Pak. j. sci. ind. res. Ser. B: biol. sci. 2019 **62B**(3) 178-182

Alternative Media Based on Papaya and Fish Extract for Glutathione Production in *Saccharomyces cerevisiae*

Nurul Dluha, Sri Widyarti and Widodo
Biology Department, Faculty of Mathematics and Natural Sciences,
Brawijaya University, Indonesia

(received April 27, 2017; revised September 24, 2017; accepted October 25, 2017)

Abstract. Glutathione (GSH) is an antioxidant that functions to protect cells from oxidative stress. It is used for medical purposes, as an additive in foods and cosmetics industry. The magnitude of these applications results in increased demand for glutathione every year, however, the cost of glutathione is high. The production of glutathione using an alternative source for the medium and the amino acids used in the media might be the solution for managing the high cost of glutathione production in yeast. This study uses an alternative media based on papaya and fish extract to reduce production costs. The fish extract contains glutamate, cysteine and glycine that can be utilised as a source of amino acid. This study suggested that media based on papaya extract could be employed to produce glutathione in yeast *Saccharomyces cerevisiae*. Moreover, administration of 5 mg/mL of fish extract could increase the glutathione production up to 36.36% as compared to a control. The optimum production of glutathione was obtained in a harvest time of 44 h culture. Therefore, further investigation by modifying the medium is warranted to produce glutathione in a cost friendly manner in the *S. cerevisiae*.

Keywords: glutathione, *Saccharomyces cerevisiae*, fish extract, papaya extract, yeast media

Introduction

Glutathione (GSH) is a thiol compound that is abundantly found in eukaryotes and can be used as an antioxidant to protect cells from oxidative stress (Dickinson and Forman, 2002; Penninckx, 2002). Moreover, glutathione also acts as an immune booster, a detoxifying agent for higher organisms, and a cell proliferation regulator (Pasternak *et al*., 2014; Pastore *et al*., 2003). Glutathione is not only used for medical purposes but also as an additive in foods and cosmetics industry (Li *et al*., 2004). The magnitude of these benefits results in increased demand for glutathione every year. The supply of glutathione was not equal to its demand and this is thought to be due to the high cost of glutathione production. Glutathione production needs an optimum medium, addition of amino acids and high quantities of ATP. Glutathione production using an alternative source of medium, amino acids and organism might be the solution to manage the high cost of glutathione production.

Saccharomyces cerevisiae is often used to produce glutathione. According to Wen *et al*. (2005), this is because the yeast has the ability to accumulate glutathione efficiently for commercial production. The organism has been classified by the FDA as GRAS (generally regarded as safe) because of its presence as a human pathogen and because it is pyrogens free (Glick *et al*., 2010; Nevoigt, 2008). *S. cerevisiae* has ATP regeneration ability through the glycolytic pathway, so it does not require additional ATP (Li *et al*., 2004; Murata *et al*., 1981). However, it requires essential amino acids as a substrate for glutathione production in the fermentation process. The addition of these amino acids can increase the production cost. Therefore, as a solution to this issue, the amino acids were substituted by adding fish extract.

The fish extract contains several amino acids that are essential for glutathione production (Medikalink, 2012; Mustafa *et al*., 2012). Their concentrations were adjusted based on research by Wen *et al*. (2005). Thus, fish extract was selected to be used as a source of amino acids in glutathione production. The optimum medium also played a pivotal role in glutathione production and cell growth as stated by Zhang *et al*. (2007). In this study, an alternative source of nitrogen and carbon from meat broth extract and papaya extract was utilised to obtain cost friendly glutathione production in *S. cerevisiae* combined with the fish extract.

*Author for correspondence; E-mail: widodo@ub.ac.id

Materials and Methods

Basic media selection for optimum yeast growth. ***Meat broth extract.*** Five hundred grams of beef was boiled in 2.5 litres of aquades for 1.5 h. Following this step, the broth was filtered and poured into bottles. The extract was then autoclaved at 121 °C for 20 min then freeze dried overnight.

Papaya extract. Two hundred grams of papaya were blended with 1 litre of aquades. The extract was then filtered and poured into bottles. The extract was freeze dried overnight.

Fish extract. A stock of fish extract (No. Cat P140116A02 & P150415A09: VipAlbumin®) was made by dissolving 12 capsules (500 mg) into 120 mL of sterile distilled water and the mixture was homogenised. The solution was then centrifuged at 4000 rpm for one hour. The supernatant was collected and centrifuged again for 10 min. The resulting supernatant was filtered through a 0.45 µm filter and stored at 20 °C.

S. cerevisiae W303-1b was obtained from the Department of Microbiology, Kässel University, Germany. The culture was grown in 40 mL culture medium that contained three types of papaya extract concentration. The first media contained yeast extract 1% (w/v) (Bacto™), meat broth extract 0.04% (w/v), and papaya extracts 0.13% (w/v). The second media contained yeast extract 1% (w/v), broth extract 0.04% (w/v), and papaya extracts 0.26% (w/v), and the third media contained yeast extract 1% (w/v), broth extract 0.04% (w/v), and papaya extracts 0.39% (w/v). The pH of each medium was 6.5. The yeast was cultured at 30 °C, 200 rpm for 48 h. Cell concentration was measured using spectrophotometer at the absorbance OD600 every 2 h for 24 h then every 3 h up to 36 h and every 4 h until 48 h. The absorbance result was then used to establish the growth curve and to determine the optimum media for yeast growth. The most beneficial media was then used to produce glutathione by fish extract induction.

Glutathione production in yeast by inducing fish extract. Glutathione production in yeast was carried out in the basic medium from the previous selection. The culture was prepared in a 75 mL flask containing 25 mL of the basic medium, then a further 10% of medium with seed culture was added (OD600 = 0.6). The yeast culture was incubated at 30 °C, 200 rpm for 24 h. After 24 h, the culture was enriched with fish extract at concentrations of 0, 5, 10 and 15 mg/mL (VipAlbumin®), according to Wen *et al*. (2005). Fish extract from *Ophiocephalus striatus* contains essential amino acids. 100 g fish albumin contains 15 g of glutamic acid, 1.11 g of glycine and 1.07 g of cysteine as indicated by Medicalink (2012). The process of yeast culture was then continued by incubating at 30 °C, 200 rpm for 44 h as stated by Liang *et al*. (2008).

Glutathione measurement. The yeast culture was harvested at 36 h and 44 h after inoculation. The culture broth was centrifuged at 4000 rpm for 10 min. The cells were then washed twice with sterile distilled water. Cells were extracted with 40% (v/v) ethanol at 30 °C for 2 h and centrifuged at 4000 rpm for 10 min. The supernatant was used for the GSH assay using the DTNB method (GBioscience, 2012; Xiong *et al*., 2009; Wei *et al*., 2003).

Statistical analysis. Data were statistically analysed using one-way ANOVA (P value ≤ 0.05) and continued with Duncan test. All statistical analyses were performed using SPSS version 16.0 for Windows.

Results and Discussion

Basic media selection was conducted to obtain the best concentration of papaya extract within which to grow the yeast. The data suggested that the three variations of papaya extract could be used as growth media for yeast. Therefore, the media could be used to substitute the standard media of yeast that consists of yeast extract. Based on research by Gross and Acosta (1991), papaya contains galactose that can be a source of carbon, and it can therefore be used as a carbon source. Media number 2 and 3 showed similar patterns in the growth curve (Fig. 1) that warrants their use as media to produce glutathione. Of the two, media number 2 was selected for use in glutathione production since the medium contained a lower concentration of papaya (0.26% (w/v)).

Further study to produce glutathione was carried out using media number 2 and induced by fish extract at different concentrations (0, 5, 10 and 15 mg/mL). The induction of glutathione production by fish extract was carried out after 24 h of culture or in phase III. Wen *et al*. (2005) stated that there are three phases in the process of glutathione production by *S. cerevisiae* i.e. Phase I (0-8 h), Phase II (8-14 h) and phase III (18-24 h). Phase III is the stationary phase in which cell growth stops, but during which glutathione production begins to increase significantly. Research conducted by Liang

et al. (2008) demonstrated that the best time to add fish extract is at the late log and early stationary phase because it can avoid inhibition of cell growth while also increasing the production of glutathione. Fish extract serves as a source of essential amino acids in the production of glutathione because it contains cysteine, glutamic acid, and glycine as shown by Medikalink (2012). Those amino acids are essential amino acids in the production of glutathione according to Wen *et al*. (2005). Besides that, the fish extract also contained serine, which is an amino acid that can form cysteine. Therefore, the addition of serine can also increase glutathione production (Suzuki *et al*., 2011; Wen *et al*., 2004).

This study demonstrated that fish extract at a concentration of 5 mg/mL was capable of increasing glutathione production while the addition of extract at concentrations of 10 mg/mL and 15 mg/mL did not show any significant difference (Fig. 2). The increasing yield of glutathione in medium induced by 5 mg/mL of fish extract reached a level that was 36.36% higher than the control. Wen *et al*. (2005) used 10 mM glutamate which is equivalent to the glutamate content in 10 mg/mL fish extract. However, the concentration of fish extract that increased glutathione production was 5 mg/mL which in turn means that the glutamate content in 5 mg/mL of fish extract can be assumed to be equivalent to 5 mM glutamate. This data corresponds to data from Tang *et al*. (2015) where the addition of 5 mM glutamate, 5 mM glycine, and 5 mM cysteine to the culture medium can increase glutathione production. Tang *et al*. (2015) used the same strain of *S. cerevisiae* i.e. W303-1b. Based on the statistical analysis results, these demonstrated that there was no significant difference in harvest time, but there was the tendency to increase GSH to 44 h compared to 36 h after inoculation (Fig. 2). This indicates that the GSH optimum harvest time is 44 h which is the end of the stationary phase. This is because in the stationary phase, the cell number has reached the maximum number of cells that can grow, and reduced the nutritional content of the culture medium. This reduction in the nutritional content places *S. cerevisiae* under stressful conditions that can trigger the production of glutathione according to Wen *et al*. (2005).

Previous research on the optimisation of media for glutathione production used a high quantity of supplements. In this study, however, only a simple medium was used as listed in Table 1. This medium still resulted in the production of glutathione although the yield was low. The results suggested that the use of media based

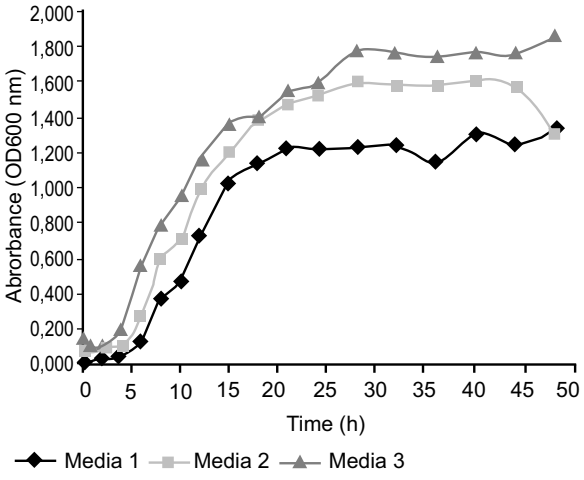

Fig. 1. Growth curve of *S. cerevisiae* W303-1B using media containing yeast extract 1% (w/v) (Bacto™), broth extract 0.04% (w/v) and supplemented by three different quantities of papaya extract, 0.13% (w/v) (Media 1), 0.26% (w/v) (Media 2) and 0.39% (w/v) (Media 3).

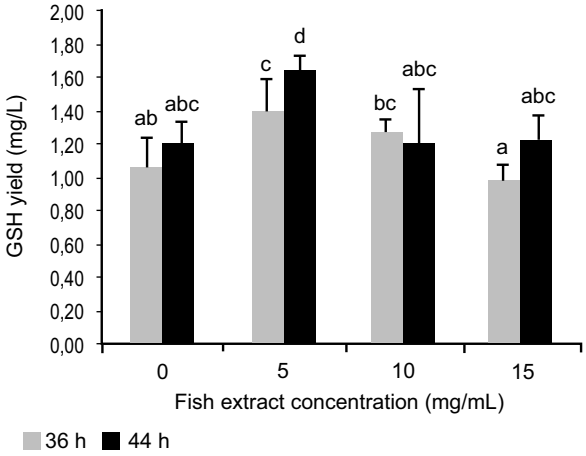

Fig. 2. The GSH yield using the papaya extract media supplemented with fish extract. Administration of 5 mg/mL fish extract could increase the production of GSH, with the highest concentration of GSH being harvested in culture after 44 h. The histogram presents the mean ± SD of the GSH concentration and the analysis of variance was conducted at P-value ≤ 0.05.

Table 1. Comparison of media used and the GSH yield

Media used	GSH yield	*S. cerevisiae*	References
70 g/L glucose, 3 g/L yeast extract, 5 g/L peptone, 70 g/L malt extract, 20 g/L molasses, 5.6 g/L $MgSO_4$, 16 mg/L $ZnSO_4$, 7 g/L $(NH_4)_2HPO_4$ and 0.2 mg/L thiamine	74.6 ± 0.00 mg/L	*S. cerevisiae* T65	Zhang *et al*. (2007)
10 g/L yeast extract, 20 g/L tryptone and 20 g/L glucose	98.7 ± 7.30 mg/L	*S. cerevisiae* W 303-1b	Tang *et al*. (2015)
Yeast extract 1% (w/v), broth extract 0.04% (w/v), and papaya extracts 0.26% (w/v)	1.65 ± 0.08 mg/L	*S. cerevisiae* W 303-1b	Present study

on papaya extract supplemented with fish extract could be used for the production of glutathione. The media requires further investigation to ensure the production of glutathione in a cost manner in *S. Cerevisiae*.

Conclusion

This study indicated that media based on papaya extract could be used to produce glutathione in yeast. The supplementation of this medium with 5 mg/mL of fish extract could increase glutathione production up to 36.36% in quantity as compared to the control. Therefore, further investigation by modifying the medium is warranted to produce glutathione in a cost friendly manner in *S. cerevisiae*.

Acknowledgement

The authors would like to thank Prof. Raffael Schaffrath and Prof. Wolfgang Nellen for providing *S. cerevisiae* W303-1b for this research.

Conflict of Interest. The authors declare no conflict of interest.

References

Dickinson, D.A., Forman, H.J. 2002. Cellular glutathione and thiols metabolism. *Biochemical Pharmacology*, **64:** 1019-1026.

GBioscience, 2012. *Ellman's Reagent*. Available at: www.gbioscience.com.

Glick, B.R., Pasternak, J.J., Patten, P.C.L. 2010. *Molecular Biotechnology Principles and Applications of Recombinant DNA*. 4th editions, ASM Press, Washington, USA.

Gross, K.C., Acosta, P.B. 1991. Fruits and Vegetables are a source of galactose: Implications in Planning the Diets of Patients with Galactosaemia. *Journal of Inherited Metabolic Disease*, **14:** 253-258.

Li, Y., Wei, G., Chen, J. 2004. Glutathione: a review on biotechnological production. *Applied Microbiology and Biotechnology*, **66:** 233-242.

Liang, G., Liao, X., Du, G., Chen, J. 2008. Optimization of amino acids addition for efficient production of glutathione in *Candida utilis*. *Biochemical Engineering Journal*, **41:** 234-240.

Murata, K., Tani, K., Kato, J., Chibata, I. 1981. Glycolytic pathway as an ATP generation system and its application to the production of glutathione and NADP. *Enzyme and Microbial Technology*, **3:** 233-242.

Mustafa, A., Widodo, M.A., Kristianto, Y. 2012. Albumin and zinc content of snakehead fish (*Channa striata*) extract and its role in health. *IEESE International Journal of Science and Technology*, **1:** 1-8.

Nevoigt, E. 2008. Progress in metabolic engineering of *Saccharomyces cerevisiae*. *Microbiology and Molecular Biology Reviews*: *MMBR*, **72:** 379-412.

Pasternak, T., Asard, H., Potters, G., Jansen, M.A. 2014. The thiol compounds glutathione and homoglutathione differentially affect cell development in alfalfa (*Medicago sativa* L.). *Plant Physiology et Biochemistry*, **74:** 16-23.

Pastore, A., Federici, G., Bertini, E., Piemonate, F. 2003. Analysis of glutathione: Implication in redox and detoxification. *Clinica Chimica Acta*, **333:** 19-39.

Penninckx, M.J. 2002. An overview on glutathione in Saccharomyces versus non-conventional yeasts. *FEMS Yeast Research*, **2:** 295-305.

Suzuki, T., Yokoyama, A., Tsuji, T., Ikeshima, E., Nakashima, K., Ikushima, S., Kobayashi, C., Yashida, S. 2011. Identification and characterization of genes involved in glutathione production in yeast. *Journal of Bioscience and Bioengineering*, **112:** 107-113.

Tang, L., Wang, W., Zhou, W., Cheng, K., Yang, Y.,

Liu, M., Cheng, K., Wang, W. 2015. Three-pathway combination for glutathione biosynthesis in *Saccharomyces cerevisiae*. *Microbial Cell Factories*, **14:** 139.

Wei, G., Li, Y., Du, G., Chen, J. 2003. Effect of surfactants on extracellular accumulation of glutathione by *Saccharomyces cerevisiae*. *Process Biotechnology*, **38:** 1133-1138.

Wen, S.H., Zhang, T., Tan, T.W. 2005. Optimization of the amino acid composition in glutathione fermentation. *Process Biochemistry*, **40:** 3474-3479.

Wen, S.H., Zhang, T., Tan, T.W. 2004. Utilization of amino acids to enhance glutathione production in *Saccharomyces cerevisiae*. *Enzyme and Microbial Technology*, **35:** 501-507.

Xiong, Z.Q., Guo, M.J., Guo, Y.X., Chu, J., Zhuang, Y.P., Zhang, S.L. 2009. Efficient extraction of intracellular reduced glutathione from fermentation broth of *Saccharomyces cerevisiae* by ethanol. *Bioresource Technology*, **100:** 1011-1014.

Zhang, T., Wen, S., Tan, T. 2007. Optimization of the medium for glutathione production in *Saccharomyces cerevisiae*. *Process Biochemistry*, **42:** 454-458.