because of the considerable peak pattern in the early minutes of retention time (minutes 1.5 - 6.0) (Figure 2). Also, the chromatogram showed that all three extracts had the same compound because the HPLC chromatogram showed that the three extracts both had peaked at a retention time of 5.8 minutes. Other peaks at different retention times, showing different compounds. Further analysis is needed to determine the type of compound and determine whether the compound at the peak of retention time is an active compound.

Elicitor works by triggering the formation of secondary metabolites through the activation of secondary pathways in response to stress [19]. The number of dominant peaks of the three extracts varies, so the possible number of extract compounds in the form of secondary metabolites also varies. The difference in the number of extracts of the dominant compound produced indicates that there are differences in the types of compounds produced by the extracts. The difference between elicitor addition time and incubation time in the production of the fungus bioactive compound *Penicillium sp* LBKURCC34 shows the differences in the compounds produced.



a. detected with UV 210 nm b. detected with UV 254 nm

Figure 2. HPLC chromatogram of ethyl acetate extracts of antibiotic production media *Penicillium sp.* LBKURCC34 with the elicitor *S. aureus* on several elicitor addition days with 2 UV wavelength detectors.

3.4. Summary of analysis parameters

In the three different treatments, the yield produced, the peak number of HPLC chromatograms and the number of pathogenic bacteria giving inhibition were compared. Table 3 shows that the three treatments gave different results. It was seen that the highest yield was produced in the H4P14 treatment with the amount of activity against three pathogenic bacteria from 4 tested. It also shows that incubation for 14 days is better to produce bioactive compounds as antibiotics.

Table 3. Results of performance parameters to produce fungal antibiotics *Penicillium sp.* LBKURCC34 with the elicitor *S. aureus*.

Observed parameters	H2P14	H4P6	H4P14	H3P14 [9]
Yield (mg)	27.05	70.2	100.9	99
HPLC Peaks	5	4	4	3
the number of inhibited bacteria (from 4 tested bacteria)	3	0	3	4

The results of the analysis with several production treatments carried out were compared with previous studies [9]. The results show that extract productivity - which is the amount of activity in inhibiting some pathogenic bacteria and the production of antibiotic bioactives - is best conducted when adding the elicitor *S. aureus* on the 3rd day in 14 days incubation (Table 3). However, the yield produced is still limited. Other parameter optimizations are needed to increase the number of extract yields, such as optimization of incubation time, pH, the concentration of nutrients such as carbon and nitrogen, or the ratio of the fungus and its bacterial elicitor.

4. Conclusions

The addition of biotic elicitor *S. aureus* extends the log phase of fungal growth *Penicillium sp.* LBKURCC34". The best production of antibiotic bioactive compounds from this fungus is obtained on the addition of elicitor *S. aureus* on the 3rd day in 14 days incubation.

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References

[1] Dattu Singh, Vandana Rathod, Shivaraj Ninganagouda, Jyothi Hiremath, Ashish Kumar Singh and Jasmine Mathew, Optimization and

characterization of silver nanoparticle by endophytic fungi *Penicillium sp.* isolated from *Curcuma longa* (turmeric) and application studies against MDR E. coli and S. aureus, *Bioinorganic chemistry and applications*, 2014, Article ID 408021, (2014), 1-8 https://doi.org/10.1155/2014/408021

[2] Cheng Gu and K. G. Karthikeyan, Sorption of the Antibiotic Tetracycline to Humic-Mineral Complexes, *Journal of Environmental Quality*, 37, 2, (2008), 704-711 https://doi.org/10.2134/jeq2007.0030

[3] Catherine M. Yule and Lalita N. Gomez, Leaf litter decomposition in a tropical peat swamp forest in Peninsular Malaysia, *Wetlands Ecology and Management*, 17, 3, (2009), 231-241 https://doi.org/10.1007/s11273-008-9103-9

[4] WR Utamy, Isolasi fungi penghasil kitinase dari tanah hutan primer Pangkalan Bukit Cagar Biosfer Giam Siak Kecil - Bukti Batu Riau, *Undergraduate thesis*, Universitas Riau, 2012, Pekanbaru

[5] Hendra Saputra, F Puspota and T Tjandrawati, Production of an antibacterial compound against the plant pathogen *Erwinia carotovora* subs. carotovora by the biocontrol strain *Gliocladium sp.* T.N.C73, *International Journal of Agricultural Technology*, 9, 5, (2013), 1157-1165

[6] Annisa Fitri, Fraksinasi Metabolit Sekunder Dan Uji Antimikroba Media Fermentasi Batch *Penicillium sp.* LBKURCC34, *Undergraduate thesis*, Universitas Riau, 2019, Pekanbaru

[7] Eka Rahayu Utami, Antibiotika, Resistensi, dan Rasionalitas Terapi, *El-Hayah*, 1, 4, (2012), 191-198 http://dx.doi.org/10.18860/elha.v1i4.1783

[8] Kenneth Todar, *Staphylococcus aureus* and Staphylococcal Disease, in: *Todar's Online Textbook of Bacteriology*, University of Wisconsin, 2005,

[9] Khairullinas, C Hardiyanti and AP Anugerah, Produksi antibiotik *Penicillium sp.* LBKURCC34 isolat lokal tanah gambut Riau dengan stimulasi bakteri *Staphylococcus aureus*, in, Universitas Riau, Pekanbaru, 2018,

[10] Hee Jung Lee, Jeong Hyung Lee, Bang Yeon Hwang, Hang Sub Kim and Jung Joon Lee, Anti-angiogenic activities of gliotoxin and its methylthio-derivative, fungal metabolites, *Archives of Pharmacal Research*, 24, 5, (2001), 397 https://doi.org/10.1007/BF02975182

[11] Raina M. Maier, Ian L. Pepper, Charles P. Gerba and Terry J. Gentry, Environmental Microbiology, 2nd edition ed., Elsevier Science, 2009

[12] Kathleen Park Talaro and Barry Chess, Foundations in Microbiology: Basic Principles, 9th edition, McGraw-Hill Education, 2014

[13] Lud Waluyo, Mikrobiologi Umum, Universitas Muhammadiyah Malang Press, Malang, 2019

[14] Asep Awaludin Prihanto, Perbandingan aktivitas antibakteri *Penicillium notatum* ATCC 28089 dengan *Penicillium sp.* R1M yang diisolasi dari mangrove Sonneratia caseolaris, *Jurnal Pengolahan Hasil Perikanan Indonesia*, 15, 1, (2012), 66-70 https://doi.org/10.17844/jphpi.v15i1.5336

[15] Supartono Supartono, Nanik Wijayati, Lina Herlina and Enny Ratnaningsih, Produksi Antibiotika Oleh *Bacillus subtilis* M10 Dalam Media Urea-Sorbitol, *Reaktor*, 13, 3, (2011), 185-193 https://doi.org/10.14710/reaktor.13.3.185-193

[16] Vania Aprilina Tanuwijaya, Produksi Penisilin oleh *Penicillium chrysogenum* dengan Penambahan Fenilalanin, in, Program Studi Biologi, Fakultas Teknobiologi, Universitas Atma Jaya Yogyakarta, Yogyakarta, 2015,

[17] Titania Tjandrawati Nugroho, Jasril, A.M Chainulfiffah, Saryono, M.R. Tanzil and Muzeliati, Perbandingan dua metode ekstraksi antibiotik dari media fermentasi *Gliocladium sp.* TNC73., *Jurnal Natur Indonesia*, 9, 1, (2006), 16-21

[18] Meenakshi Thakur and Baldev Singh Sohal, Role of elicitors in inducing resistance in plants against pathogen infection: a review, *ISRN biochemistry*, 2013, Article ID 762412, (2013), 1-10 https://doi.org/10.1155/2013/762412

[19] Indah Yulia Ningsih, Pengaruh Elisitor Biotik dan Abiotik pada Produksi Flavonoid Melalui Kultur Jaringan Tanaman, *PHARMACY: Jurnal Farmasi Indonesia (Pharmaceutical Journal of Indonesia)*, 11, 2, (2014), 117-132 http://dx.doi.org/10.30595/pji.v11i2.829