CHAPTER V

DISCUSSION

The results of the present study yield substantial information for the protective effects of fresh, blanched, and fermented *Agaricus bisporus, Lentinus edodes, Pleurotus ostreatus* and *Pleurotus abalonus* against *in vivo* induction of somatic mutation and mitotic recombination by urethane. The overall findings from this work suggest that both fresh and fermented shiitake mushroom, as well as blanched and fermented oyster mushroom acted against the mutagenicity induced by urethane in simultaneous feeding study. Fresh and blanched button mushroom promoted the mutagenicity of urethane in simultaneous feeding study. Interestingly, all fermented samples demonstrated weak or moderate antimutagenicity effect in pre- feeding studies.

5.1 The Survival and Mutagenicity of the Extracts Fed to *Drosophila* Larvae

The survival rates of adult flies suggest that all the samples were not toxic since they were not much different from that of the negative control group. People in Asia consume mushrooms as both food and medicine for thousands of years (Chang, 1996). Nowadays, mushrooms have been incorporated into health tonics, tinctures, teas, soups, and health food dishes as well as herbal formulas (Chang, 1996; Sugui *et al*, 2003). Recently, commercial mushrooms were found to be antitumors, antivirals, antioxidants, and immunomodulator (Lima *et al*, 2001; Oliveira *et al*, 2002; Yang *et al*, 2002; Wasser, 2002; Cheung, Peter, and Ooi, 2003). Therefore, this study has confirmed that mushrooms are safe for the consumer and may protect them from some mutagens.

5.2 Mutagenic Modulation on Urethane of Mushroom extracts

Urethane found in fermented foods, especially in alcoholic beverage such as wine (Dennis *et al*, 1989), is an indirect mutagen that is metabolically activated by the cytochrome P-450 enzyme system (Schlatter and Lutz, 1990). Park *et al* (1993) demonstrated that vinyl carbamate, the metabolite of urethane and ultimately responsible for its mutagenic effects, was detoxified by conjugation with glutathione-*S*-transferase. Many scientific data indicated that antimutagens and anticarcinogens found in food and beverage could decrease the mutagenic effect induced by urethane (Abraham, Sigh, and Kesavan, 1998).

The aim of simultaneous feeding study was to elucidate whether each extracts could inhibit the mutagenicity of urethane. If number of spots per wing reduces, it is postulated that the extracts may act as a scavenger of urethane or as an inducer of glutathione-*S*- transferase (GST) or an inhibitor of cytochrome P-450 system. To clarify such hypothesis, the pre-feeding types I and II were performed. Contacting only on the early stage of larval period that is supposed to induce or inhibit the activity of enzyme in biotransformation system is designed as pre-feeding type I study. While continuous feeding of extracts to the larvae in type II study allowed them to contact the possible inducer or inhibitor of enzymes in each extracts for whole period of larvae stage. In case that the reduction of urethane induced spots per wing in simultaneous feeding study is nearly the same as of the result of pre-feeding type II study and the inhibition of both studies were stronger than that of the pre-feeding type I, it seems that extracts can scavenge urethane and/or induce the enzymatic system detoxify urethane.

The data indicated that the fresh button mushroom extract and blanched button mushroom extract slightly increased the mutagenicity of urethane in simultaneous feeding study and expressed moderated mutagenic potentiator in both trials 1 and 2 of type II pre- feeding study. These results suggest that it might be due to agaritine (β-*N*-[γ-L (+)-glutamyl]-4-(hyroxymethyl) phenylhydrazine), the principal hydrazine found in edible mushroom *A. bisporus*. Hydrazines comprise a class of chemical carcinogens, which express their carcinogenicity following metabolism to reactive intermediates, catalyzed by enzyme systems such as the cytochrome P-450 (Tosk, Schmeltz, and Hoffmann, 1979; Walton, Coomb, and Catteral *et al*, 1997; Walton, Coomb, and Walker

et al, 1997). Extract from this mushroom could induce a mutagenic response in the Ames mutagenicity test. Walton *et al*, (2001) found that whole homogenates of *A. bisporus* metabolized the mushroom hydrazine agaritine to generate at least three metabolites. The three metabolites of agaritine displayed weak mutagenic activity towards *Salmonella typhimurium* strain TA 104. Moreover, mutagenic response was suppressed by the nucleophile glutathione and the hydroxyl radical scavenger dimethyl sulfoxide (Walton *et al*, 1998). The data show that blanching of mushroom did not significantly influence the antimutagenic response compared with that of fresh mushroom. In 1998, Walton *et al*. evaluated the effect of baking and freeze-drying on the direct and indirect mutagenicity in Ames test of ethanolic extracts from *A. bisporus*. Direct mutagenicity was not influenced by baking for 10 min at 225 °C, but more prolong baking, for example 4 hr at 100 °C reduced mutagenicity. It suggested that mutagenic and promutagenic compounds present in mushroom were generally not heat labile. However, blanching for 5 min at 100 °C could not decrease the mutagenicity of agaritine. Contrastingly, fresh and blanched button mushroom extracts seemed to reduce mutagenicity in type I pre- feeding study. In pre-feeding study, it was proposed that continuous feeding may either inhibit cytochrome P- 450 or enhance phase II detoxification enzyme. On the other hand, fresh mushroom expressed moderate mutagenic potentiating effect in both trials 1 and 2 of type II study, and blanched mushroom had a tendency to promote mutagenicity in type II pre- feeding study.

In simultaneous feeding study, fresh shiitake mushroom presented weak antimutagenicity in trial 1 and moderate antimutagenicity in trial 2, while blanched shiitake mushroom had no effect in both trials. Fresh oyster mushroom exhibited no inhibitory effect against the mutagenicity of urethane in this study. However, blanched oyster mushroom had a trend to reduce the mutagenicity of urethane. On the other hand, fresh and blanched abalone mushroom seemed to increase mutagenicity of urethane. Turkmen, Sari, and Velioglu (2005) found that cooking processes brought about a number of changes in physical characteristic and chemical composition of vegetables and after cooking, total antioxidant activity increased or remained unchanged depending on the type of vegetable but not type of cooking. Therefore, the

reasonable possibility to explain the mechanism of antimutagenicity of the mushroom extracts was that they could induce the activity of GST that catalyzes the conjugation of urethane with glutathione. In 1999, Kim, Kacew, and Lee found that a plant polysaccharide from *Lentinus edudes* (LPS) produced both anti-genotoxic and anti-tumor promoting activities in *in vitro* models. LPS significantly increased GST activity; it might be possible that plant polysaccharides could be effective in the induction of other detoxification enzymes. The antimutagenicity of urethane in shiitake mushroom extracts in type II experiment of pre- feeding study seemed to be stronger than that of type I experiment. It was proposed that the role of some components in this sample as inhibitor for cytochrome P- 450 enzyme system or enhancement of detoxifying enzyme as mentioned above might be a continuous effect. The results of this study were unable to compare with simultaneous feeding study because of the difference in concentration of shiitake mushroom extract.

In pre-feeding study, fresh oyster mushroom extract and blanched oyster mushroom extract showed strong mutagenicity in both type I and type II experiments. It may be suggested that in pre-feeding study a continued treatment in young larvae for 3 days in sample medium, the imaginal discs were growing and thus increasingly more target cell become exposed. Also, prolong treatment with fresh and blanched oyster mushroom may induce the catalytic activities of cytochrome P-450 enzyme system as well as inhibit glutathione-S-transferase activity. It was found that there was no significant difference on number of total spots per wing induced by urethane with fresh and blanched oyster mushroom in pre- feeding study. The result in this study was contrary with that of Bobek, Ozdi´n, and Galbavy´ (1998) which showed that the highest dose of dried oyster mushroom reduced the levels of conjugated diene in erythrocytes and in liver stimulated the activities of superoxide dismutase, catalase and glutathione peroxidase in liver. In type I pre-feeding study, fresh abalone mushroom extract exhibited moderate antimutagenicity but showed no effect against mutagenicity in type II pre-feeding experiment. Blanched mushroom extract showed weak antimutagenicity in type I and type II experiments. These results suggest that in pre-feeding study prolong treatment with fresh and blanched oyster mushroom might inhibit the catalytic activities of cytochrome P-450 enzyme system of phase I or induction of glutathione-S-

transferase activity or increasing amount of glutathione- S- transferase activity of phase II detoxifying in *Drosophila*.

The present study demonstrated that fermentation could induce the antimutagenicity of the samples. The result that fermented button mushroom seemed to reduce mutagenicity of urethane and slightly reduced mutagenicity in both types I and type II pre-feeding study achieve. Fermented shiitake mushroom exhibited strong antimutagenicity in both trials in simultaneous feeding study and fermented oyster mushroom presented weak antimutagenicity against urethane in simultaneous feeding study and all of them demonstrated the weak and moderate antimutagenicity in type I and type II experiments respectively. The results suggest that some biochemical changes occured during fermentation of edible mushroom might inhibit the activity of cytochrome P-450 enzyme system as well as increase glutathione-*S*-transferase activity. Caplice and Fitzgerald (1999) revealed that food fermentation would most probably be extended to the generation of functional components like vitamins, antioxidants and other compounds in a variety of different fermented foods. Furthermore, most vegetable fermentations occur as a consequence of providing growth conditions (such as adding of sodium chloride) that favor the lactic acid bacteria (Buckenhuskes, 1997). Sanchez, Palop, and Ballesteros (2000) found that lactic acid bacteria rapidly increased within a few hours and then holding constant at that level until the end of eggplant spontaneous fermentation. Lactic acid bacteria are well known to posses a variety of beneficial functions for humans such as antimicrobial, antitumor, and antimutagenic activities (Rhee and Park, 2001). In 1998, Lankaputhra and Shah found that live and dead cells of *Lactobacillus acidophilus* were antimutagenic against mutagens. Live bacteria cells bound or inhibited the mutagens permanently whereas dead bacteria released mutagens upon extraction with dimethyl sulfoxide. Since the data were unclear, further exploration on antimutagenicity mechanism is required. Overall the results show that the antimutagenic effect of mushroom may vary among type of mushroom.

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