UPN "VETERAN" JATIM

STIKOM BALI

PROCEEDINGS

BALI INTERNATIONAL SEMINAR ON SCIENCE AND TECHNOLOGY (BISSTECH) II 2014

"Fundamental and Applied Research for Industrial Sustainability: Food, Agrochemical, and Information and Communication Technology (ICT)"

September 2 - 4, 2014
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FACULTY OF INDUSTRIAL TECHNOLOGY - UPN "VETERAN" JAWA TIMUR
STIKOM BALI

PREFACE

Thank God that Faculty of Industrial Technology of University of Pembangunan Nasional "Veteran" Jawa Timur and STIKOM Bali are successful in conducting International Seminar on Science and Technology (BISSTECH) 2014 in Bali, September 2- 4, 2014.

We would also like to extend our gratitude to all reviewers, plenary speakers, keynote speakers, and session moderators for their cooperation and valuable suggestions. Our appreciation also goes to the organizing committees of all events during the seminar.

Finally, we wish you success and new friendship between us for better and bigger collaboration. Thank you.

Editorial Board,

BISSTECH 2014

KEYNOTE SPEAKERS

1. Marthen Kayoi (Indonesia)
2. Haryanto Wardoyo (Indonesia)

PLENARY SPEAKERS

1. Edison Effendi (Industry, Indonesia)
2. Siti Mariyam binti Hj.Shamsuddin (Malaysia)
3. Anthony Halog (Queensland University, Australia)
4. Prof. Takuya Sugahara (Ehime University, Japan)
5. Prof Jui-Hsiang Liu (Chemical Engineering Department of National Cheng-Kung University, Taiwan)
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NO	PAPER TITLE	PRESENTER	INSTITUTION
1	Effects Of 1-Methylcyclopropene And Chitosan Applications On Fruit Shelf-Life And Qualities Of 'Crystal' Guava Fruits	Soesiladi E. Widodo, Zulferiyenni, Arisha Azima	University of Lampung, Bandar Lampung, Indonesia
2	Improvement of Yeast Strains trough Irradiation and Fast Adaptation for Bioethanol Fermentation of Kappaphycus alvarezii	Dwi Setyaningsih, Gayuh Rahayu, Dheasinta Nadya Suprapto, Ahmad Fauzi	Bogor Agricultural University (IPB), Indonesia
3	Determining the Various Ratio of Rasi Flour with Mixed Flour to Produce Nutritious Extrudate Product as an Alternative Staple Food in Indonesia	Marleen Sunyoto, Tri Yuliana	Padjadjaran University, Bandung
4	Physiological Effects of Pre-cooked Breadfruit and Seeded Breadfruit (Artocarpus altilis) and Their Application for Functional Foods	Rosida	Universitas Pembangunan Nasional "Veteran" Jawa Timur, Indonesia
5	Immunostimulatory and Prebiotic Activities of Inulin Extracted from Lesser Yam Tuber (Dioscorea esculenta)	1 Sri Winarti, 2 Eni Harmayani, 2 Yustinus Marsono, 2 Yudi Pranoto, 3 Kosuke Nishi, 3 Takuya Sugahara	1 Universitas Pembangunan Nasional "Veteran" Jawa Timur, Indonesia 2 Universitas Gadjah Mada, Yogyakarta, Indonesia 3 Ehime University, Matsuyama, Japan
6	Characteristics Of Sweet Bread Using Protein Concentrate Of Lamtoro Gung (Leucaena Leucocephala) And Soybean As Emulsifier	Dedin F. Rosida, Dedid CH, Nur Hapsari	Universitas Pembangunan Nasional (UPN) "Veteran" Jawa Timur

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ARTICLES OF BALI INTERNATIONAL SEMINAR ON SCIENCE AND TECHNOLOGY (BISSTECH) II 2014
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IMPROVEMENT OF YEAST STRAINS TROUGH IRRADIATION AND FAST ADAPTATIONFOR BIOETHANOL FERMENTATION OF KAPPAPHYCUS ALVAREZII

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Abstract

Kappaphycusalvarezii has polysaccharides which consist of galactose as monomer unit. After acid hydrolysis, galactose can be used as carbon and energy source by Pachysolentannophilus IPBCC Y111149 and Saccharomyces cerevisiae IPBCC Y03545, but the ability of these strains to convert K. alvareziihydrolysates into bioethanol was low. Those strains have to be improved to achieve better performance of substrate and fermentation efficiency. The research aimed to get improved strains of S. cerevisiae IPBCC Y03545 and P. tannophilus IPBCC Y111149 which had better activity in Kappaphycusalvareziihydrolysate. Strain improvement methods were gamma irradiation and fast adaptation on Yeast Malt Peptone Galactose (YMP-galactose) broth. Gamma irradiation was done at 10 Gy, followed by adaptation on YMP-galactose for 9 times. Strains improvement through fast adaptation was done by growing the wild strains in fresh YMP-galactose (0.1% galactose) medium for 264 times. Subculturing was done every 4 hours, when the cells were at lag phase. Gamma irradiation (10 Gy) resulted in 1 isolate of P. tannophilus which had higher substrate efficiency (37.12%) and fermentation efficiency (1.16%) than its wild type (24.31% and 0.39%, respectively). While for S. cerevisiae isolates, ethanol production and substrat efficiency on K. alvareziihydrolisate were relatively the same as its wild type. Improved isolates of P. tannophilus were varied in their galactose fermentation ability, while S. cerevisiae's performance was increased with the increase of adaptation cycles. The fermentation characteristics of all improved isolates in YMP-galactose media were in accordance with their fermentation ability on K. alvareziihydrolysate. P. tannophilus isolate of 88 adaptation cycles was better than other isolates and wild strains to ferment K. alvareziihydrolysates. All adapted S. cerevisiae isolates, had better fermentation characteristics than its wild strain, except for fermentation efficiency. The shape of P. tannophilus cell was similar with the wild type, but for S. cerevisiae, the cell shape became oval-like and had bigger size.

Keywords: *P. tannophilus, S. cerevisiae, irradiation, adaptation, bioethanol, K. alvareziihydrolysates*

1. INTRODUCTION

Bioethanol is an alternative fuel which is potentially to be developed in Indonesia based on the availability and diversity of biomass. Nevertheless, the development of bioethanol is considered to endanger the food supply. Therefore many researchers then developed a bioethanol from non-food raw materials.*Kappaphycusalvarezii*is one of the red algae species that can be used as a bioetanol feedstock. It was containing kappa-*carrageenanpolysaccharides whichis composedof3,6-anhidrogalactose by54-73%* whichcan beconverted intobioethanolby several yeast (Winarno, 1996). *Saccharomycescerevisiae*canuseglucoseandgalactoseas a carbon source directly and convert it into etanol through fermentation process.*Pachysolentannophilus*also been reported can usegalactoseas a carbon source (KurtzmanandFell2000).

Fermentation techniques in the macroalgaebioethanolproduction has not been efficient with low productivity. The development of bioethanol production process from *K.alvarezii*acid hydrolyzates, conducted by Setyaningsih et al (2011) have produce ethanol with the highest levels of around 2%v/v. It can be caused by several factors, including the low fermentation activity of yeast in macroalgae acid hydrolyzate medium.

Yeast strain improvement can be done through several methods, there are genetic engineering, biological adaptations, and mutagenesis(Rowlands 2010). Biological adaptation and mutagenesis was done in this research.Mutagenesis is a mutation processes that is expected to changes the genotype and phenotype of the organism by giving a mutagen. Gamma-ray irradiation can be used as a mutagen which can change the natural properties of

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microorganisms(Gonzales *et al.* 2003). Meanwhile, the adaptation process as much as 264 times reported can change the physiology character of *S. cerevisiae* strains (Adams et al., 1985).This research conducted to improve*S. cerevisiae*and *P. tannophilus*fermenting ability in *K. alvarezii*hydrolysatemedium through fast adaptation in YMP-Galactose media(264 times) and gamma irradiation (10 Gy).

2. METHODOLOGY

2.1 Materials

*S. cerevisiae*IPBCC Y03545 and *P. tannophilus*IPBCC Y111149 isolate, *K. alvarezii*, DNS reagent,Yeast Malt Peptone Galactose 0.1% (YMP-Galactose 0.1%) broth and agar, H_2SO_4, methylene blue, and aquadest.

2.2 Methods

1. Fast Adaptation

a. Inoculant preparation

One loop of each yeast culture that had been grown for 48 hours in YMP-Galactose agar media inoculated into 5 ml YMP-Galactose broth medium and incubated for 48 hours at room temperature. Totalyeastcellsat the end of incubation timewere calculated byhaemacytometer. Thisculture wereused asthe workingculture at the next step.

b. Determination of Growth Curve

This activities conducted to determine the optimum incubation time of yeast cells. As much as 2loop of eachyeastcultureon YMP-galactose agarput into10mL ofYMP-galactose broth then incubatedfor 360 minutes at room temperature. Total yeast cell were calculated every 30 minutesusinghaemacytometer. The optimum incubation time determined by the time required for yeast cells tocomplete the 3rd generation life cycle.

c. Yeast Adaptation and Validation

Adaptation process which carried out in this study is fast adaptation. One loopof yeast cells that had been grown on YMP-Galactoseagar media was inoculated into 5 ml of YMP-Galactose broth and incubated based on the optimum time.At the end of incubation time, as much as 1 mL yeast culture was moved into 4 mL new medium of YMP galactose broth. This adaptation steps were done for 264 times.

Validation process carried out to analyze the ability of adapted yeast to ferment galactose. Adapted yeast from 88th, 176th, 264th adaptationprocess reculture on YMP galactose agar media and then incubated for 48 hours. As much as 1 loop of yeast culture put into 5 mL of YMP galactose broth in test tube which contain Durham tube and incubated for 48 hours. At the end of incubation time, presence of CO_2 bubles at Durham tube were measured and yeast cell were calculated. The morphology of 264th yeast cell were observed using microscope.

d. Irradiation

One loop of*P. tannophilus*and *S. cerevisiae*culture on YMP Galactoseagar medium was suspended into 5 mL of steril distilled water as a radiation inoculant.Irradiation carried out by 10 Gy gamma rays.Viability test of each culture after irradiation performed by methylene blue staining.As much as0,1mL inoculums withthe highestviabilityinoculatedinYMP-Galactose agar media andincubated for48hours at room temperature. Five cell colonies then selected and tested their ability to ferment galactose. Twoloopof each irradiated and controlled (without irradiation, wild type) isolate were inoculatedon 10 mL of YMP Galactosebrothat test tube which contain Durham tube, then incubated for 48 hours. The ability of the yeast to ferment galactose is indicated by the presence of CO_2 bubbles in Durham tubes.

e. Adaptation of Irradiated Culture

Irradiated cultures which capable of fermenting galactose into ethanol then adapted in YMP-Galactose broth media.Fast adaptation process for irradiated cultures performed9 times.Culture from the last adaptation then selected again their fermenting ability in the YMP Galactosebroth media by measuring CO_2 which captured at Durham tube. Control (irradiated cultures without adaptation process) and adapted isolate which had biggest fermenting ability were cultured on YMP Galcatose Agar and incubated for 48 hours. This culturewere used for bioethanol production.

f. Bioethanol production

As much as 2 loop from each culture selected from irradiated and adaptation processes were inoculated in 10 ml of YMP Galactosebroth media and incubated for 48 hours at room temperature. This is a starter culture for macroalgae bioethanol fermentation.Adapted cultures used in fermentation processes derived from 88th, 176th and 264^{th}fast adaptation. Starter cultures then put into 90 ml of *K. alvarezi*acid hydrolyzate, incubated at room temperature for 96 hours for *P. tannophilus* and 144 hours for *S. cerevisiae*. Ethanol was separated from fermentation media by distillation. Ethanol concentration was measured by density meter, whilereduction sugar was determined by DNS method. Efficiency of substrate and fermentation were calculated according to formulation below.

$$Efficiency\ of\ Substrate\ (\%) = \frac{So - S}{So} x100\%$$

$$Efficiency\ of\ Fermentation\ (\%) = \frac{ethanol\ actual}{ethanol\ teoritic} x100\%$$

3. RESULTS AND DISCUSSION

Determination of Optimum Incubation Time

Cells number of*P. tannophilus*and *S. cerevisiae*were $1.10x10^7$ cell mL^{-1} and $1.05x10^7$ cell mL^{-1} at the beginning.Both cell number has doubledinitially at 90minute, that is equal to $2.40x10^7$ cells mL^{-1} and $2.15x10^7$ cells mL-1. Lag phase of yeast growth occurs before 90 minute and after that it began to enter the exponential phase (Fig. 1). The first generation complete its life cycle after 90 minute, second generation completes its life cycle approximately at 150 minutes, while the 3rd generation complete its life cycle at minute 330. Based on growth curves determination of both types of yeast, the incubation period for the adaptation time is set to 240 minutes.

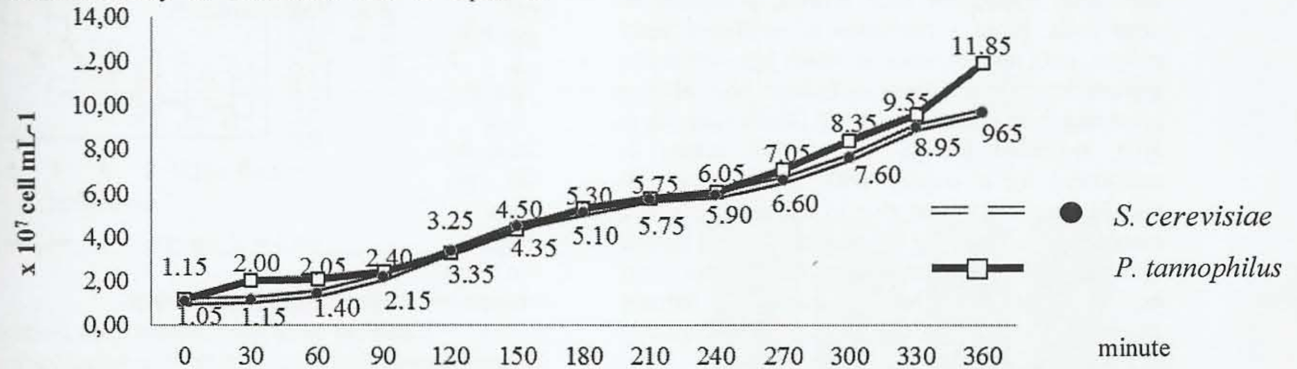

Figure 1. The population of cells on inoculants *P. tannophilus*and *S. cerevisiae*for 360minutes

Changes in Physiology and Morphology of Adapted Yeast Cell

The fermenting ability of each cultures from 88th, 176th, 264thadaptation were qualitativelyevaluated in the galactose media. *S. cerevisiae*have better galactose fermenting ability than *P.tannophillus*based on the CO_2 production and cells number (Table 1).

Adaptation process gives significant impact on the shape and size of *S. cereviceae*cells, but not with *P. tannophilus*. The wild type of*P. tannophilus*cell have anaveragelength 14.11µm and width 7.29µm. After 264 times adaptations, itbecome 14.65µm and 7.58µm. Meanwhile, wild type of *S. cerevisiae*havean average length 10.03 µm and width 5.30µm, after 264 times adaptations,it become 16.02µm and width 8.73µm (Table 2).

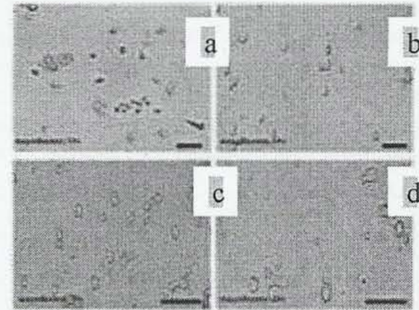

Figure 2 *P. tannophilus*cells before (a) and after (b) 264 adaptation, *S. cerevisiae*cells before (c) and after (d) 264 adaptation

Table 1.CO_2 production and cell number of yeast before and after adaptation process

Species	Before adaptation		After adaptation					
	CO_2	Cell number	88th		176th		264th	
			CO_2	Yp	CO_2	Yp	CO_2	Yp
P. tannophilus	+	-	+++	3.50	+	2.93	++	3.35
S. cerevisiae	+	-	+	2.35	++	3.28	+++	3.78

Note : Yp = Yeast population ($x10^9$ cell mL^{-1})

Table 2Size of *P. tannophilus* and *S. cerevisiae*cellsbefore and after 264thadaptation

Species	Mean of size (µm	Ratio of changes in cell

	Before adaptation		After adapttion		size before andafter adaptation
	Length	Width	Length	Width	
P. tannophilus	14.11	5.3	14.65	7.58	1:1.03
S. cerevisia	10.03	5.3	16.02	8.73	1:1.60

Gamma Irradiation

Viability of yeast cells of *S. cerevisiae* and *P. tannophilus* after gamma-ray irradiationshown by the number of living cells, indicated by blue cells after methylene blue stained, compared to dead cells. From 3 replicates of irradiated yeast culture, only one replications which has viability to gamma irradiations. These cells have been able to grow and form colonies on YMP Galactosemedium after incubated for 48 hours. This colony is considered as a colony with the highest cell viability and have been able to use galactose as a carbon source.From the fermentation test, it known that the fifth irradiated and control isolate from each species has the capability to ferment galactose, showed by CO_2productionwere captured at Durham tube (Table 3).

Table 3 showed that at test 1 and 2 the bubble formed of irradiation isolates varied than control. Bubble of *P. tannophilus*controlat test 2 is bigger than test 1, whereas bubble of *S. cerevisiae*control at test 1and 2as great.Increased of bubble size by irradiation was accured in *P. tannophilus*R3, R4, R5 and *S. cerevisiae*R2, R4. Increased or decreased of bubble size is thought to be due to different ability of yeast cells to fermentgalactose on medium to be ethanol and CO_2. It could be affected by irradiation in two yeasts species.

Adaptation of yeast

Fifth isolates irradiated had been adapted on galactose had low cells count (Figure 3). Cells amount of isolates were then compared with cells amount control. Cells amount of *P. tannophilus*and *S. cerevisiae*control were 10^7 cells/mL. It showed that transfer to the new medium was not necessarily stimulated cell growth.

Based on cells amount from each isolates, there showed that increased of *P. tannophilus*was occured at 4th stage adaptation (R1 and R2) and 5th stage adaptation(R3, R4, and R5), whereas increased of *S. cerevisiae*was occured at 4th stage adaptation(R4 and R5), 5th stage adaptation (R1 and R3), and stage adaptation 9th (R2) (Table 3). The largest increase occured in *P. tannophilus* R5 and *S. cerevisiae*R1 and R3. It showed that irradiated isolates were able to using galactose as a carbon source. Low cell count increase could be due to low availability of galactose on medium, only 0.1%.

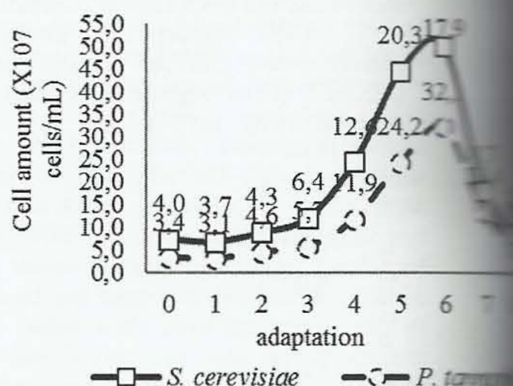

Figure 3 Average of cells amount from fifth irradiated isolates of *P. tannophilus* and *S. cerevisiae*during adaptation

Table3 The result of qualitative test on YMP Galactose Broth[a]

Yeast	Treatment	CO_2 Production	
		Before Adaptation	After Adaptation
P. tannophilus	Control	+	++
	R1	+++	++
	R2	+++	++
	R3	++	+++
	R4	+	++
	R5	+	+++
		0 hour	After 432 hours of incubation
S. cerevisiae	Control	++	++
	R1	++	+
	R2	+	++

	R3	+++	+
	R4	+++	+++
	R5	+	+

Information: [a]Volume of YMP Galactose Broth was 10 mL
[b]Bubble size: +++ = biggest; ++ = big; and + = small

Table4 Cells ratio of *P. tannophilus*and*S.cerevisiae*(control and irradiated)

Species	Treatment	Adaptation	Cell ratio
P. tannophilus	R1	4	1 : 3.5
	R2	4	1 : 3.2
	R3	5	1 : 3.6
	R4	5	1 : 2.8
	R5	5	1 : 4.9
S. cerevisiae	R1	5	1 : 4.1
	R2	9	1 : 2.9
	R3	5	1 : 4.6
	R4	4	1 : 2.4
	R5	4	1 : 2.4

One of irradiated isolate from each species are *P. tannophilus* R3 (Figure 4a) and *S. cerevisiae* R4 (Figure 4b) showed the biggest bubble than control and other isolates. Those isolates were selected and subcultured on medium YMP Galactoseagar media as inoculant to fermentation*K. alvarezii*hydrolysate. The selection is based on argument that isolate biggest bubble had better ability of using galactose as a carbon source. It could be due to isolates were able to convertgalactose into ethanol and CO_2.

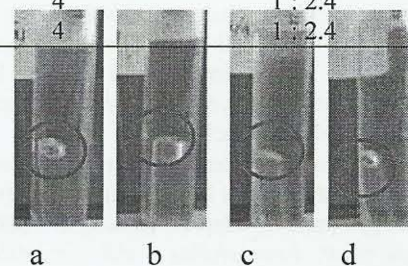

Figure4The bubble formed from test 2 of *P. tannophilus*: a) control, dan b)R3 and *S. cerevisiae*: c) control, dan d) R4

Fermentation character of chosen isolate on *K. alvarezii*hydrolysate

Table 5 Ethanol volume of yeast with some treatment

Yeast species	Ethanol Volume (% v/v)				
	Native	Adaptation process at			Gamma irradiation
		88th	176th	264th	(10 Gy)
P. tannophilus	0.02	0.06	0.05	0.03	0.06
S. cerevisiae	0.01	0.02	0.04	0.10	0.01

Table 6 Substrate efficiency of yeast with some treatments

Yeast species	Substrate efficiency (%)				
	Native	Adaptation process at			Gamma irradiation
		88th	176th	264th	(10 Gy)
P. tannophilus	24.31	22.79	26.99	30.92	24.96
S. cerevisiae	30.07	31.21	21.80	26.27	32.30

Table 7 Ethanol production of yeast with some treatments

Yeast species	Ethanol production (% w/w sugar)				
	Native	Adaptation process at			Gamma irradiation
		88th	176th	264th	(10 Gy)
P. tannophilus	0.94	3.05	2.43	1.09	2.72
S. cerevisiae	0.49	0.73	2.11	4.39	0.26

Table 8 Fermentation efficiency of yeast with some treatments

Yeast species	Fermentation efficiency (%)				
	Native	Adaptation process at			Gamma irradiation
		88th	176th	264th	(10 Gy)
P. tannophilus	0.50	1.49	1.24	0.73	1.47
S. cerevisiae	0.32	0.50	1.01	2.48	0.17

DISCUSION

Adaptation process and galactose fermentation testin this research showed *P. tannophilus*dan*S. cerevisiae* can be used galactose as carbon and energy source, although both those yeast (Kurztman& Fell 2000) or only *S. cerevisiae* was chosen glucose (Timson 2007). Galactose is nutrition non-conventional for yeast and can be used only one carbon source when glucose not available in medium (Frey 1996).

Time of regeneration of yeast was affected by growth medium. The growth of *P. tannophilus* and *S. cerevisiae* in YMP Galactose medium show that on 90 minutes first time, cell population increase to be 2 times bigger than beginning population. These research is different from what Adams *et al.* (1985) do it. They are said that time regeneration to increase population until 2 times bigger is approximately 115-120 minutes on minimum medium glucose 0.08%. Hong *et al.* (2010) were obtain 400 generations of *S. cerevsiae* in 62 days adaptation, that's mean in one generation needs approximately 3-4 hours on minimum medium galactose 2%.

Estimation adaptation period was based on the growth pattern. In 4 hours periode was expected form 3 generations adapted galactose. Adaptation process of *P. tannophillus* and*S. cerevisiae*were produced new strains with fermentation traits better than its wild type. Adaptation process did not modify the shape of *P. tannophilus* cell, but modifying the shape and size of *S. cerevisiae* cell to become oval-like and bigger. Adapted 88thof *P. tannophilus*showed that galactose fermentation ability relatively better than other strain and its wild type, while adapted 264thof *S. cerevisiae* showed that galactose fermentation ability relatively better than other strain and its wild type. These results showed that respone cell of *P. tannophilus*toward galactose adaptation different from *S. cerevisiae*.

The impact of gamma irradiation (10 Gy) to *P. tannophilus* and *S. cerevisiae* were strung out living cells and dead cells could be identified. According to Ikmalia*et al.* (2008) that the irradiation effects were only two possibilities, the cells remain alive or deathdue to essentialmolecules, cells or tissues undergo various changes, disruption or damage to biological systems such as DNA molecules, molecular enzymes, protein molecules, fat, and carbohydrates. Identificationof cells was done by giving of methylene blue dye.

The cells were also able to growth and formed colonies on YMP Galactose medium. Akacha*et al* . (2007), treatment of gamma rays at 10-20 Gy dose could increased the activity of several enzymes of *S. cerevisiae*. Therefore, in this research gamma irradiation was carried out with 10 Gy dose.

Growth of cells that had been irradiated on YMP Galactose medium were produced many large colonies. Of many colonies only selected 5 of large colonies. Colony formation showed that the yeast was tolerant to irradiation and supposedly capable of using galactose as a carbon source. Therefore, large colonies were later adapted to a series of galactose liquid medium (0.1 %) for 9 times. *P. tannophilus* and *S. cerevisiae* were yeasts glucose user, but could be used galactose when glucose was not available on medium (Kurtzman and Fell 2000). However, galactose must be converted to glucose through Laloir pathway for metabolic processes (Timson 2007). Genes were needed to utilized galactose in this yeast on both of existing and induced to be expressed. Microevolution through adaptation was one way to obtained isolates capable of using galactoseefficiently .

The result of galactose fermentation test was showed that both *P. tannophilus* R3 and *S. cerevisiae* R4 had bigger bubble than control and each other of isolates. The bigness of bubble formed was indicated that yeast cells were able to using galactose as a carbon source and convert it into ethanol and CO_2. This bubble was indicated a CO_2 conversion result of galactose. According to Radesiyani (2013) that both *P. tannophilus* IPBCC Y111149 and *S. cerevisiae* Y03545 IPBCC had capability of galactose fermentation that better than *Pi. Anomala* on galactose fermentation ability into bubbles formation.

This adaptation was obtained a yeast which capable of using galactose as a carbon source. However, increased of cells amount were low during adaptation. Fifth of irradiated isolates, the biggest increased of cells amount were occurred in *P. tannophilus* R5 (4.9x) and *S. cerevisiae* R1 (4.1x), and R3 (4.6x). This increased was occurred at adaptation. This could be due to low availability of galactose on medium. Therefore, the cells had difficulty in metabolism process.

4. CONCLUSION

Adaptation and gamma irradiation (10 Gy) in *P. tannophilus* and *S. cerevisiae* were produced isolates which capable of using galactose as a carbon source. Adaptation process for 264 times was obtained new strains which ability fermentation better than native. Adaptation process did modify a shape cell of *S. cerevisiae*. Adapted strain 88th of *P. tannophilus*had fermentation trait better than other adaptation and its native. The

otherwise, fermentation trait except substrate efficiency of adapted 264 *S. cerevisiae*was better than other adaptation and its native. Adaptation in irradiated yeasts were obtained one isolate of *P. tannophilus* which substrate efficiency and fermentation efficiency were higher than native, although only a slight increased. On the other hand, the same way was not obtained more potent *S. cerevisiae*. Compared of both adaptation process and gamma irradiation were showed that adapation process more effective because all of fermentation characteristic like ethanol production, substrate efficiency, and fermentation efficiency was higher than gamma irradiation.

5. DAFTAR PUSTAKA

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