The Role of Mushroom Nutrition as A Delivery Agent for Enzyme Therapy in Cancer Care?-Chemical and Biological Properties in Mushroom Nutrition

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Recently, mushrooms have become the target of studies trying to determine if fungi have nutritional benefits which could improve the body´s immune function (1).

In Japan, when faced with a cancer patient, Japanese medical doctors not only use radical surgery, as well as radiation or chemotherapy, but also nutritional management (immunotherapy). The use of mushroom nutrition as part of nutritional management to enhance the body´s immune function is considered standard practice in Japan and in other Asian cultures.

Several researchers have demonstrated that protein-bound polysacharide complexes such as PSK or PSP, derived from *Coriolus versicolor* ; or Lentinan derived from *Letinula edodes* are the most important component responsible for immunoenhancing and anti-tumour activities. In fact PSP is responsible for oxidative stress relief in cancer patients because it mimicks superoxide dismutase activity due to a small peptide (10KD) present in PSP. (2)

Furthermore, it is a biological response modifier because it induces increased levels of gamma-interferon, interleukin-2, superoxide dismutase activity (SOD) and T-cell proliferation in experimental animals (3, 4). On the other hand, SOD plays an important role in protecting cells against superoxide radical (O_2) damages and overproduction of O_2 *in vivo*.

However, other factors may be also involved in these biological processes which have not been fully investigated. Mushrooms contain a number of enzymes which may participate in several clinical conditions such as tumour and cancer invasion and cardiovascular disorders. It has been known that enzyme therapy plays an important role in several clinical conditions such as in cancer treatment, malignant lymphoma and cardiovascular disorders (5, 6). These mushroom enzymes mentioned below are thought to prevent oxidative stress as well as to inhibit cell growth in several diseases.
We have investigated the enzyme, protein and sugar contents in MRL products by simulating the intestinal tract of the human body. Therefore, we treated the MRL products with the following proteolytic enzymes:

1. Pepsin (500IU/tablet) at pH2 for 30 min. at 37ºC in an incubator with orbital shaking
2. Trypsin (500IU/tablet) at pH 7.6 for 30 min. at 37ºC in an incubator with orbital shaking.

Upon analyzing the enzyme, protein and sugar content in *Coriolus versicolor, Cordyceps sinensis, Ganoderma lucidium* (Reishi) and *Grifola frondosa* (Maitake*)* we found the following results:

Table 1- In the absence of proteolytic enzymes

	Enzyme, Protein and Sugar Analysis Per Tablet of MRL Product	Maitake MRL	Reishi MRL	Coriolus MRL	Cordyceps MRL
1	Protein content	20.2 mg	22.2 mg	17.3 mg	8.4 mg
2	Reducing sugars	12.6 mg	24.0 mg	14.8 mg	265.6 mg*
3	Protein-bound polysaccharide	79.5 mg	69.5 mg	91.5 mg	82.1 mg
4	Peroxidase activity	40.2 mU	11.2 mU	67.2 mU	57.2 mU
5	Laccase activity	411.5 mU	451.5 mU	521.5 mU	-------
6	Glucoamylase / / Beta-glucansase activity	1.6U	2.7U	6.9U	--------
7	Protease activity	4.9 U	4.4 mU	5.9 U	5.6 U
8	Glucose 2-oxidase activity	-----------	8.2 U	49.5 mU	----------

*Reduced sugars due to use of 200 mg of maltodextrin in Manufacturing process.

Table 2- In the presence of pepsin

	Enzyme, Protein and Sugar Analysis Per Tablet of MRL Product	Maitake MRL	Reishi MRL	Coriolus MRL	Cordyceps MRL
1	Protein content	18.5 mg	19.7 mg	15.7 mg	7.6 mg
2	Reducing sugars	12.4 mg	23.1 mg	14.5 mg	258.0 mg*
3	Protein-bound polysaccharide	71.3 mg	63.1 mg	80.5 mg	80.5 mg
4	Peroxidase activity	37.3 mU	10.1 mU	60.4 mU	50.9 mU
5	Laccase activity	370.3 mU	465.1 mU	511.6 mU	-------
6	Glucoamylase / / Beta-glucansase activity	1.4U	2.4U	6.5U	--------
7	Protease activity	4.8 U	4.5 mU	5.0 U	5.5 U
8	Glucose 2-oxidase activity	-----------	3.7 U	27.2 mU	----------

*Reduced sugars due to use of 200 mg of maltodextrin in Manufacturing process.

Table 3 – In the presence of trypsin

	Enzyme, Protein and Sugar Analysis Per Tablet of MRL Product	Maitake MRL	Reishi MRL	Coriolus MRL	Cordyceps MRL
1	Protein content	19.3 mg	21.0 mg	16.6 mg	8.1 mg
2	Reducing sugars	12.2 mg	23.5 mg	14.1 mg	261.0 mg*
3	Protein-bound polysaccharide	75.2 mg	65.2 mg	82.1 mg	78.1 mg
4	Peroxidase activity	36.9 mU	10.6 mU	64.5 mU	52.6 mU
5	Laccase activity	420.1 mU	461.3 mU	535.1 mU	-------
6	Glucoamylase / / Beta-glucansase activity	1.5U	2.5U	6.2U	--------
7	Protease activity	4.6 U	3.7 mU	5.2 U	5.7 U
8	Glucose 2-oxidase activity	-----------	8.4 U	45.0 mU	----------

*Reduced sugars due to use of 200 mg of maltodextrin in Manufacturing process.

The data in these tables reveals that the simulation of intestinal tract (pepsin and trypsin) decreases the enzyme levels by a factor in the range of 10-20%, except in the case of glucose 2-oxidase. In fact, the effect of pepsin reduces glucose 2-oxidase levels by about 50% whereas trypsin does not alter the levels of this enzyme.

Background on the aforementioned enzymes is provided below:

a) **Laccase (benzenediol:oxygen oxidoreductase; EC 1.10.3.2)** is present in active form and catalyses the reduction of dioxygen to water as well as the oxidation of a wide range of phenolic and related compounds. This enzyme also catalyses the oxidation of 3-hydroxyanthranilic acid (3-HAA) into cinnabarinic acid (CA) which is of great clinical interest because 3-HAA is produced in large quantities by interferon-y primed mononuclear phagocytes (7). Furthermore, 3-HAA has been shown to act as a powerful scavenger of reactive oxygen species. On the other hand, cinnabarinic acid (CA) is one of the major products of oxidation of 3-HAA suggesting that laccase may prevent oxidative damage in mammalian tissues. In a similar manner, the mammalian protein, ceruloplasmin which like laccase, is a member of the blue copper oxidase class of enzymes also catalysed the conversion of 3-HAA into CA.

On the other hand, this enzyme also plays an important role in biodegradation of environmental pollutants such as dechlorination of chlorophenolic compounds.

b. **Pyranose oxidase also known as glucose 2 oxidase pyranose:oxygen 2-oxidoreductase; EC 1.1.3.10)** catalyses the oxidation of several aldopyranoses producing hydrogen peroxide and 2-keto-D-glucose (8,9). Several species of basidiomycetes express this enzyme which also catalyses

one-electron reduction of several different classes of xenobiotic compounds. On the other hand, this enzyme plays an important role in clinical diagnosis of diabetes as well as in production of fine chemicals and antibiotics (i.e cortalcerone).

c. **Peroxidases (EC 1.11.1.7**) . These are a family of isoenzymes produced during the secondary metabolism in white-rot basidiomycetes . These enzymes catalyse hydrogen peroxide –dependent one-electron oxidation of a wide range of phenolic and related compounds which result in the formation of aryl cation radicals. These radicals are converted non-enzymatically in several end-products. There is a great interest in these enzymes because they can be used in the detoxification of a broad range of environmental pollutants namely PCBs and dioxins

d. **Protease activity**. The white-rot basidiomycete fungi *Coriolus versicolor* produces a significant amount of proteolytic activity. These fungi synthesize intracellular and extracellular proteases which are involved in the regulation of laccase and peroxidase activity in cultures of *Coriolus versicolor*. A protease is synthesized which cleaves protein substrates (i.e. fibrinogen and casein) specifically by hydrolysing some peptide bonds. This enzyme is of interest for two main reasons: First of all, it has a high fibrinolytic activity and hence it could be used as a potential therapeutic agent in the treatment of thrombosis. Secondly, this enzyme could be used in protein sequencing due to its unique specificity.

Furthermore, mushrooms have been known to possess a large number of different secondary metabolites (i.e. lectins, terpenoids, antibiotics and metal chelating agents) which may play an important role in immune function of the host and hence could be used in immunotherapy of several pathological states (1).

Conclusions:

The immunotherapeutic properties in mushroom nutrition are due to the delivery of:

i) protein-bound polysacharide complexes responsible for immunoenhancing and anti-tumour activities;

ii) enzymes that both prevent oxidative stress and inhibit cell growth.

iii) secondary metabolites involved in several biological processes.

Further research is required to confirm whether mushroom nutrition delivers the enzymes and secondary metabolites, with "absorption" in the lower intestine or whether the presence of the enzyme activity and secondary metabolites in the digestive tract provokes a "sympathetic response" by body, providing the same therapeutic impact.

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