Prebiotic Test of Three Variety of Mushrooms (*Auricularia polytricha, Agaricus bisporus*, and *Peluretus cystidiosus*) Towards "*Lactobacillus casei*" Bacteria

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Abstract. One formulation of maintenance of normal intestinal microbiota for diarrhea therapy in infants and children can use mushroom prebiotics. Mushroom polysaccharides are a potential source of prebiotics because they contain nutrients such as chitin, hemicellulose, α & β-glucans, mannas, xylans and galactose. Prebiotics cannot be separated from probiotics because the prebiotic target stimulates selective growth of probiotic bacteria. One of the probiotic bacteria is Lactobacillus casei. The purpose of this study is to determine the prebiotic activity of several fungi against Lactobacillus casei. The mushrooms used are black ear mushroom (Auricularia polytricha), white button mushroom (Agaricus bisporus) and brown oyster mushroom (Pleurotus cystidiosus) with 4 variations of concentration (3.125% b / v, 6.25% b / v, 12.5% b / v and 25% b / v) affecting the growth of Lactobacillus casei bacteria measured by absorbance of bacteria using a spectrophotometer at a wavelength of 434 nm. Black ear mushroom (Auricularia Polytricha), white button mushroom (Agaricus bisporus) and brown oyster mushroom (Pleurotus cystidiosus) have the potential to be a prebiotic to the Lactobacillus casei bacteria. The highest prebiotic activity is brown oyster mushroom (Pleurotus cystidiosus). Result analysis in this research is using Two-Way Anova.

1. Introduction

Diarrhea is a major public health problem in developing countries such as Indonesia, because of its high morbidity and mortality. In Indonesia diarrhea becomes the second most killer of babies after Acute Respiratory Infection (ISPA) and often lead to Extraordinary Events (KLB) (Lolopayung, et al., 2014). According to data from the World Health Organization (WHO) in 2006, every 1 in 5 deaths of children under the age of five years worldwide die of diarrhea with a total of around 760,000 deaths each year. And According to data from the Riau Provincial Health Office in 2012 diarrhea is the second most common cause of under-five mortality by 17.2% after neonatal problems (asphyxia, low birth weight, infection) by 36%.

Several attempts have been made to overcome acute diarrhea, especially among children, namely by consuming prebiotics and probiotics. According to Moller and Vrese (2004) some research results prove that certain probiotic bacteria such as bifidobacteria and lactobacillus can strengthen the immune system and overcome diarrhea, both by rotavirus and by bacteria and overcome constipation. In Alasir's (2007) study, it was shown that administration of probiotics in non-specific acute diarrhea of infants and children can shorten the duration of diarrhea, reduce the frequency of diarrhea, and increase weight gain significantly.

Prebiotics cannot be separated from probiotics because the prebiotic target is to stimulate selective growth of probiotic bacteria (Roberfroid, 2000). Therefore, the benefits of using prebiotics are

inseparable from the role of probiotics to regulate and modulate the population microbiology of probiotic bacteria.

The World Health Organization (WHO) defines probiotics as living microorganisms which when given in sufficient quantities can provide health benefits to humans. The following types of probiotics are good for the health of the human body, namely Bifidobacterium, Lactobacillus, Saccharomyces and Streptococcus species (Praja, 2011) and L. casei Shirota strain, L. reuteri, L. acidophilus LA-1, L. gasseri, L. rhamnosus, Bifidobacterium lactis Bb-1, B. breve, B. longum (Harti, 2009).

The fungi as a biological source before being used optimally, in addition to being consumed has a potential prebiotic mushroom because it contains carbohydrates such as chitin, hemicellulose, β- and α-glucans, mannas, xylans and galactants (Aida, et al., 2009).

Mushroom plants that contain carbohydrates are Black Ear Mushrooms 38.4% (Liana, et al., 2015), button mushrooms 3.26 g (Valverde, et al., 2015). Oyster mushrooms contain 56.6% carbohydrates (Yunita, 2011). Also polysaccharides of oyster mushrooms can stimulate the growth of intestinal microorganisms (probiotics), namely as prebiotics (Widyastuti, et al., 2011). Prebiotics are indigestible food ingredients that have beneficial effects by stimulating the growth of bacteria that naturally live in the intestine. Prebiotics are also said to be carbohydrates that are not digested by the body, but can be digested by microbes that are beneficial for improving health (Praja, 2011). Most carbohydrates found in nature are found as high molecular weight polysaccharides (Thenawidjaja, 1982).

For that reason, in this study, prebiotic compounds were isolated from 3 varieties of mushrooms (black ear mushrooms, white button mushrooms and brown oyster mushrooms). Then an activity test was conducted to see their effect on increasing the growth of probiotic bacteria. This study applies the prebiotic polysaccharide concept of fungi to the formula for the maintenance of the normal intestinal microbiota ecosystem for diarrhea therapy in infants and children as a series of studies in order to utilize natural ingredients, abundant mushrooms, cheap and easily obtained as a source of prebiotic compounds in the implementation of strategies treatment that does not involve antibiotics and antidiarrheal.

2. Methodology

Test material. The materials used in this study are black ear mushrooms, white buttons and brown oysters obtained from mushroom cultivation (Jamur Riau Mitra Mushroom) Pekanbaru.
Chemical material. The chemicals used are MRS Agar, MRS Borth, phosphate buffer, 70% alcohol, distilled water.
Isolation of Mushroom Polysaccharides. Each fresh mushroom was blended plus 70% alcohol and then squeezed and filtered until a solvent was obtained, then decanted and centrifuged at 3000 rpm. Then the extract obtained was tested for prebiotic activity (Kusumawati, et al., 2005).
Making Test Solvent. Test solvent is made from mushroom extract. The main liquor was made by dissolving 2.5 grams of extract for chocolate oyster mushroom and black ear mushroom and 1.5 grams of white button mushroom extract each extract plus phosphate pH ± 7 buffer of 5 ml, then each solution was diluted with double dilution made 4 series of concentration of 25% b / v; 12.5% b / v; 6.25% b / v; 3.125%, b / v and blank.
Rejuvenation of the Lactobacillus casei bacteria. It was obtained from PAU UGM in a freeze so it needed rejuvenation. Rejuvenation of pure cultures of Lactobacillus casei bacteria on MRS media was incubated for 48 hours at 37 ° C (Rahayu, 2014).
Prebiotic Activity Test for Fungi. A total of 2500 µl of MRS Borth were added with 100 µl of Lactobacillus casei suspension and 200 µl of test preparation, then incubated at 35-37^{0}C for 24 hours. The absorbance measurement was carried out at the maximum wavelength of the Lactobacilus casei suspension, which was 434 nm. Work was done in duplo (Kusumawati, et. Al., 2005).
Result Analysis. The data obtained were analyzed using Anova Completely Randomized Design (Anova CRD) / Anova Two Way at a 95% reliability level. From the calculation results, if F count is greater than F table or the significance is less than 0.05, it indicates a significant difference between treatment groups (Kusumawati, et al., 2005). And the calculation continued with the Tukey Test to find

out whether the difference between each treatment group is significant (significant) or insignificant (Santoso, 2006).

3. Result

The mushrooms were cleaned and weighed each weighing 500 grams. Each mushroom was finely blended and added with 70% alcohol, then filtered and squeezed to get sediment. After that, each sediment obtained was decanted. Then each sediment was centrifuged at 3000rpm for 15 minutes, each extract was obtained and then weighed the results of each extract. Next the test of prebiotic activity in each extract obtained was carried out (Kusumawati, et al., 2005).

What the researchers did in the organoleptic test for black ear mushrooms was in the form of black colored extracts such as jelly, odorless, insoluble in water. The color of white button mushroom is yellowish white color extract, odorless, insoluble in water and brown oyster mushroom has a grayish brown color extract, odorless and insoluble in water.

Polysaccharides are polymeric monosaccharide molecules that can chain straight or branch and can be hydrolyzed with specific enzymes that work. The results of hydrolysis will partly produce oligosaccharides and can be used to determine the molecular structure of polysaccharides (Winarno, 1997). Some polysaccharides function as storage forms for monosaccharides, while others function as structural elements in cell walls and binding tissues (Thenawidjaja, 1982). Polysaccharides are potential mushrooms as prebiotics that can prevent viral infections and can stimulate the growth of probiotic bacteria in the large intestine (Bakhta, 2014).

For the activity test, a 50% solution was made from the main source then double dilution was done. Making this solution and dilution is done aseptically in ‘laminar air flow.

Then the dilution series of test preparation was used for testing in vitro activity with liquid media. A total of 2.5 ml of liquid medium was added with 100 µl of Lactobacillus casei suspension and 200 µ of the test. Then it was incubated at 35-370C for 24 hours. The measurement of the absorbance is carried out with the maximum wavelength of the 434 nm Lactobacilus casei suspension using a UV-VIS spectrophotometer (Kusumawati, et al., 2005). The results are listed in table 1.

Table 1. Absorbance of each concentration group measured using a spectrophotometer at a wavelength of 434 nm.

Sample	Control	Group I	Group II	Group III	Group VI
Brown Oyster Mushroom	0.418	0.687	0.997	1.420	1.638
	0.418	0.695	0.966	1.460	1.600
Black Ear Mushroom	0.463	0.769	0.827	0.945	0.984
	0.463	0.756	0.845	0.943	0.969
White Button Mushroom	0.344	0.354	0.447	0.553	0.983
	0.344	0.365	0.445	0.536	1.029
	X = 0,408	X = 0,604	X = 0,775	X = 0,976	X = 1,201

Description. Control I: media + phosphate buffer; Control II: media + phosphate buffer + bacterial suspension; Group I: liquid medium + bacterial suspension + test amount (3.12% b / v); Group II: liquid medium + bacterial suspension + test amount (6.25% b / v); Group III liquid medium + bacterial suspension + test (12.5% b / v); Ex IV: liquid medium + bacterial suspension + test (25% b / v).

From the data above, statistical analysis is performed using Anova Completely Randomized Design (CRD) / Anova Two Way at a 95% reliability level. The results of which are listed in table 2 and table 3.

Table 2. Summary of Two Way Anova statistics from the absorbance data of each dilution group

Source of Variation	Sum of Squares (SS)	Degree of Freedom (df)	Average Square (MS)	F Count	Significant
Between samples and concentration	0,636	8	0,079	374,882	0,000

Table 3. Summary of Tukey HSD calculations from the absorbance of each concentration group

K	K	I	II	III	IV
I	-0.19600*	0.19600*	0.35117*	0.56783*	0.79217*
II	-0.35117*	-0.15517*	0.15517*	0.37183*	0.59617*
III	-0.56783*	-0.37183*	-0.21667*	0.21667*	0.44100*
IV	-0.79217*	-0.59617*	-0.44100*	-0.22433*	0.22433*

(*).There are significant differences between concentration groups

Description. Control I: media + phosphate buffer; Control II: media + phosphate buffer + bacterial suspension; Group I: liquid medium + bacterial suspension + test amount (3.12% b / v); Group II: liquid medium + bacterial suspension + test amount (6.25% b / v); Group III liquid medium + bacterial suspension + test (12.5% b / v); Ex IV: liquid medium + bacterial suspension + test (25% b / v)

From the calculation of Anova above, it is known that significant 0.00 means smaller than 0.05, so it can be concluded that there is at least one set of groups that have significant differences. The calculation is continued with the Tukey HSD test in which the results can be seen in table 3.

From the results of the statistical analysis above, it can be seen that there are significant differences between the control group and the test solution group with a concentration of 3.125% w / v, 6.25% w / v, 12.5% w / v and 25% b / v. So it can be concluded that these four concentrations are able to increase the growth of Lactobacillus casei bacteria. From the results of calculating the concentration of isolates in liquid media carried out in duplicate on the average absorbance data, brown oyster mushrooms is 0.691, 0.982, 1.440 and 1,619, black ear mushroom is 0.763, 0.836, 0.943, and 0.997, and white button mushrooms is 0.360, 0.446, 0.545 and 1.006. It can be concluded that the higher the sample concentration, the higher the average absorbance Lactobacillus casei.

4. Conclusion

From the research that has been done, it can be concluded that black ear mushroom (Auricularia polytricha), white button mushroom (Agaricus bisporus) and brown oyster mushroom (Pleurotus cystidiosus) have prebiotic activity against the Lactobacillus casei bacteria. The results of data analysis performed using Two-Way Anova at 95% reliability level shows that the results of all test samples are 0.000 (Significant <0.05), so all test samples have significant differences and influence on the Lactobacillus casei bacteria.

The highest prebiotic activity is brown oyster mushroom (Pleurotus cystidiosus).

5. BIBLIOGRAPHY

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