Preliminary Identification Of Banana Peel Indigenous Yeasts With Proteolytic Activity

Gemilang Lara Utama, Halimah, Nandi Sukri, Roostita Lobo Balia

Abstract: Banana peel having high content of nutrient such as carbohydrat and protein, can be a source of indigenous yeast with proteolytic activity. This research aims to identify indigenous yeasts with proteolytic activity and antimicrobial activity from banana peel. Banana peel was inoculated into Potato Dextrose Agar (PDA) media modified with 3% yeast extract and 10 ppm Amoxicillin and were incubated for 48 hours in room temperature. Each colony formed were then differentiated macrospically and purified in Yeast Mould Agar (YMA). Purified colonies were further identified under microscope and only the colonies having yeasts morphology were identified using Vitek 2 bioMérieux and tested for its proteolytic activity with paving block method using Nutrient Agar plus 3% Skim Milk, with formation of clear zone were measured as proteolytic activity. Antimicrobial activity tested using well diffusion methods using Nutrient Agar against Salmonella spp. and Escherichia coli. The results showed 2 yeasts isolates found and proteolytic activity was only found in 1 isolate with ±15 mm diameter of clear zone amd antimicrobial activity has showed negative result. The isolate reveales as Candida spp.

Index Terms: antimicrobial, banana peel, biochemical activity, Candida spp., indigenous yeast, proteolytic, Vitek 2

1. INTRODUCTION

BANANA peel is included in the type of organic waste, which is waste that can degraded by microorganisms. Microorganisms that are found naturally and have adapted to the environmental conditions in which they found as indigenous microorganisms have the ability to accelerate the process of breaking down organic matter [1]. Lehar [2] states that the process of decomposition of organic material, one of which is the decay begins with the secretion of extracellular enzymes by microorganisms that can hydrolyze large complex molecules into smaller molecules, so that they can be utilized by other microorganisms. The decay process will cause changes in some chemical components in food, including increasing sugar levels due to the hydrolysis of starch. In addition, protein degradation also occurs, namely the breakdown of complex molecules into simpler molecules caused by the activity of the protease enzyme [3]. The protease enzyme can be produced by microorganisms contained in food because the sugar content is high enough in food waste can be used as a growth substrate for microorganisms that produce protease enzymes. Roostita et al., [4] states that extracellular proteases produced by indigenous microorganisms can act as antimicrobials so that they can used in extending the shelf life of a food product. One type of indigenous microorganisms that can found in banana peel waste with the ability to produce proteases is yeast. This supported by Fleet in Spencer & Spencer [5] and Al Falih [6], which states that some types of yeast have the ability to secrete extracellular proteases that are quite high. Antimicrobial activity resulting from extracellular protease of indigenous yeast from banana peel waste can also play a role in killing pathogenic microorganisms that found in food. Pathogenic microorganisms can cause various diseases. One of the pathogenic microorganisms that are found is Salmonella spp. which found in nuts, salad dressing, mayonnaise, milk, and other foods [7]. Supardi & Sukamto [8] mentioned that foods that are often contaminated with Salmonella spp. namely eggs and their processed products, fish and their processed products, chicken meat, beef, and milk and their processed products such as ice cream and cheese. In addition to Salmonella spp., Balitbang [9] also showed that pathogenic microorganisms found in fresh vegetables are Escherichia coli. Some strains of E. coli can cause disease in humans and animals by producing enterotoxins. The difference in the substrate will determine the type of indigenous yeast that can be isolated. Indigenous yeasts originating from banana peel waste have not identified and its ability to produce proteases has the potential as an antimicrobial. Based on this background, further studies needed to determine the types of indigenous yeast in banana peel waste that produces extracellular proteases and their ability as antimicrobials against pathogenic microorganisms (Salmonella spp. and E. coli).

2 MATERIALS AND METHODS

2.1 Isolation and Identification of Indigenous Yeasts

Indigenous yeast were isolated from 5 g of inoculated banana peel waste using modified Potato Dextrose Agar (PDA) with the addition of 3% Yeasts Extract / YE (Kraft Foods) and 10 ppm amoxicillin, then incubated for 2 days at room temperature. Isolates then purified using modified Yeast Mould Agar (YMA) with the addition of 10 ppm kloramfenikol [10]. Isolates that grow on modified YMA then identified under microscope for size and shape then tested with Vitek 2 bioMérieux [11].

2.2 Proteolytic Activity Test

Each isolates was propagated by swabbing 1 loopful on the surface of modified PDA, incubated for 48 hours at room temperature. Meanwhile, NA with 3% skim milk was pour into a petri dish, let harden, and a hole was punched on the agar. Isolate formed on the modified PDA were collected by the same way as the hole formed in NA and the collected agar was put inside the hole of NA skim milk. Incubation was done for 48 hours at room temperature and proteolytic activity was described as the formation of clear zone, which later being measured [12].

2.3 Antimicrobial Activity Test

Yeast colony from each isolated species was sub cultured into 10 mL modified NB with the addition of 3% Yeasts Extract / YE (Kraft Foods) and 10 ppm amoxicillin and was incubated at 27°C for 48 h. Dilute the yeast solution. Escherichia coli and Salmonella spp. sub cultured into 10 mL of Nutrient Broth (NB) and incubated at 37°C for 24 h. Nutrient Agar (NA) plates aseptically sweep until covered with Escherichia coli and Salmonella broth using sterile swabs, and a hole was punched on the agar. Put in the yeast solution onto each NA plates. Incubate the NA plates at 37°C for 48 h then diameter of clear

zones were measured [4].

3 RESULTS AND DISCUSSIONS

3.1 Indigenous Yeasts Characterization

After characterizing, the cells observed under a microscope. The results (Table 1) showed that the isolate I – 3 has cylindrical cell with width 3.98 µm and length 6.77 µm, while isolate I – 5 has apiculate cell with width 2.41 µm and length 5.18 µm. Macroscopically, the colonies of isolate I -3 are broken white, rounded, wrinkled surface, and aerobic while isolate I – 5 are broken white, rounded, and aerobic. All colonies are categorized in to yeast according to Fardiaz [13] where yeast cell has length around 1-5 µm until 20-50 µm, and width sized 1-10 µm. The selected isolates were identified by Vitek 2 bioMérieux (Table 2.).

TABLE 1

CHARACTERISTICS OF INDIGENOUS YEASTS

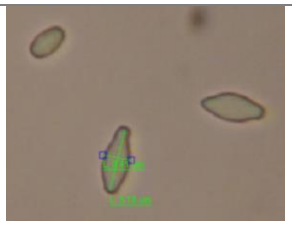
Isolate	Cell Morphology	Colony Morphology
I - 3		
I - 5		

TABLE 2

BIOCHEMICAL IDENTIFICATION OF ISOLATE I-3 (A) AND I-5 (B)

(A)

(3	LysA	-	4	lMLTa	+	5	LeuA	+	7	ARG	+	10	ERYa	-	12	GLYLa	(+)*
13	TyrA	+	14	BNAG	-	15	ARBa	-	18	AMYa	-	19	dGALa	+	20	GENa	-
21	dGLUa	+	23	LACa	+	24	MadGa	-	26	dCELa	-	27	GGT	+	28	dMALa	-
29	dRAFa	-	30	NAGA1	-	32	dMNEa	+	33	dMELa	-	34	dMLZa	-	38	lSBEa	-
39	lRHAa	-	40	XLTa	-	42	dSORa	-	44	SACa	-	45	URE	-	46	AGLU	-
47	dTURa	-	48	dTREa	-	49	NO3a	-	51	lARAa	+	52	dGATa	+	53	ESC	-
54	lGLTa	+	55	dXYLa	-	56	LATa	-	58	ACEa	+	59	CITa	(-)*	60	GRTas	+
61	lPROa	-	62	2KGa	+	63	NAGa	-	64	dGNTa	-						

(B)

3	LysA	-	4	lMLTa	+	5	LeuA	+	7	ARG	+	10	ERYa	-	12	GLYLa	+
13	TyrA	+	14	BNAG	-	15	ARBa	-	18	AMYa	-	19	dGALa	+	20	GENa	-
21	dGLUa	+	23	LACa	+	24	MadGa	-	26	dCELa	-	27	GGT	+	28	dMALa	-
29	dRAFa	-	30	NAGA1	-	32	dMNEa	+	33	dMELa	-	34	dMLZa	-	38	lSBEa	-
39	lRHAa	-	40	XLTa	-	42	dSORa	-	44	SACa	-	45	URE	-	46	AGLU	-
47	dTURa	-	48	dTREa	-	49	NO3a	-	51	lARAa	+	52	dGATa	+	53	ESC	-
54	lGLTa	+	55	dXYLa	-	56	LATa	-	58	ACEa	+	59	CITa	-	60	GRTas	+
61	lPROa	-	62	2KGa	+	63	NAGa	-	64	dGNTa	-						

*Reactions that appear in parentheses are indicative of weak reactions that are too close to the test threshold.

LysA = Lysine Arylamidase. TyrA = Tyrosine Arylamidase. dGLUa = D-glucose assimilation. dRAFa = D-raffinose assimilation. lRHAa = L-rhamnose. dTURa = D-turanose assimilation. lGLTa = L-glutamate assimilation. lPROa = L-proline assimilation. lMLTa = L-malate assimilation. BNAG = β-n-acetyl-glucosaminidase. LACa = Lactose assimilation. NAGA1 = PNP-N-acetyl-BD-galactosaminidase. XLTa = Xylitol assimilation. dTREa = D-trehalose assimilation. dXYLa = D-xylose assimilation. 2Kga = 2-keto-D-gluconate assimilation. LeuA = Leucine-Arylamidase. ARBa = Arbutin assimilation. MadGa = Methyl-A-D-Glucopyranoside. dMNEa = D-mannose assimilation. dSORa = D-sorbitol assimilation. NO3a = Nitrate assimilation. LATa = L-lactate assimilation. NAGa = N-acetyl-glucosamine assimilation. ARG = Arginine GP. AMYa = Amygdalin assimilation. dCELa = D-cellobiose assimilation. dMELa = D-melibiose assimilation. SACa = Saccharose/Sucrose assimilation. lARAa = L-arabinose assimilation. ACEa = Acetate assimilation. dGNTa = D-gluconate assimilation. ERYa = Erythriol assimilation. dGALa = D-galactose assimilation. GGT = Gamma-Glutamyl-Transferase. dMLZa = D-melezitose assimilation. URE = Urease. dGATa = D-galacturonate assimilation. CITa = Citrate assimilation. GLYLa = Glycerol assimilation. GENa = Gentibiose assimilation. dMALa = D-maltose assimilation. lSBEa = L-sorbose assimilation. AGLU = α-Glucosidase. ESC = Esculin Hydrolisis. GRTas = Glucoronate assimilation.

The result showed that isolate I – 3 and isolate I – 5 were identified as yeast belonging to genus Candida sp. Candida has cell characteristiscs with various shapes of cell like rounded, elongated, oval, cylindrical, rarely apiculate, ogival, triangular, or bottle shaped. The diameter and length range from (2-5) × (2.5 -10) µm [14] (Nurhariyati et al., 2004). In media culture, the growth of Candida spp. has the characteristic smooth and shiny surface and cream-colored colonies [15]. The results of identification using Vitek 2 showed that isolate I–3 and I–5 have same biochemical ability to assimilate several carbon compounds, there are galactose, glucose, mannose, malate, arabinose, sucrose, and lactose. Brooks [16] isolated yeast from banana peel were identified as Saccharomyces cerevisiae, Saccahromyces kluyveri, and Debaryomyces hansenii. Saccharomyces cerevisiae has a similar ability in assimilating galactose, glucose, and sucrose,

while Debaryomyces hansenii has the ability to assimilating glucose and galactose [17], [18]. This results indicates that indigenous yeasts isolated from banana peel waste use glucose as a carbon source for their metabolic processes.

3.2 Proteolytic Activity of Indigenous Yeasts

The result showed (Table 3) only isolate I – 3 that has proteolytic activity with the diameter of clear zone 15 mm. The clear zone around yeast isolate formed of casein hydrolysis by protease produces by yeast, while the non-hydrolyed looks darker in media agar [12].

TABLE 3
PROTEOLYTIC ACTIVITY OF INDIGENOUS YEASTS

Isolate	Clear Zone Diameter	Figure
I – 3	15 mm	
I - 5	-	

Candida spp. which has the proteolytic activity are Candida albicans, Candida tropicalis, Candida parapsilosis, and Candida krusei [19], [20]. Several Candida spesies also didnt have proteolytic activity, such as Candida guilliermondii and Candida moris [21]. The type of protease produced by Candida spp. is aspartyl protease (SAP). Candida spp. that produces this enzyme are Candida albicans, Candida dubliniensis, Candida tropicalis, and Candida parapsilosis [22]. SAP is included in the acid proteolytic enzyme because it consists of two aspartic acid residues [23]. One of these aspartic acids has a negative charge. Negative aspartic acid is close to a nuceophilic water molecule (tends to attract a positive charge). Negative aspartic acid activates nucleophilic water molecules and attacks the carbonyl group in the scissile bond on the substrate causes hydrolysis and structural changes of the substrate [24].

3.3 Antimicrobial Activity of Indigenous Yeasts

Table 4 showed both isolates had negative antimicrobial activity against Escherichia coli and Salmonella spp.. This result indicate that there are no correlation between protealytic activity with antimicrobial activity.

TABLE 4
ANTIMICROBIAL ACTIVITY OF INDIGENOUS YEASTS

Isolate	E. coli	Salmonella spp.
I - 3		
	0 mm	0 mm
I - 5		
	0 mm	0 mm

The mechanism of protease as an antimicrobial can occur due to the interaction between peptide and bacterial cell membrane. Followed with membrane damage, cell wall biosynthesis, and cell translocation causes the break down of protein in the bacterial cell wall and allow nucleotides and amino acids to break out and inhibit the entry of active ingredients into the cell, this condition can cause bacterial [25], [26]. The type of bacteria target will be the factors that affect antimicrobial activity. Gram-negative bacteria more resistant to antimicrobial. This is because the outer cell membrane of gram-negative bacteria can be the first defense against penetration of antimicrobial caused reducing the uptake of antimicrobial compounds in the intracellular target [27], [28]. Yeast antimicrobial activity not only caused by proteolytic activity. Antimicrobials activity in yeast can also be obtained from organic acids (hexnoic, octanoic, decanoic) and proteins inhibitor for bacterial and mold growth, immunoglobulin, a stimulation and killer toxins secretion [29], [30], [31]. Yeast also has the ability to produce sulfur dioxide which can inhibit the growth of lactic acid bacteria [32].

4 CONCLUSION

The results showed 2 indigenous yeasts isolates found from banana peel waste. The isolate reveales as Candida spp.. Proteolytic activity was only found in 1 isolate with ±15 mm diameter of clear zone and antimicrobial activity has showed negative result.

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