CONFIDENTIAL

NURILAN: AGARICUS BLAZEI MURILL (ABM) CLAIMS SUBSTANTIATION

June 2007

Food or Food Component	Health Relationship	Conditions (if any)	Nature of evidence	Grade of evidence	References*	Example of wording
Agaricus Blazei Murill (ABM)	Immune health	Minimum amount to achieve health effect: 2 grams dried Agaricus Blazei Murill per day *Product should be equivalent to this amount.*	Individual study	Passclaim 3	1-7	Support the immune response. Supports the human complement system Support the immune system. Helps to maintain an active immune system ABM is rich in beta-glucans that contributes to the immune activity

Reference List

No.	Reference
1	Cristina Lull, Harry J. Wichers and Huub Savelkoul. Antinflammatory and Immunomodulating Properties of Fungal Metabolites. Mediators of Inflammation 2005:2 (2005) 63–80.
2	S. Shimizu, H. Kitada, H. Yokota, J. Yamakawa, T. Murayama, K. Sugiyama, H. Izumi, and N. Yamaguchi. Activation of the alternative complement pathway by Agaricus blazei Murill. Phytomedicine 9: 536–545, 2002
3	Kasai H, He LM, Kawamura M, Yang PT, Deng XW, Munkanta M, Yamashita A, Terunuma H, Hirama M, Horiuchi I, Natori T, Koga T, Amano Y, Yamaguchi N, Ito M. IL-12 Production Induced by Agaricus blazei Fraction H (ABH) Involves Toll-like Receptor, Evid Based Complement Alternat Med 2004 Dec;1(3):259-267.
4	Uchiyama Shoji, Use of Agaricus Blazei Murill to prevent or treat skin and other disorders, World Patent Application WO 02/15917 A1.
5	F. Firenzuoli, L. Gori and G. Lombardo, The Medicinal Mushroom Agaricus blazei Murrill: Review of Literature and Pharmaco-Toxicological Problems. eCAM Advance Access published March 27, 2007.
6	Ying Liu, Yasushi Fukuwatari, Ko Okumura, Kazuyoshi Takeda, Ken-ichi Ishibashi, Mai Furukawa, Naohito Ohno, Kazu Mori, Ming Gao and Masuro Motoi, Immunomodulating Activity of Agaricus brasiliensis KA21 in Mice and in Human Volunteers. eCAM Advance Access published April 12, 2007
7	Tjakko Stijve, Maria Angela L. De A. Amazonas, AGARICUS BLAZEI MURRILL - A NEW GOURMET AND MEDICINAL MUSHROOM FROM BRAZIL. Australasian Mycologist 21 (1) 2002: report. **Starting at page 29 of the document (reference 7)**

Summaries of studies

Reference 1	**Individual Study**
Authors	Cristina Lull, Harry J. Wichers and Huub Savelkoul.
Title of study	Antinflammatory and Immunomodulating Properties of Fungal Metabolites.
Journal Reference	Mediators of Inflammation • 2005:2 (2005) 63–80.
Aim of the study	Discussion of current information on the ability of extracts and isolated metabolites from mushrooms to modulate immune responses.
Results	Water extracts of the mycelia culture and fruiting bodies of Agaricus blazei Murill induced TNF-α secretion by macrophages derived from rat bone marrow. Fractions B-4 and B-5 obtained from ethanol precipitation of fruiting bodies markedly induced TNF-α secretion. Similar effects were observed in IL-8 secretion by macrophages. Regarding NO, fraction B-5 induced a significant increase in NO secretion and fractions B-4 and B-6 slightly induced NO secretion. Northern blot analysis showed that the increases in cytokine and NO secretion were due to an increase in cytokine mRNAs or NO synthase mRNA [65]. **Thus A blazei Murill contains certain components which activate macrophages contributing to the immune response in vitro.**

Reference 2	**Individual Study**
Authors	S. Shimizu, H. Kitada, H. Yokota, J. Yamakawa, T. Murayama, K. Sugiyama, H. Izumi, and N. Yamaguchi.
Title of study	Activation of the alternative complement pathway by Agaricus blazei Murill
Journal Reference	Phytomedicine 9: 536–545, 2002
Aim of the study	The study was designed to evaluate the effect of A. blazei Murill upon activation of the complement system in human serum in vitro. Additional studies were performed to determine the cytotoxic effect of complement-opsonized particles of A. blazei Murill against human tumor cells in culture.
Definitions	The alternative pathway of the complement system is a humoral component of the immune system's natural defence against infections which can operate without antibody participation.
Results	A fine particle of A. blazei Murill (ABP), prepared by mechanical disruption, was used throughout the experiments. **ABP activated the human complement system via the alternative pathway in human serum.** Activation of the alternative pathway was both time- and dose-dependent. When the particles from fruiting bodies of A. blazei Murill (ABP-F) were reacted with human serum, the formation of complement-opsonized ABP, iC3b-ABP-F complexes, and binding of the complexes to human peripheral blood monocytes, were demonstrated in vitro by immunofluorescence. Further, the resident human peripheral nucleated cells incubated in the presence of iC3b-ABP-F complexes inhibited the proliferation of human tumor cell line TPC-1 in vitro.

Reference 3	**Individual Study**
Authors	Kasai H, He LM, Kawamura M, Yang PT, Deng XW, Munkanta M, Yamashita A, Terunuma H, Hirama M, Horiuchi I, Natori T, Koga T, Amano Y, Yamaguchi N, Ito M.
Title of study	IL-12 Production Induced by Agaricus blazei Fraction H (ABH) Involves Toll-like Receptor
Journal Reference	Evid Based Complement Alternat Med 2004 Dec;1(3):259-267.
Aim of the study	Agaricus blazei Murill is an edible fungus used in traditional medicine, which has various well documented medicinal properties. In the present study, we investigated the effects of hemicellulase derived mycelia extract (Agaricus blazei fraction H: ABH) on the immune system. First, we examined the cytokine-inducing activity of ABH on human peripheral mononuclear cells (PBMC).
Results	human tumor cell line in vitro (38). These reports indicated that components of A.blazei have various activities. **It was reported that A.blazei extract acts mainly through modulation of the immune system, activating macrophages, neutrophils and lymphocytes (3,4,8).** The results indicated that ABH induced expression of IL-12, a cytokine known to be a critical regulator of cellular immune responses. Flow cytometric analysis demonstrated the induction of IL-12 production by the CD14-positive cell population, consisting of monocytes/macrophages (Mo/M□). Furthermore, the elimination of Mo/M□ attenuated IL-12 production in PBMC. ABH-induced IL-12 production was inhibited by anti-CD14 and anti-TLR4 antibodies but not by anti-TLR2 antibody. The activity of ABH was not inhibited by polymyxin B, while the activity of lipopolysaccharide used as a reference was inhibited. Oral administration of ABH enhanced natural killer (NK) activity in the spleen. These findings suggest that ABH activated Mo/M□ in a manner dependent on CD14/TLR4 and NK activity.

Reference 4	**Individual Study**
Authors	Uchiyama, Shoji; (Tokyo, JP) ; Haramaki, Kenji; (Palos Verdes Estates, CA).
Title of study	Use of Agaricus blazei murill to prevent or treat skin and other disorders
Journal Reference	United States Patent Application
Aim of the patent	Agaricus blazei in whole, particulate, or extracted form, are useful as a barrier when applied to the skin against harmful effects of environmental toxins, pollution, chemicals, and radiation. Taken internally, whole, particulate, or extracted Agaricus blazei offer protection from various disorders such as autoimmune disorders, and alone or in conjunction with other therapies, can be beneficial to treat such disorders.
Results	As with the topical compositions, additional appropriate active and inactive ingredients may be used, such as herbal extracts and/or vitamins such as vitamin C. **Appropriate dosages for maximum therapeutic benefit are preferably equivalent to an amount extracted from between about 2 g to about 10 g of dried Agaricus blazei per day.** Larger or smaller doses can be taken as well without adverse affect. The extract can be taken daily in one or more doses, but a preferred regimen of dosing is a 10 to 30 day course of therapy, preferably with intervals of between 1-10 days on the extract followed by 1-10 days off of the extract.

Reference 5	**Individual Study**
Authors	F. Firenzuoli, L. Gori and G. Lombardo
Title of study	The Medicinal Mushroom Agaricus blazei Murrill: Review of Literature and Pharmaco-Toxicological Problems.
Journal Reference	eCAM Advance Access published March 27, 2007
Aim of the study	Aim this study is to make a review of Literature and Pharmaco-Toxicological Problems.
Results	Careful clinical studies comparing the activity of isolated compounds, whole mushroom extracts and epidemiological data are still necessary to determine whether ABM provide real clinical benefits. Dose-response studies and isolation, as well as chemical identification and quantification of specific compounds responsible for the potential benefit from ABM mushroom ingestion should be fully developed, **although there seems to be clear evidence that ABM extracts are rich in b-glucans that presumably contribute to the observed immunostimulatory activity**. Other substances are probably involved as well, the immunostimulation following ingestion of polysaccharides is possible and probably useful in cancer patients if it does not give rise to pharmacological interferences. A main safety concern is represented by the toxicity and cancerogenicity of agaritine and its derivatives that should be completely evaluated; and probably would be useful for these mushrooms like other herbal remedies, to completely define the problem of heavy metal contents. Due to the large consumption of ABM in popular medicine, probably more data are needed on action mechanisms of its component and safety before counselling the assumption for prevention and treatment of cancer and immunodepressive disorders.

Reference 6	**Individual Study**
Authors	Ying Liu, Yasushi Fukuwatari, Ko Okumura, Kazuyoshi Takeda, Ken-ichi Ishibashi, Mai Furukawa, Naohito Ohno, Kazu Mori, Ming Gao and Masuro Motoi
Title of study	Immunomodulating Activity of Agaricus brasiliensis KA21 in Mice and in Human Volunteers
Journal Reference	eCAM Advance Access published April 12, 2007
Aim of the study	Aim of the study was to evaluate the immunoenhancing effects of the naturally outdoor-cultivated fruit body of Agaricus brasiliensis KA21 (i.e. Agaricus blazei).
Results	In clinical research on human volunteers (3 grams of dried A. brasiliensis per day, we found that A. brasiliensis decreased body weight, BMI, percentage body fat, percentage visceral fat and blood glucose level significantly, and reduced obesity. It also decreased blood cholesterol level and neutral fat level, normalized liver function and **activated the immune function in mibyou patients (people with poor health)**. In the absence of epidemiological data, no evaluation of carcinogenicity of agaritine to humans could be made.

Reference 7	**Individual Study**
Authors	Tjakko Stijve, Maria Angela L. De A. Amazonas
Title of study	AGARICUS BLAZEI MURRILL - A NEW GOURMET AND MEDICINAL MUSHROOM FROM BRAZIL
Journal Reference	Australasian Mycologist 21 (1) 2002: report
Aim of the study	Evaluation of the history and traditional use of ABM and projecting the potential of it to European markets.
Results	In 1945 the American mycologist W.A. Murrill discovered an unknown representative of the genus Agaricus on the lawn of his friend R.W. Blaze, who lived in Gainsville, Florida. In honour of his friend, he described the new species as Agaricus blazei in a rather obscure scientific journal. For years, this new mushroom which is unknown in Europe and far from common in North America remains in the dark until it is rediscovered in the 1960s by Japanese coffee growers working in Brazil. It is told that one of them, the scientist Takatoshi Furumoto is intrigued by the observation that the inhabitants of the Piedade/Ibiuna district suffer far less from geriatric afflictions than the rest of the Brazilian population. When investigating the causes of this phenomenon, he discovers that the Piedade people regularly consume a kind of mushroom that is unknown elsewhere. The story is probably a latter date fabrication to render the healing powers of this mushroom more plausible. In reality, the inhabitants of Piedade have never eaten this mushroom, which is, today, not even common in their area. Furumoto was rather captivated by its excellent organoleptic properties which reminded him of the famous Matsutake, a delicious edible but rare mushroom in Japan. He therefore sent samples of the Brazilian mushroom to several Japanese universities, and he also consulted the well-known Belgian agaricologist, Dr Paul Heinemann, who identified the species as A. blazei Murrill. As already mentioned, **A. blazei contains high levels of beta glucans, immunostimulating polysaccharides** which are selectively cytotoxic on tumor cells. Consequently, many companies on the Internet advertise and sell not only the dried mushroom, but also preparations containing enriched fractions of the active principles. The accompanying publicity often exaggerates their healing powers, but its beneficial action in the treatment of various forms of cancer, arteriosclerosis, diabetes and chronic hepatitis seems to have been well established by clinical research. **In Japanese pharmacies one already finds a whole array of medicinal drugs based on mushrooms**.