Antibacterial Activity of Mold Isolate from “Wikau Maombo” Based on Incubation Period

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Antibacterial Activity of Mold Isolate from "Wikau Maombo" Based on Incubation Period

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Abstract. The aims of this study is to determine the antibacterial activity of the type of mold isolates obtained from "Wikau Maombo", the effect of incubation period of the mold isolates against antibacterial activity and the influence of the type and incubation period of the mold isolate against antibacterial activity. This research was experimental research which was to know the antibacterial activity by molds against bacterial growth of *Esherichia coli* ATCC 35218 and *Staphylococcus aureus* ATCC 25923. The Testing of antibacterial activity of the mold isolates were conducted using *Well Diffusion method*. The analysis of data used SAS software (Statistical Analysis System). The result showed that the type of mold isolates obtained from "Wikau Maombo" fermented significant effect on the antibacterial activity. The highest activity found in WM4 isolates which have antibacterial activity as much as 16.7 mm for *E. coli* and 17.4 mm for *S. aureus*. The incubation period of mold isolates significant effect on the antibacterial activity. The highest antibacterial activity found on the tenth day with activities as much as 9.8 mm for *E. coli* and 10.2 mm for *S. aureus.* Interaction between species and incubation period of mold isolates significant effect on the antibacterial activity. The best antibacterial activity found in WM4 with ten days of incubation period.

1. Introduction

Antimicrobials are the substances that kill or inhibit the growth of microorganisms such as bacteria, fungi or protozoans [1]. Antibacterials are the substances that kill or inhibit the growth of bacteria specially. The history of antimicrobials begins with the observations of Pasteur and Joubert, who discovered that one type of bacteria could prevent the growth of another. The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world; hence, antimicrobial drugs are used in the treatment of various diseases [1]. Antibacterials are the substances that kill or inhibit the growth of bacteria specially [1].

Most traditional fermentations employ a whole array of natural microflora that could function under the varied environmental and non-sterile conditions presented by the different processes. Such fermentations are characterized by numerous microorganisms of varying functions that could be beneficial or detrimental to the fermentation processes; mixed cultures that produce the blend of rich flavours and aromas of the product [2]. Traditional fermented food products or beverages which are the result of enzyme activity from microorganisms, are found in many areas in Indonesia. Southeast Sulawesi province, for example, has traditional fermented food known as "kabuto" and "Wikau

Maombo". "Wikau Maombo" is a traditional food made from a bitter cassava root (*Manihot aipi* Phol.) by a fermentation process. The process of making "Wikau Maombo" through the anaerobic fermentation process that utilizes microorganisms from the environment spontaneously . Many microorganisms that play a role in the fermentation process, namely from groups of molds, yeasts and bacteria. Both foods involve the activity of spontaneous natural microorganisms. Mold is one of the microorganisms that play a role in the process [3]. Many microorganisms that play a role in the fermentation process, namely from groups of molds, yeasts and bacteria. The mold from "Wikau Maombo" fermentation was been isolation. Result of the research indicate that from five obtained mould isolates of " Wikau Maombo" fermented, there are three isolates that is FT2.1, FT3.2 and of FT3.4 owning mould characteristic of Rhizopus [4]. Furthermore, the five of mold isolates was observed its antibacterial activity.

2. Experimental Details

Each of the mold isolates that had been prepared as a starter subsequently incubated in *shaker-incubator* at room temperature for 10 days. Sampling the product of culture metabolite molds done per two days, starting before the incubation period (0 hours) to the tenth day. Suspension of mold culture was centrifuged at 3800 rpm for 20 min. The suspension is filtered using Whatman's disc paper to separate the spores with the supernatant. Supernatant was taken and used in testing of antibacterial activity against *E. coli* bacteria and *S. aureus* bacteria.

Test of antibacterial metabolite activity produced by mold isolates against *S. aureus* ATCC 25923 bacteria and *E. coli* ATCC 35218 using *well diffusion method* [1,5]. Antibacterial testing in this study used aquades as a negative control and penicillin antibiotics as a positive control. Petri dishes containing indicator bacteria, supernatant of mold isolate, aquadest and penicillin were put into the refrigerator for 60 minutes, so that the supernatant can diffuse on the media. The indicator bacteria in the petri dish were incubated for 24 hours at 37°C. The clear zone formed around the well, is determined by measuring the vertical diameter zone and the horizontal diameter by unit (mm) using the calipers. The subsequent inhibitory zone was compared with the positive control of penicillin antibiotics [1,6]. The data of antibacterial activity obtained were analyzed using SAS (*Statistical Analysis System*) program with two factorial variance analysis. If the treatment had significant effect on the observed parameter, then continued with Duncan test at 95% confidence level [7].

3. Results and Discussion

The antibacterial activity determined based on observation of inhibit zone diameter. Antibacterial activity of mold isolates based on the incubation period are listed in Table 1.

Table 1. Antibacterial Activity of Mold Isolates by Incubation Period

Incubation Time (day) / Isolates Code		Dry biomass mold (g / mL) and Antibacterial activity (mm)					
		0	2	4	6	8	10
WM1	BK	0,0019	0,0065	0,0101	0,0073	0,0066	0,0062
	Ec	0,0	0,0	9.7	11.2	12.5	14.4
	Sa	0,0	0,0	10.7	12.4	13.1	14.6
WM2	BK	0,0006	0,0057	0,0075	0,0079	0,0095	0,0081
	Ec	0,0	0,0	2.3	3.9	5.8	2.1
	Sa	0,0	0,0	5.2	5.7	5.8	2.6
WM3	BK	0,0023	0,0067	0,007	0,0071	0,0072	0,0080
	Ec	0,0	0,0	5.7	6.0	6.6	14.2
	Sa	0,0	0,0	6.7	7.4	8.6	14.3
WM4	BK	0,0035	0,0058	0,0097	0,0132	0,0067	0,0066
	Ec	0,0	0,0	9.8	11.2	14.8	16.7
	Sa	0,0	0,0	11.9	13.2	15.7	17,4
WM5	BK	0,0025	0,0061	0,0106	0,0084	0,0071	0,0054
	Ec	0,0	0,0	7.3	10.2	12.3	16.2
	Sa	0,0	0,0	5.3	8.7	11.3	15.1
Ct(+)	Ec	5.6	5.5	5.3	5.6	5.5	5.4
	Sa	8.5	8.6	8.4	8.6	8.7	8.5
Ct(-)	Ec	0,0	0,0	0,0	0,0	0,0	0,0
	Sa	0,0	0,0	0,0	0,0	0,0	0,0

The activity of antibacterial producing molds to the growth of indicator bacteria is known based on the diameter of the inhibit zone formed around the wells containing the antibacterial compounds of the molded isolates on the NA medium. The indicator bacteria used were *E. coli* representing Gram negative bacteria and *S. aureus* representing Gram positive bacteria. The results showed that the fifth isolates had the ability to produce antibacterial compounds.

The observational data in Table 1 indicates that the isolates WM1, WM3, WM4 and WM5 are isolates of mold capable of producing antibacterial compounds with interpretation of the inhibitory zone of the intermediate category, since they have a inhibitory zone diameter of above 11 mm which has a respective inhibitory zone diameter of 14, 4 mm, 14.2 mm, 17.4 mm and 16.2 mm in *E. coli* indicator bacteria and 14.6 mm, 14.3 mm, 16.4 mm and 15.1 mm in *S. aureus*. The observed diameter of the antibacterial producing antibacterial block resistor zone is shown in Figure 1.

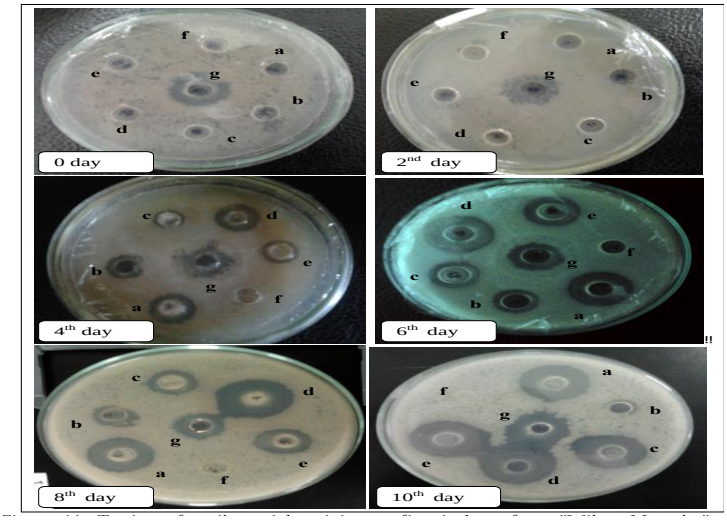

Figure 1. Testing of antibacterial activity on five mold isolates from"Wikau Maombo"; a) Isolate of WM1, b) isolate of WM2, c) isolate of WM3, d) Isolate of WM4, e) Isolate of WM5, f) negative control of aquadest, g) positive control of penicillin.

Antibacterial is one of the natural chemical substances of secondary metabolism of microorganisms. In general, antibacterial compounds can be classified by spectrum of activity, bacterial effects, and inhibitory mechanisms. Based on the spectrum of activity, antibacterial compounds are divided into broad spectrum (*broad spectrum*) and narrow spectrum (*narrow spectrum*). Spectrum-wide antibacterial compounds are able to inhibit the growth of Gram-negative bacteria and Gram-positive bacteria, while narrow-spectrum antibacterial compounds are only able to inhibit the growth of Gram-negative or Gram-positive bacteria [8,9]. The results of Fig. 1 show that the antibacterial compounds produced by the five isolates from "Wikau Maombo" are classified as broad-spectrum antibacterials because they inhibit the growth of *E. coli* and *S. aureus* bacteria.

The result of the observation in Figure 1 shows that the five molds isolates from Wikau Maombo which can produce bactericidal antibacterial compounds in *E. coli* and *S. aureus*, this is based on the formation of total inhibitory zone area on bacteria growth media indicator. Bactericidal means antibacterial compounds have the ability to kill bacterial cells. The total zone is marked with a perfectly clear inhibitory zone [9,10,11].

Based on the results of anava, it can be concluded that there are differences in the influence of type of mold and incubation period to antibacterial activity. Further Duncan test results for interaction type of mold isolate and incubation time on antibacterial activity are listed in Table 2.

Table 2. Duncan Test Results Interaction Type of Mold and Incubation Time of Antibacterial Activity of *E. coli*

Type of isolate	Incubation period (days)			
	4	6	8	10
WM4	9,8 at	11,2 au	14,8 av	**16,7 aw**
WM1	9,7 at	11,2 au	12,5 bv	14,4 bw
WM5	7,3 bt	10,2 bu	12,3 bv	16,2 aw
WM3	5,7 ct	6,0 cu	6,6 cv	14,2 bw
WM2	2,3 dt	3,9 eu	5,8 dv	2,1 dw
Ct (+)	5.3 ct	5,6 du	5,5 dv	5,4 cw
Ct (-)	0 et	0 fu	0 fv	0 ew

Description: The numbers accompanied by different letters are significantly different in the Duncan test α 0.05

Table 2 shows that the observations of the fourth and sixth days of the isolates WM4 and WM1 inhibitory activity against *E. coli* were not significantly different. The eighth day observations of WM1 and WM5 isolates were not significantly different. The tenth day in isolates WM4 and WM5 inhibitory activity against *E. coli* was not significantly different. Based on Duncan test results, the influence of isolate type and incubation period interaction of the most excellent isolate as antibacterial producer was isolated WM4 with ten days incubation period.

Based on the results of anava, it can be concluded that there are differences in the influence of mold type and incubation period to antibacterial activity. Further Duncan test results for interaction type of mold isolate and incubation period on antibacterial activity are listed in Table 3.

Table 3. Duncan Test Results of Interaction Type of Mold Isolates and Incubation Time on Antibacterial Activity

Type of isolate	Incubation period (days)			
	4	6	8	10
WM4	11,9 at	13,2 au	15,7 av	**17,4** aw
WM1	10,7 bt	12,4 bu	13,1 bv	14,6 cw
WM5	5,3 et	8,7 cu	11,3 cv	15,1 bw
WM3	6,7 dt	7,4 du	8,6 dv	14,3 cw
WM2	5,2 et	5,7 eu	5,8 ev	2,6 ew
Ct (+)	8,4 ct	8,6 cu	8,7 dv	8,5 dw
Ct (-)	0 ft	0 fu	0 fv	0 fw

Description: The numbers accompanied by different letters are significantly different in the Duncan test α 0.05

Table 3 shows that observation of the fourth day, antibacterial activity of WM5 and WM2 isolates was not significantly different. The antibacterial activity of WM5 isolates and the positive control of penicillin antibiotics did not differ significantly on the sixth day. The antibacterial activity of WM3 isolates and the positive control of penicillin antibiotics did not differ significantly on the eighth day. The antibacterial activity of WM1 and WM3 isolates did not differ significantly on the tenth day. Based on Duncan test results, the influence of isolate type interaction and incubation time of the best isolate as antibacterial producer was isolated WM4 with ten days incubation time.

The result of this research showed that the antibacterial isolates of WM4 had a larger inhibitory zone diameter against *Staphylococcus aureus* than *Escherichia coli.* This indicates that the antibacterial compounds produced by WM4 molds are more effective against *Staphylococcus aureus* which is a Gram-positive bacteria than *Escherichia coli* which is a Gram-negative bacterium. Nevertheless, the antibacterial compounds produced by WM4 isolate belong to bacteriostatic and broad spectrum because they are able to suppress the growth of both indicator bacteria. Some previous researchers have also reported the presence of several types of mold that can produce antibacterial compounds.

4. Conclusion

In conclusion, this study shows that the type of mold isolate obtained from "Wikau Maombo" fermented has a significant effect on antibacterial activity. The highest activity was WM4 isolates with antibacterial activity of 16.7 mm in *Escherichia coli* and 17.4 mm in *Staphylococcus aureus*. The incubation period of fungi isolate has significant effect on antibacterial activity. The highest antibacterial activity on the tenth day with activity was 9.8 mm in *Escherichia coli* and 10.2 mm in *Staphylococcus aureus*. The interaction between type of mold and incubation period of had significant effect on antibacterial activity. The best antibacterial activity was WM4 isolate type with ten days incubation period. Thus the mold isolate of WM4 from "Wikau Maombo" can be developed for antibacterial production with a ten days incubation period.

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