



Mycology News

Volume 1, Edition 7

€ 2.90 \$ 3.50 £ 2.40

Welcome to the seventh issue of Mycology News, a newsletter for health care professionals dedicated to the dissemination of information on the clinical use of mushroom-nutrition. In this edition, we present the results of Dr. Julian Kenyon's recent clinical study on the use of *Coriolus versicolor* supplementation in Stage III and Stage IV cancer patients and look at the potential importance of enzymes in mushroom nutrition, as well as reporting on the possible application of mushroom nutrition in inflammatory bowel disorders.



Mushroom Nutrition Comes of Age in the West

With the availability for the first time of significant clinical studies carried out in the West, such as those by Dr Julian Kenyon published in this issue and Dr Jean Monro ⁽¹⁾, as well as increasing amounts of research on areas such as the role played by enzymes in mushroom nutrition, the clinical importance of mushroom nutrition in the West is becoming ever clearer.

No other group of natural substances exhibit such a profound effect on the health and balance of the immune system and thus the body's ability to maintain homeostasis in the face of multiple challenges.

This immuno-modulatory action is perhaps best understood in terms of the impact of mushroom nutrition on the balance between the cellular and humoral immune responses. As these two arms of the immune system are mutually inhibitory, through the actions of cytokines produced by the so-called TH1 and TH2 cells, a strong TH2 (pro-inflammatory) immune response, such as that induced under conditions of stress or chemical exposure, will suppress the production of TH1 cytokines, which play a vital role in strengthening the cellular immune response and thus the body's ability to defend itself against multiple pathogens, including bacteria, viruses, fungi and carcinogenic agents.

Factors such as stress and chemical exposure thus weaken our body's ability to defend itself, not through impairing the cellular immune response (the ability of the body to recognize and destroy foreign bodies) per se but through leading to a chronic elevation of the humoral immune response, which normally predominates in cases of local wound healing.

This chronically elevated pro-inflammatory immune response is termed a TH2 immune state and it is in the quest for effective agents to restore the normal balance between the TH1 and TH2 immune states that it is now clear that mushroom nutrition has a significant contribution to make in terms of efficacy, cost and safety.



(1) See Mycology News 5 for background on reversing TH1 to TH2 shifts with *Coriolus versicolor* nutrition in Chronic Fatigue Syndrome patients by Dr. Jean Monro - Breakspear Hospital-Tel:44-1442-261-333.

TH1 vs. TH2 Immune responses

Cytokine TH1 Immune Response		Cytokine TH2 Immune Response
Cellular Immune response	Type	Humoral Immune response
10:00 to 20:00	Timing	20:00 to 10:00
Anti-Viral Anti Bacterial Anti-Parasitic	Function	Pro-inflammatory
Interleukin 2 (IL2) Interleukin 12 (IL12) Interferon Gamma (INF)	Cytokines	Interleukin 4 (IL4) Interleukin 6 (IL6) Interleukin 10 (IL10)
Low	Cortisol Levels	High
High	Natural Killer Cell Activity	Low

OBSERVATIONAL NON-CONTROLLED STUDY OF THE USE OF CORIOLUS VERSICOLOR SUPPLEMENTATION IN 30 CANCER PATIENTS

Dr. Julian Kenyon (MD, MB ChB)*



**Dr. Kenyon was the founder and Chairman of the British Medical Acupuncture Society, and founder of the Centre for the Study of Complementary Medicine. He runs the Dove Clinic for Integrated Medicine in London and Twyford, Tel: 44-1962-718000 (www.doveclinic.com)*

INTRODUCTION

PSP, a polysaccharopeptide obtained from cultivated mycelia of the mushroom *Coriolus versicolor*, is a biological response modifier capable of showing diverse biological activities. It is a chemically homogenous substance possessing a molecular weight of approximately 100 kilodaltons. PSP is composed of 90% polysaccharides and 10% peptides. In addition to glucose, its polysaccharide constituents consist of five other sugars including arabinose, galactose, mannose, rhamnose and xylose. The polypeptide constituents contain more than twenty different amino acids, notably aspartic and glutamic acids. PSP exhibits immunomodulatory and anti-tumour activities with low cytotoxicity. It has been used in Asia, particularly in China, as an adjuvant in the clinical treatment of cancer to boost the immunological status of patients undergoing chemotherapy and/or radiotherapy. In addition, PSP exhibits analgesic, anti-viral and hepato-protective effects.

Cancer is the result of changes in key regulatory genes which control cell proliferation, differentiation and survival. Cancer development is a complex multi-step process in which normal cells gradually progress to malignancy. Both the activation of the oncogenes and the inactivity of the tumour suppresser genes are critical steps in tumour initiation and progression. The failure of cancer cells to undergo programmed cell death, known as apoptosis, is a critical factor in the development of tumours. The immune response mounted by the body is of major importance in preventing this process happening, or if it has happened, moving it towards the direction of normal apoptosis. (Rudin C M & Thompson C B 1997).

The activity of the immune system is firstly non-specific, mediated by natural killer cells, and secondly tumour antigen specific by mounting a cell mediated immune response known as a thymic helper cell one (TH1) response. Most commonly cancer patients mount a marked thymic helper cell two (humoral immunity), a so-called TH2 response, which involves the production of large quantities of antibodies (Kenyon 2001, Gotos et al 1999).

TH1 cells produce one set of cytokines whilst the TH2 cells produce another set of cytokines. The cytokines produced by the two cell groups both influence the anti-cancer defence mechanism in a different way. Amongst the cytokines produced by the TH1 cells there is Tumour Necrosis Factor Beta, which is known for its ability to destroy cancer cells. However, if the TH1 response is suppressed, Tumour Necrosis Factor Beta can be produced by natural killer cells. An effective anti-tumour response is a cell mediated TH1 immune response. If the TH2 humoral response is excessively activated then a set of cytokines, amongst which is Interleukin 5, will be produced and these can negatively affect the anti-cancer defence mechanism either directly, or indirectly. A recent study has shown that medium and high cytotoxic activity of peripheral blood lymphocytes (mediated by TH1 lymphocytes and also natural killer cells in a non-specific way), is associated with reduced cancer risk. Whereas low

activity is associated with increased cancer risk, suggesting a role for natural immunological host defence mechanisms against cancer (Kazue Imai et al 2000).

Telomerase is a ribonucleo protein polymerase enzyme whose function is to maintain the essential genetic element of telomeres, the eukaryotic ends of chromosomes. Telomerase activity becomes suppressed in the ageing process, but activation of telomerase is regarded as essential to most cancers. This means that there is a specific association of human telomerase activity with cancer and usually it is high in cancer patients.

There is clinical evidence to show that patients' who have tumours that do not display telomerase activity are likely to eliminate the cancer, quite often spontaneously. It is considered that the repression of telomerase activity could be one of the mechanisms for cancer regression (Shay J W & Wright W E 1998).

Several mushrooms are available for medical use at the present time. More than 50 mushroom species exhibit anti-cancer activity in-vitro, or in animal models, and of these, 6 have been investigated in human cancers. All are non-toxic and very well tolerated. Two proteoglycans from *Coriolus versicolor*-PSK (Polysaccharide/K) and PSP (Polysaccharide-peptide) – have demonstrated the most promise. Both have been subject to Phase II and Phase III trials in China, and PSP significantly extended 5 year survival in oesophageal cancer. PSP also significantly improved quality of life, provided substantial pain relief and enhanced immune status in 70-97% of patients with cancers of the stomach, oesophagus, lung, ovary and cervix. PSK and PSP boosted immune cell production, ameliorated chemotherapy symptoms, and enhanced tumour infiltration by dendritic and cytotoxic T-cells. They have extremely high tolerability, proven benefits to survival and quality of life, and their compatibility to chemotherapy and radiation therapy makes them well suited for cancer management regimens (Kidd, P.M. "The use of mushroom glucans and proteoglycans in cancer treatment". P.M. *Altern Med Red* 2000; 5 (1): 4-27).

MATERIALS & METHODS

A biomass powder of *Coriolus versicolor* was chosen as it has a significantly higher content of PSP's compared to other mushroom preparations, specifically the biomass equivalents of *Grifola frondosa* (Maitake), *Ganoderma lucidium* (Reishi) and *Cordyceps sinensis*. This biomass form of *Coriolus versicolor* also has significantly greater peroxidase activity than biomass equivalents of *Grifola frondosa* and *Ganoderma lucidium* specifically. Lastly, it has higher beta-glucanase activity than *Grifola frondosa* (Maitake) and *Cordyceps sinensis* as well as increased glucose 2-oxidase activity (Karmali A, Instituto Superior de Engenharia de Lisboa (ISEL), Lisbon Portugal 2002).

The biomass powder contained the mycelium and the primordia (young fruitbody) of *Coriolus versicolor*, grown on a sterile substrate.

Condition	Patient Numbers	Secondary Tumors	Number of Secondaries
Hodgkin's Lymphoma	1	None	0
Prostate Cancer	8	Bone	6
Bowel Carcinoma	10	Liver	9
Breast Cancer	8	Mestastisis	8
Died During Study	3	Not Applicable	0
Total		30	23

TABLE I: PATIENTS BY TUMOUR TYPE AND NUMBER OF SECONDARY TUMORS

The biomass powder was then manufactured, into 500 mg tablets under Pharmaceutical GMP standards in the United Kingdom.

Thirty (30) patients were observed from the author's clinical practice. They had a variety of solid tumours, mostly stage 3 or stage 4. The breakdown by tumour type is given in Table I:

Interleukin 5, interleukin 12 (both at gene expression level), tumour necrosis factor beta (also at gene expression level) and telomerase were recorded at day 0, day 60 and day 120.

The supplementation schedule for *Coriolus versicolor* was three tablets (500 mg each tablet) three times a day (9 tablets) for the first month (4.5 grams per day), six tablets three times a day (18 tablets) for the second month (9.0 grams per day) and nine tablets three times a day (27 tablets) for the third and fourth (13.5 grams grams per day). Supplementation intake was 30 minutes prior to meals.

See table II below: ⁽¹⁾

Table II <i>Coriolus versicolor</i> Supplementation Schedule		
	Grams per day	Tablets Per day
1 Month 1	4.5	9
2 Month 2	9.0	18
3 Month 3	13.5	27
4 Month 4	13.5	27

(1) *Coriolus versicolor* was supplied by Mycology Research Laboratories Ltd., Brough, United Kingdom (<http://www.mycologyresearch.com> or <http://www.aneid.pt>)

RESULTS

Of the group of 30 patients who were selected to enter this study, 3 patients have died. This therefore leaves us with 27 study results.

Based on the aforementioned four immunological parameters, over the 120 day supplementation period, the average change in Interleukin 5, interleukin 12 (both at gene expression level), tumour necrosis factor beta (also at gene expression level) and telomerase was encouraging.

The summary of results based on the four (4) recorded immunological parameters of the 27 patients is outlined in Table III.

DISCUSSION

These results show a significant drop in telomerase activity (-75.9%) in the group with the exception of four cases (Patients 3, 8, 11 and 15). The average decrease in Interleukin 5 was -80.1%, and the majority showed an increase in Interleukin 12 (111.7%) and slight increase in tumour necrosis factor beta (14.2%). This shows that there is a general move towards a TH1 immune response in the majority of cases studied here.

It is suggested that, In order to provide for greater ease of use, the use of tablet presentations of *Coriolus versicolor* should be replaced with a powder presentation in months 3 and 4 (13.5 gram per day).

CONCLUSION

This observational study on the use of *Coriolus versicolor* shows that there appears to be a differentiating effect on cancer cells by lowering telomerase activity and encouragement of an immune function move towards a cell mediated TH1 immune response, which is a more effective anti-tumour response. This is indeed remarkable, as the majority of these cases were stage 3 and 4 cancers, many of them chemotherapy and radiotherapy failures.

The use of *Coriolus versicolor* supplementation as adjuvant nutritional therapy to support the immune system in Stage 3 and 4 cancer patients should be further studied.

Table III: Summary of Results**	Day 0	Day 60	Day 120	Average Change
1 Telomerase	1727	1034	417	-75.9%
2 Interleukin 5 *	22540	28516	4482	-80.1%
3 Interleukin 12 *	13931	20968	29489	111.7%
4 Tumour Necrosis Factor Beta*(1)	27777	26113	31713	14.2%
*Gene Expression Level (1) only 26 data points				

Table IV: Statistical Data

Interleukin 6

0 – 60 days	0.589691
0 – 120 days	0.039371 Significant p< .05
60 – 120 days	0.007972 Significant p< .01

Telomerase

0 – 60 days	0.039307 Significant p< .05
0 – 120 days	1.78E-05 Significant p<.00001
60 – 120 days	0.018498 Significant p< .05

Interleukin 12

0 – 60 days	0.478791
0 – 120 days	0.133005
60 – 120 days	0.439141

0 = 60 days starting value <3001 0.003306

Note: When there is a cut off of evaluation of interleukin 12 using only those patients who had a starting value of less than 3,001, there was a significant increase in production of interleukin 12.

References:

Gotos et al 1999 "Analysis of TH1 and TH2 cytokine production by peripheral blood mononuclear cells as a parameter of immunological dysfunction in advanced cancer patients." Cancer Immunol.Immunother.48:435

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Coriolus versicolor

The Use of Coriolus versicolor Supplementation in Inflammatory Bowel Conditions

Pro-inflammatory auto-immune bowel conditions such as Crohn's disease and Ulcerative Colitis are increasingly seen in clinical practice and present particular challenges for practitioners, often being managed with the use of steroids, to the long term detriment of the patient. It is therefore of note that mushroom nutrition may offer a possible approach to auto-immune bowel conditions.

Case Study A: Ulcerative Colitis

A 57 year old woman who had suffered from Ulcerative Colitis for over 2 years presented with pain in the abdomen, mucus and frequent blood in the stool.

Supplementation Schedule: Coriolus-MRL at 6 tablets per day for 14 days-(3 tablets 30 minute prior to breakfast-3 tablets 30 minutes prior to dinner).

Observations: After 2 weeks supplementation with Coriolus-MRL at 6 tablets a day the symptoms had cleared with normal bowel movements. Supplementation was continued for a further 4 weeks and improvement was maintained when last seen at the end of this time.

Practitioner: Mr. Nur Mohammed. Contact point: +44 131 6220058

Case Study B: Crohn's Disease

A 44-year old woman diagnosed with Crohn's with severe abdominal pain and diarrhoea.

Supplementation Schedule: Coriolus-MRL at 6 tablets per day for 28 days (3 tablets 30 minutes prior to breakfast and 3 tablets 30 minutes prior to dinner.) Patients were simultaneously treated with acupuncture during the 28 day period.

Observations: Patient had noted marked reduction in pain and a restoration of normal bowel function.

Practitioner: Mr. Tom Lawrence. Contact point: thomas.lawrence@virgin.net

Discussion: Conditions such as Crohn's and Ulcerative Colitis are further examples of conditions where a chronic TH2 immune state persists, requiring (as with chronic fatigue syndrome (M.E.) and many types of cancer) an agent to encourage the body back to a TH1 pattern. It is likely that the ability of Coriolus versicolor supplementation to encourage a reversal of such a TH1-TH2 immune shift (as demonstrated by Dr. Jean Monro and by Dr. Julian Kenyon), is responsible for Coriolus versicolor's effectiveness in the above cases.

THE USE OF CORIOLUS VERSICOLOR SUPPLEMENTATION IN BREAST CANCER PATIENTS – CASE STUDY



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The Use of Coriolus versicolor supplementation in Breast Cancer Patients - Case Study Submitted to the 4th International Symposium on Mushroom Nutrition, June 14th 2002 at Westminster University.



Paula Boaventura

Background:

Immune enhancement properties of select plants and mushrooms have been studied by Japanese researchers in the 1960's, with the majority of mycological research focused on extracts derived from both *Ganoderma lucidium* (reishi) and *Lentinula edodes* (shiitake).

In the late 1960's, a hot water extract of *Lentinula edodes* (Berk) Sing. edible mushroom, completely inhibited the growth of sarcoma 180 implanted subcutaneously in ICR mice.

From the extract, Professor Goro Chihara isolated and purified a polysaccharide, which showed marked antitumour activity, and named the polysaccharide Lentinan (1). However, Lentinan proved to be too toxic for long term clinical use (2).

It was the search for a mycological extract that had less toxicity and fewer side effects than Lentinan that led researchers at Kureha Chemical Industry Company to focus on the effectiveness of the oral administration of Polyporaceae (one of the Basidiomycetes) on stomach cancer patients.

Kureha screened over 200 species of the fruit bodies of the Basidiomycetes for their antitumor activity against various tumour cells, including sarcoma 180, and found several promising Polyporaceae strains (3). Among these strains *Coriolus versicolor* (Fr.) Quel (kawaratake), was considered to be the most suitable for further fractionation due to its high antitumour activity and stability during serial cultivation (4)

Immune Parameter Recordings

Extracts of cultured mycelia of *Coriolus versicolor* demonstrated antitumour activity comparable to that of the fruitbody. In 1971, the active principle was precipitated from extracts of cultured hyphae of *Coriolus versicolor* (Fr.) Quel (CM-101 strain) with saturated ammonium sulfate, desalted and named PSK or Krestin (5). PSK has been reported to induce host-mediated antitumor activity (6).

Aim of Study

To assess the efficacy of non-fractionalized *Coriolus versicolor* supplementation in supporting the immune system of patient diagnosed with breast cancer. The principal parameters being a set of (four) immune parameters taken at baseline, 50 days and 90 days after initiation of *Coriolus* supplementation.

Subject

Dr. I, aged 57, profession medical doctor. Diagnosed with breast cancer and began chemotherapy on April 18th, 2001. She underwent 12 sessions from July to September of 2001. Chemotherapy was then repeated from December 2001 to February 7th 2002 for pulmonary metastasis.

Supplementation Schedule

On January, 2002, non-fractionalized *Coriolus versicolor* supplementation was provided to support the immune system. Patient I began with 4.5 grams per day of non-extracted *Coriolus versicolor* for 30 days, followed by 3.0 grams per day for 20 days, followed by 4.5 grams per day for an additional 30 days.

Patient Dr. I			
Testing Dates	Jan 1 2002	Mar 1 2002	April 4 2002
Supplementation Period	Baseline	50 days after	90 days after
1 Total Leukocyte Count (x 10 ⁶ /ml)	1.63	4.07	6.39
2 % Linf	58.5% (950/ul)	18.4% (748/ul)	17% (1086/ul)
3 % linf CD3-CD56	9% (85/ul)	17.3% (129/ul)	12.0% (130/ul)
4 Proportion of NK cells (mature)/NK cells ++ (imature)	92.4%/ 7.6%	90.8% /9.2%	59% /11.0%
5 Supplementation Level of <i>Coriolus versicolor</i>	9 tablets/day	6 tablets/day	9 tablets/day
6 Hemoglobin			13.6
7 Hematocrit			39%

continued...

OBSERVATIONS:

After 50 days of Coriolus supplementation, Patient I felt better and physically looked better. After taking 90 days of Coriolus supplementation, Patient I was happy with progress and stopped iron supplementation during chemotherapy sessions.

CONCLUSION

While this is only one case, there is enough evidence to suggest a curiosity does exist and that further open label clinical development should be carried out to confirm the immune supportive function of Coriolus versicolor nutrition in patients diagnosed with breast cancer. In future studies, it is suggested that the Coriolus supplementation schedule be maintained at 4.5 grams per day.

(1) "Medical Aspects of Lentinan Isolated From Lentinus Edodes (Berk) Sing" - Goro Chihara, Biotechnology Research Centre, Teikyo University, Nogawa 907, Miyamae-ku, Kawasaki 213, Japan. Chapter 27-Mushroom Biology and Mushroom Products-Precedings of the Second International Conference-University Park, Pennsylvania June 9-12, 1996. Edited by D.J. Royce.

(2) Translation of "Cancer Immunotherapy 1977" -Takeo Mori, Tadaaki Sakai, Ichiji Itoh, Tokyo Metropolitan Komagome Hospital, Published by Life Science August 5th, 1977.

(3) "Diverse Biological Activity of PSK (Krestin), A Protein-Bound Polysaccharide from Coriolus versicolor (Fr.) Quel-Hiroshi Sakagami and Minoru Takeda-First Department of Biochemistry, School of Medicine, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142 Japan, Page 237 Chapter 25-Mushroom Biology and Mushroom Products-Precedings of the Second International Conference-University Park, Pennsylvania June 9-12, 1996, Edited by D.J. Royce.

(4) Ibid Page 237

(5) Ibid Page 237

(6) Ibid Page 237

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

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The use of mushroom nutrition as part of the nutritional management of cancer patients is standard practice in Japan and other Asian cultures. Recently, there have been a number of studies looking at the nutritional benefits of fungi in enhancing immune function (1).

Several researchers have demonstrated that protein-bound polysaccharide complexes such as PSK or PSP, derived from *Coriolus versicolor*, or Lentinan, derived from *Lentinula edodes* are the most important component responsible for the immune enhancing and anti-tumour activities of these fungi. However, other components of fungi also play an important part in their immuno-modulatory activity. For instance a 10KD peptide from *Coriolus* has been shown to mimic the activity of Superoxide dismutase, thus helping reduce oxidative stress.

It has been known for some time that enzyme therapy has important benefits for several clinical conditions, including cancer and

cardiovascular disorders (5, 6). From the data presented below it is apparent that mushrooms are particularly rich sources of enzymes which may participate in these conditions through reduction in oxidative stress and inhibition of cell proliferation.

We measured the enzyme content of *Coriolus versicolor*, *Cordyceps sinensis*, *Ganoderma lucidium* (Reishi) and *Grifola frondosa* (Maitake), both in whole mushroom extract and in the presence of pepsin (at pH2, 37°C for 30 min.) and trypsin (at pH 7.6, 37°C for 30 min.) in order to simulate conditions in the digestive tract. The results showed that in the simulated intestinal tract conditions there is a 10-20% decrease in enzyme levels, except in the case of glucose 2-oxidase, which pepsin reduces by about 50% while trypsin has no effect.

TABLE 1- Analysis of Enzyme, Protein and Sugar per tablet of MRL Product in the absence of proteolytic enzymes

	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	-----	-----

*The presence of reducing sugars is due to the use of maltodextrin in the manufacturing process.

TABLE 2 - Analysis of Enzyme, Protein and Sugar per tablet of MRL Product in the presence of Pepsin

	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	-----

*The presence of reducing sugars is due to the use of maltodextrin in the manufacturing process.

TABLE 3:
Analysis of Enzyme, Protein and Sugar per tablet of MRL Product in the presence of trypsin.

	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	-----

*The presence of reducing sugars is due to the use of maltodextrin in the manufacturing process

a) Laccase (benzenediol:oxygen oxidoreductase; EC 1.10.3.2) is present in active form and catalyses the reduction of di-oxygen 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 cinnabaric acid (CA) which is of great clinical interest because 3-HAA is produced in large quantities by interferon- γ primed mononuclear phagocytes (7). Furthermore, 3-HAA has been shown to act as a powerful scavenger of reactive oxygen species. On the other hand, cinnabaric 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.

This enzyme also plays an important role in the biodegradation of environmental pollutants including the 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. This enzyme plays an important role in the clinical diagnosis of diabetes as well as in the production of fine chemicals and antibiotics (i.e. corticosterone).

c) Peroxidases (EC 1.11.1.7) are a family of isoenzymes produced during 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 into several end-products. There is great interest in these enzymes because they can be used in the detoxification of a broad range of environmental pollutants including PCBs and dioxins.

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

In addition to protein bound polysaccharides and enzymes, mushrooms have been shown to possess a large number of secondary metabolites (i.e. lectins, terpenoids, antibiotics and metal chelating agents), which may play an important role in the immune function of the host and hence could be used in immunotherapy of several disease states (1).

Conclusions:

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

- protein-bound polysaccharide complexes responsible for immune enhancement and anti-tumour activity.
- enzymes that both prevent oxidative stress and inhibit cell growth.
- secondary metabolites involved in several biological processes.

Further research is required to determine whether the enzymes and secondary metabolites are actually absorbed in the lower intestine or whether the presence of the enzyme activity and secondary metabolites in the digestive tract provokes a "sympathetic response" by immune tissue in the bowel, providing the same therapeutic impact.

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- Background on Professor Karmali**
1980 BSc.Hons. Biochemistry - Polytechnic of North London, 1983 Ph.D Biochemistry (Enzymology), King's College London, 2001 Professor - Enzyme Technology, Evora University, Portugal.
- In terms of publications focused on mycology, Professor Karmali's publications have included:
- One-Step Purification and Properties of Glucose 2-Oxidase from *Coriolus versicolor* - P. Oliveira, A Karmali, A Clemente-JBC, (1996), Vol. 1, pp.273-283
 - Chromatographic Behaviour of Glucose 1-and 2-Oxidase from fungal strains on immobilized metal chelates-V Pacheco and A. Karmali-Journal of Industrial Microbiology & Biotechnology (1998) 21, 57-64
 - Glucose 1- and 2-Oxidases from Fungal Strains:Isolation and Production of Monoclonal Antibodies-Amin Karmali & Paulo Oliveira-Journal of Biotechnology 69 (1999) 151-162.