Antimicrobial Production Using Endophytic Fungus *Sporothrix sp*. LBKURCC43 by Carbon and Nitrogen Modification

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Antimicrobial Production Using Endophytic Fungus *Sporothrix sp*. LBKURCC43 by Carbon and Nitrogen Modification

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Abstract. Endophytes are endosymbiont organisms that can produce antimicrobial compounds. Media composition is one of the main components to produce industrial compounds. This study investigated the optimal media composition for antimicrobial production by endophytic fungus *Sporothrix sp*. LBKURCC43. The media were modified by applying composition combination between corn, potato, and sweet potato as carbon sources, and soybean and beef meat as nitrogen sources; incubation times of the fermentation were 5, 10, 15, 20, and 25 days. Antibacterial activity against pathogenic microorganisms (*Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*) was examined by using the agar diffusion method. A phytochemical test was conducted for determining the highest antibacterial activity. The results showed compounds produced in modified medium MM 03 (potato and soybean) had the highest inhibition zone diameter (8.09 ± 0.62 mm) at 20 days of incubation against *E.coli* (the ratio between potato and soybean for the medium was 3.60: 0.24). While MM 05 (corn, potato, and soybean at a ratio of 1.8: 1.8: 0.24) indicated the highest inhibition zone against *S. aureus* (6.03 ± 0.82 mm) at day 20. None of the compound in the modified medium was active against *C. albicans*. In conclusion, there were varies abilities of endophytic fungus in producing antimicrobial compounds based on their growth medium composition. The highest activity against *E. Coli* was obtained in the medium consisted of potato and soybean. The phytochemical test indicated the modified medium (MM 05) contained a compound that was categorized as saponin.

1. Introduction

Endophytes are a type of organisms that living inside a plant's tissues and used them as their host in the form of mutualistic symbiosis. Several studies reported that the endophyte microorganisms have a potency to produce bioactive compounds such as antibacterial, antifungal, antivirus, anticancer, and antimalaria [1]. Modifications of fermentation parameters are applied for increasing the efficiencies of bioactive compounds production [2]. Bioactive compounds produced by microorganisms are influenced by fermentation condition such as agitation, aeration, pH, temperature and media composition [3]. The fermentation culture media have different effects on the growth of several

species of microorganism. Some substrates (precursors) influence the biosynthesis mechanism of antibacterial compounds such as carbon and nitrogen [4], therefore, the difference in media composition used for culture production resulted in the differences in the bioactive compounds producing process and their final products [5].

Media for bioactive compounds production need to fulfill the nutritional prerequisite and technical objective of the fermentation process. Nutrition has to be formulated to meet a targeted product [6]. In general, fungi can degrade carbohydrate and other organic matters by enzymatic reactions, so it can ease the assimilation. The requirements of carbon source are bigger than other nutrients [7]. In addition to the carbon source, nitrogen has also be included in fermentation media. Organic nitrogen is the best nitrogen sources to produce antibiotic compounds compare to the inorganic nitrogen sources [8]. Nitrogen is required to synthesize amino acid and to build a protein that is required for protoplasm formation [7].

Fermentation media that are commonly used to produce bioactive compounds are including Malt Extract Broth (MEB), Saboraud Dextrose Broth (SDB), and media made by Huang et. al., [9], whereas media that use natural carbon and nitrogen sources are very rarely found. Research conducted by Fitriyah et. al., [10] found that antimicrobial activity of crude extract of fungi *Fusarium sp*. and *Sporothrix sp.* on MEB media indicated an inhibition zone of 20.00 ± 0.00 mm toward *Escherichia coli* at day-20. This research focused on optimization of the antimicrobial compounds production from endophytic fungus *Sporothrix sp*. LBKURCC43 by using modified media prepared from natural materials; these were corn, potato and sweet potato as carbon sources as well as soybean and beef meat as nitrogen sources which are modified into 14 media. Fermentation time was set for 5, 10, 15, 20, and 25 days. Media showed the highest inhibition activity against pathogenic microorganism was continued to phytochemical test including terpenoid, steroid, alkaloid, flavonoid, phenolic and saponin tests aimed to identify the category of secondary metabolite produced.

2. Experimental Section

2.1. Preparation of fermentation media

Compositions of fermentation media to produce antimicrobial compound as shown in table 1**.**

Table 1. The composition of fermentation media.

Media	Composition (g/200 mL)
MM 01	Corn (3.6) + soybean (0.24)
MM 02	Sweet potato (3.6) + soybean (0.24)
MM 03	Potato (3.6) + soybean (0.24)
MM 04	Corn (1.8) + sweet potato (1.8) + soybean (0.24)
MM 05	Corn (1.8) + potato (1.8) + soybean (0.24)
MM 06	Sweet potato (1.8) + potato (1.8) + soybean (0.24)
MM 07	Corn (1.2) + sweet potato (1.2) + potato (1.2) + soybean (0.24)
MM 08	Corn (3.6) + beef meat (0.24)
MM 09	Sweet potato (3.6) + beef meat (0.24)
MM 10	Potato (3.6) + beef meat (0.24)
MM 11	Corn (1.8) + sweet potato (1.8) + beef meat (0.24)
MM 12	Corn (1.8) + potato (1.8) + beef meat (0.24)
MM 13	Sweet potato (1.8) + potato (1.8) + beef meat (0.24)
MM 14	Corn (1.2) + sweet potato (1.2) + potato (1.2) + beef meat (0.24)

All materials (fresh condition) were dried, finely ground, and sifted. Those materials were then weighed according to its media compositions and were diluted in 200 mL of distilled water, heated until dissolved and followed by sterilizing in an autoclave at 121 ^{o}C and 15 lbs for 20 minutes. Those media were kept for one day and ready to use if it is not contaminated.

2.2. Endophytic fungus fermentation

Endophytic fungus inoculum was inoculated into each of 200 mL modified media (MM 01-MM 14) and then was incubated for 25 days at room temperature and speed of 150 rpm in a *rotary shaker*. Every 5 days the production media was harvested. The secondary metabolite of the fungi was collected by filtering using vacuum Erlenmeyer to separate the supernatant and the cell mass. The supernatant (filtrate) was evaporated in a vacuum rotary evaporator to get the solid extract. Then, the evaporated solid extract was kept in a desiccator and ready for use for the antimicrobial test using *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. The solid extract that shown an indication of the highest activity was continued to the process of a phytochemical test; this was conducted in order to identify group types of the secondary metabolite compound.

2.3. Maintenance of tested bacteria and fungi

The tested bacteria, such as *Escherichia coli* and *Staphylococcus aureus* that were previously grown in slant agar were aseptically reinoculated in fresh Nutrient Agar (NA) for 24 hours. The grown colony was inoculated in Nutrient Broth (NB) media and incubated for 24 hours at 37 ^{o}C. Afterward, the bacterial absorbance was observed. Meanwhile, the slant culture of tested *Candida albicans* was aseptically reinoculated on fresh Potato Dextrose Agar (PDA) and was incubated for 4 × 24 hours. Then, the fungi were re-inoculated in Sabouroud Dextrose Broth (SDB) and were incubated for 48 hours. The bacteria and fungi were ready for the antibacterial test when the optical density (OD) has reached 0.08 - 0.1 (equal to 10^{7} CFU/mL). If the OD value higher than 0.1, the culture is diluted in 0.85% of NaCl solution.

2.4. Test of antibacterial and antifungal

Pathogenic inoculum of *E. coli*, *S. aureus* and *C. albicans* (OD_{600nm}~0,1), which each of them was equal to 10^{7} CFU/mL (Martins et. al., 2011), was inoculated as much as 1 mL in a test tube containing 15 mL NB media (temperature of 50 ^{o}C) and was vortexed. The broth culture was poured into a petri dish and let to solidify. Each of solid extract of the endophytic fungus (50 μL) was dropped on a sterile disc paper (diameter of 6 mm) and dried. Positive control to be used was Amoxsan for bacteria and Ketoconazole for fungi at a concentration of 30 μg, and negative control to be used was sterile fermentation media. Then, a paper disc was put on the NA media containing tested microorganism. The petri dish was then incubated at 37 ^{o}f in reversed condition. Finally, the diameter of the clear zone surrounding the disc paper was measured after 24 hours incubation for bacteria and 2 × 24 hours for fungi.

2.5. Phytochemical test

Sterile solid extract containing secondary metabolite produced by endophytic fungus *Sporothrix sp.* LBKURCC43 performing antimicrobial activity was continued for phytochemical test [11]. The objective was to identify secondary metabolite compound produced. The conducted tests were tests of terpenoid/steroid, alkaloid, phenolic, flavonoid, and saponin.

3. Results and Discussion

3.1. Screening antimicrobial activity of the solid extract of endophytic fungus

The composition of solid extracts of endophytic fungus from 14 modified media were coded as MM 01, MM 02, MM 03, MM 04, MM 05, MM 06, MM 07, MM 08, MM 09, MM 10, MM 11, MM 12, MM 13, and MM 14; those media were tested for antimicrobial activity against *E. coli*, *S. aureus*, and

C. albicans. The ability of the produced secondary metabolite compounds in inhibiting the pathogenic organisms was observed from the clear zone appeared on the assayed media. The ability of each solid extracts of endophytic fungus *Sporothrix sp*. in inhibiting the growth of pathogen *E. coli* was presented in table 2.

Table 2. Antimicrobial activity of the solid extract of endophytic fungus against *E. Coli.*

Media	The diameter of Inhibition Zone (mm)				
	Day 5	Day 10	Day 15	Day 20	Day 25
MM 01	0.64 ± 0.35	4.63 ± 0.20	1.86 ± 0.26	1.30 ± 0.48	0.97 ± 0.67
MM 02	0.00 ± 0.00	6.27 ± 0.41	0.99 ± 0.22	4.46 ± 0.96	1.08 ± 0.26
MM 03	**0.99 ± 0.55**	**7.39 ± 0.98**	**3.12 ± 0.49**	**8.09 ± 0.62**	**1.17 ± 0.12**
MM 04	0.00 ± 0.00	1.50 ± 0.73	0.88 ± 0.49	1.13 ± 0.87	0.81 ± 0.70
MM 05	2.09 ± 0.31	3.41 ± 0.47	2.84 ± 0.30	1.47 ± 0,20	1.12 ± 0.45
MM 06	1.62 ± 0.26	1.23 ± 0.42	1.02 ± 0.98	0.52 ± 0.31	0.51 ± 0.62
MM 07	0.00 ± 0.00	0.63 ± 0.50	2.18 ± 0.20	0.83 ± 0.13	0.35 ± 0.22
MM 08	0.00 ± 0.00	0.64 ± 0.31	1.67 ± 0.56	1.82 ± 0.66	1.28 ± 0.33
MM 09	0.70 ± 0.20	0.16 ± 0.10	0.73 ± 0.79	0.22 ± 0.11	2.30 ± 0.26
MM 10	0.60 ± 0.30	0.89 ± 0.78	0. 73 ± 0.70	2.43 ± 0.75	1.07 ± 0.29
MM 11	0.95 ± 0.28	1.53 ± 0.25	1.23 ± 0.72	0.67 ± 0.53	0.61 ± 0.29
MM 12	2.41 ± 1.06	2.67 ± 0.44	1.72 ± 0.84	0.77 ± 0.26	0.57 ± 0.60
MM 13	0.58 ± 0.19	2.08 ± 0.20	1.23 ± 0.51	1.41 ± 0.38	0.46 ± 0.12
MM 14	1.40 ± 0.09	1.45 ± 0.53	0.38 ± 0.18	1.79 ± 0.36	0.63 ± 0.50

The result showed that the modified media (MM 01, MM 02, and MM 03) had higher activity than other tested media. The inhibition zone on media MM 01 was 4.63 ± 0.20 mm at day-10, while for MM 02 was 6.27 ± 0.41 mm at day-10, and MM 03 was 8.09 ± 0.62 mm at day-20. Meanwhile, activities of media MM 04 until MM 14 were low. Media MM 03 also has highest inhibition zone at day-10 with 7.39 ± 0.98 mm. These results suggest that the optimum media with highest activity of the endophytic fungus *Sporothrix sp*. LBKURCC43 toward *E. coli* is the MM 03 which was consisted of potato and soybean (3,6 : 0,24).

Fermentation is carried out with a batch system with nutrition at the beginning of the fermentation process. The endophytic fungus will adapt to the environment and existing nutrients that lead to changes in morphological and physiological properties of fungi [12]. Differences in carbon and nitrogen sources contained in the fermentation media cause differences in the growth or inhibition of fungi. Antimicrobial activity test toward *S. aureus* indicated a relatively high value on several media as shown in table 3. MM 05 was a modified medium indicating optimum activity that the average of inhibition zone was 6.03 ± 0.82 mm at day-20 in comparison to other modified media (MM 01, MM 02, MM 04, MM 08, MM 13 and MM 14) which the inhibition were

5.81, 4.32, 4.07, 5.72, 4.78, and 6.17 mm. MM 05 composed of 1.8 g of corn, 1.8 g of potato, and 0.24 g soybean in 200 mL of distilled water.

Table 3. Antimicrobial activity of the solid extract of endophytic fungus against *S. aureus*

Media	The diameter of Inhibition Zone (mm)				
	Day 5	Day 10	Day 15	Day 20	Day 25
MM 01	0.52 ± 0.35	0.93 ± 0.61	5.81 ± 0.46	4.46 ± 0.61	3.20 ± 0.22
MM 02	0.39 ± 0.24	2.28 ± 0.38	4.32 ± 0.96	0.31 ± 0.19	0.43 ± 0.30
MM 03	0.58 ± 0.37	0.78 ± 0.45	1.02 ± 0.70	0.76 ± 0.41	0.43 ± 0.19
MM 04	0.63 ± 0.18	3.28 ± 0.39	4.07 ± 0.05	2.62 ± 0.98	1.17 ± 0.55
MM 05	**2.26 ± 0.18**	**4.28 ± 0.33**	**5.61 ± 0.57**	**6.03 ± 0.82**	**3.62 ± 0.62**
MM 06	0.55 ± 0.37	1.86 ± 0.39	1.71 ± 0.67	1.67 ± 0.73	0.53 ± 0.22
MM 07	0.63 ± 0.33	0.38 ± 0.23	1.58 ± 0.90	1.48 ± 0.77	1.93 ± 0.74
MM 08	0.24 ± 0.03	2.31 ± 1.02	2.29 ± 0.25	2.20 ± 0.62	5.72 ± 0.79
MM 09	0.74 ± 0.92	1.00 ± 1.24	0.32 ± 0.09	0.31 ± 0.03	1.12 ± 0.31
MM 10	0.33 ± 0.15	0.42 ± 1.10	0.29 ± 0.21	1.33 ± 0.35	0.26 ± 0.19
MM 11	0.00 ± 0.00	0.56 ± 0.06	1.19 ± 0.20	0.76 ± 0.03	0.43 ± 0.06
MM 12	1.22 ± 0.83	1.73 ± 0.86	2.53 ± 1.23	1.09 ± 0.55	0.93 ± 0.48
MM 13	0.43 ± 0.45	3.68 ± 0.69	0.68 ± 0.22	4.78 ± 0.32	0.25 ± 0.26
MM 14	0.68 ± 0.20	0.70 ± 0.65	6.17 ± 0.11	2.48 ± 0.33	1.93 ± 0.45

Antimicrobial activities of endophytic fungus (*Sporothrix sp*. LBKURCC43) indicated that there was an influence of 14 varied media on the growth of *E. coli* and *S. aureus* with varieties of values. Ability comparison between extract of endophytic fungus *Sporothrix sp*. LBKURCC43 at day 20 toward *E. coli* and *S. aureus* was presented in figure 1.

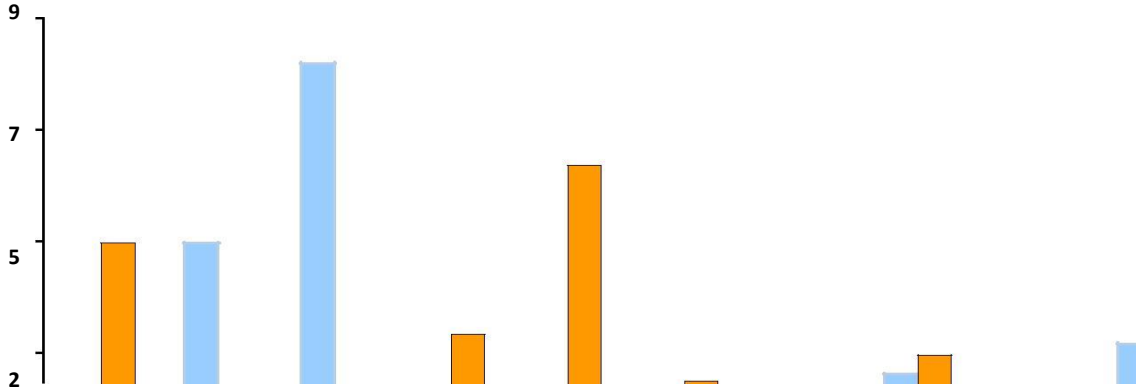

Figure 1. Comparison between activities of the solid extract of endophytic fungus toward *E. coli* and *S. aureus* at day-20

MM 03 and MM 05 had optimum activities in comparison to other 12 modified media at day-20 as optimum incubation time. The optimum antimicrobial activity was dependent on media used. All modified media did not indicate an antimicrobial activity caused by the extract of endophytic fungus *Sporothrix sp*. LBKURCC43 against *C. albicans*.

3.2. Determination of optimum media of endophytic fungus Sporothrix sp. LBKURCC43

There were some differences of antimicrobial activities of the compounds that were found in each modified media in this study; these have occurred might be due to the complexity of the used nutrient sources. The different carbon source was also meant the variations of fermentation, and also the enzymatic reaction for producing different compounds. The carbon sources were more varies can be caused by the possible carbon requirement is bigger than other nutrients [7] such as it is required in the reproduction biosynthesis, cells maintenance for producing the compounds and energy sources required for all the processes. The complexity of the carbon sources and the presence of other compounds in natural material results in critical condition for microorganisms so that secondary metabolite being produced is important to maintain their lives [13]. Therefore, optimum antimicrobial activity and incubation time are influenced by the media being used.

Carbon sources from organic compounds are used as the energy supply for the cell during the oxidative and biosynthesis processes. Several carbon sources that can be used are carbohydrate (monosaccharide, alcohol sugars, polysaccharide, and oligosaccharide), organic acids and carbon dioxide [14]. Apart from the carbon sources, nitrogen is also required for amino acid and protein synthesis. Nitrogen sources that can be used are corn, soybean, yeast extract, and meat. Liquid media commonly used, so that it can be produced in big amount, easier treatment and more efficient [15], and produced compounds have more optimal activity.

In the antimicrobial test against Gram-negative bacteria, *E. coli,* modified media MM 03 was media that showed the highest inhibition zone diameter (8.09 ± 0.62 mm) at day-20 in comparison to other media. The composition of the MM 03 media consisted of potato (3.6 g) and soybean (0.24 g) in 200 mL distilled water. The average antimicrobial activity of modified media against *E. coli* as indicated in figure 2. Modified Media Number 3 had the highest average of inhibition zone 19.42%.

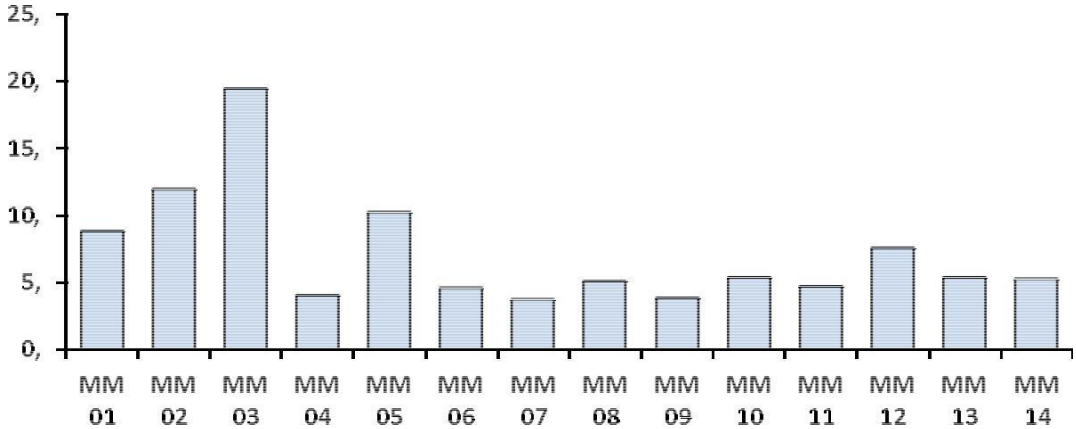

Figure 2. Comparison graph of inhibition zone of the extract of endophytic fungus *Sporothrix sp*. LBKURCC43 against *E. coli*

The results of the antimicrobial test against Gram-positive bacteria in this study, such as *S. aureus,* the MM 05 media showed the highest activity; the inhibition zone diameter for the media was 6.03 ± 0.82 mm at day-20. MM 05, the media consisted of corn, potato, and soybean with the ratio in 200 mL of

distilled water were 1.8 g, 1.8 g, and 0.24 g, respectively. In relation to this, a previous research that was reported by Shinta et. al., [16] suggested that, their crude extract which was isolated from endophytic fungus *Sporothrix* sp. LBKURCC43 showed antibacterial activity against *S. aureus* with ranges started from 20.65 mm to 23.30 mm, the result showed the highest value was at day-5. A similar result to that, was one that reported by Saryono et. al., [17] their study used endophytic fungus *Fusarium sp*. LBKURCC41 on media similar to that was conducted by Huang et. al., [9]; the activity of the metabolite compound was 19.33 mm on *E. coli* and 17.00 mm toward *S. aureus* at day -15. Based on a study done by Merlin [18], that used endophytic fungus *F. Solani* LCPANCF01 that was grown in media M2D to produce biomass and active metabolites, the result showed that, the addition of dextrose resulted in a higher yield than the other carbon sources such as lactose, sucrose, starch, fructose, galactose, mannose, and glycerol. Whereas for the nitrogen source that they used which was yeast extract showed the highest value compared to the other N sources such as peptone, soytone, and meat extract. The highest antimicrobial activity was in *Enterococcus faecalis* with inhibition zone 25 mm. In this study, the average antimicrobial activity of modified media toward *S. aureus* was presented in figure 3. The result showed that the modified media MM 05 had the highest average zone inhibition with the value of 17.69%.

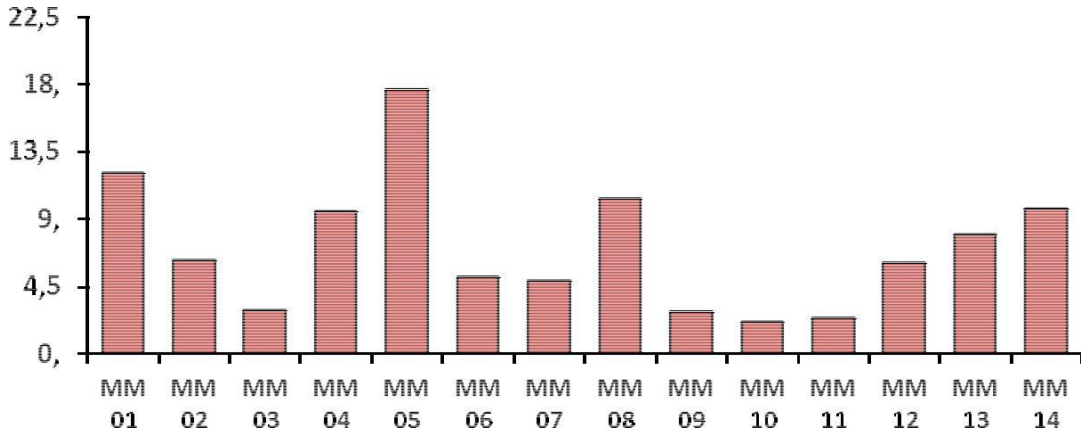

Figure 3. Comparison graph of inhibition zone of the extract of endophytic fungus *Sporothrix sp*. LBKURCC43 against *S. aureus*

The current research did not indicate an antimicrobial effect of modified media on pathogenic fungi *C. albicans*. This suggested that modified media could not produce antifungal from the endophytic fungus *Sporothrix sp.* from the use of natural carbon and nitrogen sources. Meanwhile, Nurhidayah et. al., [19] reported that endophyte plant *Cotylelobium melanoxylon* could inhibit fungi *C. albicans* with inhibition zone 10.30 mm that the metabolite compounds were alkaloid and flavonoid. In addition to carbon source complexity, temperature also influenced the efficiency of substrate conversion to cell mass [20]. In general, maximum conversion occurred at the temperature lower than the temperature of maximum growth speed. Optimum pH could also enhance growth and formation of bioactive compounds which generally occurred at pH 7-8 [21]. The difference in optimum pH was caused by each isolate produces several different antimicrobial compounds. [22], and pH change on media could stimulate the formation of a new compound that could influence the formation of the antimicrobial compound.

3.3. Phytochemical test

The result of the phytochemical test in this study indicated that endophytic fungus *Sporothrix sp*. LBKURCC43 that was grown in modified media consisted of carbon sources of corn, potato, and

sweet potato, and the nitrogen sources of soybean and beef meat, contained secondary metabolite compound belong to the group of saponin (MM 05); the results are shown in table 4.

Table 4. Antimicrobial activity of the solid extract of endophytic fungus against *E. Coli*

Media	**Terpenoid**	**Steroid**	**Alkaloid**	**Fenolik**	**Flavonoid**	**Saponin**
MM 03	-	-	-	-	-	-
MM 05	-	-	-	-	-	+

Saponin is a secondary metabolite which has surface active, it is able to inhibit microbial growth by inhibiting cell protein synthesis. Saponin molecule can absorb water (hydrophilic) and lipophilic so that disturb permeability of bacterial cell membrane, and change the membrane structure functions which results in cell damage and lysis [23]

4. Conclusion

This research concluded that for the growth of endophytic fungus *Sporothrix sp*. LBKURCC43, the variation of corn, potato, sweet potato, soybean, and beef can be used as the source of carbon and nitrogen sources. Optimum media for the production of antimicrobial compound optimum was modified media MM 03 of which composed of potato and soybean. This medium indicated inhibition zone composition 8.09 ± 0.62 mm against bacteria *E. coli*. Antimicrobial activity resulted from endophytic fungus *Sporothrix sp.* LBKURCC43 inhibit the growth of *E. coli* and *S. aureus* which indicated the inhibition zone diameters at day 20 were 8.09 ± 0.62 mm and 6.03 ± 0.82 mm, respectively. However, the media did not affect *C. albicans.* Secondary metabolite compound produced by endophytic fungus *Sporothrix sp*. LBKURCC43 in inhibiting the growth of *E. coli* and *S. aureus* was categorized into saponin.

References

[1] Strobel G and Daisy B **2003** Bioprospecting for Microbial Endophytes and Their Natural Products *Microbiology and Molecular Biology Reviews* **67** 491-502

[2] Prihatiningtias W and Wahyuningsih M S H **2006** *Prospek Mikroba Endofit sebagai Sumber Senyawa Bioaktif* (Yogyakarta: Universitas Gadjah Mada)

[3] Pratiwi S T 2008 *Mikrobiologi Farmasi* (Jakarta: Penerbit Erlangga)

[4] Margino S 2008 Produksi Metabolit Sekunder (Antibiotik) oleh Isolate Jamur Endofit Indonesia *Majalah Farmasi Indonesia* **19** 86-94

[5] Razak R A, Patong A R, Harlim T, Djide M N, Haslia and Mahdalia 2011 *Produksi Senyawa Bakteriosin Secara Fermentasi Menggunakan Isolat BAL Enterococcus faecium DU55 dari dangke* (Makasar: Universitas Tadulako)

[6] Waites M J, Morgan N L, Rockey J S, and Higton G 2001 *Industrial Microbiology: An Introduction* (London: Blackwell Science Ltd)

[7] Taurisia P P, Proborini M W, and Nuhantoro I 2015 Pengaruh Media Terhadap Pertumbuhan dan Biomassa Cendawan *Alternaria alternata* (Fries) Keissler *Jurnal Biologi* **19(1)** 30-33

[8] Narayana K J P, and Vijayalakshmi M 2008 Optimization of Antimicrobial Metabolites Production by *Streptomyces albidoflavus*. *Res. J. Pharmacol* **2(1)** 4-7

[9] Huang W Y, Cai Y Z, Hyde K D, Corke H, and Sun M 2007 Endophytic Fungi from *Nerium oleander L* (Apocynaceae) main constituents and antioxidant activity *World J Microbiol Biotechnol* **23(9)** 1253-1263

[10] Fitriyah S, Jose C, and Saryono 2013 Skrining aktivitas Antimikroba dan Uji Fitokimia dari Kapang Endofitik Tanaman Dahlia (Dahlia variabilis) *J. Ind. Che. Acta.* **3(2)** 50-55

[11] Devi N N, Prabakaran J J, and Wahab F 2012 Phytochemical analysis and enzyme analysis of endophytic fungi from *Centella asiatica Asian Pac J Trop Biomed* 2012 1-5

[12] Gandjar, Indrawati, Sjamsuridzal W, and Oetari A 2006 *Mikologi Dasar dan Terapan* (Jakarta: Yayasan Obor Indonesia)

[13] Kumala S, Agustina E, and Wahyudi P 2007 Uji aktivitas antimikrobial metabolit sekunder kapang endofit tanaman trengguli (*Cassia fistula* L) *Jurnal Bahan Alam Indonesia* **2(6)** 46-48

[14] Moore E and Landecker 1972 The Fungi (Toronto: Prentice-Hall of Canada, Ltd)

[15] Pokhrel C P and Ohga S 2007 Submerged Culture Conditions for Mycelial Yield and Polysaccharides Production by *Lyophyllum decastes Food Chemistry* **105** 641-646

[16] Shinta D Y, Yusmarini, Sonata H, Teruna H Y, and Saryono 2015 The media variance of production for antimicrobe homogeny from the endophyte fungi of dahlia plant seed (*Dahlia variabilis*) *J. Chem. Pharm. Res.* **7(9S)** 239-245

[17] Saryono, Hendris S, Fitriyah D, Jose C, Nugroho T T, and Ardhi A 2015 Antimicrobial activity and molecular characterization of endophytic fungi strain isolated from dahlia (*Dahlia variabilis*) *J. Chem. Pharm. Res.* **7(9S)** 201-208

[18] Merlin J N, Christhudas N, Kumar P P, and Agastian P 2013 Optimization of Growth and Bioactive Metabolite Production: *Fusarium Soloni*. *Asian J Pharm Clin Re*s **6(3)**.

[19] Nurhidayah, Hasanah U, and Idramsa 2014 Pengaruh Ekstrak Metabolit Sekunder Jamur Endofit Tumbuhan *Cotylelobium melanoxylon* dalam Menghambat Pertumbuhan Mikroba Patogen. *Prosiding Seminar Nasional Biologi dan Pembelajarannya* 308-317

[20] Jaelani A 2007 Optimalisasi Fermentasi Bungkil Inti Sawit (*Elaeis guineensis Jacq*) oleh Kapang *Trichoderma reesei Jurnal Ilmu Ternak* **7(2)** 87-94

[21] Ulya J 2009 *Kemampuan Penghambatan Streptomyces spp. Terhadap Mikroba Patogen Tular Tanah pada Beberapa Kondisi Pertumbuhan: Jenis Media, Waktu Produksi, pH, dan Suhu*. (Bogor: Sekolah Pasca Sarjana Institut Pertanian Bogor)

[22] Augustine S K, Bhaysar S P, and Kapadis B P 2005 Production of Growth Dependent Metabolite Active Against Dermatophytes by *Streptomyces rochei Ak 39 Indian Journal of Medicine* **121** 164-170

[23] Arabski M, Ciuk W, Czerwonka G, Lankoff A, and Kaca W 2012 Effects of Saponins against Clinical *E. Coli* Strains and Eukaryotic Cell Line *J Biomed Biotechnol* **2012** 1-6