Effect of Zinc-Enriched Yeast Supplementation on Serum Zinc and Testosterone Concentrations in Ethanol Feeding Rats

Jae-Young Cha[1], Jin-Sun Heo, Bo-Kyung Park and Young-Su Cho*

Department of Biotechnology, College of Natural Resources and Life Science, Dong-A University, Busan 604-714
[1]*Technical Research Institute, Daesun Distilling Co. Ltd., Busan 619-934, Korea*

Received June 10, 2008 / Accepted July 22, 2008

Zinc is an essential trace element participating in many physiological functions and notably an important function for sperm physiology. Zinc-enriched yeast strain, *Saccharomyces cerevisiae,* isolated from tropical fruit rambutan. This strain contained 306 ppm zinc concentration and other components contained by K 28,640 ppm, Mg 2,342 ppm, Na 1,048 ppm, Ca 366 ppm, Fe 236 ppm and Mn 4 ppm. The serum concentration of testosterone was decreased in ethanol treatment rats. As compared with ethanol treated control rats, the zinc-enriched yeast strain supplementation showed significantly increased the testosterone concentration in serum. In addition, zinc concentration in serum was decreased in alcohol treatment, but this reduction was significantly increased by zinc-enriched yeast strain supplementation in ethanol feeding rats. These results indicate that zinc-enriched yeast *Saccharomyces cerevisiae* strain could play an important role in the sperm physiology by the marked elevation of serum testosterone concentration.

Key words : Zinc, yeast, ethanol, testosterone, rat

Introduction

Bioaccumulation capacity in microorganisms, mushrooms and plants has been studied for the purpose of obtaining organisms enriched in certain essential minerals for human health [11,26]. The high bioactive components producing yeast strains have been found in some species of yeast, such as *Saccharomyces cerevisiae* and *Candida utilis* [22,23]. The determination of various inorganic elements in yeast is useful in pharmaceutical industry and is useful for biological, nutritional and toxicological studies [12]. *S. cerevisiae* strain contains a number of the essential trace elements, mainly selenium, zinc, glutathione and beta-glucan [23,30,35]. Recently our study demonstrated that dietary supplemented with glutathione- and zinc-enriched *S. cerevisiae* strains protected against carbon tetrachloride- and alcohol-induced hepatotoxicity and oxidative stress in rats [4,29].

Zinc is an essential mineral participating in many physiological functions in human [14]. Zinc is involved in lipid metabolism, insulin resistance, hepatoprotective effect and body weight loss, and then also plays an important role in sperm physiology [8,13,14,33]. As a consequence, nature has developed an array of elaborated processes for the absorption, storage, and transport of zinc within the body, and a homeostatic balance between these mechanisms is essential for good health [17]. It was reported that alcoholics frequently showed zinc deficiency [28]. Zinc deficiency decreased serum testosterone levels in rats [25]. Recent studies have been shown that zinc supplementation to the diet was significantly increased plasma testosterone concentrations in animals [1,16]. Therefore, we expected that a highly zinc-accumulating yeast supplementation in the diet would enhance plasma testosterone concentrations in ethanol feeding rats. Our present study reported that zinc-enriched yeast *S. cerevisiae* was isolated from the juice of the rambutan fruit [5]. Thus, the objective of this study was to investigate whether the administration of the zinc-enriched yeast *S. cerevisiae* causes an increase of serum testosterone concentration in ethanol feeding rats.

*Corresponding author
Tel : +82-51-200-7586, Fax : +82-51-200-7505
E-mail : choys@dau.ac.kr

Materials and Methods

Isolation of zinc-enriched yeast strain

Highly zinc-containing yeast strain, *S. cerevisiae,* was isolated from the juice of tropical fruit rambutan used in this study. Yeast strain was aerobically cultured in the YM medium containing glucose 1% (w/v), peptone 0.5%, yeast extract 0.3% and malt extract 0.3% at 30°C under the agitation at 200 rpm for 48 hr. The harvested yeast cells were lyophilized to prepare experimental diet.

Animal and experimental design

Sprague-Dawley strain male rats were purchased from the Hyochang Science Animals Co. (Daegu, Korea) and was housed individually in the suspended wire-mesh stainless steel cage under room temperature (21~24℃) and lighting (12 hr). Animals were allowed to access freely to semi-purified basal diet for 1 week before the experiment. Animals were then randomly divided into three experimental groups based on dietary categories: the normal rats fed with water and basal diet, the ethanol control rats fed with ethanol and basal diet, ethanol+yeast feeding rats fed with ethanol and 5% (w/w) zinc-enriched yeast strain contained basal diet. A normal diet consisting of (% by wt) casein 20, corn starch 15, sucrose 55, cellulose 5, corn oil 10, AIN 93 G-MX mineral mix (MP Biomedicals, Germany) 3.5, AIN 93 VX vitamin mix (MP Biomedicals, Germany) 1, DL-methionine 0.3 and choline bitartrate 0.2. The equal amount of zinc-enriched yeast strain supplementation was replaced with casein into the ethanol+ yeast feeding rats. Overall, each group consisted of six rats that were fed appropriate diet for a 4-week period.

Zinc determinations in yeast strain and serum

Zinc concentrations in yeast strain were analyzed by ICP-AES (Inductively coupled plasma-atomic emission spectrophotometer) [39]. Zinc concentrations in the serum were analyzed by a Perkin-Elmer (Uberlingen) Model 300 Atomic Absorption Spectrophotometer [39]. The determination concentration was shown as ppm.

Testosterone determination in serum

Total testosterone analyses in the serum were conducted using the commercial test Immulite (Catalogue no: L2KTT2) in accordance with competitive immunoassay method in Neodin Medicinal Institute (Seoul, Korea). The results were shown as μg/l.

Statistical analysis

The data from animal experiments are presented as the mean±S.E., and were analyzed using one way analysis of variance (ANOVA), with the differences analyzed using the Duncan's new multiple-range test [10]. A p value <0.05 was accepted as being a statistical significance of difference.

Results and Discussion

Isolation of zinc-enriched yeast *S. cerevisiae* strain

Our previous study reported that highly zinc-containing strain, yeast *S. cerevisiae* strain, was isolated from the juice of tropical fruit rambutan, because zinc shows hepatoprotective effect and also plays an important role in sperm physiology [14,18]. The zinc concentration in yeast strain was 306 ppm (Table 1). Other components contained by K 28,640 ppm, Mg 2,342 ppm, Na 1,048 ppm, Ca 366 ppm, Fe 236 ppm and Mn 4 ppm (Table 2). The zinc content in zinc-enriched yeast strain was comparatively higher than other results obtained previously that the zinc concentration was active dry yeast by 52~67 ppm [39], beer brewing dried yeast by 40 ppm [31] and yeast *S. cerevisiae* strain by 5.5 ppm [3], and waste brewery yeast was identified as a trace element [6]. In addition, zinc concentrations in other plants was wheat germ by 135~191 ppm [20], wheat by 51.1~131 ppm [15], wheat flour by 14.6~21.2 ppm [15] and rice by 14.5~48.6 ppm [32]. Therefore, we expected that a highly zinc-accumulating yeast supplementation in the diet would enhance serum testosterone concentrations in ethanol feeding rats.

Changes of body weight gain and total and relative testis weights

As expected, ethanol treatment showed a significant

Table 1. Zinc concentrations in various yeast and cereal foodstuffs

	Foodstuff	Zinc (ppm)	Ref.
Yeast	Zinc-enriched yeast	306	This study
	Active dry yeast	52~67	20
	Beer brewing dried yeast	40	23
	S. cerevisiae	5.5	24
	Waste brewery yeast	trace element	25
Cereal	Wheat germ	135~191	26
	Wheat	51.1~131	27
	Wheat flour	14.6~21.2	27
	Rice	14.5~48.6	28

Values are means of the duplicates.

Table 2. Concentrations of minerals in zinc-enriched yeast strain used in the present study

Ingredients	K	Mg	Na	Ca	Zn	Fe	Mn
Concentration (ppm)	28,640	2,342	1,048	366	306	236	4

Table 3. Changes of the body weight gain, testis weights and serum zinc concentrations in ethanol feeding rats receiving zinc-enriched yeast strain

Group	Normal	Ethanol feeding	
		Control	Yeast
Total body weight gain (g)	69.2±2.12[a]	31.0±2.61[ab]	15.9±1.89[b]
Testis weight (g)	3.31±0.08[NS]	3.48±0.10	3.43±0.12
Relative testis weight (%, g/100 g b.w.)	0.79±0.02[b]	0.93±0.05[a]	0.96±0.04[a]
Serum zinc concentration (ppm)	0.0238±0.00[ab]	0.0197±0.00[b]	0.0290±0.00[a]

Yeast: Zinc-enriched yeast strain.
Values with different letters are significantly different at $p<0.05$. (mean±S.E., n=6).
NS: not significant differences.

decrease in body weight gain compared with normal rats (Table 3). However, zinc-enriched yeast strain supplementation in ethanol feeding rats was significantly lowered the body weight gain. The physiological effects of Brewer's yeast were observed previously that feeding of a diet containing 5% levels prevented obesity in mice [38]. It was also reported that dried yeast dose-dependent reduced in body weight [31]. Thus, a significant decrease in body weight gain by zinc-enriched yeast strain supplementation was more pronounced, in comparison with the ethanol feeding rats (Table 3). Both ethanol treatment rats resulted in a significant increase of the ratio weight between testis and whole body weight (Table 3). But total testis weights were not statistically significant different in the experimental groups.

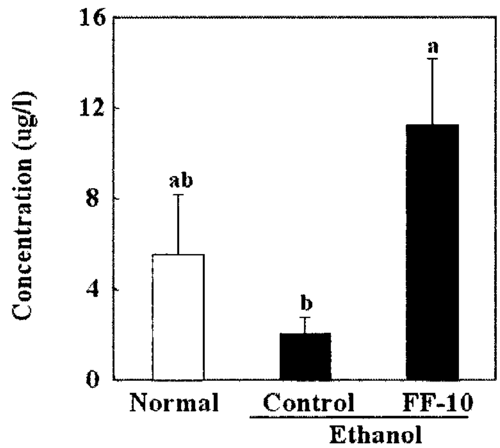

Fig. 1. Changes of the serum testosterone concentration in ethanol feeding rats receiving zinc-enriched yeast strain. Values with different letters are the significantly different statistically at $p<0.05$ (mean±S.E., n=6).

Changes of zinc and testosterone concentrations in serum

The adults recommended daily dosage of elemental zinc for healthy subjects is 1.5~1.7 μM/day (100~150 μg/day) [24]. Zinc, an essential trace element, is found in almost all enzyme classes [34] and is involved in lipid metabolism, insulin resistance and body weight loss [8,33]. Zinc is linked to important functions for sperm physiology [1,3]. Zinc is also a micronutrient crucial for normal growth and development. Alcoholics frequently show zinc deficiency [25]. Zinc deficiency decreased testosterone levels in rats [25]. Ethanol feeding showed a significant decrease in the values of serum testosterone in rats [21]. In the present study, ethanol treatment was also significantly reduced serum testosterone level (Fig. 1). However, zinc-enriched yeast strain supplementation was significantly increased testosterone concentration in alcohol feeding rats. Recent studies have been shown that zinc supplementation to the diet was significantly increased plasma-free and total testosterone concentrations in rat and camel [1,16]. Yeast *S. cerevisiae* feeding was also slightly increased by the action of the antiandrogen moiety present in serum testosterone levels in rats [27].

It was previous reported that the highest concentrations of zinc was found in the cell wall and membrane debris in yeast *Yarrowia lipolytica* by electrochemical methods [30]. Present study also used the whole cell containing the highest concentration of zinc. It is well known that ethanol consumption damages the gastrointestinal tract and influences absorption of various metals. Dinsmore *et al.* reported a lower absorption of zinc in alcoholic patients [9]. Wilson and Hoyumpa also observed in chicks that ethanol decreased duodenal absorption of zinc [36].

Serum zinc concentrations were slightly lowered in rats fed ethanol compared to the normal rats, but this reduction was significantly increased by zinc-enriched yeast strain supplementation in ethanol feeing rats (Table 3). The concentrations of serum zinc in healthy subjects have been reported to peak 4 weeks after administration of a daily zinc supplement of 2.3~7.7 μM/day (150~500 μg/day) and then decreased to initial values after ceasing supplementation [37]. Recent rat studies reported an increase in serum zinc due to an increase in the zinc absorption values [21]. Zinc absorption from a supplement administered three or more hr after ingestion of a meal has been shown to range between 40% and 90%, while a supplement administered with a meal results in an absorption rate of 8% to 38% [19]. The average daily intake of zinc in a well-balanced American diet is approximately 1.8 to 2.3 μM/day (120 to 150 μg/day), assuming a 40% absorption rate from food [24]. Zinc metabolism is altered by ethanol at several levels, including reduced dietary zinc intake and increased zinc excretion in urine [7]. Lower plasma zinc levels are also present in infants with fetal alcohol syndrome, and increased urinary zinc excretion appears to be responsible for decreased plasma zinc concentrations [2].

In summary, we expected that zinc-enriched yeast strain isolated from tropical fruit rambutan could play an important role in the sperm physiology by the marked elevation of serum testosterone concentration.

Acknowledgement

This study was supported by research funds from Dong-A University.

References

1. Al-Qarawi, A., A. H. Abdel-Rahman, M. S. El-Belely and S. A. El-Mougy. 2000. Age-related changes in plasma testosterone concentrations and genital organs content of bulk and trace elements in the male dromedary camel. *Animal Reproduction Sci.* **62**, 297-307.
2. Assadi, F. K. and M. Ziai. 1986. Zinc status of infants with fetal alcohol syndrome. *Pediatric Research* **20**, 551-554.
3. Baltaci, A. K., R. Mogulkoc and A. Ozturk. 2006. Testosterone and zinc supplementation in castrated rats: Effects on plasma leptin levels and relation with LH, FSH and testosterone. *Life Sci.* **78**, 746-752.
4. Cha, J. Y., J. S. Heo and Y. S. Cho. 2008. Effect of zinc-enriched yeast FF-10 strain on the alcoholic hepatotoxicity in alcohol feeding rats. *Food Sci. Biotechnol.* (in press)
5. Cha, J. Y., J. S. Heo, J. W. Kim, S. W. Lee and Y. S. Cho. 2008. Isolation and identification of zinc-enriched yeast *Saccharomyces cerevisiae* FF-10 from the tropical fruit rambutan. *J. Life Sci.* **18**, 447-457.
6. Choi, H. T., S. K. Rhee and J. Y. Son. 2001. Studies on the processing of yeast extract by waste brewery yeast. *Korean J. Food Nutr.* **14**, 161-166.
7. Coudray, C., J. C. Tressol, C. Feillet-Coudray, J. Bellanger, D. Pepin and A. Mazur. 2000. Long-term consumption of red wine does not modify intestinal absorption or status of zinc and copper in rats. *J. Nutr.* **130**, 1309-1313.
8. Díaz-Gómez, N. M., E. Doménech, F. Barroso, S. Castells, C. Cortabarría and A. Jiménez. 2003. The effect of zinc supplementation on linear growth, body composition, and growth factors in preterm infants. *Pediatrics* **111**, 1002-1009.
9. Dinsmore, W., M. E. Callender, D. McMaster, S. J. Todd and A. H. Love. 1985. Zinc absorption in alcoholics using zinc-65. *Digestion* **32**, 238-242.
10. Duncan, D. B. 1957. Multiple range test for correlated and heteroscedastic means. *Biometrics* **13**, 164-176.
11. Elless, M., M. Blaylock, J. Huang and C. Gussman. 2000. Plants as a natural source of concentrated mineral nutritional supplements. *Food Chem.* **71**, 181-188.
12. George, A. Z., S. R. Efthymia, G. T. Demetrius and A. S. John. 2002. Determination of mineral content of active dry yeast used in pharmaceutical formulations. *J. Pharm. Biomedical Analysis* **28**, 463-473.
13. Goel, A., V. Dani and D. K. Dhawan. 2005. Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in chlorpyrifos-induced toxicity. *Chem. Biol. Interact* **156**, 131-140.
14. Hambidge, M. 2000. Human zinc deficiency. *J. Nutr.* **130** (Suppl), 1345-1349.
15. Hinesly, T. D., V. Sudarski-hack, D. E. Alexander, E. L. Ziegler and G. L. Barrett. 1977. Effect of sewage sluge applications on phosphorous and metal concentrations in fractions of corn and wheat kernels. *Cereal Chem.* **56**, 283-287.
16. Kang, G. D., K. H. Lee, S. G. Do, C. S. Kim, J. G. Suh, Y. S. Oh and J. H. Nham. 2001. Effect of silk fibroin on the protection of alcoholic hepatotoxicity in the liver of alcohol preference mouse. *Int. J. Indust. Entomol.* **2**, 15-18.
17. Krebs, N. F. 2000. Overview of zinc absorption and excretion in the human gastrointestinal tract. *J. Nutr.* **130**, 1374S-1377S.
18. Kumar, N., R. P. Verma, L. P. Singh, V. P. Varshney and R. S. Dass. 2006. Effect of different levels and sources of zinc supplementation on quantitative and qualitative semen attributes and serum testosterone level in crossbred cattle (*Bos indicus* x *Bas Taurus*) bulls. *Reprod. Nutr. Dev.* **46**, 663-675.
19. Mertz, W. 1986. Zinc in Trace Elements in Human and Animal Nutrition. New York. Academic press.
20. Motoi, H. and Y. Suzuki. 2004. A water-soluble natural zinc source prepared from defatted wheat germ. *Food Sci. Biotechnol.* **13**, 17-20.
21. Murillo-fuentes, M. L., R. Artillo, M. L. Ojeda, M. J. Delgado, M. L. Murillo and D. O. Carreras. 2007. Effects of prenatal or postnatal ethanol consumption on zinc intestinal absorption and excretion in rats. *Alcohol Alcoholism* **42**, 3-10.
22. Ohtake, Y. and Y. Okumura. 1992. Establishing a high glutathione producing yeast species. *Biosci. Ind.* **50**, 29-34.
23. Park, J. C., M. Ok, J. Y. Cha and Y. S. Cho. 2003. Isolation

and identification of the glutathione producing *Saccharomyces cerevisiae* FF-8 from Korean traditional rice wine and optimal producing conditions. *J. Korean Soc. Agric. Chem. Biotechnol.* **46**, 348-352.
24. Prasad, A. S. 1985. Clinical and biochemical manifestations of zinc deficiency in human subjects. *J. Am. Coll. Nutr.* **4**, 65-72.
25. Prasad, A. S. 1991. Discovery of human zinc deficiency and studies in an experimental human model. *Am. J. Clin. Nutr.* **53**, 403-412.
26. Rabinovich, M., D. Figlas, S. Delmastro and N. Curvetto. 2007. Copper- and zinc-enriched mycelium of *Agaricus blazei* Murrill: Bioaccumulation and bioavailability. *J. Med. Food* **10**, 175-183.
27. Rana, S., D. Bisht and P. K. Chokraborti. 1998. Activation of rat androgen receptor by androgenic ligands is unaffected by antiandrogens in *Saccharomyces cerevisiae*. *Gene* **209**, 247-254.
28. Rodriguez, M. F., R. E. Gonzalez, F. F. Santolaria, M. L. Galindo, T. O. Hernandez, L. N. Batista and P. M. Molina. 1997. Zinc, copper, manganese, and iron in chronic alcoholic liver disease. *Alcohol* **14**, 39-44.
29. Shon, M. H., J. Y. Cha, C. H. Lee, S. H. Park and Y. S. Cho. 2007. Protective effect of administrated glutathione-enriched *Saccharomyces cerevisiae* FF-8 against carbon tetrachloride (CCl_4)-induced hepatotoxicity and oxidative stress in rats. *Food Sci. Biotechnol.* **16**, 967-974.
30. Strouhal, M., R. Kizek, J. Vacek, L. Trnkova and M. Nemec. 2003. Electrochemical study of heavy metals and metallothionein in yeast *Yarrowia lipolytica*. *Bioelectrochemistry* **60**, 29-36.
31. Tachiyashiki, K. and K. Imaizumi. 1994. Effects of dried yeast on the body weight, serum cholesterol levels, and perirenal and periepididymal adipose tissue cells in rats. *J. Jpn. Sco. Nutr. Food Sci.* **47**, 219-226.
32. Take, A., K. Yano, Y. Suzuki and K. Noda. 1997. Magnesium, manganese, zinc and copper contents in Japanese foodstuffs. *J. Jpn. Soc. Nutr. Food Sci.* **30**, 381-393.
33. Tallman, D. L. and C. G. Taylor. 2003. Effects of dietary fat and zinc on adiposity, serum leptin and adipose fatty acid composition in C57BL/6J mice. *J. Nutr. Biochem.* 14, 17-23.
34. Vallee, B. L. and K. H. Falchuk. 1993. The biochemical basis of zinc physiology. *Physiological Review* 73, 79-118.
35. Vlckova, V., S. Nadova, V. Duhova, K. Zavodan, Z. Moranova, P. Rauko, G. Kogan and E. Miadokova. 2006. Natural microbial polysaccharide sulphoethyl glucan as antigenotoxic and cancer preventing agent. *Neoplasma* **53**, 524-529.
36. Wilson, F. A. and A. W. Hoyumpa. 1979. Ethanol and small intestinal transport. *Gastroenterology* **76**, 388-403.
37. Wilson, P. and H. Greene. 2003. Importance of zinc in human nutrition. *Nutr. MD* **2**, 1-8.
38. Yasue, M. 2003. Brewer's yeast may prevent obesity. *Bioindustry* **20**, 38-43.
39. Zachariadis, G. A., E. S. Raidou, D. G. Themelis and J. A. Stratis. 2002. Determination of mineral content of active dry yeast used in pharmaceutical formulations. *J. Pharm. Biomed. Anal.* **28**, 463-473.

초록 : 알코올 급여 흰쥐의 혈중 성호르몬 및 아연 농도에 미치는 아연 고함유 효모 *Saccharomyces cerevisiae* 급여의 영향

차재영[1] · 허진선 · 박보경 · 조영수*
(동아대학교 생명자원과학대학 생명공학과, [1]대선주조(주) 기술연구소)

아연은 많은 생리학적인 기능을 가지는 필수미량 원소로, 특히 성기능에 있어서 중요한 역할을 하는 것으로 잘 알려져 있다. 열대 과일 Rambutan으로부터 분리된 아연 고함유 효모의 미네랄 성분은 아연 306 ppm, K 28,640 ppm, Mg 2,342, Na 1,048 ppm, Ca 366 ppm, Fe 236 ppm 및 Mn 4 ppm이 함유되어 있었다. 혈청 중 성호르몬인 테스토스테론(testosterone) 농도는 정상군에 비해 에탄올 급여 실험군에서 감소 경향을 나타내었다. 에탄올 급여에 의한 성호르몬 테스토스테론의 농도 감소는 아연 고함유 효모 투여로 현저하게 증가하였다. 또한, 혈청 중 아연 함량은 에탄올 급여 실험군에서 감소경향을 보였고, 이러한 감소는 아연 고함유 효모 투여로 현저하게 증가하였다. 따라서, 에탄올 투여에 의한 혈중 아연 및 테스토스테론 농도의 감소효과는 아연 고함유 효모 투여로 상쇄되는 것으로 나타나 본 실험에 사용된 아연 고함유 효모는 성기능 관련 건강기능식품의 소재로서 산업적으로 유용하게 사용될 것으로 사료되어 진다.