J. Microbiol. Biotechnol. (2011), **21**(8), 791–795
doi: 10.4014/jmb.1103.02039
First published online 15 June 2011

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Effect of Fermented Sea Tangle on the Alcohol Dehydrogenase and Acetaldehyde Dehydrogenase in *Saccharomyces cerevisiae*

Cha, Jae-Young[1], Jae-Jun Jeong[1], Hyun-Ju Yang[1], Bae-Jin Lee[2], and Young-Su Cho[3]*

[1]*Technical Research Institute, Daesun Distilling Co., Ltd., Busan 619-951, Korea*
[2]*Marinebioprocess Co., Ltd., Busan 619-912, Korea*
[3]*Department of Biotechnology, College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea*

Received: March 2, 2011 / Revised: May 10, 2011 / Accepted: May 11, 2011

Sea tangle, a kind of brown seaweed, was fermented with *Lactobacillus brevis* BJ-20. The gamma-aminobutyric acid (GABA) content in fermented sea tangle (FST) was 5.56% (w/w) and GABA in total free amino acid of FST was 49.5%. The effect of FST on the enzyme activities and mRNA protein expression of alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) involved in alcohol metabolism in *Saccharomyces cerevisiae* was investigated. Yeast was cultured in YPD medium supplemented with different concentrations of FST powder [0, 0.4, 0.8, and 1.0% (w/v)] for 18 h. FST had no cytotoxic effect on the yeast growth. The highest activities and protein expressions of ADH and ALDH from the cell-free extracts of *S. cerevisiae* were evident with the 0.4% and 0.8% (w/v) FST-supplemented concentrations, respectively. The highest concentrations of GABA as well as minerals (Zn, Ca, and Mg) were found in the cell-free extracts of *S. cerevisiae* cultured in medium supplemented with 0.4% (w/v) FST. The levels of GABA, Zn, Ca, and Mg in *S. cerevisiae* were strongly correlated with the enzyme activities of ADH and ALDH in yeast. These results indicate that FST can enhance the enzyme activities and protein expression of ADH and ALDH in *S. cerevisiae*.

Keywords: Sea tangle, *Saccharomyces cerevisiae*, GABA, alcohol dehydrogenase (ADH), acetaldehyde dehydrogenase (ALDH)

Sea tangle (*Laminaria japonica*) is an edible brown seaweed that is a popular dietary supplement and traditional marine foodstuff in Korea [2, 15]. Sea tangle is enriched in protein, amino acids, minerals, polyphenols, and dietary fiber [1]. Its potential biological actions include antioxidation [14, 15], antimutagenic [22], and antiobesity activities [27]. Recently, sea tangle has been used as the raw material for industrial products such as functional and pharmaceutical foods and cosmetics [17]. Gamma-aminobutyric acid (GABA)-enriched sea tangle that is fermented by *Lactobacillus brevis* BJ20 has been studied as a new source of potentially biologically protective action against ethanol- and carbon tetrachloride-induced hepatic damage in rats [18] and having antioxidant activity [17]. However, there is little information regarding GABA-enriched fermented sea tangle (FST) on the alcohol metabolism enzymes such as alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH).

GABA-enriched food can be used as a dietary supplement to help treat chronic alcohol-related symptoms [21]. In particular, the yeast *Saccharomyces cerevisiae* possesses higher ADH and ALDH activities than do rats [13, 16]. For these reasons, attempts to apply *S. cerevisiae* as a suitable model system of alcohol metabolism have been reported [17]. To further this research, this study examined the effects on the enzyme activities and mRNA protein expression of both ADH and ALDH in *S. cerevisiae* cultured in medium supplemented with different contents of FST.

Materials and Methods

Yeast Strain and Culture Conditions

S. cerevisiae KCCM 11350 was aerobically cultured in a 0.5 l flask containing 0.1 l of YPD medium (yeast extract 10 g/l, peptone 20 g/l, and dextrose 20 g/l) that was unsupplemented or supplemented with 0.4%, 0.8%, or 1% (w/v) of lyophilized FST powder at 30°C with agitation (200 rpm) for 18 h. The cells were harvested by centrifugation at 7,000 ×*g* for 15 min, the supernatant was removed, and the yeast cells were washed three times with distilled water. Cell growth was

**Corresponding author*
Phone: +82-51-200-7586; Fax: +82-51-200-7536;
E-mail: choys@dau.ac.kr

determined by measuring the dry cell weight. FST fermented with *L. brevis* BJ20 (KCTC 11377BP) was generously provided by Marinebioprocess Co., Ltd. (Busan, Korea).

Preparation of Crude Extract from *S. cerevisiae*

The washed yeast cells were suspended in 50 mM sodium phosphate buffer (pH 8.0) containing 1 mM EDTA and 2 mM dithiothreitol, and the suspended cells were disintegrated by sonication with three 30 s cycles at medium intensity (40% duty cycle) with a Ultrasonic Processor VCX-750 (Sonics& Materials, Newtown CT, USA). The sonicated cells were centrifuged at 4°C for 10 min at 10,000 ×*g*. Protein concentration of the supernatant was determined by the method of Bradford [4] using a commercial assay (Bio-Rad, Hercules, CA, USA) as specified by the manufacturer.

GABA Analysis

The change in GABA quantity before and after yeast cell cultivation in FST-supplemented medium was determined, as was the amount of GABA absorbed into yeast cells. Cultivated yeast cells were washed with deionized water and disintegrated as described above. The sonicated cells were analyzed for absorbed GABA using a model 1200 high-performance liquid chromatography system (Agilent Technologies, Santa Clara, CA, USA) consisting of a binary pump, a column oven, a fluorescence detector, and an autosampler. A Zorbax Eclipse C_{18} column AAA (4.6 × 150 mm, 3.5 μm; Agilent Technologies) was used for the chromatographic separation. The mobile phases were A (40 mmol/l Na_2HPO_4 at pH 7.8) and B (45% acetonitrile, 45% methanol, 10% water). According to the injector program, each sample was derivatized with *o*-phthaldealdehyde 3-mercaptopropionic acid (OPA-3MPA) [23]. OPA-3MPA derivatized samples were separated at a column temperature of 40°C and a flow rate of 2.0 ml/min according to the gradient method [23]. GABA derivatized with OPA-3MPA was detected by fluorescence with excitation and emission wavelengths of 340 nm and 450 nm, respectively, and a photomultiplier gain of 10. The GABA content was calculated using commercial GABA (Sigma-Aldrich, St. Louis, MO, USA) as the standard based on a standard curve. The retention time of GABA standard was 10.42 min in the present study condition (Fig. 1).

Mineral Analysis

Yeast cells were ashed in porcelain crucibles at 500°C for 12 h and diluted with deionized water and filtered using Toyo 2A filter paper (Toyo Roshi, Tokyo, Japan) and a 0.45 μm Milex-HA membrane filter (Nihon Millipore Japan, Tokyo, Japan). The Zinc (Zn) content was measured using an AA6700F flame atomic absorption spectrophotometer (Shimadzu, Kyoto, Japan) and was expressed as ppm (mg/kg).

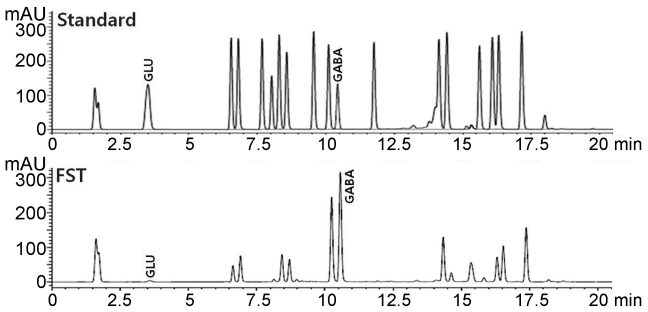

Fig. 1. HPLC chromatograms of GABA in standard sea tangle and FST.
GLU: Glutamate; GABA: γ-aminobutyric acid.

Alcohol Metabolizing Enzyme Activity Assays

ADH and ALDH activities were assayed as described previously [3 and 16, respectively]. The ADH and ALDH activity assays determined the formation of NADH in the reaction system by fluorescence absorbance at 340 nm for 5 min after the initiation of the enzyme reaction at 25°C. The activities of ADH and ALDH were expressed as percent (%) of the control values by the specific activity as micromoles of NADH produced per minute per milligram of protein.

Western Blot Analysis

Proteins in the cell-free extracts containing ADH or ALDH of *S. cerevisiae* were separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE; 20 and 100 μg for ADH and ALDH proteins per lane, respectively) as described previously [25]. The separated proteins were transferred electrophoretically to a nitrocellulose membrane at 4°C for 16 h at a constant current of 300/240 mA/cm^2. After blocking with TBST (10 mM Tris pH 7.5, 100 mM NaCl, 0.1% Tween 20) containing 10% nonfat milk for 1 h at room temperature, the membranes were incubated with a 1:5,000 dilution of rabbit polyclonal ADH or ALDH1A1 conjugated with horseradish peroxidase (HRP) antibody (Abcam, Cambridge, UK). The equivalent amount of protein was loaded and reacted with a 1:250 dilution of HRP-conjugated goat anti-actin (I-19; Santa Cruz Biotechnology, Santa Cruz, CA, USA), which was used as a control for protein loading. Bound antibody was detected using the SuperSignal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL, USA). The relative density of the ADH or ALDH in each reaction was quantified with a Lumi-Imager F1 densitometer (Roche, Basal, Switzerland) and Lumianalyst 3.1.0 software (Roche).

Statistical Analysis

The data from animal experiments are presented as the mean±SE, and were analyzed using one-way analysis of variance (ANOVA), with the differences analyzed using the Duncan's new multiple-range test [9]. A p-value <0.05 was accepted as being a statistically significant difference.

Results and Discussion

Saccharomyces cerevisiae Cell Growth

S. cerevisiae has been successfully used to analyze the mechanisms of cytotoxicity for a variety of drugs [20]. The possible toxic effect of *S. cerevisiae* was presently tested at different concentrations of FST powder (0–1%) for 18 h. There were no significant differences in the cell growth of *S. cerevisiae* (Fig. 2).

Content/Activity of ADH and ALDH, and Contents of GABA and Minerals

Alcohol is readily absorbed from the gastrointestinal tract, circulates rapidly, and becomes distributed uniformly throughout the body [11]. Thereafter, 80–90% of the alcohol absorbed

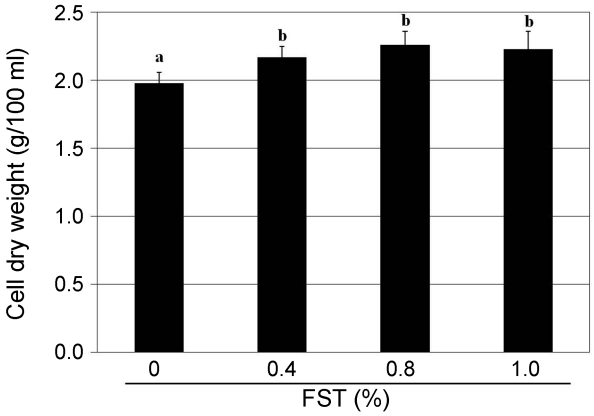

Fig. 2. Cell growth of *Saccharomyces cerevisiae* cultured in YPD medium supplemented with different concentrations of FST at 30°C for 18 h.
Cell growth was determined by measuring the dry cell weight (DCW). Values with different letters are significantly different statistically at $p < 0.05$ (mean±SE, n=3).

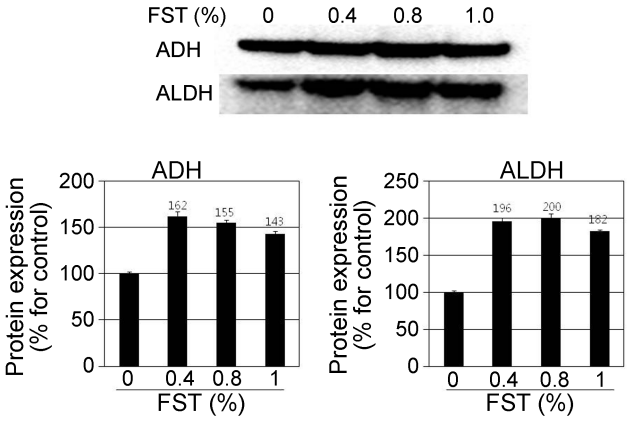

Fig. 4. Effect of FST on the protein expression of ADH and ALDH from cell-free extracts of *Saccharomyces cerevisiae*.

is rapidly oxidized in the liver to acetaldehyde by ADH and to acetate by ALDH [10]. ADH and ALDH enzymes are essential for alcohol metabolism; it has been hypothesized that these enzymes are induced by components such as amino acids. Yeast ADH1 and ADH2 perform two cytoplasmic activities involved in the production as well as the oxidation of alcohol, but mitochondrial-encoded ADH3 seems not to be important in alcohol metabolism [28].

Presently, the expressions and activities of ADH and ALDH in cell-free extracts of *S. cerevisiae* were higher in all FST-supplemented groups, as compared with the non-supplemented group (Fig. 3 and 4). The highest activities of ADH and ALDH were evident in the cell-free extracts of *S. cerevisiae* cultured in medium supplemented with 0.4% and 0.8% FST, respectively (Fig. 3). The most exuberant protein expressions of ADH and ALDH from the cell-free extracts of *S. cerevisiae* were also induced by the supplementation of 0.4% and 0.8% FST, respectively (Fig. 4). These results showed a good correlation between the activity and protein expression of ADH or ALDH from cell-free extracts of *S. cerevisiae*.

Some amino acids play an important biological role for alcohol metabolism enzymes [6, 7, 24]. The GABA content in FST was 5.56% (w/w) and the GABA content in total free amino acid of FST was 49.5% (w/w), by HPLC analysis (Fig. 1). The amount of GABA in yeast cells cultured in the basal medium without FST was 6 ppm, although GABA was not contained in its cultivation medium, whereas this amount was 30–35 ppm in yeast cells cultured in FST-supplemented medium (Fig. 5). This result suggested that the exogenous GABA in GABA-enriched FST-supplemented medium passed into the yeast cells during cultivation. The highest amount of GABA was observed in both yeast cells and medium supplemented with 0.4% (w/v) FST (Fig. 5). The results reflected a positive correlation between ADH

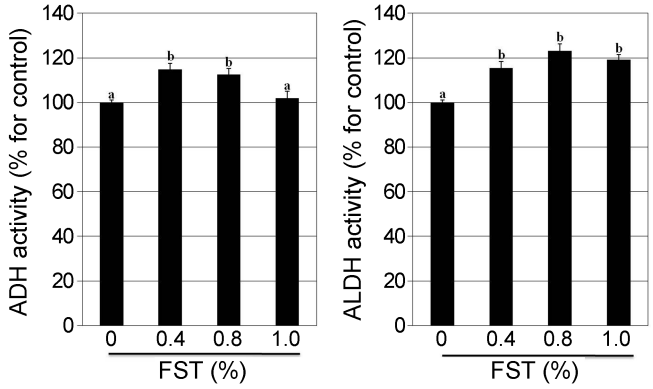

Fig. 3. Effect of FST on the activities of ADH and ALDH from cell-free extracts of *Saccharomyces cerevisiae*.
Values with different letters are significantly different statistically at $p < 0.05$ (mean±SE, n=3).

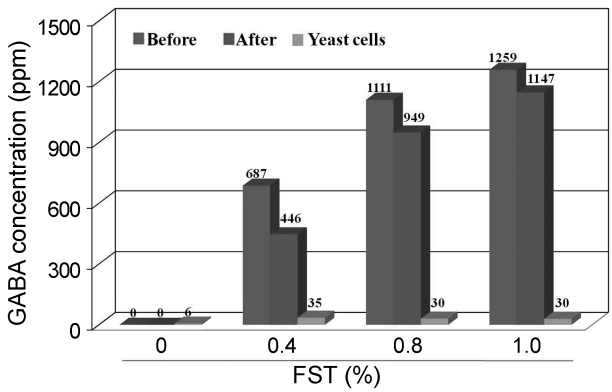

Fig. 5. GABA content in *Saccharomyces cerevisiae* cultivated in medium with or without FST and in the medium before and after yeast cultivation.

Table 1. Effect of FST on the concentration of minerals in *Saccharomyces cerevisiae*.

Mineral (ppm)	FST treatment content (%)			
	0	0.4	0.8	1.0
Zn	1.87±0.02[a]	2.84±0.02[b]	2.59±0.00[c]	1.94±0.00[d]
Ca	1.28±0.04[a]	1.33±0.04[a]	1.53±0.06[b]	1.82±0.01[c]
Mg	12.23±0.03[a]	17.33±0.17[b]	17.83±0.44[c]	21.17±0.17[d]

Values with different letters are significantly different statistically at $p < 0.05$ (mean±SE, n=3).

activity or its mRNA expression and intracellular GABA level.

Zn has many important physiological and biological functions in humans and microorganisms [6, 12]. Zn acts as a functional, structural, and regulatory cofactor for a large number of enzymes [5]. The yeast ADH Zn-containing enzyme is activated directly dependent on the presence of catalytic Zn [19]. Hence, the Zn concentration of *S. cerevisiae* was measured as one of the enzyme stimulating cofactors in this study. Zn contents in *S. cerevisiae* were increased in the medium supplemented with FST compared with nonsupplemented medium (Table 1). The highest content of Zn in *S. cerevisiae* was evident at 0.4% (w/v) FST, indicating a high correlation of Zn content with ADH expression and activity in *S. cerevisiae*.

ALDHs are multisubunit enzymes that catalyze the oxidation of acetaldehyde to acetate efficiently and rapidly during ethanol metabolism. *S. cerevisiae* has at least three major classes of ALDH isoenzymes (ALDH1, 2, and 5) [8, 13]. *S. cerevisiae* cytosolic ALDH1 appears to play a more major role in acetaldehyde oxidation than does the mitochondrial ALDH2 [13, 26]. *S. cerevisiae* cytosolic ALDH has been shown to be essentially NADP-dependent and its activity is stimulated by divalent cations such as Mg^{2+}, Ca^{2+}, and Mn^{2+} [8, 13, 26]. In one study, the activity of ALDH1 was stimulated 2.8-, 3.8-, and 2.1-folds with 20 mM $MgCl_2$, 30 mM $CaCl_2$, and 2 mM $MnCl_2$, respectively [26]. The contents of Ca and Mg in *S. cerevisiae* increased in parallel with increased FST supplementation (Table 1).

Presently, the levels of GABA, Ca, and Mg were highly related with ALDH expression and activity in cell-free extracts of *S. cerevisiae*.

In conclusion, GABA-enriched FST stimulates the expression and activity of both ADH and ALDH in *S. cerevisiae*. Thus, FST could be beneficial in alleviating the symptoms of excessive alcohol consumption.

Acknowledgment

This study was supported by the Dong-A University Research Fund.

References

1. Ahn, S. M., Y. K. Hong, G. S. Kwon, and H. Y. Sohn. 2010. Evaluation of *in vitro* anticoagulation activity of 35 different seaweed extracts. *J. Life Sci.* **20:** 1640–1647.
2. Athukorala, Y., K. W. Lee, S. K. Kim, and Y. J. Jeon. 2007. Anticoagulant activity of marine green and brown algae collected from Jeju Island in Korea. *Bioresour. Technol.* **98:** 1711–1716.
3. Bergmeyer, H. U. 1983. *Methods of Enzymatic Analysis*, 3rd Ed., Vol. 11. p. 139. Verlag Chemie, Weinheim, Germany.
4. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248–254.
5. Brown, P. H., I. Cakmak, and Q. Zhang. 1993. Form and function of zinc plants, pp. 93–106. *In* A. D. Robson (ed.). Zinc in Soils and Plants, 1st Ed. Kluwer Academic Publishers, Dordrecht.
6. Cha, J. Y., J. S. Heo, B. K. Park, and Y. S. Cho. 2008. Effect of zinc-enriched yeast supplementation on serum zinc and testosterone concentrations in ethanol feeding rats. *J. Life Sci.* **18:** 947–951.
7. Cha, J. Y., H. J. Jung, J. J. Jeong, H. J. Yang, Y. T. Kim, and Y. S. Lee. 2009. Effects of amino acids on the activities of alcohol metabolizing enzyme alcohol dehydrogenase and acetaldehyde dehydrogenase. *J. Life Sci.* **19:** 1321–1327.
8. Dickinson, F. M. 1996. The purification and some properties of the Mg^{2+}-activated cytosolic aldehyde dehydrogenase of *Saccharomyces cerevisiae*. *Biochem J.* **315(Pt2):** 393–399.
9. Duncan, D. B. 1957. Multiple range test for correlated and heteroscedastic means. *Biometrics* **13:** 164–176.
10. Gill, K., Z. Amit, and B. R. Smith. 1996. The regulation of alcohol consumption in rats: The role of alcohol, metabolizing enzymes, catalase and aldehyde dehydrogenase. *Alcohol* **13:** 347–355.
11. Gmel, G., J. C. Givel, B. Yersin, and J. B. Daeppen. 2007. Injury and repeated injury – what is the link with acute consumption, binge drinking and chronic heavy alcohol use? *Swiss Med. Wkly.* **137(45–46):** 642–648.
12. Hambidge, M. 2000. Human zinc deficiency. *J Nutr.* **130 (Suppl):** 1344–1349.
13. Jacobson, M. K. and C. Bernofsky. 1974. Mitochondrial acetaldehyde dehydrogenase from *Saccharomyces cerevisiae*. *Biochim. Biophys Acta* **350:** 277–292.
14. Jin, D., G. Li, J. S. Kim, C. S. Yong, J. A. Kim, and K. Huh. 2004. Preventive effects of *Laminaria japonica* aqueous extract on the oxidative stress and xanthine oxidase activity in streptozotocin-induced diabetic rat liver. *Biol. Pharm. Bull.* **27:** 1037–1040.
15. Kim, S. J., S. N. Woo, H. Y. Yun, S. S. Yum, E. S. Choi, and J. R. Do. 2005. Total phenolic contents and biological activities of Korean seaweed extracts. *Food Sci. Biotechnol.* **14:** 798–802.
16. Koivula, T., M. Koivusalo, and K. O. Lindros. 1975. Liver aldehyde and alcohol dehydrogenase activities in rat strains genetically selected for their ethanol preference. *Biochem. Pharmacol.* **24:** 1807–1811.
17. Lee, B. J., J. S. Kim, Y. M. Kang, J. H. Lim, Y. M. Kim, M. S. Lee, M. H. Jeong, C. B. Ahn, and J. Y. Je. 2010. Antioxidant

activity and γ-aminobutyric acid (GABA) content in fermented sea tangle by *Lactobacillus brevis* BJ20 isolated from traditional fermented foods. *Food Chem.* **122:** 271–276.
18. Lee, B. J., M. Senevirathne, J. S. Kim, Y. M. Kim, M. S. Lee, M. H. Jeong, *et al*. 2010. Protective effect of fermented sea tangle against ethanol and carbon tetrachloride-induced hepatic damage in Sprague–Dawley rats. *Food Chem. Toxicol.* **48:** 1123–1128.
19. Magonet, E., P. Hayen, D. Delforge, E. Delaive, and E. Remacle. 1992. Importance of the structural zinc atom for the stability of yeast alcohol dehydrogenase. *Biochem. J.* **287(Pt 2):** 361–365.
20. Nitiss, J. L. and J. Heitman. 2007. *Yeast as a Tool in Cancer Research*. Springer, Dordrecht, The Netherlands.
21. Oh, S. H., J. R. Soh, and Y. S. Cha. 2003. Germinated brown rice extract shows a nutraceutical effect in the recovery of chronic alcohol-related symptoms. *J. Med. Food* **6:** 115–121.
22. Okai, Y., K. Higashi-okai, and S. Nakamura. 1993. Identification of heterogenous antimutagenic activities in the extract of edible brown seaweeds, *Laminaria japonica* (Makonbu) and *Undaria pinnatifida* (Wakame), by the gene expression system in *Salmonella* Typhimurium (TA1535/pSK1002). *Mutat. Res.* **303:** 63–70.
23. Schwarz, E. L., W. L. Roberts, and M. Pasquali. 2005. Analysis of plasma amino acids by HPLC with photodiode array and fluorescence detection. *Clin. Chim. Acta* **354:** 83–90.
24. Shon, J. R., T. Yamamoto, and Y. S. Cha. 2003. The effects of carnitine and/or GABA supplementation on the recovery of chronic ethanol administered rats. *J. Food Sci. Nutr.* **8:** 119–123.
25. Vasiliou, V., J. Lee, A. Pappa, and D. R. Petersen. 2000. Involvement of p65 in the regulation of NF-kappaB in rat hepatic stellate cells during cirrhosis. *Biochem. Biophys Res. Commun.* **273:** 546–550.
26. Wang, X., C. J. Mann, Y. Bai, L. Ni, and H. Weiner. 1998. Molecular cloning, characterization, and potential roles of cytosolic and mitochondrial aldehyde dehydrogenases in ethanol metabolism in *Saccharomyces cerevisiae*. *J. Bacteriol.* **180:** 822–830.
27. You, J. S., M. J. Sung, and K. J. Chang. 2009. Evaluation of 8-week body weight control program including sea tangle (*Laminaria japonica*) supplementation in Korean female college students. *Nutr. Res. Pract.* **3:** 307–314.
28. Young, E. T. and D. Pilgrim. 1985. Isolation and DNA sequence of ADH3, a nuclear gene encoding the mitochondrial isozyme of alcohol dehydrogenase in *Saccharomyces cerevisiae. Mol. Cell Biol.* **5:** 3224–3334.