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Potential and Enzymatic Characterizations of Marine Yeast for Pufas from Balai Taman Nasional Karimunjawa

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Abstract. Potential of huge mangrove ecosystem in Indonesia, especially in Balai Taman Nasional Karimunjawa, for exploration of potential microorganism. One of them were yeast. PUFA is beneficial as a source of food for low and high-level organisms. The aim of this study is to explore the potential of yeast by enzymatic methods, differences of culture media and molecular identification in Balai Taman Nasional Karimunjawa. The research flow included isolation of yeast from several mangrove ecosystem located in Karimunjawa by purposive sampling methods. The pine pollen used to bait, identification colony morphology, Protease, amylolytic, cellulolytic, Lipase Enzymatic Test, mass culture used a variety of different media, molecular identification used 18S rDNA. The results of the studied obtained 4 isolates with the code (T2, KM1, KM2, KM3) which have the potential on the lipase and protease enzyme. Molecular identification used 18S rDNA analysis showed that all isolates were the yeast. Differences in the use of media also produce various yeast biomass.

1. Introduction

42% of 89 genus yeasts are found in Indonesia [7]. Yeast is a fungus that not consist mycelium. Fuel, water, plants, animals and insects can produce yeast. Yeast is one that hard to find in nature. Yeast is facultative aerobic microorganism [14]. The shape of yeast are circle, oval, cylinder and rod. Yeast has capability resistant to the stress environment [12]. Yeast can be divided into two groups based on its metabolisms are fermentative and oxidative [8]. Yeast particle size is bigger than bacteria. Microorganism can produce enzymatic activity, include yeast.

There are a lot of enzymatic test among others proteolytic, amilolytic, cellulolytic, and lipolytic. Breaking the peptide bond in the protein is what protease enzyme do. Protease is needed physiologically for the life of organisms. Protease does not only play a role in cellular metabolic processes, but can also be applied in the industrial field [15]. Lipase or also called triacylglycerol hydrolase, is an important enzyme in the field of biotechnology. Lipases include serine hydrolase and have high stability in organic solvents. Lipase include hydrolase enzymes that act in the water environment in the carboxyl ester bonds found in triacylglycerols to separate fatty acids and glycerol [6]. Cellulose is an enzyme used to hydrolyze cellulose. Cellulose is a subunit of glucose which is



insoluble in water [9]. Amylase is an enzyme that has the ability to break down glucoside bonds in starch polymers [5].

2. Research Methods

2.1. Sampling of mangrove leaf litter and sediment samples

Take mangrove sediment samples from 2 locations, namely Mangrove Tracking, Kemojan Village, Karimunjawa. Then it brought to the Laboratory of Tropical Marine Biotechnology, FPIK, Diponegoro University to be observed.

2.2. Isolation and Purification

0.2 grams of mangrove sediment samples were inserted into Erlenmeyer with 20 ml of sterile sea water added pine pollen to attract and then incubated 7 days [3]. Yeast isolates that have been purely cultured with a composition of agar 12 g / L, Yeast extract 3 g / L, Pepton 5 g / L, Glysecol 3ml / L [13]. Furthermore, each Yeast isolate was grown on a 50 ml Erlenmeyer bottle containing media, then shake it with 150 rpm, 24 ° C temperature.

2.3. Molecular studies

Lysis buffer is used for extraction that cells in liquid culture are carried out by using Centrifuge (8000 rpm, 5 minutes) [4], then Pellet is taken and added with 200 lysis buffer and homogenized. Electrophoresis is done with a 1% concentrate agarose gel, the device is run with a 100 V voltage for 30 minutes. The electrophoresis results were observed with UV Illuminator. Sequencing is done to see the composition of bases that form DNA sequences. The results of sequential isolates were then compared with DNA sequences in the GeneBank data base. Search is done with the internet system through the Basic Local Alignment Search Tool (BLAST) data tracking program at the National Center for Biotechnology Information, National Institute for Health, USA (www.ncbi.nlm.nih.gov) [1]. Phylogenetic analysis was carried out by comparing 10 Yeast sequences and 1 Yeast outgroup in the Gen Bank database when conducting BLAST analysis.

2.4. Enzymatic test

a. Proteolytic Test

The activity test of protease enzyme production was carried out by procedure. Isolates obtained from the isolation were made on the growing medium which was enriched with skim milk (1%) then incubated. Proteolytic activity is indicated by the formation of a clear zone around the dot with a white background [11].

b. Amilolytic Test

Test activity of amylase enzyme production activity is carried out by procedure. The isolates obtained from the results of isolation were made on the spice media enriched with starch (1%) then incubated. The 1% lugol's iodine solution was poured onto the culture to identify amylase activity, the activity of amylase enzyme was shown by the formation of clear zones around the paper disc with a dark blue background [11].

c. Cellulolytic Test

Cellulase production activity test was carried out by procedure. Isolates obtained from the isolation were made dot on growing media enriched with 1% CMC. Congo red solution is poured onto culture to identify cellulolytic activity. The presence of cellulase enzyme activity is indicated by the formation of clear zones around the paper disc with a pink background [11].

d. Lipolytic Test

Test of lipase enzyme production activity was carried out by a procedure which was modified. Isolates obtained from the isolation were made spot (dotting) on the growing medium and enriched with Tween 80 then incubated. The activity of lipase is indicated by the formation of a cloudy white fatty acid precipitate around the paper disc [11].

3. Result and Discussion

Molecular identification of 4 isolates was carried out by the chelex method. The results obtained were then amplified by PCR (Polymerase Chain Reaction). Based on the results of the amplification of the ITS area, it is known that isolates have a size between 500 - 520 bp. Size of amplification of ITS (Internal Transcribed Spacer) fragment area that use primers ITS1 and ITS4 is about 380 - 900 bp [2]. Then products that obtained from PCR determine the sequence of nucleotides of the isolates.

Table 1. Results of Homology Analysis with BLAST

No.	Isolate Code	Length (bp)	Closest Spesies	Similarity	Accession Number
1.	T 2	863	<i>Kluyveromyces siamensis</i> isolate SY3-1	99%	KY963105
2.	KM 1	424	<i>Candida tropicalis</i> strain NN4	100%	MH260384
3.	KM 2	643	<i>Candida tropicalis</i> voucher A.Fth_180	100%	MG818800
4.	KM 3	612	<i>Meyerozyma caribbica</i> strain UFLA CWFY11 (<i>Candida fermentati</i>)	99%	KM402049

The results of 4 isolates analyzed using Basic Local Alignment Search Tool (BLAST). BLAST results from 4 isolates with primers ITS1 and ITS4 each have length T 2 863 bp; KM 1 424 bp; KM 2 643 bp and KM 3 612 bp. The results of isolation and purification of isolates T2, KM1, KM2, and KM3 were obtained sequentially, namely *Kluyveromyces siamensis*, *Candida tropicalis*, and *Candida fermentati*. The same species will have 99 - 100% similarity [10]. KM 1 and KM 2 obtained are same species based on NCBI database because they have 99 – 100% similarity.

Enzyme is catalysts that can accelerate chemical reactions that produced by cells where enzymes can be work. Through its activity, enzyme arrange coordination with the enzyme system well, with the result that there will be good relation between a number of different metabolic activities.

Table 2. Enzymatic Test Results

Substrate	Enzyme Tested	KM 1	KM 2	KM 3	T 2
Starch	Amylase	-	-	-	-
Trybutirin Agar	Lipase	+	+	+	+
Milk	Protease	-	+	+	-
CMC	Cellulase	-	-	-	-

In the amylolytic test, starch is used as a microbe nutrient, but first hydrolyzed into a simple form of glucose with amylase enzyme helps. The amylase enzyme breaks the glycosidic bond from starch. The indicator that used in this amylolytic test is iodine, where starch will react with iodine on the media and showed black-blue color. The black-blue color occurs when iodine get into an empty part of the starch. So there will be clear zones around the colony. With this clear zone, it shows the activity of the amylase enzyme in the process of hydrolyzing starch. However, the results obtained were negative that means there were no clear zones surrounding the colony. Lipolytic test is intended to determine the ability of microbes to produce lipase enzymes from microbial metabolism. To get nutrients from lipids, first hydrolyze the lipids into a simple form of glycerol and fatty acids. With the appearance of yellow spots around the colony, there is lipase enzyme activity in the media. As if the result obtained, all of the isolates produce lipase enzyme. The function of proteolytic test to determine the ability of

microbes to produce protease enzymes. The protein that used is skim milk. The process of protein hydrolysis will gradually produce a simpler form, namely amino acids. If there is protease enzyme activity, there must be a clear zone around the colony. Only isolate coded KM 2 and KM 3 produce protease enzyme. Cellulolytic test show the clear zone around the colonies in CMC media. The test results showed that no one of the isolates were able to produce cellulose enzyme. This enzyme testing use duplo technique.

4. Conclusion

Molecular identification shows isolates with T2 code is *Kluyveromyces siamensis*, KM1 and KM2 are *Candida tropicalis*, and KM3 is *Candida fermentati*. KM 2 and KM 3 are potential isolates for PUFAs

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