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Cancer Chemopreventive Potential of *Scenedesmus* spp. Cultured in Medium Containing Bioreacted Swine Urine

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Abstract *Scenedesmus* spp. were cultured for 51 days in newly developed medium, KEP I (Kim and Ecopeace: initials of corresponding author and environmental company) made with Bacterio-Mineral-Water (3%, v/v) that had been bio-reacted with swine urine medium to 10% (v/v) Bold's Basal medium, and investigated for cancer chemopreventive potential by measuring the induction of quinone reductase (QR), glutathione S-transferase (GST), and reduced glutathione (GSH), and inhibition of cytochrome P450 (CYP) 1A1 activity. The activitives of QR and GST of *Scenedesmus* spp. cultured in KEP I medium were increased by 3.0-fold and 1.5-fold, respectively. However, *Scenedesmus* spp. cultured in control medium (CT) increased the activitives of QR and GST by 1.8-fold and 1.3-fold, respectively. *Scenedesmus* spp. in KEP I medium strongly inhibited CYP 1A1 activity. These results show that *Scenedesmus* spp. in KEP I medium has cancer chemopreventive potential and may be a candidate for further development as a chemopreventive agent.

Key words: *Scenedesmus*, cancer chemoprevention, quinone reductase, glutathione S-transferase, glutathione, cytochrome P450 1A1

Cancer chemoprevention is the administration of natural or synthetic substances to prevent neoplastic disease or to inhibit its progression. Chemoprevention has received growing consideration as a means of cancer control. The chemopreventive agents may function by a variety of mechanisms, directed at all major stages of carcinogenesis. One mechanism of particular note involves the induction of phase II detoxification enzymes, such as NAD(P)H: (quinone-acceptor) oxidoreductase [quinone reductase (QR)] or glutathione S-transferase (GST) [20]. The induction of glutathione (GSH) is also used to test potential chemopreventive agents. GSH has a variety of cellular functions, and one role of particular importance is the protection of cellular macromolecules against reactive intermediates [10]. Cytochrome P450 (CYP) catalyzes the biotransformation of drugs and endogenous compounds and bioactivation of procarcinogens and toxins [9]. CYP enzyme has been identified as a potential target for cancer chemoprevention.

Aquatic microalgae such as *Scenedesmus*, a green alga, have great potential as bioresources for fishes [13, 22], alternative and health foods [7, 14], and pharmaceutical products [11]. It was previously demonstrated that KEP I (Kim and Ecopeace: initials of corresponding author and environmental company) medium made by adding bacteria mineral water that had been bio-reacted with swine urine medium to 10% (v/v) Bold's Basal medium [12] accelerated the physiological and biochemical activities of the cells of *Scenedesmus* and delayed the beginning time of stationary phase of their cells. In this study, the cancer chemopreventive potential of *Scenedesmus* spp. cultured in KEP I medium was examined by measuring the induction of QR, GST, and GSH, and the inhibition of CYP 1A1 activity.

Materials and Methods

Preparation of Sample

The microalgae originated from the Yeungnam Culture Collection of Algae (YCCA) at Yeungnam University, South Korea. The strains were mixed cultures of *Scenedesmus acutus*, *Scenedesmus spinosus*, and *Scenedesmus quadricauda* in control (10% Bold's Basal medium, v/v) [12] or KEP I media. KEP I medium was newly developed in the laboratory by adding Bacterio-Mineral-Water (3%, v/v) that had been bio-reacted with swine urine to 10% Bolds

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Basal medium. They were cultured at 24°C with a light: dark photoperiod of 14 h:10 h, light intensity of 115 mol $m^{-1}s^{-1}$, and stirred with a magnetic stirrer for aeration. These samples were frozen at -50°C for 48 h and then powdered.

Determination of QR Activity in Cell Culture

The inducer potency for QR was measured in Hapa 1c1c7 murine hepatoma cells grown in 96-well microtiter plates as described earlier [15]. The induction of QR activity was calculated from the ratio of the specific enzyme activity of sample-treated cells in comparison with the solvent control.

GST Activity

GST enzyme activity was determined by measuring the conjugation of glutathione to 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate by the method of Habig *et al.* [6]. Protein content was monitored in duplicate plates using a bicinchoninic acid protein assay kit (Sigma, St. Louis, MO, U.S.A.) with bovine serum albumin (BSA) as a standard. The GST activity was expressed as the slope/min/mg of protein. The induction of GST activity was calculated from the ratio of the enzyme activity of sample-treated cells in comparison with the solvent control.

Determination of GSH Levels

The standard protocol described by Griffith [5] was coupled to a NADPH-generating system (glucose-6-phosphate/glucose-6-phosphate dehydrogenase) to determine total GSH levels in cells cultured in 96-well plates. GSH was sequentially oxidized by 5,5'-dithiobis-(2-nitrobenzoic acid) and reduced by NADPH in the presence of glutathione reductase. The extend of 2-nitro-5-thiobenzoic acid formation is monitored at 405 nm. GSH standard curve was constructed. The protein content was determined in duplicate plates prepared and treated as described above, using a bicinchoninic protein assay kit (Sigma, St. Louis, MO, U.S.A.) with bovine serum albumin as a standard. The GSH levels were expressed as nmol/mg of protein. The induction of GSH levels was calculated from the ratio of the GSH content of the sample-treated cells in comparison with the solvent control.

Cytochrome P450 1A1 Activity

Sprague-Dawley rats were treated with 7,12-dimethylbenz[*a*]anthracene (DMBA), and liver microsomes were isolated from homogenized liver by differential centrifugation [16]. Ethoxyresorufin *O*-deethylation (EROD) activity was determined for the measurement of cytochrome P4501A1 activity. Reaction mixture contained microsomal protein, 0.05 M Tris-HCl buffer (pH 7.5), bovine serum albumin, 0.25 M $MgCl_2$, NADPH-generating system (NADP, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase), ethoxyresorufin, and various concentrations of samples. After incubating at 37°C for 4 min, the reactions were terminated by the addition of 2 ml of methanol. The formation of resorufin was determined fluorometrically with a BIO-TEK SFM25 spectrofluorometer (550 nm excitation and 585 nm emission) (BIO-TEK, Vermont, U.S.A.). Assays were conducted in triplicate and the percent of inhibition was calculated as: [1-(sample A-blank)/(solvent A-blank)]×100.

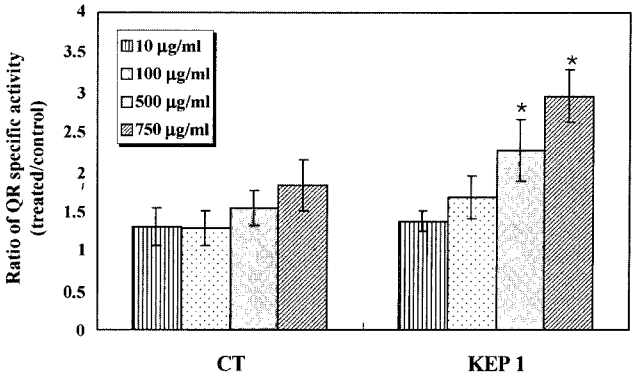

Fig. 1. Induction of quinone reductase (QR) activity by *Scenedesmus* spp. cultured in control (CT) and KEP I media. Values represent mean±SD (n=3).
*p<0.05 compared with control.

RESULTS

The effect of *Scenedesmus* spp. on the induction of QR activity is shown in Fig. 1. *Scenedesmus* spp. cultured in KEP I medium was a more effective inducer of QR than the *Scenedesmus* spp. cultured in control (CT) medium. Maximum enzyme induction mediated by the *Scenedesmus* spp. cultured in KEP I medium was observed at a concentration of 750 µg/ml (Fig. 1).

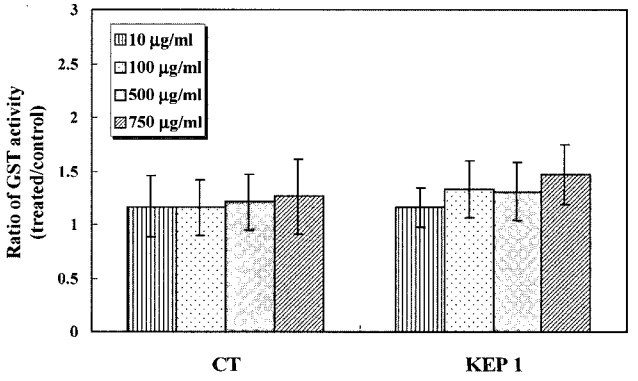

Fig. 2. Induction of glutathione S-transferase (GST) activity by *Scenedesmus* spp. cultured in control (CT) and KEP I media. Values represent mean±SD (n=3).

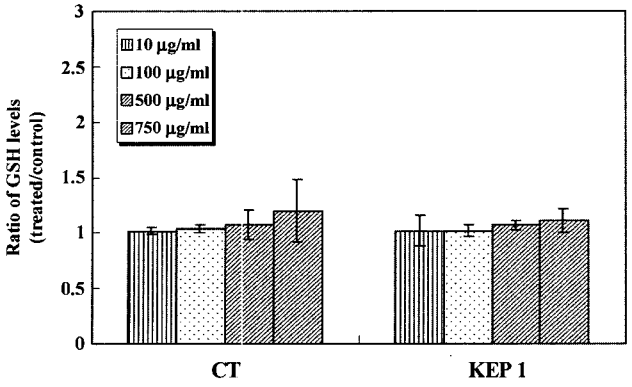

Fig. 3. Induction of reduced glutathione (GSH) levels by *Scenedesmus* spp. cultured in control (CT) and KEP I media. Values represent mean±SD (n=3).

The ability to induce GST activity is a property common to many chemopreventive agents, and the induction of glutathione-conjugating enzymes appears to be an important mechanism for diminishing carcinogenicity. The potential of *Scenedesmus* spp. to induce the activity of GST in Hepa1c1c7 cells was further tested. For the *Scenedesmus* spp. cultured in KEP I medium, increased GST activity was found, as indicated in Fig. 2. However, only a slight increase in GST activity was observed by the *Scenedesmus* spp. cultured in CT medium.

The level of induced total GSH was measured in mouse hepatoma Hapa1c1c7 cells. *Scenedesmus* spp. cultured in CT or in KEP I media had no effect on GSH induction (Fig. 3).

The effect of *Scenedesmus* spp. on CYP 1A1-mediated ethoxyresorufin *O*-deethylase activity in DMBA-treated rat liver microsomes is presented in Fig. 4. It was evident that *Scenedesmus* spp. cultured in KEP I media showed

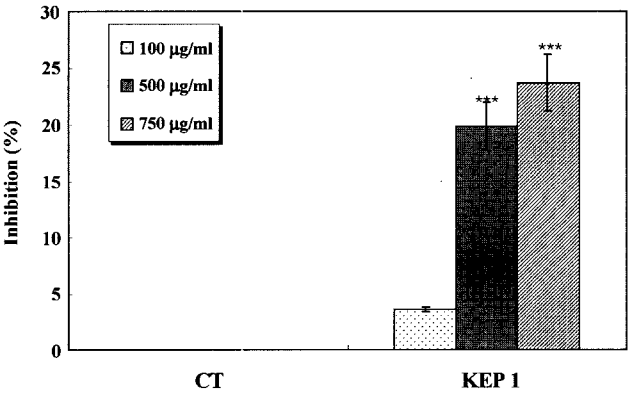

Fig. 4. Inhibition of rat liver microsomal cytochrome P450 1A1-dependent ethoxyresorufin *O*-deethylase (EROD) activity by *Scenedesmus* spp. cultured in control (CT) and KEP I media. Values represent mean±SD (n=3).
***$p<0.005$ compared with control.

dose-dependent decrease in the activity of CYP 1A1. However, *Scenedesmus* spp. cultured in CT medium did not inhibit the DMBA-induced CYP 1A1 activity at all concentrations examined.

DISCUSSION

A number of previous studies suggest that induction of phase II detoxification enzymes, such as QR and GST, is a relevant mechanism for cancer chemoprevention [4, 8, 16, 17, 18, 21]. Quinone reductase functions as an inducible protective device against quinone toxicity by reducing quinones to relatively stable hydroquinones. These resulting hydroquinones can be conjugated and excreted through mercapturic acid pathways. GST catalyzes the conjugation of electrophilic compounds with glutathione, resulting in soluble complexes that are generally more hydrophilic and less cytotoxic. Many types of compounds, such as sulforaphane, ethoxyquin, and phenolic, have been reported to protect against carcinogens and other toxic effects of a variety of chemical agents at multiple sites in animal models via the induction of phase II detoxification enzymes [2, 3, 23], such as QR and GST. The colon tumor-inhibitory effect of organosulfur compounds is associated with an increase of GST, QR, and UDP-glucuronosyl transferase activities in the colon [1]. Singh *et al.* [19] also found that chlorophyllin significantly elevates GST activity and GSH levels in the liver. These inducible enzymes facilitate the metabolic detoxification of xenobiotics in mammals. Therefore, it is reasonable to assume that the increased activities of QR and GST in *Scenedesmus* spp. may play an important role in relation to cancer chemopreventive effect.

Phase I (CYP) enzyme induction is considered a potential cancer risk factor due to the activation of pro-carcinogens to their ultimate reactive forms. Therefore, modulation of this enzyme can affect chemical carcinogenesis. CYP 1A1/1A2 isoforms are involved in the activation of polycyclic aromatic hydrocarbons and nitrosamines, major carcinogens present in tobacco smoke.

The current results suggest that *Scenedesmus* spp. can achieve cancer chemopreventive activity by inducing QR and GST activities to increase carcinogen excretion and to decrease carcinogen-DNA interactions, and by inhibition of CYP 1A1 activity for the conversion of procarcinogens into the ultimate carcinogens. Furthermore, *Scenedesmus* spp. cultured in KEP I medium was a more effective inducer of QR and GST activities and inhibitor of CYP 1A1 activity than the *Scenedesmus* spp. cultured in CT medium. Partially modified medium by Bacterio-Mineral-Water that originated from swine urine may produce more effective agents from the same species of microalgae for physiological and biochemical modifications and for the development of functional foods, medicines or feeds.

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REFERENCES

1. Bandaru, S. R., V. R. Chinthalapally, R. Abraham, and K. Gary. 1993. Chemoprevention of colon carcinogenesis by organosulfur compounds. *Cancer Res.* **53:** 3493-3498.
2. Cabral, J. R. P. and G. E. Neal. 1983. The inhibitory effect of ethoxyquin on the carcinogenic action of aflatoxin B_1 in rats. *Cancer Lett.* **19:** 125-132.
3. De Long, M. J., H. J. Prochaska, and P. Talalay. 1985. Tissue-specific induction patterns of cancer-protective enzymes in mice by tert-butyl-4-hydroxyanisole and related substituted phenols. *Cancer Res.* **45:** 546-551.
4. Faulkner, K., R. Mithen, and G. Williamson. 1998. Selective increase of the potential anticarcinogen 4-methylsulphinylbutyl glucosinolate in broccoli. *Carcinogenesis* **19:** 605-609.
5. Griffith, O. W. 1980. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal. Biochem.* **106:** 207-212.
6. Habig, W. H., M. J. Pabst, and W. B. Jakoby. 1974. Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* **249:** 7130-7139.
7. Kang, K., C.-Y. J. Lee, and C. H. Lee. 2002. Comparison of rheological properties of powder *Chlorella* sp. cultivated in fermentor and pond. *J. Microbiol. Biotechnol.* **12:** 740-745.
8. Kim, T. H., Y. J. Jo, Y. M. Ha, Y. H. Shon, B. J. Bae, and K. S. Nam. 2001. Effect of chitosan oligosaccharide on enzymes for cancer chemoprevention. *J. Korean Cancer Assoc.* **33:** 64-70.
9. Lee, H. J. and M. B. Gu. 2003. Effect of benzo[a]pyrene on genes related to the cell cycle and cytochrome P450 of *Saccharomyces cerevisiae*. *J. Microbiol. Biotechnol.* **13:** 624-627.
10. Molders, P. and B. Jernestrom. 1983. Interaction of glutathione with reactive intermediates, pp. 99-108. *In* A. Larson, S. Orrenius, A. Holgren, and B. Mannervik (eds.), *Functions of Glutathione: Biochemical, Physiological, and Clinical Aspects*. Raven Press, New York, U.S.A.
11. Morimoto, T., N. Murakami, A. Nagatsu, and J. Sakakibara. 1993. Studies on glycolipids. 7. Isolation of 2 new sulfoquinovosyl diacylglycerols from the green alga *Chlorella vulgaris*. *Chem. Pharm. Bull.* **41:** 1545-1548.
12. Nichols, H. W. 1973. Isolation and purification, pp. 7-158. *In* J. R. Stein (ed.), *Handbook of Phycological Methods-Culture Methods and Growth Measurements*, Cambridge University Press, England.
13. Olguin, H. F., A. Salibian, and A. Puig. 2000. Comparative sensitivity of *Scenedesmus acutus* and *Chlorella pyrenoidosa* as sentinel organisms for aquatic ecotoxicity assessment. *Environ. Toxicol.* **15:** 14-22.
14. Ortegacalvo, J. J., C. Mazuelos, B. Hermosin, and C. Saizjimenez. 1993. Chemical composition of *Spirulina* and eukaryotic algae food products marketed in Spain. *J. Appl. Phycol.* **5:** 425-435.
15. Prochaska, H. J. and A. B. Santamaria. 1988. Direct measurement of NAD(P)H:Quinone reductase from cells cultured in microtiter wells: A screening assay for anticarcinogenic enzyme inducers. *Anal. Biochem.* **169:** 328-336.
16. Shon, Y. H., S. Y. Kim, J. S. Lee, and K. S. Nam. 2000. Enhancement of phase II and antioxidant enzymes in mice by soybeans fermented with basidiomycetes. *J. Microbiol. Biotechnol.* **10:** 851-857.
17. Shon, Y. H. and K. S. Nam. 2000. *In vitro* cancer chemopreventive activities of polysaccharides from soybeans fermented with *Phellinus igniarius* or *Agrocybe cylindracea*. *J. Microbiol. Biotechnol.* **11:** 1071-1076.
18. Shon, Y. H. and K. S. Nam. 2001. Antimutagenicity and induction of anticarcinogenic phase II enzymes by basidiomycetes. *J. Ethnopharmacol.* **77:** 103-109.
19. Singh, A., S. P. Singh, and R. Bamezai. 1996. Modulatory influence of chlorophyllin on the mouse skin papollomagenesis and xenobiotic detoxification system. *Carcinogenesis* **17:** 1459-1463.
20. Talalay, P., J. W. Fahey, W. D. Holtzclaw, T. Prestera, and Y. Zhang. 1995. Chemoprotection against cancer by phase II enzyme induction. *Toxicol. Lett.* (Amst.) **82:** 173-179.
21. Williamson, G., G. W. Plumb, Y. Uda, K. R. Price, and M. J. C. Rhodes. 1996. Dietary quercetin glycosides: Antioxidant activity and induction of the anticarcinogenic phase II marker enzyme quinone reductase in Hepa1c1c7 cells. *Carcinogenesis* **17:** 2385-2387.
22. Yashveer, S. 2003. Photosynthetic activity, and lipid and hydrocarbon production by alginate-immobilized cells of *Botryococcus* in relation to growth phase. *J. Microbiol. Biotechnol.* **13:** 687-691.
23. Zhang, Y., T. W. Kensler, C. G. Cho, G. H. Posner, and P. Talalay. 1994. Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. *Proc. Natl. Acad. Sci. USA* **91:** 3147-3150.