Antioxidative Activity of Zinc-Enriched *Saccharomyces cerevisiae* FF-10 in *In vitro* Model Systems

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Received December 16, 2008 / Accepted February 10, 2009

Zinc is an essential trace element for human and plays an important biological role in antioxidant properties. We have been reported that zinc-enriched *S. cerevisiae* FF-10 contained 392 mg% in the YM basal and 3,193 mg% in the YM optimal medium. Antioxidative activity of FF-10 was tested *in vitro* models by DPPH (α,α'-diphenyl-β-picrylhydrazyl) radical scavenging activity and lipid peroxidation using linoleic acid (LA) and rat liver homogenate. DPPH radical scavenging activity was higher in the cell-free extract of FF-10 cultured in the YM optimal medium (YMOM) than that in the YM basal medium (YMBM). The inhibition activity of lipid peroxidation using rat liver homogenate was shown in the following order: BHT > YMOM > YMBM and these values were dose dependently. The lipid peroxidation of the control mixture by ferric thiocyanate and TBA methods using LA was increased rapidly as typical peroxidation curve of LA from one day and the antioxidation activity of the cell free extracts by cultivating FF-10 in the YMOM were higher than that of the YMBM. Result of this study indicate that the cell-free extracts containing a high intercellular zinc of *S. cerevisiae* FF-10 cultured in YMOM showed strong antioxidation capacities in DPPH radical scavenging activity and lipid peroxidation using LA and rat liver homogenate.

Key words : Antioxidation, zinc, *S. cerevisiae* FF-10, DPPH, lipid peroxidation

Introduction

Zinc is an essential trace element of all organisms [31]. Zinc plays important roles in various physiologic functions in growth, development, and sexual maturity in males [16,21]. Zinc also plays biological roles in antioxidant properties, lipid metabolism, insulin resistance, immune response, sperm physiology and hepatic injury [3,26,34,39]. The recommended dairy intake of zinc is 10 mg per day in Japan and 15 mg in the USA [23,37]. The current estimate is that a nutritional deficiency of zinc may affect over 2 billion subjects in the developing world and 18% of people in the USA consume less than half the recommended levels of zinc [21]. Clinical zinc deficiency causes various clinical problem, such as growth retardation and taste impairment [29,37]. The main cause of zinc deficiency in human is nutritional.

Yeast has long been utilized in areas of food, medicine and cosmetic industry due to their bioactive and nutritional components, such as amino acids, peptides, zinc, beta-glucan, glutathione, cerebroside, and terrein [8,10,25,28]. Zinc has shown hepatoprotective effect against hepatotoxin and antioxidant properties against oxidative damage in organisms [4,19,34,35]. We also recently have been reported that highly zinc containing yeast strain isolated from the tropical fruit rambutan and this strain was protected against alcohol-induced hepatotoxicity and oxidative stress in rats [10,11].

The many kinds of natural antioxidants from microorganisms have been isolated by using various screening systems [12,24]. The antioxidative substances were also produced intercellularly into *S. cerevisiae* IFO 2114 and extracellularly into the culture medium by *Aspergillus soja* and *Aspergillus oryzae* [22,36]. We also have been reported that an intercellularly antioxidative activities found in the *Bacillus* sp. FF-7 and a glutathione-enriched producing yeast strain *Saccharomyces cerevisiae* FF-8 [12,27]. However, there is very limited information on the capacities of this high zinc-containing yeast strain *S. cerevisiae* FF-10 on the antioxidation properties against lipid peroxidation. Thus, the antioxidation activity of the zinc-enriched cell-free extracts from *S. cerevisiae* FF-10 was tested *in vitro* experimental models, DPPH radical scavenging activity, ferric thiocyanate and thiobarbituric acid reacting substances (TBARS) methods against the lipid peroxidation of rat liver homogenate and LA.

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Materials and Methods

Yeast strain culture conditions

We have been reported that highly zinc containing yeast strain, *S. cerevisiae* FF-10 (KACC 93071), was isolated from the tropical fruit rambutan [13] and this strain contained 392 mg% and 3,193 mg% zinc concentrations in the YM basal and optimal mediums, respectively. Zinc-enriched yeast strain FF-10 was aerobically cultivated either in the YMBM (glucose 1.0%, peptone 0.3%, yeast extract 0.3%, malt extract 0.3%) and YMOM (2.0% (w/v) glucose, 5.0% yeast extract, and 0.04% $ZnCl_2$) with 100 rpm shaking at 30°C for 72 hr. After incubation, the culture was centrifuged at 7,000× *g* for 15 min, the supernatant was removed, and the yeast cells was washed with distilled water three times. The harvested yeast cells were suspended in 0.2 M phosphate buffer (pH 7.2) and disrupted by sonication. The disrupted cells were then removed by centrifugation, and the cell-free extract was concentrated by an evaporator, followed by lyophilization. The powder obtained was dissolved in water and used for *in vitro* antioxidation activity measurement.

DPPH radical scavenging activity assay

Antioxidation activity was determined based on the free radical scavenging activity of the experimental compounds [6,7]. DPPH (16 mg) was dissolved in 100 ml ethanol and this solution was filtered with Whatman filter paper No. 2. After mixing 1 ml of DPPH solution with 5 ml of 0.05 % (w/v) the FF-10 cell-free extract, for 30 min incubation period at room temperature, the optimal density (OD) was measured at 528 nm. The antioxidant activity was expressed as the absorbance and were calculated according to the following equation: [DPPH radical scavenging activity (%)= (control OD-sample OD)/control OD×100]. 0.05% (w/v) BHT was used as positive control and, which is one of the most widely used chemicals to retard oxidation [15,27].

Determination of lipid peroxidation using rat liver homogenate

The liver homogenate prepared from the normal diet feeding rats was used to analyze the concentrations of TBARS. The lipid peroxidation in rat liver homogenate induced with 0.2 mM Fe^{2+} and 0.25 mM ascorbate was measured as concentrations of TBARS by the previously described method with a minor modification [32]. Rat liver homogenates containing Fe^{2+} and ascorbate with or without the 0.05 % (w/v) cell-free extract, were placed in a shaking water bath at 37°C for 30 min. And then equal volumes of 0.75% TBA and 15% trichloroacetic acid were added. Reaction mixtures were heated at boiling water for 30 min, and after cooling in ice-water, centrifuged for 10 min at 3,000 rpm to separate corpusculated particles. The absorbance of the supernatant was read at 532 nm. The antioxidant activity for TBARS formation was expressed as the absorbance. 0.05% BHT was used as positive control.

Determination of lipid peroxidation using linoleic acid

Lipid peroxidation using linoleic acid was determined by TBA bound malondialdehyde. The TBA method was used for lipid peroxidation of the LA in Fe^{2+}-ascorbate system as the oxidative catalysis [17,18]. The 0.05% (w/v) FF-10 cell-free extract was added to a solution of LA 0.13 ml, ethanol 10 ml, 0.01% $FeSO_4$ 0.2 ml, 0.01% ascorbate 0.2 ml, and 0.2 M phosphate buffer (pH 7.0) 10 ml. The total reaction volume was adjusted to 20 ml with deionized distilled water, and the reaction mixture was incubated at 40°C in the dark during up to 7 days. Every day, 0.2 ml of incubated reaction solution was mixed with 0.05 ml of 7.2% butylated hydroxytoluene and 2 ml of 20 mM TBA/15% trichloroacetic acid. The sample was mixed using a vortex, and incubated in a boiling water bath for 30 min to develop color. After cooling in cold water, the samples were centrifuged at 3,000× *g* for 15 min and the absorbance of the resulting upper layer was measured at 500 nm. Deionized distilling water was substituted for FF-10 cell-free extracts in the blank samples.

Results and Discussion

DPPH-free radical scavenging activity

The ability of zinc to retard oxidative processes has been recognized for many years. The FF-10 cell free extract containing zinc as antioxidative substance cultured in both the YMBM and the YMOM were evaluated for antioxidant activity by *in vitro* experimental methods. The free radical scavenging activity measurement using discoloration of DPPH has been widely used due to its stability, simplicity, and reproducibility [1,2]. Generally, natural antioxidants such as glutathione, tocopherol, phenolic-compounds, demonstrated greater antioxidant action in DPPH free radical scavenging activity [7,14,15,27,40]. A significant positive relationship between these compounds and DPPH radical scavenging activ-

ity was observed [40]. In this regard, we also reported that the DPPH radical scavenging activity of the glutathione produced by *S. cerevisiae* FF-8 is as high as 0.05% (w/v) BHT. The DPPH radical scavenging activity of the cell free extracts of zinc-enriched FF-10 cultured in the YMOM was higher than that of the YMBM (Fig. 1). However, BHT (0.05%) used as the most widely a positive control showed the highest antioxidation activity under this study conditions.

Inhibition of lipid peroxidation using the rat liver homogenate

Zinc plays a sacrificial defense role against oxidative damage in organisms [19,34]. The inhibition activity of metal ion Fe^{2+}-catalyzed lipid peroxidation by the ferric TBA method using tissues has been widely used due to *in vivo* membrane damage attributed to lipid oxidation [9,15,27,32]. Hence, present study examined the inhibitory effect of zinc-enriched FF-10 on lipid peroxidation in Fe^{2+}-catalyzed system using rat liver homogenate. An inhibition activity was shown in the following order: BHT > YMOM > YMBM and these were dose-dependent (Fig. 2). As expected, BHT used as positive control showed the highest antioxidation activity. This antioxidation effect was more pronounced in strain cultured in the YMOM than that in the YMBM. This result indicate that zinc in FF-10 strain is an antioxidation substance and functioned effectively as natural antioxidant.

Inhibition of lipid peroxidation using linoleic acid

The antioxidation activity of the cell-free extract of FF-10

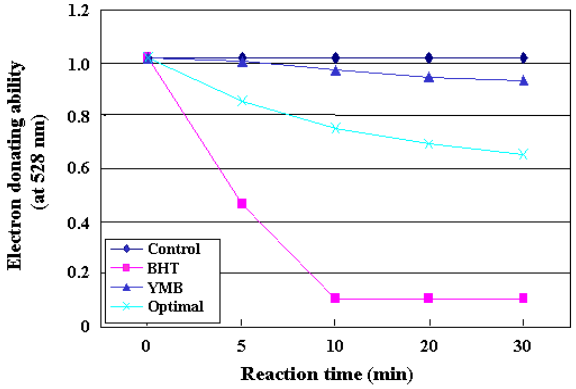

Fig. 1. The DPPH free radical scavenging activity of the cell-free extracts of a high zinc *S. cerevisiae* FF-10 cultured in both the YMBM and the YMOM. BHT: butylated hydroxytoluene (0.05%) YMBM was contained 1.0% (w/v) glucose, 0.3% peptone, 0.3% yeast extract, and 0.3% malt extract. YMOM was contained 2.0% (w/v) glucose, 5.0% yeast extract, and 0.04% $ZnCl_2$.

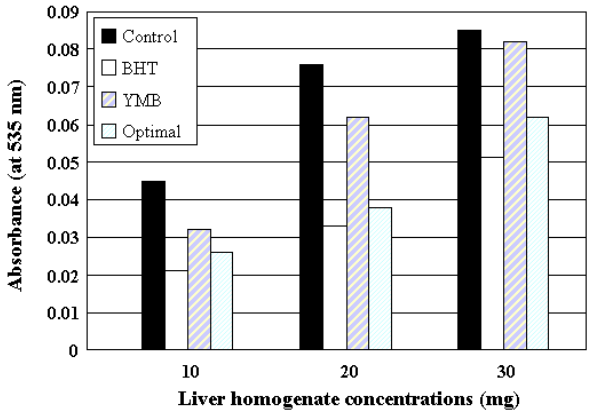

Fig. 2. Antioxidative activity of the cell-free extracts of a high zinc *S. cerevisiae* FF-10 cultured in both the YMBM and the YMOM against the lipid peroxidation of the rat liver homogenate as measured by the TBARS method. BHT: butylated hydroxytoluene (0.05%) YMBM was contained 1.0% (w/v) glucose, 0.3% peptone, 0.3% yeast extract, and 0.3% malt extract. YMOM was contained 2.0% (w/v) glucose, 5.0% yeast extract, and 0.04% $ZnCl_2$.

was measured based on the inhibition of LA peroxidation by the ferric thiocyanate and TBA methods in this study. As shown in Fig. 3 and 4, the production of LA peroxide in the control increased rapidly from the first day and this increase continued until upto seven days. However, the cell-free extract of FF-10 cultured in the YMOM showed an antioxidation effect of inhibiting lipid peroxidation compared with the control. BHT showed the highest antioxidation activity under this study conditions. Present

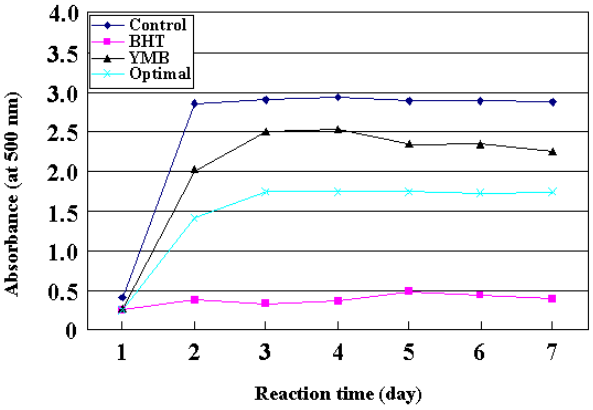

Fig. 3. Antioxidative activity of the cell-free extracts of a high zinc *S. cerevisiae* FF-10 cultured in both the YMBM and the YMOM against the linoleic acid oxidation measured by the ferric thiocyanate method. BHT: butylated hydroxytoluene (0.05%) YMBM was contained 1.0% (w/v) glucose, 0.3% peptone, 0.3% yeast extract, and 0.3% malt extract. YMOM was contained 2.0% (w/v) glucose, 5.0% yeast extract, and 0.04% $ZnCl_2$.

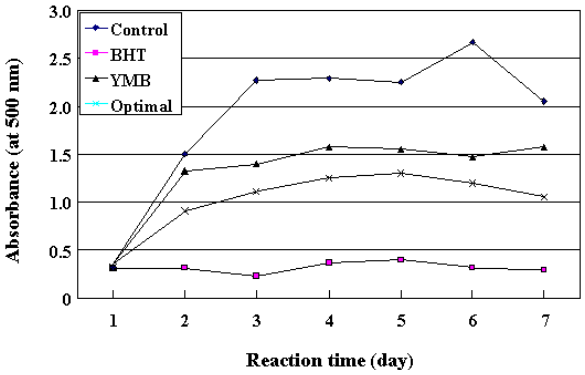

Fig. 4. Antioxidative activity of the cell-free extracts of a high zinc *S. cerevisiae* FF-10 cultured in both the YMBM and the YMOM against the lipid peroxidation measured by the ferric TBA method. BHT: butylated hydroxytoluene (0.05%) YMBM was contained 1.0% (w/v) glucose, 0.3% peptone, 0.3% yeast extract, and 0.3% malt extract. YMOM was contained 2.0% (w/v) glucose, 5.0% yeast extract, and 0.04% $ZnCl_2$.

results concluded that the cell-free extract of *S. cerevisiae* FF-10 cultured in the YMOM had stronger antioxidative capacity than that of the YMBM against the LA peroxidation.

Results of this study suggest the utility of the zinc-enriched *S. cerevisiae* FF-10 for various nutritional products including antioxidant for use as health food or nutraceutical supplement as an antioxidant.

Acknowledgement

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

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초록 : 아연-고함유 효모 *Saccharomyces cerevisiae* FF-10 세포액의 항산화효과

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아연 고함유 효모 *S. cerevisiae* FF-10의 항산화능을 검토하기 위하여 DPPH 전자 공여능, linoleic acid을 이용한 ferric thiocyanate법과 TBA법에 의한 과산화지질 생성 정도 및 흰쥐 간 조직 생체막을 이용한 TBARS법에 의한 과산화지질 생성 정도를 측정하였다. 본 실험은 효모 생육배지인 YM 기본배지와 아연 생산량을 증대시키는 YM 최적배지에서 각각 배양된 *S. cerevisiae* FF-10의 세포 파쇄액의 항산화 활성을 비교하였다. DPPH 전자 공여능은 양성 대조구로 사용한 BHT에서 가장 높았고, YM 기본배지 보다는 YM 최적배지에서 배양된 FF-10 세포 파쇄액에서 항산화 활성이 높게 나타났다. 간 조직 생체막 과산화지질 생성 정도는 BHT > 최적 생산배지 > 기본배지 순으로 저해되었다. Linoleic acid를 이용한 과산화지질 생성정도는 음성 대조구에서 반응 1일째부터 급격히 증가한 후 반응종료일까지 계속 그 수준이 유지되었고, 양성 대조구인 BHT 처리구에서는 과산화지질 생성이 억제되어 높은 항산화활성이 확인되었으며, YM 기본배지 보다는 YM 최적배지에서 높은 과산화지질 생성 저해활성을 보였다. 이상의 결과에서 *in vitro* 항산화 실험계인 DPPH radical scavenging activity, 간 조직 생체막과 linolic acid 지방산을 이용한 ferric thiocyanate and TBARS 측정에서 항산화 활성은 양성 대조구인 BHT 보다는 낮았으나 최적배지에서 배양된 아연 고함유 효모 *S. cerevisiae* FF-10 균주의 세포 파쇄액에서 모두 높게 나타나 *in vivo* 항산화 실험계에서도 확인이 필요한 것으로 사료되어 진다.