Summary of Doctoral Thesis

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Title	Studies on the effects of edible mushroom *Grifola frondosa* on intestinal homeostasis

Intestinal homeostasis plays a vital role in maintaining not only gastrointestinal health but also the overall systemic health. The complex interactions between epithelial cells, immune cells, stroma cells, and gut inhabitant regulate a number of diverse mechanisms in order to maintain intestinal homeostasis. A breakdown in these pathways can disrupt the homeostatic mechanisms and lead to chronic intestinal inflammation and inflammatory-related health consequences, such as inflammatory bowel disease, ulcerative colitis, and bowel cancer. Gut inflammation is frequently accompanied by imbalance in the intestinal microflora. Particularly, probiotic bacteria, that can be regarded as part of the natural human microbiota, engage in improving homeostasis through counteracting the inflammation process by stabilizing the gut microbial environment and gut barrier function and mediating pro and anti-inflammatory cytokines. Several mushroom bioactive components including polysaccharides, glycoproteins, proteins, lipids, and secondary metabolites, are known to be involved in the induction and maintenance of intestinal homeostasis. *Grifola frondosa,* commonly known as maitake, is an edible mushroom, that has been shown to possesses various biological activities against several metabolic disorders including cardiovascular disease, diabetes, hypertension, inflammatory bowel disease, and cancers of the stomach, breast, and pancreas. However, the effects of maitake on the maintenance of intestinal homeostasis are still unclear. Therefore, the purpose of our study was to identify the effects of maitake on maintaining gut homeostasis, colorectal cancer development, and growth of probiotics. The effects of maitake and the synergistic inhibitory effect of maitake when combined with milk on aberrant

crypt foci (ACF) formation, colonic inflammation, and suppression of colorectal cancer were evaluated using 1,2-dimethylhydrazine (DMH)-induced mouse model of colon carcinogenesis. In addition, the impact of maitake fruiting bodies, maitake extracts, and purified fractions on the growth of colon probiotic microorganisms were assessed and specific maitake glucans were isolated and characterized.

In chapter 1, the chemopreventive properties of maitake, and the synergistic effects when combined with milk against colon carcinoma in Balb/c mice treated with DMH colon carcinogenesis were investigated. Further, the possible mechanisms by which maitake and milk may exert their actions together or individually were also studied. Animals fed with AIN-76 standard diet were used as positive and negative controls, while AIN-76 supplemented with either 10% milk (10MK), 10% maitake (10MM), 5% milk + 5% maitake (5Mix), or 10% milk + 10% maitake (10Mix) diets were used for experimental groups. Supplementation with maitake, milk, and their combinations showed significant effect on suppressing DMH-induced ACF formation compared to mice treated with DMH positive control with the highest effect observed in the 10Mix group. Cecal pH was significantly lower in the 10MM and 10Mix groups than that of control and, maitake and, its combinations were able to remarkably increase cecum short chain fatty acid levels (SCFA) which were reduced by DMH treatments. Although, no significant difference was observed among samples regarding the liver weight, the levels of thiobarbituric acid reactive substances (TBARS) in the liver which were significantly increased by DMH treatment, were remarkably lower in the dietary treatment groups, and the effect was more pronounced in the 10Mix group. Further, all the tested diets were able to modulate pro-inflammatory cytokines; TNF-α and IL16, anti-inflammatory cytokine; IL-1ra, and chemokine; IP 10. Particularly, TNF-α level was considerably lower in the 10Mix group and was comparable with the DMH negative control showing the highest suppression of colon inflammation, induced by DMH administration. On the other hand, supplementation with these food components significantly reduced anti-apoptotic proteins Bcl-2, Bcl-x, MCL1, XIAP,

and p27/Kip while increasing apoptosis-related protein p53, cytochrome c, Bad, TRAIL R2, TNF R1, and Fas in comparison to the DMH positive control, thus suggesting the involvement of apoptosis in suppression of DMH-induced colon carcinogenesis. Compared to all the other dietary treatments, the 10Mix combination treatment showed the highest protection against colon carcinogenesis. Taken together, these results suggest that maitake, dietary milk, and the combination diets can suppress colon inflammation and upregulated muted cell apoptosis to suppress ACF formation and colorectal cancer development. Importantly, the synergistic inhibitory effect of maitake in combination with milk may be an effective nutritional chemoprevention agent against colon carcinogenesis.

In chapter 2, the synbiotic effect of maitake, maitake extractions and further purified fractions were evaluated using *in vitro* bacterial culture models and the responsible maitake polysaccharides were identified and elucidated. Maitake glucans were isolated from fruiting bodies through subsequent boiling water, ethanol, and alkali extraction steps. The water soluble (ER1), ethanol soluble (ER2), cold alkali soluble (ER3), hot alkali soluble (ER4,) and insoluble (ER5) extracts were purified, characterized by various analytical methods and further tested for prebiotic activity. The NMR spectroscopic analysis identified the glucans in all the extracts as belonging to the β type. Evaluation of the synbiotic effect of maitake extracts showed that all extracts (ER1-ER5) facilitated the growth of seven tested colon probiotic microorganisms; *Lactobacillus. rhamnosus* (ATCC 53103), *Lactobacillus acidophilus* (JCM 1132), *Lactobacillus delbrueckii* subsp bulgaricus (ATCC 11842), *Lactobacillus casei* (ATCC 393), *Lactobacillus fermentum* (ATCC 14931), *Bifidobacterium longum* (BB 536), *Bifidobacterium adolescentis* (ATCC 1275), and *Bifidobacterium animalis* subsp animalis (ATCC 1253). Among the maitake extracts, ER1 showed remarkably different growth characteristics, which were similar to that of positive control (glucose). The ER1 extact was further characterized by methylation analysis and was identified as 1,3-β D-glucan and the molecular weight

as revealed by HPLC was around 2.2×10^4 Da. The ER1 extract was further purified using anion exchange chromatography and divided into three sub-fractions (ER1P-F1, ER1P-F2, and ER1P-F3) based on the carbohydrate contents, and tested for prebiotic activity. Even though, all three sub-fractions were able to stimulate the growth of all probiotics tested, ER1P-F2 and ER1P-F3 showed a remarkably different effects compared to ER1P-F1. The ER1P-F2 and ER1P-F3 sub-fractions were further divided using a molecular weight cutoff value of 10 kDa. The ER1P-F2 and ER1P-F3 fractions with molecular weight >10 kDa showed significant effects on the growth of *L. rhamnosus* (ATCC 53103), *B. longum* (BB 536) and *B. adolescentis* (ATCC 1275). These findings indicate that maitake can be used as potential prebiotic agent to facilitate the growth of colonic probiotic bacteria and 1,3-β D-glucan from maitake water extracts may be responsible for the observed synbiotic effect.

In conclusion, the findings of this study suggest that fruiting bodies and polysaccharide fraction of maitake may be involved in facilitating the growth of colon probiotics microorganisms, suppression of ACF development and colon carcinogenesis and regulation of intestinal homeostasis.