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THE DIVERSITY OF PAPAYA (*CARICA PAPAYA*) SEEDS INDIGENOUS YEASTS AND ITS ANTIMICROBIAL ACTIVITIES TOWARDS *E.coli* AND *S.typhimurium*

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Abstract: Papaya seeds has various utilization that can be obtained from its bioactive compound and indigenous microorganism activity. The aim of this study is to identify the diversity of yeasts isolated from papaya seeds and determine the antimicrobial activity towards *E.coli* and *S.typhimurium*. Indigenous yeast isolated from papaya seeds using Modified Potato Dextrose Agar (PDA) which is added by yeast extract (Kraft foods) and antibiotics (Amoxicillin). The microscopic and macroscopic characterization has been done by observing shape, texture, color, and size. The species of indigenous yeasts are rapidly checked by using RapID Yeast Plus System that confirmed through the Electronic Code Compendium (www.remel.comeric). The testing of antimicrobial activity of indigenous yeast towards pathogenic bacteria were done by using plug method. The research done experimentally and all of the data were analyzed descriptively. The results showed that there are seven yeasts isolated which three species identified from papaya seeds. The isolates are *Candida krusei* (1 strain), *Candida tropicalis* (5 strains) and *Cryptococcus albidus* (1 strain). Antimicrobial test found that all of the isolate shown antimicrobial activities against *E. coli* and *S. tyhphimurium,* except *Candida krusei*. *Candida tropicalis*1 shown the highest antimicrobial activities against *E. coli*, while *Candida tropicalis*5 has the highest antimicrobial activities towards *S. typhimurium.*

Keywords: Papaya seeds, Identification, Isolation, Antimicrobial.

INTRODUCTION

One of the most popular fruit found in tropical country is papaya (*Carica Papaya L.)*, which every part of the fruit can be utilized. In West Java - Indonesia, the production of papaya reaches 86.576 ton in 2016 and keep increasing every year (Badan Pusat Statistik, 2017). Papaya seeds usually being thrown away because of its spicy and bitter flavor that comes from its phytochemical compound such as flavonoids (Nofitriyani, 2016; Sukadana, et al., 2008; Suyanto, 2009). Nonetheless, papaya seeds can be potentially developed.

Papaya seeds generally used as diarrhea medication, diabetic medication, contraception, antiinflamation, and antimicrobial substances (Purwaningdyah, et al., 2015; Juárez-Rojop, et al., 2012; Venkateshwarlu, et al., 2013; Uche-Nwachi, et al., 2011; Amazu, et al., 2010; Efunwole, et al., 2014). Various utilization of papaya seeds can be obtained from not only its bioactive compound, but also could be obtained from microorganism activity. Indigenous microorganism has the potential in the biodegradation, bioleaching, antibiotic, bioremedation, nitrogen fixation, etc (Kumar & Gopal, 2015).

Yeast as a indigenous microoganism is evenly distributed, not only have a high tolerance towards antibiotics, acid, salt, sugar, and also have antimicrobial activity (Putranto, et al., 2010). In food industry, the utilization of yeast is found in wine, beer, bread, fermented foods (pickels, butter, cheese, yoghurts, etc), and as a food preservation (Mukerji, et al., 2000). Yeast as a microorganism that can live in stress environment, commonly isolated from a high sugar content substrate, such as fruits (Tsegaye, 2016).

Maragatham & Panneerselvam (2011) found 64 yeast isolates in seven rotten papayas, which are *Saccharomyces italicus*, *Saccharomyces pasteuriamus*, *Schizosaccharomyces pombe*, *Saccharomyces bayanus*, *Saccharomyces uvarum*, *Saccharomyces ceresvisceae*, and *Zygosaccharomyces* that have the ability to produce ethanol. Isolation of *Candida famata* and *Rhodotorula mucilaginosa* from rotten and fresh papaya have a antagonistic characteristics and parasitism activity against anthracnose disease in papaya fruit cause by *Colletotrichum gloeosporioides* fungi (Magallon-Adalon, et al., 2012). The aim of this work is to identify and add a new information about the biodiversity and antimicrobial activity of indigenous yeasts from papaya seeds.

MATERIALS AND METHODS

Isolation of Indigenous Yeasts

5 gram of papaya seeds were mashed using sterile mortar, then diluted in 45 mL NaCl 0.85 %. Dilution was done serially up to 10^{-3} and 1 mL of diluted suspension from 10^{-2} and 10^{-3} was taken and plated using potato dextrose agar added by 3 % of yeast extract and 5 ppm of amoxicillin to inhibit bacterial growth. Inoculated plates then incubated in 30 ^{0}C for 48 hours. Yeast isolates were

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subcultured on modified potato dextrose agar and incubated in 30 ^{0}C for 48 hours. Purified yeast isolates were routinely maintained and kept in refrigerator at -4 ^{0}C ± 1 ^{0}C.

Macroscopic and Microscopic Characterization of Indigenous Yeasts

Macroscopic characterization was done by observing colony color, elevation, shape, and other distinct features. Microscopic characterization was done by smearing yeast colony, added by a drop of sterile distilled water and observed under microscope using x40 objective lenses.

Indigenous Yeasts Identification using RapID Yeast Plus Kit

Yeasts identification was done by enzyme based biochemical test using RapID Yeast Plus Kit. Purified yeast isolate was swabbed from agar plate to RapID Innoculation Fluid until the visual turbidity obliterated the inoculation card. The suspension was mixed thoroughly using vortex then poured evenly in RapID Yeast Plus Kit inoculating trough. The kit panel was tilted 45^0 until the suspension distributed evenly in the reaction cavities and incubated for 4 hours at 30 ^{0}C. When the incubation was done, cavities 7 through 14 was added by 1 drop of RapID Yeast Plus Reagent A, and 1 drop of Rapid Yeast Plus Reagent B added to cavities 16 through 18.

Antimicrobial Activity of Indigenous Yeast towards Pathogenic Bacteria

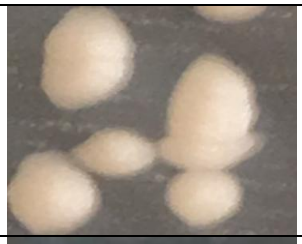
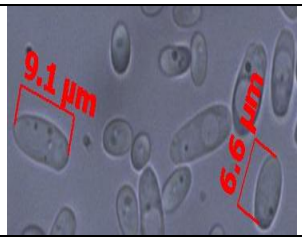
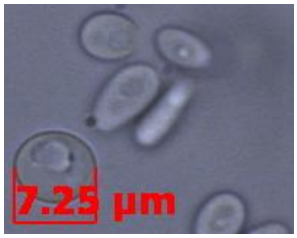
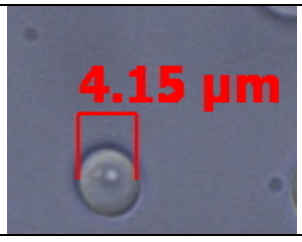
Yeast colony were swabbed aseptically using sterile swab into Potato Dextrose Agar (PDA) added with 3% yeast extract and incubated at 30^{o}C for 48 hours. *Escherichia coli* and *Salmonella typhimurium* were subcultured and swabbed aseptically into Nutrient Agar (NA) using sterile swabs. Both yeast agar plate and bacterial agar plate were plug aseptically. Yeast agar plate plug then placed carefully into bacterial agar plates. The bacterial plates then incubated at 37^{o}C for 48 hours and diameter of the clear zones were measured every 24 hours.

RESULT AND DISCUSSION

Indigenous Yeast Macroscopic and Microscopic Characterization

Table 1.

Indigenous Yeast Characterization

Code	Macroscopic characteristics		Microscopic Characteristics	
Ph 2	White, irregular form, rough surface, entire, flat elevation, entire margin.		Oval, size: 6,6 – 9,1µm	
Ph 3	White, round, ambonate elevation, entire margin, rough surface.		Round, size: 4,15 – 7,51 µm	
Ph 4	White, round, ambonate elevation, entire margin, rough surface.		Oval, size: 4,15 – 6,48 µm	

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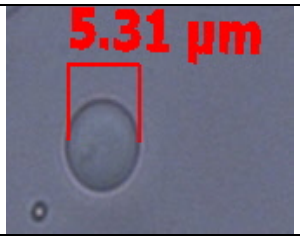
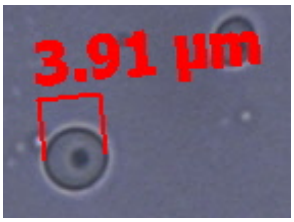
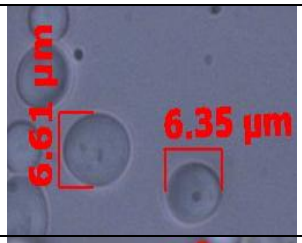
Ph 5	White, round, convex elevation, entire, glistening texture		Oval, size: 5,31 µm	
Ph 6	White, round, ambonate elevation, entire margin, rough surface.		Round, size: 3,56 – 3,91 µm	
Ph 7	White, round, ambonate elevation, entire margin, glistening texture.		Oval, size: 5,99 – 6,61 µm	
Ph 8	White, round, ambonate elevation, entire margin, rough surface.		Oval, size: 4,67 – 6,49 µm	

Macroscopic characterization from **Table 1**. all have yeast-like characteristics which could be identified by its morphological characteristics, such as shape, texture, surface, color, elevation, and margin. Yeast characterization by its shapes are round, filamentous, irregular, and rhizoid. Characterization by its elevation are convex, raised, flat, crateriform, umbonate. Yeast margin characterization are entire, undulate, filliform, lobate, and curled (Leung & Liu, 2005). Microorganism microscopic characteristics observed had a yeast-like characteristics, such as round, oval, cylinder, ovoid, spherical, and spheroid cell shape, with its length range from 1-5 µm to 20-50 µm and width 1-10 µm (Pelczar & Chan, 2005).

Purified isolates then identified using RapID Yeast Plus Kit based on yeasts biochemical reactivity. Identification using RapID Yeast Plus Kit examine yeasts ability to utilize carbohydrates, hydrolyze fatty acids esters, enzymatic hydrolysis of substituted glycoside and phosphor-esther, hydrolyze urea and aryl-amide substrate (ThermoFisher-Scientific, 2016).

Table 2.

Yeast Identification by RapID Yeast Plus System

Test	Isolate						
	Ph.2	**Ph.3**	**Ph.4**	**Ph.5**	**Ph.6**	**Ph.7**	**Ph.8**
GLU	+	+	+	+	+	+	+
MAL	-	+	+	+	+	+	+
SUC	-	+	+	-	-	+	+
TRE	-	+	+	+	+	+	+
RAF	-	-	-	-	-	-	-
LIP	-	-	-	-	-	-	-
NAGA	-	-	+	-	+	+	+
αGLU	-	+	+	+	+	+	+

Test	Isolate						
	Ph.2	**Ph.3**	**Ph.4**	**Ph.5**	**Ph.6**	**Ph.7**	**Ph.8**
βGLU	-	-	+	+	-	-	-
ONPG	-	-	-	-	-	-	-
αGAL	-	-	-	-	-	-	-
βFUC	-	-	-	+	-	-	-
PHS	-	+	-	+	-	+	+
PCHO	-	-	+	-	-	-	-
URE	-	-	-	-	-	-	-
PRO	-	-	-	+	-	-	-
HIST	+	+	+	+	+	+	+
LGY	+	-	+	-	+	-	-
Yeasts Species	*Candida krusei*	*Candida tropicalis*	*Candida tropicalis*	*Cryptococcus albidus*	*Candida tropicalis*	*Candida tropicalis*	*Candida tropicalis*

GLU (Glucose), MAL (Maltose), SUC (Sucrose), TRE (Trehalose), RAF (Raffinose), LIP (Fatty Acid Ester), NAGA (ρ-Nitrophenyl-N-acetyl-β,D-galactosaminide), αGLU (ρ-Nitrophenyl-α,D-glucoside), βGLU (ρ-Nitrophenyl-β,D-glucoside), ONPG (σ-Nitrophenyl-β,D-galactoside), αGAL (ρ-Nitrophenyl-α,D-galactoside), βFUC (ρ-Nitrophenyl-β,D-fucoside), PHS (ρ-Nitrophenyl phosphate), PCHO (ρ-Nitrophenyl phosphorychlorine), URE (urea), PRO (Prolin-β-naphtylamide), HIST(Histidine- β-naphtylamide), LGY (Leucyl-glycin- β- naphtylamide)

Yeast identification shown at **Table 2.** obtained three isolates, which are *Candida krusei* (1 strain), *Candida tropicalis* (5 strain), and *Cryptococcus albidus* (1 strain). The yeast obtained usually found in fruits, such as papaya (Kurtzman, *et al*., 2011; Deak dan Beuchat, 1996; Barnett, *et al*., 1991). The existence of yeast in papaya fruits could also be found in papaya's seeds because of vacuumed condition inside papaya fruit and also the availability of nutrients for the growth of yeasts.

Differences in the ability of hydrolysis of chemical compounds are shown in Table 3., where isolate Ph.3 cannot hydrolyze substituted glycosides and phosphoester (NAGA, β-GLU, PCHO, and LGY) enzymatically. While isolate P.4 can hydrolyze compounds NAGA, β-GLU, PCHO, and LGY, but cannot hydrolyze PHS compounds. As well as, isolates P.6, P.7 and P.8 which can only hydrolyze some substituted and phosphoester glycosides, namely PHS and α-GLU.

Candida tropicalis is a rounded, vegetative and osmotolerant yeast with a size of 2-10 µm (Murray, et al., 2003). All identified *Candida tropicalis* isolates can metabolize carbohydrates except raffinose and histidine-β-naphthylamide. In accordance with the research of Silva, et al. (2012) and Zuza-Alves, et al., (2017), *Candida tropicalis* has the ability to oxidize and assimilate glucose sucrose, galactose, trehalose and maltose oxidatively. Meanwhile, raffinose sugar cannot be metabolized by yeast *Candida tropicalis* (Kurtzman and Fell, 1998). Yeast isolates *Candida tropicalis* cannot metabolize urea and auxotrophic compounds against histidine compounds (Murray, et al., 2003; Gleeson, et al., 1990).

Candida krusei is a single cell yeast species that does not produce spores and is elongated in shape like rice (Hafiz, 2011). Based on Table 3., *Candida krusei* isolates cannot hydrolyze all carbohydrate compounds, except glucose (Kurtzman, 2011). *Candida krusei* can also hydrolyze the HIST and LGY columns which are amino acid groups. The yeast has an active region of the enzyme that can bind to the hydroxyl ion as its ligand. The hydroxyl ion then binds to histidine and aspartate that make up water (Fateh, et al., 2015).

Cryptococcus albidus is not a fermentative yeast, but can assimilate inositol and hydrolyze urea (Barnett, et al., 1990; Viviani & Tortorano, 2009). *Cryptococcus albidus* cells form are round to ovoid (rounded elongated, like egg shape) and encapsulated by layers of glycoprotein compounds that have characteristics such as gelatin (Refai, et al., 2014). Yeast isolates of *Cryptococcus albidus* can use carbohydrate compounds and substituted glycosides such as glucose, maltose, trehalose, citric acid, salicin, cellobiosa, inositol, except sucrose as a carbon source for the assimilation process for example nitric assimilation (Casadevall and Perfect, 1998; Refai, et al. , 2014; Viviani and Tortorano, 2009).

Candida krusei, *Candida tropicalis*, *and Cryptococcus albidus* isolates can be found in fruit products such as papaya (Kurtzman, et al., 2011; Deak and Beuchat, 1996; Barnett, et al., 1991). Three isolates in papaya are found because of the glucose content as one of the nutritional substrates that support yeast growth. The type of yeast contained in papaya fruit can also be found in papaya seeds because of the vacuum conditions in papaya fruit and the availability of nutrients for yeast growth.

Antimicrobial Activity of Indigenous Yeast towards Pathogenic Bacteria

Even though the identification result showed the same species, the ability of each yeast strain to hydrolysis chemical compound is different (Juszczyk, 2005). So, all

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strains were used in antimicrobial test. Yeast antimicrobial activity was showed by the formation of clear zone area around the plug. The bigger the clear zone area formed, the higher the inhibition of pathogenic bacteria growth (Prasaja, *et al*., 2014).

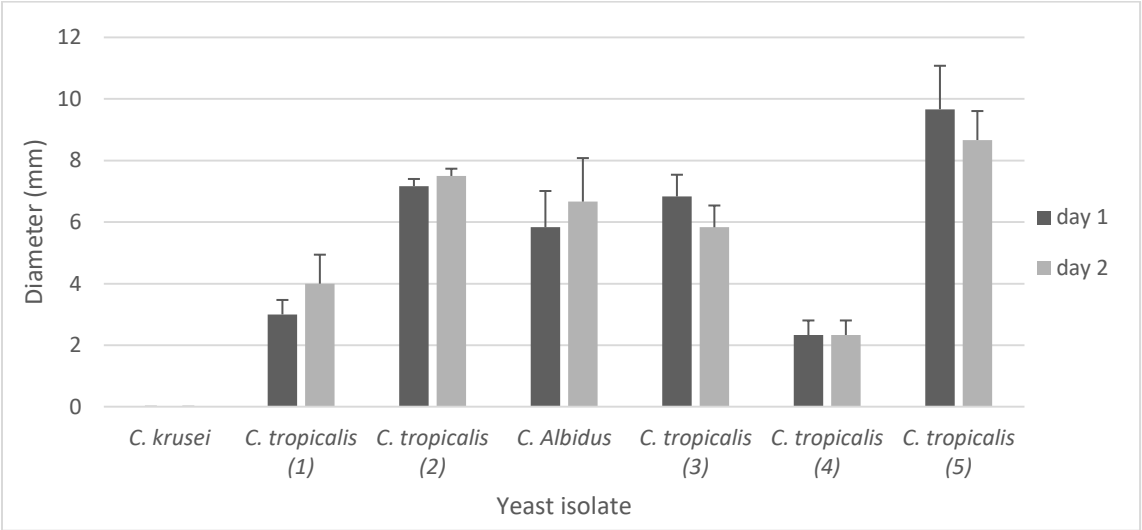

Fig. 1. Indigenous Yeasts Antimicrobial Activities towards *E.coli*

The yeast that has the highest antimicrobial activity against *E. coli* is *Candida tropicalis* (5). *Candida krusei* isolate didn't have any antimicrobial activity because it didn't form any clear zone area around the plug. While *Candida tropicalis* (4) was classified as weak antimicrobial activity (d = 0-3 mm), *Candida tropicalis* 1 and *Cryptococcus albidus* were classified as moderate antimicrobial activity (d = 3-6 mm), and *Candida tropicalis* 3 and *Candida tropicalis* 5 were classified as strong antimicrobial activity (d > 6mm) (Pan, et al., 2009).

All strains of *Candida tropicalis* showed different antimicrobial activity, because the ability to hydrolysis organic compounds were different which could affect the yeast metabolites production that can be used as antimicrobial agents (Roostita, *et al*., 2011).

On second day, isolate *Candida tropicalis* 1 and *Candida tropicalis* 2 had increased diameter clear zone than day one. This shows an increase in yeasts antimicrobial activity. The increased in clear zone diameter can be caused by the attachment of *E. coli* to *C. tropicalis* hyphae, and entered the *C. tropicalis* cells, so the number of bacterial cells decreases (Tarifa, et al., 2014)

While isolate *Candida tropicalis* 3, *Candida tropicalis* 4, and *Candida tropicalis* 5 had decreased diameter clear zone than day one. Decreased clear zone diameter can be caused by the formation of LPS (Lipopolysaccharide) as bacterial endotoxins by *E. coli* that could inhibit the formation of biofilm by *Candida spp.* and the growth of *Candida tropicalis* (Bandara, et al., 2009).

Cryptococcus albidus could produce extracellular enzymes, such as protease and phospholipase that have proteolytic and virulence characteristics. Extracellular proteolytic enzymes could hydrolyze bacterial cell wall which made of peptidoglicans, so that water could enter the cell and caused the lysis of cell (Nascimento, et al., 2017; Madigan, et al., 2006).

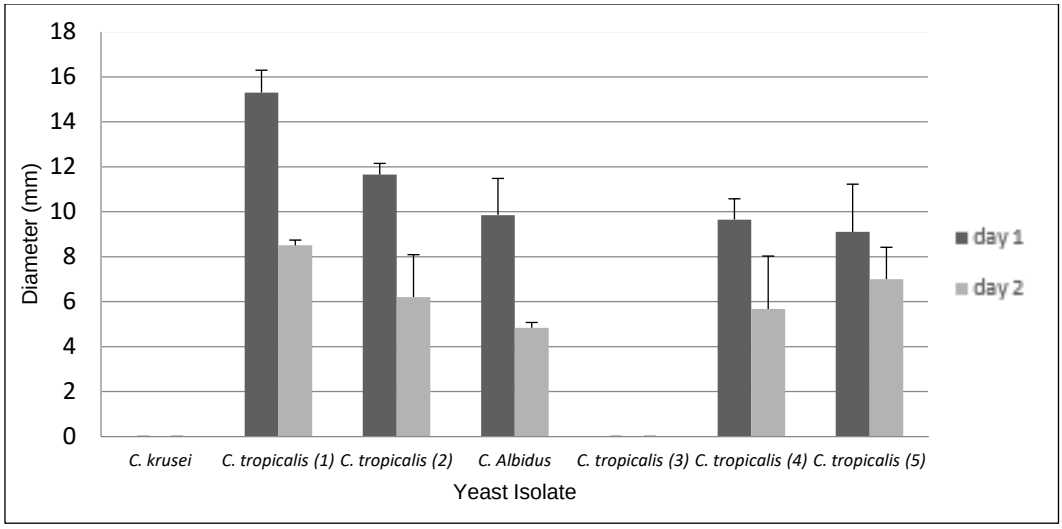

Fig. 2. Indigenous Yeasts Antimicrobial Activities towards *S.typhimurium.*

The yeast that has the highest antimicrobial activity against *S. typhimurium* is *Candida tropicalis* 1. *Candida krusei* and *Candida tropicalis* 3 didn't have any antimicrobial activity because it didn't form any clear zone area around the plug. *Candida tropicalis* 1, *Candida tropicalis* 2, *Candida tropicalis* 4, *Cryptococcus albidus, Candida tropicalis* 5 were classified as strong antimicrobial activity (d > 6mm) (Pan, et al., 2009).

The formation of clear zone area can be caused by the metabolites produced by yeasts, such as organic acids. Organic acids compounds produced were acetic acids, lactic acids, malic acids, tartaric acids, and citric acids (Dibner, 2003). Utilization of organic acids could inhibit the growth of *Salmonella* (Van Immerseel, et al., 2005)

All of the clear zone area decreased on day two, which showed that the antimicrobial activity is also decreased. The decrease in antimicrobial activity by *Candida tropicalis* caused by the formation of *Salmonella* LPS (Bandara, et al., 2010).

CONCLUSION

There are three yeast isolate and seven strains isolated from papaya seeds. The isolates are *Candida krusei* (1 strain), *Candida tropicalis* (5 strains) and *Cryptococcus albidus* (1 strain). Antimicrobial test found that all isolate have antimicrobial activity against *E. coli* and *S. tyhphimurium*, except *Candida krusei.* Isolate *Candida tropicalis* 1 has the highest antimicrobial activity against *E. coli*, while *Candida tropicalis* 5 has the highest antimicrobial activity against *S. typhimurium.*

AUTHORS CONTRIBUTION

Conceptualization: Utama G. L., Herlina, Kayaputri I. L.; Methodology: Utama G. L. and Herlina; Data collection: Herlina; Data validation: Utama G. L. and Kayaputri I. L.; Data processing: Herlina; Writing – original draft preparation: Utama G. L. and Herlina; Writing – review and editing: Utama G. L. and Balia R. L.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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