EVP-2008-0014 MA1

SIXTH INTERNATIONAL CONFERENCE ON MUSHROOM BIOLOGY AND MUSHROOM PRODUCTS

29th September – 3rd October, 2008

BONN, GERMANY

Organizers

World Society for Mushroom Biology and Mushroom Products (WSMBMP)

Gesellschaft für angewandte Mykologie u.Umweltstudien (GAMU) GmbH

Co-organized by

Tourismus & Congress GmbH Region Bonn / Rhein-Sieg / Ahrweiler

Bund Deutscher Champignon- und Kulturpilzanbauer e.V. (BDC)

Supported by

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SCIENTIFIC PROGRAMME

Programme at a glance

Time	29/09 Monday	30/09 Tuesday	01/10 Wednesday	02/10 hursday	03/10 Friday
08.30 – 10.00		Registration (From 08.30) Opening Ceremony (09.00-10.00)	Plenary Session III Genetics & Breeding (GB) Plenary Session IV Integrated Pest Management (IPM) Oral Presentations Section III (GB) Section IV (IPM)		Plenary Session VII Mushroon Resource Development (MRD) Plenary Session VIII Environmental Implications and Applications (EIA) Oral Presentations Section VII (MRD) Section VIII (EIA)
10.00 – 10.30		Coffee break	Coffee break		Coffee break
10.30 – 12.00		Keynote Lecture (I) (10.30-11.15) Keynote Lecture (II) (11.15-12.00)	Oral Presentations (cont) Section III (GB) Section IV (IPM) Workshop #2	Excursion	Oral Presentations (cont) Section VII (MRD) Section VIII (EIA) Workshop #3
12.00 – 14.00		Lunch	Lunch		Lunch
14:00 – 15.00 15:00 - 15:30	Registration	Plenary Session I Physiology & Biochemistry (PB) Plenary Session II Cultivation Technology (CT) Oral Presentations Section I (PB) Section II (CT)	Plenary Session V Nutritional & Medicinal Aspects (NMA) Plenary SessionVI Safety, Quality Control and Regulational Aspects (SQCR) Oral Presentations Section V (NMA) Section VI (SQCR)		Plenary Session IX Mycorrhizal Mushrooms and Mycorrhization (MMM) Plenary Session X Economics and Marketing (EM) Oral Presentations Section IX (MMM) Section X (EM)
15.30 – 16.00		Tea break	Tea break		
16.00 – 18.00		Oral Presentations (cont) Section I (PB) Section II (CT) Workshop #1	Oral Presentations (cont) Section V (NMA) Section VI (SQCR)		Tea break (16.00-16.30) Closing Ceremony (16.45-17.30)
19.00	Welcome reception	River Rhine cruise (19.30)	Poster Session (18.00-19.30)		Conference Banquet

30th September, 2008, Tuesday (Morning)

Foyer

08:30-18:00 Registration/Information

Theatre

09:00-10:00 **OPENING CEREMONY**

Introductory music by the quartet of the Collegium Musicum of the University of Bonn

Welcoming address:	Professor D. J. Royse, President, World Society for Mushroom Biology and Mushroom Products
Welcoming address:	Mr Ulrich Hauschild, Deputy Mayor, City of Bonn
Welcoming address:	Mr Peter Hettlich, Member, German Federal Parliament
Message:	Mr Franz Schmaus, President, German Mushroom Growers Association
Opening address:	Professor J. I. Lelley, Chairman, Conference Organizing Committee

10:00-10:30 Coffee

10:30-11:15 Keynote lecture

11:15-12:00 Keynote lecture

12:00-14:00 Lunch

KEYNOTE LECTURES

SIXTH INTERNATIONAL CONFERENCE ON MUSHROOM BIOLOGY AND MUSHROOM PRODUCTS

29th September – 3rd October, 2008

BONN, GERMANY

PROGRAMME AND ABSTRACTS

Organizers

World Society for Mushroom Biology and Mushroom Products (WSMBMP)

and

Gesellschaft für angewandte Mykologie u.Umweltstudien (GAMU)

KEYNOTE LECTURE: K-1

30th September, 2008; 10:30-11:15. Theatre

"Mushrooms, Cause and Cure"

Professor Dr L.J.L.D van Griensven (The Netherlands)

Chairman: Professor D.J. Royse (University Park, USA)

KEYNOTE LECTURE: K-2

30th September, 2008; 11:15-12:00 Theatre

"Economic Developments in the Mushroom Industry"

Mr L.G.J. van Horen (The Netherlands)

Chairman: Professor H.-P. Molitoris (Regensberg, Germany)

PLENARY SESSIONS

Tuesday, 30th September (Afternoon) **Theatre**

I. Physiology & Biochemistry Chairperson: K. Burton

14:00-14:30	PL-1-1	Reactive oxygen species and the strategy of antioxidant defence in mushrooms J.M. Savoie (France)
14:30-15:00	PL-1-2	The influence of casing and compost moisture on sporophore quality of *Agaricus bisporus* D. Beyer (USA)

Tuesday, 30th September (Afternoon) **Lecture Hall I**

II. Cultivation Technology Chairperson: J.E. Sanchez

14:00-14:30	PL-2-1	Double cropping *Agaricus bisporus* by re-supplementing and re-casing compost D.J. Royse (USA)
14:30-15:00	PL-2-2	Cultivation of *Lentinula edodes* in China Q. Tan (China)

Wednesday, 1st October (Morning) **Theatre**

III. Genetics & Breeding Chairperson: Qi Tan

08:30-09:00	PL-3-1	Mushroom breeding: hurdles and challenges A. S. M. Sonnenberg (The Netherlands)
09:00-09:30	PL-3-2	Molecular genetics of mating system in bipolar mushroom, *Pholiota nameko* T. Aimi (Japan)

Wednesday, 1st October (Morning) **Lecture Hall I**

IV. Integrated Pest Management Chairperson: A. Geösel

08:30-09:00	PL-4-1	Challenges facing mushroom disease control in the 21st Century H. Grogan (Ireland)
09:00-09:30	PL-4-2	Factors affecting spotting and discoloration of the cultivated mushroom, *Agaricus bisporus* D. L. Rinker (Canada)

Wednesday, 1st October (Afternoon) **Theatre**

V. Nutritional & Medicinal Aspects Chairperson: U. Lindequist

14:00-14:30	PL-5-1	Nutritional and medical aspects of ergothioneine--the unique mushroom antioxidant R.B. Beelman (USA)
14:30-15:00	PL-5-2	A translation research to investigate anti-cancer activity of mushrooms S. Chen (USA)

Wednesday, 1st October (Afternoon) **Lecture Hall I**

VI. Safety, Quality Control and Regulational Aspects Chairperson: A.S.M. Sonnenberg

14:00-14:30	PL-6-1	Molecular modelling and modification of mushroom senescence and quality-loss K. Burton (UK)
14:30-15:00	PL-6-2	Standardisation and quality control in the mushroom nutriceutical industry J.A. Buswell (China)

Friday, 3rd October (Morning) **Theatre**

VII. Mushroom Resource Development Chairperson: P. Kalberer

08:30-09:00 PL-7-1 Commercial cultivation of *Lyophyllum shimeji*

K. Yamanaka (Japan)

09:00-09:30 PL-7-2 Organic button mushroom production, quality produce and pesticide residue analysis

B.L. Dhar (India)

Friday, 3rd October (Morning) **Lecture Hall I**

VIII. Environmental Implications and Applications Chairperson: J.A. Buswell

08:30-09:00 PL-8-1 Olive mill waste as a substrate for the cultivation of *Pleurotus* spp. mushrooms

D. Levanon (Israel)

09:00-09:30 PL-8-2 Energy issues related to mushroom production, and the quest for profitable use of spent mushroom compost

P. Oei (The Netherlands)

Friday, 3rd October (Afternoon) **Theatre**

IX. Mycorrhizal Mushrooms and Mycorrhization Chairperson: J.I. Lelley

14:00-14:30 PL-9-1 Problems and perspectives in the production of *Tuber* infected plants

A. Zambonelli (Italy)

14:30-15:00 PL-9-2 Mycorrhizal research applied to experiences in plantations of mycorrhizal mushrooms, especially in Central Europe

Z. Bratek (Hungary)

Friday, 3rd October (Afternoon) **Lecture Hall I**

X. Economics and Marketing Chairperson: D. Levanon

14:00-14:30 PL-10-1 The mushroom media landscape: changes and challenges

R. Dreve (The Netherlands)

14:30-15:00 PL-10-2 Development of Polish mushroom exports

T. Smolenski (Poland)

Theatre

16:30-17:15 **CLOSING CEREMONY**

GENERAL ORAL PRESENTATION SESSIONS

I. PHYSIOLOGY & BIOCHEMISTRY

Tuesday, 30th September **Theatre**

Chairperson: K. Burton

15:00-15:15 O-1-1: GROWTH AND FRUIT BODY FORMATION OF *GANODERMA LUCIDUM, LENTINUS EDODES* AND *PLEUROTUS OSTREATUS* ON MEDIA SUPPLEMENTED WITH SELENIUM
M. Savic, J.Petrovic, A. Klaus, M. Rajkovic, N.Filipovic, S.Antic-Mladenovic and M.Niksic

15:15-15:30 O-1-2: RECOGNITION AND DEGRADATION OF INSOLUBLE CRYSTALLINE CELLULOSE BY *POLYPORUS ARCULARIUS*
Tadanori Aimi[1], Yuka Ohnishi[1], Mitsutoshi Nagase[2], Tsuyoshi Ichiyanagi[1] and Yutaka Kitamoto[3]

Chairperson: T. Aimi

16:00-16:15 O-1-3: SUSCEPTIBILITY OF EUROPEAN POPULATIONS OF *AGARICUS BISPORUS* TO *TRICHODERMA* METABOLITES
J.-M. Savoie, N. Minvielle, S. Rextoueix and P. Callac

16:15-16:30 O-1-4: EXTRACELLULAR ENZYME PRODUCTION OF THE TWO CAUSATIVE AGENTS OF OYSTER MUSHROOM GREEN MOULD UNDER INDUCTIVE AND NON-INDUCTIVE CONDITIONS
L. Kredics, T. Cseh, P. Körmöczi, L. Hatvani, L. Manczinger and C. Vágvölgyi

16:30-16:45 O-1-5: THE USE OF DIRECT DPPH METHOD FOR DETERMINATION OF ANTIOXIDANT CONTENT IN MOREL MUSHROOM
Gander G and Masaphy S

16:45-17:00 O-1-6: ANTIMICROBIAL ACTIVITIES OF FOUR WILD MUSHROOM SPECIES COLLECTED FROM TURKEY
Fatih Kalyoncu and Mustafa Oskay

17:00-17:15 O-1-7: DEGRADATION OF ENDOSULFAN BY *PLEUROTUS* SPP
G.M. Mendoza, J.E. Sánchez, M.G. Nieto, and F.J. Márquez-Rocha

II. CULTIVATION TECHNOLOGY

Tuesday, 30th September **Lecture Hall I**

Chairperson: J.E. Sanchez

15:00-15:15 O-2-1: MUSHROOM COMPOST PRODUCTION – A REVIEW OF INDUSTRY QUALITY ASSURANCE FRAMEWORKS IN IRELAND
Mairead Kilpatrick, H. S. Shekhar Sharma and Gary Lyons

15:15-15:30 O-2-2: EFFECT OF AEROBIC FERMENTATION SUBSTRATE IN THE PRODUCTION OF *PLEUROTUS OSTREATUS* AND ITS RESISTANCE TO *TRICHODERMA VIRIDE*
G. Mata and F.E. Torres-Hernández

Chairperson: D.J. Royse

16:00-16:15 O-2-3: RESPONSE OF *AGARICUS BRASILIENSIS* TO SUPPLEMENTATION AT SPAWNING
E. Souza-Dias, D.L. Rinker and G. Alm

16:15-16:30 O-2-4: CHARACTERIZATION OF BACTERIAL COMMUNITY CHANGES DURING OYSTER MUSHROOM SUBSTRATE PRODUCTION
B. Vajna, D. Szili, A. Nagy and K. Márialigeti

16:30-16:45 O-2-5: THE IMPACT OF WASTE'S COMPOSITION, ON COMPOSTING AND CULTIVATION PROCEDURES OF MUSHROOMS
O. Danai, N. Ezov, and D. Levanon

16:45-17:00 O-2-6: EVALUATION OF SUBSTRATE COMPOSITION AND PRODUCTIVITY OF SHIITAKE LOGS TO ENHANCE MUSHROOM QUALITY
H. S. Shekhar Sharma, Mairead Kilpatrick, Gary Lyons, Harry Wichers, and Joris Hoozee

17:00-17:15 O-2-7: YIELD AND MUSHROOM SOLIDS OF *AGARICUS BISPORUS* AS INFLUENCED BY MOISTURE CONTENT OF SUBSTRATES
Delphina P. Mamiro and Daniel J. Royse

III. GENETICS AND BREEDING

Wednesday, 1st October **Theatre**

Chairperson: Qi Tan

09:30-09:45 O-3-1: AN ASSESSMENT OF THE GENETIC DIVERSITY OF *GANODERMA* STRAINS CULTIVATED IN CHINA USING MOLECULAR METHODS
Jingsong Zhang

09:45-10:00 O-3-2: OUTCROSSING VIA THE BULLER PHENOMENON IN A COMPOST INOCULATED WITH SPORES AND MYCELIUM OF *AGARICUS BISPORUS,* A METHOD FOR BREEDING
P. Callac, M. Imbernon and J.-M. Savoie

Chairperson: J.M. Savoie

10:30-10:45 O-3-3: MOLECULAR GENE TYPING OF COMMERCIAL STRAINS OF EDIBLE MUSHROOMS, *PLEUROTUS OSTREATUS* AND *AGARICUS BISPORUS*, CULTIVATED IN RUSSIA
A.V. Shnyreva

10:45-11:00 O-3-4: CLONING AND SEQUENCE ANALYSIS OF THE *LENTINULA EDODES* OROTIDINE-5'-MONOPHOSPHATE DECARBOXYLASE GENE
Dapeng Bao, Meiyan Zhang and Mingjie Chen

11:00-11:15 O-3-5: HETEROLOGOUS EXPRESSION IN ENOKI MUSHROOM *FLAMMULINA VELUTIPES*
C.-Y. Kuo, C.-C. Chang, R.-S. Hseu and C.-T. Huang

11:15-11:30 O-3-6: INHERITANCE PATTERNS OF A STRAIN-SPECIFIC SCAR MARKER FOR *LENTINULA EDODES* STRAIN 135
Meiyan Zhang, Chun-yan Song, Qi Tan, Ming-Jie Chen and Ying-Jie Pan

IV. INTEGRATED PEST MANAGEMENT

Wednesday, 1st October **Lecture Hall I**

Chairperson: A. Geösel

09:30-09:45 O-4-1: SPECIFIC PCR REVEALS THAT THE SUBSTRATE OF WILD GROWN *PLEUROTUS OSTREATUS* IS A POTENTIAL SOURCE OF GREEN MOULD AFFECTING OYSTER MUSHROOM PRODUCTION
L. Hatvani, S. Kocsubé, L. Manczinger, I.S. Druzhinina, C.P. Kubicek, C. Vágvölgyi and L. Kredics

09:45-10:00 O-4-2: *AGARICUS BISPORUS* INFECTION BY *VERTICILLIUM FUNGICOLA* AND INCIDENCE ON THE REGULATION OF GENES
M.L. Largeteau, C. Latapy, P. Broca and J.-M. Savoie

Chairperson: H. Grogan

10:30-10:45 O-4-3: IMMUNOLOGICAL DETECTION OF DSRNAS IN WILD *AGARICUS* SPECIES AND IN VIRUS INFECTED CULTIVATED CHAMPIGNON
András Geösel [1], Krisztián Halász[2], József Szarvas[3], Csaba Hajdú[3] and Noémi Lukács

10:45-11:00 O-4-4: CONTROL OF YELLOW ROT ON REISHI MUSHROOM (*GANODERMA LUCIDUM* KARSTEN) USING A SHELF-CULTIVATION AND MODIFIED VINYL COVER METHOD
H.J. Kang, J.T. Hwang, J.G. Noh, J.S. Choi, W.B. Chang, I.G. Song and K.B. Min

11:00-11:15 O-4-5: ISOLATION OF FUNGAL VIRUSES, CAUSATIVE AGENTS OF MUSHROOM DISEASES, AND DEVELOPMENT OF THEIR DETECTION SYSTEMS
Hyeon-Su Ro and Hyun-Sook Lee

11:15-11:30 O-4-6: BIOLOGICAL CONTROL AGAINST *TRICHODERMA* SPECIES IN *AGARICUS* CULTIVATION
Júlia Győrfi and András Geösel

11:30-11:45 O-4-7: NOVEL MYCOVIRUSES IN *AGARICUS BISPORUS*
Hyo-Kyung Won, Dong-Kyu Kim and Hyun-Sook Lee

11:45-12:00 O-4-8: GREEN MOLD DISEASE OF *PLEUROTUS OSTREATUS* IN HUNGARY AND ADVANCES IN ITS BIOCONTROL
A. Nagy, L. Manczinger, L. Kredics, L. Hatvani, J. Győrfi, Gy. Turóczi, Zs. Antal, E. Sajben and Cs. Vágvölgyi

V. NUTRITIONAL & MEDICINAL ASPECTS

Wednesday, 1st October **Theatre**

Chairperson: U. Lindequist

15:00-15:15 O-5-1: EVALUATION OF THE EFFICACY OF *CORIOLUS VERSICOLOR* SUPPLEMENTATION IN THE REGRESSION OF LOW-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS CAUSED BY HUMAN PAPILLOMA VIRUS
J. Silva Couto

15:15-15:30 O-5-2: MUSHROOMS IN TRADITIONAL EUROPEAN FOLK MEDICINE
H.-P. Hanssen

Chairperson: L.J.L.D van Griensven

16:00-16:15 O-5-3: IMMUNE STIMULATORY ACTIVITY OF GLIS: A BIOACTIVE PROTEOGLYCAN FROM *GANODERMA LUCIDUM*
H. Fan, J. Zhang, Q. Tang, C. Zhou, W. Jia, M. Zimmermann-Kordmann and W. Reutter

16:15-16:30 O-5-4: ANTIOXIDANT PROPERTIES OF *GRIFOLA* GARGAL EXTRACTS
J.P.F. de Bruijn, C. Loyola, P. Aqueveque, J.A. Cañumir, M. Cortéz and A. France

16:30-16:45 O-5-5: CANCER PREVENTION AND MANAGEMENT BY MUSHROOM SUPPLEMENTED DIET
Jean A Monro

16:45-17:00 O-5-6: MYCOTHERAPY – TREATMENT WITH MEDICINAL MUSHROOMS
Susanne Ehlers

17:00-17:15 O-5-7: INHIBITORY EFFECT OF MOUSE SARCOMA 180 BY CRUDE B–D-GLUCAN EXTRACTED FROM *HERICIUM ERINACEUS*
Yon Il Choi, Gun Woo Lee, Mi Ja Shim[1], Hyun-Su Rho[2], Hyun Sook Lee[2], Min Woong Lee[3], U-Youn Lee and Tae Soo Lee

Chairperson: H.-P. Hanssen

17:15-17:30 O-5-8: BONE CELL STIMULATING EFFECTS OF EDIBLE MUSHROOMS - A NEW PERSPECTIVE FOR PREVENTION OF OSTEOPOROSIS
K. Wende, A. Saif and U. Lindequist

17:30-17:45 O-5-9: A STUDY OF MUSHROOM NUTRIENTS AND CHEMICAL COMPOSITION ACCORDING TO TREATMENTS AND HARVEST
Necla Çağlarırmak

17:45-18:00 O-5-10:MUSHROOMS AS THE SOURCE OF NOVEL THERAPEUTIC STRATEGIES FOR THE TREATMENT OF NEURODEGENERATIVE CONDITIONS
V. Calabrese[1], C. Cornelius[1], M. Cavallaro[1], M. Cambria[1] and M.A. Toscano[2]

18:00-18:15 0-5-11: FIBRINOLYTIC ACTIVITY OF SEVERAL COPRINOID MUSHROOMS
S.M. Badalyan, L.R. Melikyan, M. Navarro-González and U. Kües

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VI. SAFETY, QUALITY CONTROL AND REGULATIONAL ASPECTS

Wednesday, 1st October **Lecture Hall I**

Chairperson: A.S.M. Sonnenberg

15:00-15:15 O-6-1: MATHEMATICALLY CHARACTERIZING VARIATIONS OF SEC-HPLC PROFILE AMONG DIFFERENT *GANODERMA* POLYSACCHARIDES
T.B. Feng, X.T. Yang, K. Mi, Q.Y. Yang and H.Q. Feng

VII. MUSHROOM RESOURCE DEVELOPMENT

Friday, 3rd October **Theatre**

Chairperson: P. Kalberer

09:30-09:45 O-7-1: *GANODERMA PFEIFFERI* – A NEW MEDICINAL MUSHROOM
W.-D. Jülich and U. Lindequist

09:45-10:00 O-7-2: INVESTIGATION OF THE POTENTIAL USE OF SPENT *PLEUROTUS OSTREATUS* (WILD TYPE, ORIGINATED FROM NEW MEXICO) SUBSTRATE IN RUMINANT FEED
Milan J. Adamović[1], Ivanka M. Milenkovic[2] and Ivana D. Adamović

Chairperson: T.S. Lee

10:30-10:45 O-7-3: EASTERN MEDICINAL MUSHROOMS GO WEST
H.-P. Hanssen and O. Neugebauer

10:45-11:00 O-7-4: MUSHROOMS AND SKIN TREATMENT – FROM TRADITIONAL USE TO MODERN APPLICATIONS
O. Neugebauer, H.-P. Hanssen and M. Kerscher

11:00-11:15 O-7-5: ORGANIC PRODUCTION OF OYSTER MUSHROOM IN INDIA
Yogendra Mahto and R.N.Verma

VIII. ENVIRONMENTAL IMPLICATIONS AND APPLICATIONS

Friday, 3rd October **Lecture Hall I**

Chairperson: J.A. Buswell

09:30-09:45 O-8-1: OYSTER MUSHROOM *PLEUROTUS OSTREATUS* (JACQ.) P.KUMM. – ITS CULTIVATION AND UTILIZING IN SLOVAK FORESTRY
M. Pavlik, M. Hrasko and A. Pavlikova

09:45-10:00 O-8-2: DEVELOPMENT OF SUBSTRATE FOR FRUITING OF *PLEUROTUS OSTREATUS* USING WASTE BED-LOG (*LENTINUS EDODES*)
Who-Bong Chang, Jae-Sun Choi, Sang-Cheul Lim, Kyoung-Beum Min and Cha, Jae-Soon[2]

10:30-10:45 O-8-3: BIOTECHNOLOGY TO GROW EDIBLE AND MEDICINAL MUSHROOMS ON VINE AND WINERY WASTES
M. Petre and A. Teodorescu

IX. MYCORRHIZAL MUSHROOMS AND MYCORRHIZATION

Friday, 3rd October **Theatre**

Chairperson: J.I. Lelley

15:00-15:15 O-9-1: MONITORING OF AN EXTENSIVE TRUFFLE ORCHARD IN HUNGARY
A. Gógán Csorbainé, Z. Bratek and J. Dimény

15:15-15:30 O-9-2: APPEARANCE OF ECTOMYCORRHIZAL MUSHROOMS AT DISTURBED FOREST STANDS
M Pavlik

Chairperson: M. Pavlik

16:00-16:15 O-9-3: ECOLOGICAL RESEARCH BASING SWEET TRUFFLE (*MATTIROLOMYCES TERFEZIOIDES* /MATTIR./ E. FISCHER) CULTIVATION
B. Drescher, Z. Illyés, J. Vikor and Z. Bratek

16:15-16:30 O-9-4: NEW PHYTOINDICATION BASED PREDICTIONAL METHOD FOR BETTER SELECTION OF BURGUNDY TRUFFLE PLANTATION SITES AND HOST PLANTS
Z. Illyés, B. Drescher, J. Vikor and Z. Bratek

X. ECONOMICS AND MARKETING

Friday, 3rd October **Lecture Hall I**

Chairperson: D. Levanon

15:00-15:15 O-10-1: WILD MUSHROOMS AND SOCIOECONOMICS IN NORTHEAST THAILAND
U. Klinhom

Oral Presentations – General Information

All speakers giving Power Point presentations should contact a member of the Technical Section at the registration desk as soon as possible after arrival to download their files. Speakers using slides should hand in their slides to the projector operator no less than 15 minutes prior to the start of the relevant session. A Preview Room will be available for speakers to view their Power Point presentation/slides and ensure they are in good order.

Speakers are requested to arrive a few minutes before the start of the relevant session and to report to the Chairperson, who will have absolute discretion to adjust the time schedule should there be any absentees. Changes to the published schedule will be displayed in advance on the notice board located outside the lecture theatres.

Poster Session

All posters should be set up in the designated Poster Exhibition Area in the Poster Hall in the Conference Centre between 09:00 and 16:00 on Tuesday, 30th September, and should remain displayed until 1200 hr on Friday, 3rd October. Poster stands will be numbered, and authors should display their posters according to the number assigned to their abstract.

The formal poster session has been scheduled for Wednesday, 1st October, from 18:00-19:30 hr. Assigned authors should attend their posters during these times for discussion with participants.

WORKSHOPS

Workshop No. 1 (Tuesday, 30th September 2008 – 16.00-18.00 h): Lecture Hall II

"Structure and medicinal properties of mushroom-derived ß-glucans".

Coordinator: Professor U. Lindequist (Germany)

Panellists: Professor T.S. Lee (Korea)
Dr. L.J.L.D. van Griensven (The Netherlands)

Workshop No. 2 (Wednesday, 1st October, 2008 – 10.30-12.30 h): Lecture Hall II

"Role of mushrooms in Chinese and western medicine: similarities and differences".

Coordinator: Dr. H.-P. Hanssen (Germany)

Panellists: Professor S. Chen (USA)
Professor Dr H.P. Molitoris (Germany)
Dr J. S. Zhang (China)

Workshop No. 3 (Friday, 3rd October, 2008 – 10.30-12.30 h) Lecture Hall II

"*Agaricus* cultivation: how to make a high quality growth substrate"

Coordinator: Dr. B. Desrumaux (Belgium)

Panellists: Professor H.S.S. Sharma (UK)
Professor D.J. Royse (USA)

Excursion

As part of the conference programme, delegates will have the opportunity to visit one of Germany's largest and most modern *Agaricus* farms owned by Mr. Hans Deckers. The farm is involved in both white and brown *Agaricus* mushroom cultivation including the entire handling process of fresh mushrooms for marketing. Additionally, the excursion includes a visit to a well-known Dutch *Agaricus* compost producer with phase I to phase III production. Delegates will also visit the world's largest company engaged in establishing complete mushroom farms, the Christiaens Group, which is active in this field in five continents. Finally, lunch will be served at Gerard van de Vossenberg`s excellent restaurant specialising in mushroom dishes. The Chef, Gerard van de Vossenberg, is a member of the Fédération Cuisinier Exclusive D´Europe and is also the bearer of the "Golden Champignon" which is only awarded to chefs with an outstanding contribution in the preparation of culinary mushroom dishes.

Exhibits

Commercial exhibits will be displayed in the Exhibits Hall within the Conference Centre from 08:30 until 18:00 daily, 30th September-2nd October, and from 08:30 until 16:00 on 3rd October, 2008.

General Meeting of the World Society for Mushroom Biology and Mushroom Products (WSMBMP)

The Triennial General Meeting (TGM) of the WSMBMP will be held on a date and at a venue (to be announced) located in the Conference Convention Centre. This meeting will be chaired by Professor D. J. Royse, President of the Society and is open to all Members of the Society.

The TGM will be preceded by a meeting of the Executive Committee.

Annual General Meeting of the German Mushroom Growers Association (BDC)

The meeting will be held on Friday, October 3rd from 8:30 to 10:00 hours in the Lecture Hall II. The meeting will be chaired by Mr. Franz Schmaus, President of BDC.

Conference Reception

The Conference Welcome Reception

will be held from

19:00 p.m., Monday, 30th September 2008

in the Foyer in the Conference Centre

Conference Banquet

The Conference Banquet

will be held on

Friday, 3rd October 2008, commencing 19:00 p.m,

in the

Conference Hall

ABSTRACTS

Keynote Lectures

K1: MUSHROOMS: CAUSE AND CURE

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Since times immemorial, mushrooms have been used as hallucinogens, in medical applications, for human consumption, and for commercial exploitation. Initially a collectable free food from nature, the cultivation of edible mushrooms began ~700 years ago in China with the cultivation of shiitake (*Lentinula edodes*) on wood logs. In Europe, the cultivation of *Agaricus bisporus* (the button mushroom), first described by the French botanist Tournefort in 1707, spread all over Europe in the 18th Century after it was discovered that the mushroom could be grown on flat cultivation beds consisting of mixtures of straw and weathered horse manure. The industry expanded when cultivation was transferred from outdoors to caves with a stable climate, and later into the "Pennsylvania doubles" indoor system consisting of large racks containing up to 12 beds positioned above each other and divided over two floors. This arrangement formed the basis of the Dutch mushroom farming system that is constructed of standardized metal beds in fully acclimatized rooms, a very efficient and expensive design that has since been introduced worldwide. Indoor composting and two-phase cultivation, developed in the 1980s, have created a complete system of incomparably efficient mushroom cultivation. According to the US Department of Agriculture, world output of industrially produced white button mushrooms in 2004 was approximately three million tonnes. At the same time, a wide range of other mushrooms including shiitake, oyster mushrooms (*Pleurotus* spp), and the rice straw mushroom (*Volvariella volvacea*), were produced elsewhere in the world on substrates ranging from wood logs and wetted non-fermented straw to complicated sterilized mixtures of various organic and inorganic materials. These cultivation systems are easily adaptable to local circumstances, can be built without enormous investment, produce a valued edible product for local trade and have created employment for hundreds of thousands in Asia, South and Middle America, and Africa. Apart from their organoleptic properties, mushrooms are a healthy food, low in calories and fat but containing high levels of protein and all the essential amino acids. They are also a good source of phytosterols, especially ergosterol, and antioxidants that can prevent malignant disease and aging effects. However, surprisingly little evidence has been collected that proves their presumed health effects beyond any doubt. Mushrooms including *Agaricus brasiliensis*, *Cordyceps sinensis*, *Coriolus versicolor, Ganoderma lucidum* , *L. edodes* and *Phellinus linteus* are also recognized for their use in the prevention and cure of a range of diseases including atherosclerosis, cancer, chronic hepatitis and autoimmune diseases such as diabetes type I. Mushroom extracts contain various bioactive components, including glucans, phenolic compounds and proteins, that exert immunomodulatory effects in various forms (e.g. pro-inflammatory activity or adjuvant effects, and also apoptosis), mostly involving chemokines as mediators. Various mushroom-derived enzymes (peroxidases, laccases) have also been shown to be effective in bioremediation processes for removing a range of recalcitrant pollutants (e.g. polychlorinated aromatics, polyaromatic hydrocarbons) from the environment. Spent mushroom substrate has also been used to remove heavy metals from aqueous solutions and odorous and toxic gaseous compounds from the exhaust air generated by industrial processes. Ideas for the further development of mushrooms and derived products, and for strengthening the position of the mushroom industry, will be proposed.

K2: ECONOMIC DEVELOPMENTS IN THE MUSHROOM INDUSTRY

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Production of mushrooms shows a decreasing trend in developed regions such as Europe, the U.S. and Canada. Growers, in Western Europe in particular, are facing increasing competition from low-cost producers such as China. In addition, mushroom companies are facing rising production costs and price pressure from increasingly powerful retail multiples. As a consequence of these developments, the number of mushroom growers in Europe and the U.S. has decreased significantly in recent years. These companies have been taken over by existing players and, consequently, mushroom companies are scaling-up, allowing them to produce in a more efficient and competitive way. In China, on the other hand, mushroom consumption is increasing in anticipation on increasing domestic demand. Mushroom consumption shows different stages of development. In emerging countries, consumption levels of cultivated mushrooms are still relatively low. The growing population is driving consumption of mushrooms in these countries. In addition, increasing incomes and consumers increasingly eating away from home, will stimulate mushroom consumption in emerging countries. In developed regions such as Western Europe and North America on the other hand, mushroom consumption is rather stable in volume. Consumption is primarily driven by health consciousness and convenience. Rather than growing in volume, the mushroom sector in developed regions has ample opportunities to create growth in value. Currently, there is a shift from consuming the traditional white button mushroom towards the consumption of higher value specialty mushrooms. In many Western countries, convenience products such as fresh-cut mushrooms and stuffed mushrooms have become popular. On a global level, mushrooms are increasingly consumed in fresh form. Consumption of processed mushrooms is gradually decreasing, in favor of fresh ones. Mushrooms are traded all over the world, but due to their perishability, fresh ones in particular are mainly traded between neighbouring countries. Processed mushrooms on the other hand, are traded globally. They are less perishable and therefore easier to trade over long distances. China dominates global trade in canned mushrooms.

PL-2-1: DOUBLE CROPPING *AGARICUS BISPORUS* BY RE-SUPPLEMENTING AND RE-CASING COMPOST

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Four mushroom crops were grown to assess the effect of supplementing first or second break "spent" mushroom compost (MC) on mushroom yield. Mushrooms were produced for one or two breaks at the Mushroom Test Demonstration Facility, the casing layer was removed and the fragmented MC was non-supplemented or re-supplemented with hydrolyzed protein, crystalline or feed grade amino acids, or commercial supplements and then re-cased for a second crop at the Mushroom Research Center. Crops 1 and 2 were designed to examine the effect of re-supplementing second-break MC with SoyPlus®, a commercial delayed release nutrient. Crops 3 and 4 evaluated the effects of non-supplementing or supplementing first-break MC with commercial delayed-release nutrients, hydrolyzed protein or amino acids. For Crops 1 and 2, BEs (two breaks) ranged from 46% on non-supplemented MC in Crop 1 to 60% on SoyPlus®-supplemented MC in Crop 2. For comparison, SoyPlus®-supplemented Phase II compost BEs (two breaks) ranged from 58% in Crop 1 to 66% in Crop 2. For Crops 3 and 4, mean (combined Crops 3 and 4) BEs ranged from a low of 72% on non-supplemented MC to a high of 101% on MC supplemented with crystalline L-isoleucine or Pro Fam H200 FG (a hydrolyzed soy isolate). Overall double crop yields were higher when first-break MC was supplemented compared to second-break MC. Re-supplementing and re-casing MC offers an opportunity for growers to obtain additional mushroom production from the same compost. As materials costs rise, double cropping would potentially increase revenues while reducing costs associated with compost preparation and disposal. Growers using a multi-zone system, i.e., bulk Phase II compost preparation and bulk spawn run (Phase III), would be in the best position to fully exploit double cropping. The multi-zone system reduces time of exposure of the compost to pests and diseases because both Phase II and Phase III tunnels are equipped with filters that prevent competitors from reaching the compost.

PL-2-2: PROGRESS OF XIANG-GU (*LENTINULA EDODES*) RESEARCH IN CHINA

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The edible/medicinal mushroom, *Lentinula edodes* (Xiang-gu in Chinese), is cultivated and consumed in many countries worldwide. In terms of total annual output, it currently ranks second, behind *Agaricus bisporus*, among all the mushrooms grown on an industrial scale. China is the major producer and exporter of Xiang-gu, and the mushroom continues to play an increasingly important role in the economic and social development of the country. This is due in large part to the many scientific breakthroughs and production improvements relating to Xiang-gu achieved in recent years by Chinese mushroom biologists and growers. This paper describes the latest advances in Xiang-gu research and development in China over a wide range of areas including germplasm resources, strain identification, breeding and breeding technologies, and pharmacological activity.

PL-3-1: MUSHROOM BREEDING: HURDLES AND CHALLENGES

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Mushroom breeding is an applied science that can contribute to a significant extent to the development of the mushroom industry. Compared to plant breeding, however, there are hardly any large breeding programs and hardly any research programs related to mushroom breeding. There are a number of reasons for this. Next to a small market compared to plant products, it seems to be difficult to protect mushroom varieties. This makes investment in mushroom breeding far from profitable. In some species, such as the button mushroom *Agaricus bisporus*, also some technical problems must be tackled, such as the peculiar life cycle and low recombination frequencies in meiosis. Also, strain instabilities can be a problem and often there is a very narrow genetic base in existing commercial strains for most species. This article will elaborate on these hurdles but will also give some possibilities to solve the problems.

PL-3-2: MOLECULAR GENETICS OF MATING SYSTEM IN BIPOLAR MUSHROOM *PHOLIOTA NAMEKO*

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Pholiota nameko is a wood-rotting edible mushroom that carries a bipolar *A* incompatibility factor. Our previous studies sequenced and characterized the gene for the homeodomain protein (*hox1*) in *P. nameko*, which is a putative homologue of *A* mating-type genes in the tetrapolar basidiomycete, *Coprinopsis cinerea*, In addition, the gene for the pheromone receptor (*rcb1*) in *P. nameko*, which is one of the homologues of *B* mating type genes in *C. cinerea*, was sequenced and characterized. Linkage analysis determined that both genes are present as a single locus in the haploid genome. Moreover, *hox1* was mapped at *A* mating type locus on linkage group I in *P. nameko*, whereas *rcb1* was mapped on the other linkage group and was not linked to the *A* mating type locus. Therefore, these results indicated that linkage between homeodomain protein and pheromone receptor genes in *P. nameko* are different from the bipolar phytopathogenic basidiomycete *Ustilago hordei* in which the two genes are located on the same chromosome. In this study we investigated the genetic structure around *hox1* and *rcb1* in *P. nameko*, and discovered a second homeodomain protein gene (*hox2*) upstream of *hox1*. Meanwhile, two pheromone receptor genes (*rcb2* and *rcb3*) and two putative pheromone genes (*phb1* and *phb2*) were discovered around *rcb1*. Therefore, DNA sequences from genomic regions around *hox1* and *rcb1* in *P. nameko* are similar with those from mating type loci in the model mushroom species. In order to confirm the function of the homeodomain, pheromone and pheromone receptor proteins, the cloned genes were introduced into *P. nameko* protoplasts using a DNA-mediated transformation system. The *P. nameko* strain into which the *hox1* gene had been introduced produced many pseudo-clamp like structures. Although it is well known that the mating system in *P. nameko* is bipolar, the function of homeodomain protein in *P. nameko* is similar to the homeodomain proteins in the tetrapolar mushrooms *C. cinerea* and *Schizophillum commune*. Therefore, it is possible that clamp formations are not regulated by homeodomain proteins alone, but also by pheromone and pheromone receptor proteins. In this presentation, relationships between clamp cell formation and compatibility in bipolar mushrooms will be discussed.

Plenary Lectures

PL-1-1: REACTIVE OXYGEN SPECIES AND THE STRATEGY OF ANTIOXIDANT DEFENCE IN MUSHROOMS

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Oxidative stress leading to an increased presence of free radicals and reactive oxygen species (ROS) plays an important role in human degenerative diseases associated with ageing, and diets rich in antioxidants are expected to help human body to reduce oxidative damage. Mushrooms are potential attractive sources of antioxidants and there have been recent investigations on the antioxidant properties of extracts from various cultivated and wild edible species. Although research has been focused mainly on the composition and putative therapeutic effects of mushrooms, little information is available about ROS generation and the strategy of antioxidant defence in mushrooms. In addition to the well established damaging effects of ROS on DNA, proteins, lipids and other cell components, there is increasing evidence supporting the contention that ROS play important physiological roles in yeasts and filamentous fungi. The level of ROS in the cell regulates the growth and differentiation of the fungal organism and the adaptation of fungi to oxidative stress is tightly connected with the redox-dependant changes in the activities of antioxidant components. As in plants and animals, both of which are capable of an oxidative burst in response to pathogen recognition and ROS production associated with defence responses, mushrooms respond to biotic interferences by accumulation of H_2O_2 or quinones and the death of either the competitor or the fungi itself. Questions relating to the involvement of ROS regulation in Basidiomycete development and defence systems have to be addressed to increase our knowledge of edible mushrooms. This review will discuss progress on ROS generation and the strategy of antioxidant defence in *A. bisporus* and *Pleurotus* spp. with emphasis on new research methods.

PL-1-2: THE INFLUENCE OF CASING AND COMPOST MOISTURE ON SPOROPHORE QUALITY OF *AGARICUS BISPORUS*

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It has been observed on commercial mushrooms farms under stressed conditions, a symptom will develop described as "window panes" on the mushroom caps and stipes. The occurrence of this symptom often results in a loss of fresh quality, and in extreme situations, an outbreak of bacterial blotch. These window panes are caused by excessive moisture in the mushroom tissue and seem to be related to environmental conditions and or physical characteristics, like moisture, of the casing layer or compost. Our research project has examined several environmental and cultural practices that influence the development of these symptoms. Phase I substrate was prepared according to standard procedures used at Penn State's Mushroom Test Demonstration Facility. However, prior to filling trays for Phase II, one treatment received no additional water, resulting in a low moisture substrate, another treatment received additional water to a point where the substrate was judged to be wetter than the standard control, but still able to be conditioned in Phase II. Additional treatments to these three compost treatments included three casing treatments: a dry, a standard control and a wet casing layer. Initial results would suggest that low compost and casing moistures enhance the occurrence of window panes. In a second experiment, the above substrate and casing treatments were prepared and held in one room until nine days after casing (the primordia were 2-4 mm in size). At that time half of the treatments replicates were moved into a room with a relative humidity of around 86-88% and the other half held in a room at a relative humidity of more than 95% until harvesting. The initial results suggest that the symptoms are further enhanced under high relative humidity. Additional experiments are being conducted that include the transplantation of casing with primordia from different substrate moistures and different casing moistures to determine if the precursors for this symptom development are formed early in primordia development.

PL-4-1: CHALLENGES FACING MUSHROOM DISEASE CONTROL IN THE 21ST CENTURY

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Mushroom production continues to be an important industry around the world with increased competition arising due to the expansion of the industry in countries with emerging growing economies. Efficient production systems are therefore required in well-established industries if they are to remain competitive. Changes in production technologies can increase output but pest and disease outbreaks can severely reduce the profitability of a farm. Many pathogens are now resistant or tolerant to the chemicals used to control them, fungicides such as carbendazim (now withdrawn for mushrooms in Europe) and prochloraz can be broken down by microbial activity in the casing thereby reducing their effectiveness. There are fewer pesticides approved for use on mushrooms now due to increased regulation and product withdrawal. New pathogens strains are emerging on a regular basis despite, or as a result of, technological advances in the mushroom industry. The shift to bulk spawn run composts, mechanised filling and emptying, industry scale growing units, wetter casings has occurred alongside serious outbreaks of new pathogens such as *Trichoderma aggressivum*, *Cladobotryum mycophilum* Type 2 and Mushroom Virus X. Recent research in France and Australia seems to indicate that more aggressive strains of Dry bubble disease (*Verticillium* spp.) may be emerging. The control of diseases therefore is increasingly dependent on ensuring that an effective disease hygiene system is in place. Growers need to be fully aware of the way in which the different diseases infect a crop and what factors enable them to spread and get out of control. Although many general hygiene measures will give good background control, specific measures are usually needed to treat specific pathogens. Water-use needs to be examined when *Verticillium* disease is present, air handling and disease treatment need to be considered when Cobweb disease is present, disinfection of spawning and casing machinery needs to be considered when Mushroom Virus X is present. Recent research indicates that alternative/biological pesticide products may be useful against mushroom pests and diseases but the costs of registering such products for mushroom use may be too high. Collaborative work between the major mushroom producing countries may be a way forward.

PL-4-2: FACTORS AFFECTING SPOTTING AND DISCOLORATION OF THE CULTIVATED MUSHROOM, *AGARICUS BISPORUS*

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Mushroom producers strive to achieve perfectly looking mushrooms for fresh market. However, diseases and horticultural anomalies can dramatically affect pre- and post-harvest mushroom quality. Species, biotype and concentration - all affect disease symptom expression. Among four *Trichoderma* species (*T. atroviride, harzianum, koningii* and *viride*) inoculated on mushrooms at difference concentrations, symptom development was earlier and most severe for *T. harzianum* and least severe for *T. koningii.* The majority of symptoms occurred after harvest (four days after inoculation). In a survey of bacterial blotch on mushroom farms, there were at least seven genetic groups that caused blotch symptoms on mushrooms. Symptoms caused by *Pseudomonas* isolates varied from dark brown, brown and yellow. Blotch symptoms caused by a unique biotype of *Pseudomonas* species developed post-harvest at refrigeration temperatures. Casing pH affects mushroom production and quality. The desirable casing pH is neutral, adjusted with lime. Increasing pH in an attempt to reduce *Trichoderma* expression lowered mushroom yield and did not reduce the effect of *Trichoderma* on mushroom production. Reducing the amount of lime in the 'normal' 1:1ratio (w:w) of lime to peat in the casing mix increased the incidence of blotch and decreased the 'whiteness' of the mushroom.

PL-5-1: FACTORS AFFECTING ERGOTHIONEINE CONTENT IN BUTTON MUSHROOMS

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L-Ergothioneine (ERGO) is a naturally-occurring 2-thio-imidazole amino acid biosynthesized primarily by fungi and a few bacteria. ERGO is a potent antioxidant and cellular protector. A preliminary survey indicated that *Agaricus bisporus* strains contained significantly lower levels of ERGO than *Lentinula edodoes*, *Pleurotus ostreatus, Pleurotus eryngii* and *Grifola frondosa*. Brown strains of *A.bisporus* were found to contain higher levels of ERGO than the more commonly consumed white strains. Hence, studies were conducted to determine if ERGO content in button mushroom could be increased to higher levels. Studies indicated that ERGO concentration in mushrooms appeared to be increased by several stress factors, such as use of dry substrate, breaking the mycelium in the substrate at casing and harvesting mushrooms from the later flushes of its crop cycle. This, research has demonstrated that button mushrooms grown in dry compost and obtained from the end of the third flush contained ERGO levels of 2.1 and 1.8 mg/g d.w. for brown and white strains, respectively. These data indicated that button mushrooms can potentially contain ERGO levels about 5-10 times higher than previously observed or as high as those found previously in specialty mushrooms. It was also determined that ERGO levels declined with levels of maturity at harvest such that buttons contained higher levels of ERGO compared to open mushrooms. ERGO levels were similar in cap and stipe tissue but were lower in gill tissue in mushrooms harvested at full maturity. A storage experiment revealed that ERGO levels in freeze-dried portabella powder declined steadily over time in storage. ERGO concentrations were reduced by 40 and 42 percent after 8 weeks at 4 and 23°C, respectively.

PL-5-2: A TRANSLATIONAL RESEARCH TO INVESTIGATE ANTI-CANCER ACTIVITY OF MUSHROOMS

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White button mushrooms have been found to contain phytochemicals that can suppress the proliferation of breast and prostate cancer cells. Two important targets of these phytochemicals are aromatase and steroid 5alpha-reductase. Aromatase is an enzyme that makes estrogen. It is commonly known that estrogen promotes the development of breast cancer, especially in postmenopausal women. Aromatase inhibitors are important drugs in the treatment of estrogen-dependent breast cancer. Steroid 5alpha-reductase converts testosterone to dihydrotestosterone (DHT) and has been shown to play an important role in the development of prostate cancer and benign prostate hyperplasia. The use of steroid 5alpha-reductase inhibitors has been found to decrease the incidence of prostate cancer. Our laboratory has found that mushrooms contain conjugated linoleic acid (CLA) that can inhibit both aromatase and 5alpha-reductase at micromolar concentrations. Cell culture experiments have demonstrated that mushroom extract suppresses the estrogen-dependent proliferation of breast cancer cells. Interestingly, mushroom extract can inhibit the proliferation of both hormone-dependent as well as hormone-independent prostate cancer cells. Importantly, oral intake of mushroom extract has been shown to slow down the growth of breast and prostate tumors in a nude mouse model. Microarray analysis of tumors identified significant changes in gene expression in the mushroom-fed mice as compared to controls. Gene profile analysis identified alterations in networks involved in cell death, growth and proliferation, lipid metabolism, the TCA cycle and immune response. These results illustrate the anti-cancer potential of phytochemicals in mushroom extract, both in *vitro* and in *vivo,* and support the recommendation of white button mushroom as a dietary component that may aid in the prevention of breast cancer in women and prostate cancer in men. Based on these preclinical findings, two clinical trials are designed and will be initiated recently at

the City of Hope Medical Center, California, to investigate the cancer preventive capabilities of mushrooms in human.

PL-6-1: MOLECULAR MODELLING AND MODIFICATION OF MUSHROOM SENESCENCE AND QUALITY-LOSS

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Mushroom quality has importance in terms of consumer perception and enjoyment which leads to economic consequences. Both industry and researchers have sought to improve quality during mushroom growth and maintain quality after harvest. First mushroom quality is defined as per consumer perceptions, and then researchers aim to develop practical methods to improve quality and to gain further understanding of the underlying causes of quality-loss.The breeding of hybrid strains of *Agaricus bisporus* has brought major quality improvement. We can expect further advances of marker-assisted breeding with the release of the *A. bisporus* genome sequence and the use of high density linkage genetic maps. The recent development and design of a mushroom bruisometer at Warwick and Coventry Universities has enabled the identification of the agronomic conditions which favour mushroom bruising and brown discolouration. These factors relate to the compost, casing and environment. Quality-loss after harvest can be controlled by low temperature storage and modified atmosphere packaging. There is a concept that the storage capabilities of mushrooms have been optimised and that any further improvements will only be determined by addressing the biological cause of quality-loss through breeding. For this reason there has been a major study to understand the biochemical and genetic causes of post-harvest quality-loss. The biochemical and chemical changes in the mushroom are discussed in relation to changes in gene expression. The up-regulated 20 genes demonstrate that different physiological processes are occurring in the mushroom after harvest. The expression of 5 of these genes has been statistically modelled which enable the identification of primary and secondary events. The key role of the urea forming gene, argininosuccinate lyase, was tested by the gene silencing technique, RNAi.

PL-6-2: STANDARDISATION AND QUALITY CONTROL IN THE MUSHROOM NUTRICEUTICAL INDUSTRY

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Recent years have witnessed an immense growth of interest in mushrooms as a source of new compounds capable of improving human biological functions. This has been supported in large part by the application of modern analytical techniques that have established scientific bases for earlier empirical observations relating to the health-promoting effects of these fungi. The term 'mushroom nutriceuticals', has been coined to embody both the nutritional and medicinal features of these biological response modifiers extractable from the fungal mycelium (and culture fluids) or fruit bodies. Since mushrooms have a long tradition as a food source, mushroom feedstocks are categorized as 'generally considered safe'. Furthermore, mushroom nutriceuticals exhibit little or no toxicity, even at high doses, and are apparently lacking in various side effects that frequently accompany the use of synthetic drugs. However, a major problem associated with mushroom-based dietary supplements is their wide variability and the current lack of standard production and testing protocols necessary to guarantee product quality. There is serious need for improvements in both quality and regulatory controls in the area. Both are essential in order to increase and maintain consumer confidence, protect public health, and to meet current and future quality and safety criteria set by the regulatory authorities.

PL-7-1: COMMERCIAL CULTIVATION OF *LYOPHYLLUM SHIMEJI*

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Lyophyllum shimeji, "Hon-shimeji" in Japanese is an ectomycorrhizal fungus that grows in association with pine and/or Oak trees. The fungus has been prized as the most delicious and the next expensive mushroom to Matsutake, *Tricholoma matsutake* in Japan. Hon-shimeji has been successfully cultivated in pure culture using the selected strains that is capable of growing saprobically by Ohta experimentally. However, the productivity was not good for commercial cultivation because of using limited wild strains and inadequate substrate. The substrate contained large amount of costly material for such as barley grains. We obtained high-quality and high-yield excellent strains by mating between monokaryons derived from wild strains or by backcross breeding. Moreover, optimum and inexpensive substrate for the commercial cultivation of Hon-shimeji was newly developed. Fruit bodies formed stably on the substrate consisting broadleaf wood-softwood mixed sawdust, nutritional additives and liquid synthetic nutrients in 800-ml plastic bottles. In Japan we have two types of pinning method, the pinning with casing and without casing, for commercial cultivation of this fungus. Hon-shimeji is cultivated in facilities provided automatic cultivation machines using plastic bottles (800 and 1,100 ml). Cultivation cycle is 90-100 days.

PL-7-2: ORGANIC BUTTON MUSHROOM PRODUCTION , QUALITY PRODUCE AND PESTICIDE RESIDUE ANALYSIS

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The collaborative project on organic button mushroom production was started in 2002. So far, 10 trials have been conducted under this project at the Centre. Scientists of this Centre worked on the cultivation technology for organic button mushroom production, whereas pesticide residue analysis was done by the residue analysts in the Department of Entomology, University of Horticulture and Forestry, Nauni, Solan, HP. The basic work centred on compost preparation without the use of fertilizers/chemicals with aim of eliminating pesticides, detected from the base material from the substrate, through high-temperature prolonged aerobic fermentation of the compost during Phase I, for possible catabolism of the pesticides to harmless by-products. In first 2 trials, normal compost turning schedule was followed, which did not result in elimination of the pesticides from the substrate. But, with improved/prolonged high temperature fermentation in Phase I, most of the pesticide residues detected from base materials were eliminated from the substrate. In the first four trials, low yields were obtained due to interference by insect pests/diseases/competitor moulds but with improvements in cultivation technology higher mushroom yields of 18-20 kg/100kg compost in 4-6 weeks of cropping were obtained, with improved quality of fruit body. The base materials used for crop raising were the cereal straws, poultry manure, decomposed Farm Yard Manure/Spent Mushroom Substrate/ Coirpith, wheat bran, cotton seed cake and brewer's grain. These materials were procured from the available market place, and not from organically produced crops. The composting schedule followed was pre-wetting: days 6, 4 and 2; Phase I in bunker/ricks 0, 3, 6, 9, 11, 13, 15 (fill); Phase II in bulk pasteurization chamber following a normal schedule of pasteurization at 57-59 ^{O}C and conditioning at 48-53 ^{O}C. Sylvan A-15 strain of *Agaricus bisporus* was used in the study. Spawn run was accomplished in 12-14 days, case run in 7 days and the first flush appeared on average 16-17 days after casing application. The crop was raised in an environmentally controlled cropping room at 15-17 ^{O}C temperature, 80-85% RH and CO_2 concentration of around 800-1000 ppm. Extra precautions had to be taken to keep the pests out and use non-chemical methods of pest control. Hanging of oil-coated polythene sheets with a yellow lamp for fly trapping was successfully used to trap flying insects with a fair amount of success. Use of common salt to isolate and destroy the stray diseased fruit bodies on the

crop bed was successfully done to keep the disease under check as and when detected. The first 3 weeks of cropping were almost healthy and trouble free, and insect pests/diseases started causing problems after three weeks of cropping when most of the produce was harvested. Mushroom yields of 18-20 kg/100 kg compost were harvested in 4-6 weeks of cropping after standardization of the cultivation technology. The samples for pesticide residues were drawn from all base materials, water, spawn, casing materials and finally the fruit body. The pesticide residue analysis was done for about 40 different pesticides or their isomers/analogues/catabolic byproducts commonly used on agri-crops and poultry. Important amongst these were hexachloro-cyclohexane (HCH) – all its isomers, DDT – different analogues, endosulpahan – different isomers, ethylene bis-dithiocarbamates, carbendazim and chloropyriphos. The quality of the fruit body was assessed on measurable quality parameters like fruit body length/weight, pileus diameter/ thickness/weight, stipe diameter/length/weight, pileus-stipe ratio, toughness of fruit body, dry weight and the N-content. Almost all the quality characteristics showed superiority when compared to mushrooms raised non-organically. The protocol for organic button mushroom production was finalized and one on-farm-trial was conducted on a progressive mushroom farm near the Centre with good results. The samples of fruit bodies have been collected from the farmer for pesticide residue analysis to confirm the freedom of the mushroom from pesticides. The cultivation protocol thus standardized will form the basis for crop certification by certifying agencies for organic button mushroom production.

PL-8-1: OLIVE MILL WASTE AS A SUBSTRATE FOR THE CULTIVATION OF *PLEUROTUS* SPP. MUSHROOMS

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Olive mill waste (OMW) is the main waste material of modern industrial production of olive oil. The addition of up to 30% OMW to cereal grains used for the production of *Pleurotus* inoculums (spawn) for mushroom cultivation, had no negative impact on spawn's quality. Slight increase in mushroom yields, with no harm to their quality, was demonstrated, when 50% OMW were added to wheat straw for *Pleurotus spp* cultivation. Exocellular activity of the ligninolytic enzymes laccase and peroxidase was higher in the OMW supplemented pure cultures, spawn and straw substrates of *Pleurotus spp*. Total peroxidase activity (TP) was higher than laccase activity in the wheat straw/OMW substrate. TP activity in liquid culture correlated with OMW content in the medium. These findings indicate that there is an option to use OMW as supplement for *Pleurotus* substrates.

PL-8-2: ENERGY ISSUES RELATED TO MUSHROOM PRODUCTION, AND THE QUESTFOR PROFITABLE USE OF SPENT MUSHROOM COMPOST

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The Productschap Tuinbouw (Horticulture Council) commissioned this study to find alternative ways to use spent mushroom compost (SMC) because the costs of disposal have risen lately, due to a change in legislation. The SMC is now regarded as animal manure, which is limited in its application as soil conditioner. The study described not only uses of SMC from *Agaricus*, but also SMC from other

mushroom species. The different options were then considered for application in The Netherlands. The most promising application is to develop a bio-refinery process, which makes optimal use of composition of the SMC. The proposed research direction is to analyse the casing soil and the underlying compost for their specific properties and to find applications for both materials. Value can be added by separating these two materials and researching new ways for commercially viable applications. One feasible solution may be to decrease the salt concentration and use the champost as a substrate for potted plants. Another option is to extract valuable components and market these separately, e.g. specific plant hormones or proteins.

PL-9-1: PROBLEMS AND PERSPECTIVES IN THE PRODUCTION OF *TUBER* INFECTED PLANTS

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The first important step in truffle cultivation is the production of *Tuber* infected plants. For this three forms of inocula can be used: spores, infected roots or pure cultures. It is the last of these which shows the most promise because it provides the opportunity for selecting fungal strains with ideal infections, affinity for the host plant and adaption to the ecological conditions and so optimising truffle production. Our initial experimental results confirm that strains can vary in infectivity and host specificity. However, before this information can be applied commercially it will be necessary to standardise the inoculation technique, perfect the preparation of the inoculum and select the most suitable medium for growing the cultures. In the future it should also be possible to select beneficial fungi and bacteria that can promote ectomycorrhizal development and fruit body formation.

PL-9-2: MYCORRHIZAL RESEARCH APPLIED TO EXPERIENCES IN PLANTATIONS OF MYCORRHIZAL MUSHROOMS, ESPECIALLY IN CENTRAL EUROPE

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All over the world experimentations are hopefully carried out for cultivating several valuable mycorrhiza-mushrooms, while in some countries nowadays it has become a practice to establish plantations of black truffles: *T. melanosporum* and *T. uncinatum.* It is an urgent task to avoid non productive truffles plantations, to obtain higher values of harvest on average, for solving these problems one uses even more knowledge of natural truffieres' ecology. Last decade, in Central Europe research on truffles plantations has been initiated. Special local climatic and pedological characters made it necessary to apply knowledge of explored natural truffieres' ecology in local plantations Phenologic and mycorrhizal research activity on plantations supplies sure knowledge in relation of host plants, soils, pedology-climate, as well as of biology and life cycles of truffles. It is important to integrate these two research trends, as we attempted to do it in this paper, in order to make plantations more successful.

PL-10-1: THE MUSHROOM MEDIA LANDSCAPE: CHANGES AND CHALLENGES

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There must be some 25 mushroom trade journals being published in the global mushroom industry right now, with Mushroom News, published since the 1950's, the oldest one still in existence. Not surprisingly,

almost every important mushroom producing country has its own history of mushroom publications. China publishes even four different mushroom journals today, and countries like Poland, Germany and The Netherlands all have a firm tradition in this respect. These mushroom magazines all differ enormously in terms of publication frequency, number of pages and advertisements, lay-out, point of view and quality. However, they have one goal in common; to create and/or channel valuable information for professional growers, traders, and suppliers in this dynamic and ever changing sector. Sadly, the 'Mother Of All Mushroom Journals', The Mushroom Journal, with its predecessors launched even before the Second World War, closed shop some time ago. Furthermore, in Korea, a popular website called MushWorld, suddenly went offline this year. This goes to illustrate that the presence and success of these publications in, for example, a national mushroom industry, is not guaranteed. The closeness of many journals with these respective industries can become a burden, when reader/member numbers start to dwindle. But there are more threats and challenges that have to be dealt with: where and how to get good quality content, bias vs. independency, limited timeframes and budgets, and the rise of the digital media. Roel Dreve, editor of international B-to-B mushroom publications for Reed Business, will shed some light on the trials and tribulations of creating journalistically and commercially sound trade journals within this industry, and share some thoughts on trends and the hurdles to be taken.

PL-10-2: THE DEVELOPMENT OF POLISH MUSHROOMS EXPORTS

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The development of Polish mushroom producers during the last decade was determined by external, hence international, factors like the enlargement of the European Union (EU) and the development of global markets. The important role played by Polish mushroom producers in the European market was supported by the territorial expansion of the EU, which had a positive impact on trade between Poland and the rest of the EU. However, additional macro-economic factors, including interest rate instability and the volatility of the foreign exchange rates, are still affecting Polish companies, particularly those with a strong presence in foreign markets. Most noteworthy among the many attributes of the Polish mushroom producers is their ability to adapt to new market rules, and to maintain a strong position based on previous experience and a long tradition of producing excellent mushrooms.

General Oral Presentations

I. PHYSIOLOGY & BIOCHEMISTRY

O-1-1: GROWTH AND FRUIT BODY FORMATION OF *GANODERMA LUCIDUM, LENTINUS EDODES* AND *PLEUROTUS OSTREATUS* ON MEDIA SUPPLEMENTED WITH SELENIUM

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Selenium (Se) is biochemically a very important microelement in food, which protects cells from free-radical damage, enables the thyroid to produce thyroid hormone, helps lower the risk of joint inflammation and whose deficiency in the diet results in serious heart diseases. Bioaccumulation of Se in order to improve the pharmacological qualities of the industrial mushrooms was investigated. The growth of mycelia and fruit body formation of different medicinal mushroom strains of *Lentinus edodes, Ganoderma lucidum* and *Pleurotus ostreatus,* over the wide range of concentrations and inorganic and organic forms of selenium were examined. Vegetative growth of mycelium was measured as colony diameter in pure cultures supplemented with organic selenium from the new synthetic compound Zn(II) complex with 2-quinolinecarboxaldehyde selenosemicarbazone [Zn(dapsesc)]. Organic selenium at concentrations of 1-50 mg/l did not significantly influence the growth of mycelia on agar based media and on sawdust substrate. Inorganic forms of Se supplements, prepared as $NaSeO_4$ and Na_2SeO_3, showed stimulatory effects (1-50 mg/l) and toxic effects (at higher concentrations) on the growth of mycelia. Media supplemented with different contents of Se showed different levels of toxicity according to the different strains and species. On a standard industrial sawdust based substrate, supplemented with 100 mg/kg $NaSeO_4$ and Na_2SeO_3, accumulation of Se in fruit bodies was determined with electrochemical atomic absorption methods. Se as Na_2SeO_4 and Na_2SeO_3 was effectively taken up from substrates and accumulated in fruit bodies. Mushroom growth on the substrate was not inhibited with this concentration of Se, and was even stimulated in the case of *G. lucidum.* The total selenium content of the frozen and dried mushrooms depended on the mushroom strain and species, and on the form of supplemented selenium. *P. ostreatus* and *L. edodes* accumulated selenium more effectively from Na_2SeO_3 then from Na_2SeO_4. *P. ostreatus* accumulated selenium between 120 and 250 mg/kg, *L. edodes* 79-130 mg/kg and *G. lucidum* about 3.7 mg/kg dry weight. *Ganoderma lucidum* better absorbed Se from Na_2SeO_4. In mushrooms cultivated without Se supplement, Se contents were only about 1 mg/kg.

O-1-2: RECOGNITION AND DEGRADATION OF INSOLUBLE CRYSTALLINE CELLULOSE BY *POLYPORUS ARCULARIUS*

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The genes encoding cellulases, which belong to glycosyl hydrolase families have been cloned from the basidiomycetous mushrooms. The transcripts of cellulase genes are strongly induced when the mycelia are grown in medium containing crystalline cellulose, and they are not expressed in medium containing glucose, but how insoluble substrates such as microcrystalline cellulose are recognized by these fungal cells is not clear. The polypore mushroom *Polyporus arcularius* is a wood-decomposing basidiomycete that produces at least three types (I, II, and IIIa) of carboxymethyl cellulase (CMCase) when the medium contains crystalline cellulose as the sole carbon source and produced mainly cellobiose in the medium. The genomic and cDNA clones encoding the family 12 endoglucanase (CMCase IIIa) gene (*cel3A*) of *P. arcularius* have been sequenced, and Cel3A has been expressed as an active enzyme in *Escherichia coli.* To determine the role and function of each type of cellulase in the degradation of crystalline cellulose by

basidiomycetous mushrooms, the structure of all of the cellulase genes should be investigated, but the nucleotide sequences of the other cellulase genes in *P. arcularius* have not yet been reported. In the current study, the genomic and cDNA clones encoding the endoglucanases (*cel4*), and the two cellobiohydrolases (*cel1* and *cel2*) of *P. arcularius* sequenced and characterized. The predicted amino acid sequence of Cel1 Cel2, Cel3a and Cel4 are similar to glycosyl hydrolase family 7, 6 12 and 5 protein, respectively. The expressions of the all cellulase genes (*cel1 cel2, cel3a* and *cel4*) were induced by Avicel (microcrystalline cellulose) and cellopentaose but repressed by glucose, cellobiose, cellotriose, and cellotetraose. There was a low level of transcription of both genes regardless of the carbon source. These results suggest that *P. arcularius* cells constitutively express a very low level of cellulase that can degrade insoluble crystalline cellulose and that the transcription of celluases in the cells is induced by products produced by these endoglucanases such as cellooligosaccharides. From our findings, we propose a possible mechanism for the recognition and degradation of insoluble crystalline cellulose by fungal cells.

O-1-3: SUSCEPTIBILITY OF EUROPEAN POPULATIONS OF *AGARICUS BISPORUS* TO *TRICHODERMA* METABOLITES

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Trichoderma aggressivum lead to severe crop losses in *Agaricus bisporus* cultures. Selection of resistant cultivars using the wild genetic resources is a challenge. Five genotypically distinct populations of *Agaricus bisporus* have been identified. In the present work, 90 wild strains from the European populations, 38 North American isolates and five cultivars were compared for their susceptibility to *Trichoderma* metabolites. The aims were to evaluate the variability of this trait between populations and to determine if subdivision exists inside a population in relation with geographical origins. Inside the European population, the Greek sub-population was previously shown to be genotypically distinctive, while retaining European characteristics. It was discriminated here for its relatively high level of resistance and adaptation to *Trichoderma* metabolites by comparison with other European populations. The litter of *Crupessus sempevirens* and the climatic conditions associated with the development of this tree appeared to select strains of *A. bisporus* with low susceptibility to *Trichoderma* metabolites.

O-1-4: EXTRACELLULAR ENZYME PRODUCTION OF THE TWO CAUSATIVE AGENTS OF OYSTER MUSHROOM GREEN MOULD UNDER INDUCTIVE AND NON-INDUCTIVE CONDITIONS

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In the latest years the green mould disease of *Pleurotus* spp. caused by *Trichoderma* has been reported in several countries. *Pleurotus ostreatus* is the third most important commercially grown basidiomycete world-wide, and its production is getting increasingly affected by green mould infections causing great crop losses. The fungi responsible for the green mould disease of *Pleurotus* have been recently described as the new species *Trichoderma pleurotum* and *T. pleuroticola*. Both of these species belong to the Harzianum clade of the genus *Hypocrea/Trichoderma* which also includes *Trichoderma aggressivum*, the causative agent of *Agaricus* green mould disease. *T. pleuroticola* and *T. pleurotum* are two genetically closely related, but phenotypically clearly divergent species. We examined the extracellular enzyme production of several isolates belonging to these two species under non-inductive conditions and upon induction by *Pleurotus* powder or wheat straw powder, or both. We measured the capability of the strains to produce extracellular trypsin-like, chymotrypsin-like and chymoelastase-like protease, aminopeptidase, β-glucosidase, cellobiohydrolase and *N*-acetyl-glucosaminidase activites and compared them with those

of *T. aggressivum* f. *aggressivum*, *T. aggressivum* f. *europaeum* and *T. harzianum*. Significant differences could be observed between the two *Pleurotus* pathogenic species in the production of proteolytic activities. *T. pleuroticola* isolates produced 3-8 times higher amounts of chymotrypsin-like protease activities on *Pleurotus* powder than *T. pleurotum* isolates. In contrast, *T. pleurotum* isolates produced significantly higher amounts of both trypsin-like, chymotrypsin-like and chymoelastase-like protease activities on wheat straw powder than *T. pleuroticola* isolates. The proteolytic system of *T. pleurotum* is highly inducible by the wheat straw substrate used for *Pleurotus* cultivation, while the proteases of *T. pleuroticola* – especially chymotrypsin-like activities – seems to be induced by the presence of *P. ostreatus* and not by the substrate. A further interesting phenomenon is the lack of constitutive *N*-acetyl-glucosaminidase production in the case of *T. pleuroticola* and *T. pleurotum*, in which these two species clearly differ from *T. aggressivum* and *T. harzianum* isolates. Our results suggest that that the two closely related *Pleurotus* pathogenic *Trichoderma* species may use different enzymatic strategies for the adaptation to the circumstances existing during *Pleurotus* cultivation.

O-1-5: THE USE OF DIRECT DPPH METHOD FOR DETERMINATION OF ANTIOXIDANT CONTENT IN MOREL MUSHROOM

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Antioxidant compounds are considered to have important role in reducing damages originated by oxidative process in the body. Therefore they have a potential to reduce the chances of development of many diseases. In recent years mushroom were proven to be important source for natural antioxidant compounds. Many studies are conducted in this respect, using different analytical methods for measuring the antioxidative activity of mushroom extracts. In the present work, a method for measuring the antioxidative activity of a whole morel fruit body's tissue, using DPPH (1,1-diphenil-2-picrylhydrazyl) method was studied. Fruiting bodies were milled, either as wet or freeze dried matter, and added into DPPH solution, incubated at 30 °C. Antioxidant activity was determined as reduction in 715 nm of DPPH color. Different aspects, such as the optimal tissue ratio to solution, the incubation duration and the moisture content of the mushroom tissue, were determined. The method was proven to fit morel ascocarps, with high linearity of the antioxidant content to increasing tissue content, but had less satisfactory results when *Pleurotus* and *Agaricus* fruiting bodies were examined.

O-1-6: ANTIMICROBIAL ACTIVITIES OF FOUR WILD MUSHROOM SPECIES COLLECTED FROM TURKEY

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Four wild mushrooms, namely *Gloeophyllum trabeum* (Pers.) Murrill, *Rigidoporus ulmarius* (Sowerby) Imazeki, *Meripilus giganteus* (Pers.) P. Karst. and *Paxillus involutus* (Batsch) Fr. collected from the southwest of Turkey, were tested for their antimicrobial activities by using the disc diffusion method and minimum inhibition concentration (MIC) method. The ethanol and n-hexane extracts from the mycelia that isolated from fresh fruit bodies of these mushrooms were assayed against 15 microorganisms. In comparison with the test antibiotics penicillin, novobiocin, nalidixic acid and ampicillin. This research has shown that four wild mushrooms revealed antimicrobial activities against some Gram (+) and Gram (-) bacteria, yeasts, filamentous fungi and actinomycetes.

O-1-7: DEGRADATION OF ENDOSULFAN BY *PLEUROTUS* SPP

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In order to select a strain of *Pleurotus* spp able to degrade the insecticide endosulfan the mycelial growth of 80 strains of *Pleurotus* spp on agar containing 0.5% peptone and 100 ppm endosulfan was observed. Three strains were selected and their growth rate with and without endosulfan was measured in low nitrogen liquid medium. Finally one strain (*Pleurotus pulmonarius* ECS-0190) was cultivated on low (2.4 mM) and high (24 mM) nitrogen liquid media with and without endosulfan and the concentration of endosulfan on each medium was determined. All the strains tested grew in peptone endosulfan agar at a rate between 0.11 and 0.38 cm/d. Three strains (ECS-0184, ECS-0185, and ECS-0190), with radial extension rates (RER) of 0.33, 0.35, and 0.34 in cm/d respectively), were selected at this point. Biomass production and growth rate in liquid medium without endosulfan were determined: *Pleurotus* sp ECS-0184 (0.99 g/l; 0.081 d-1), *P. pulmonarius* ECS-0190 (0.89 g/l ; 0.071 d-1) and *Pleurotus* sp ECS-0185 (0.287 g/l ; 0.021 d-1). In the presence of endosulfan, biomass and growth rates were as follows: *P. pulmonarius* ECS-0190 (0.61 g/l ; 0.045 d-1); *Pleurotus* sp ECS-0184 (0.522 g/l ; 0.038 d-1); and *P. ostreatus* ECS-0185 (0.23 g/l ; 0.016 d-1). Then *P. pulmonarius* ECS-0190 was cultured in high and low nitrogen media. The initial concentration of endosulfan was 82.19 mg/l (α=53.99 and β=28.19). After cultivation for eight days, the endosulfan remaining in the high-nitrogen medium was 3.25 mg/l (α=1.26 and β=1.41), while in the low-nitrogen medium, the endosulfan remaining was 0.17 mg/l (α=0.12 and β=0.05). Removal rates were 96.7 and 99.79 %, respectively, in comparison with the final concentration in the control (63.84 mg/l; α=41.68 and β= 22.16). *Pleurotus pulmonarius* ECS-0190 showed the greatest ability to degrade the insecticide, and its ability was further enhanced when the fungus grew in a low-nitrogen medium: After eight days of contact with the insecticide in liquid medium, 99.79% of the initial endosulfan was removed.

II. CULTIVATION TECHNOLOGY

O-2-1: MUSHROOM COMPOST PRODUCTION – A REVIEW OF INDUSTRY QUALITY ASSURANCE FRAMEWORKS IN IRELAND

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Irish composters have established a reputation for excellent substrate quality manufactured to BestAvailable Techniques (BAT) for the control of emissions to air from composting installations. The majority implement compost production methods that follow the principles of Hazard Analysis Critical Control Points (HACCP). The industry frameworks reported in this paper is based on Irish and UK legislations and policy documents, European Union directives, academic studies and discussions with industry members from the Republic of Ireland and Northern Ireland. Although already extensive, quality assurance (QA) protocols may need further developed with the aid of workshops, research and development and interviews with small groups of high performing industry members. The results can be used to inform government policies along with dissemination of the key findings to all members. This effort should be followed up by discussions with industry to ensure that the best practice will be widely recognized, accepted and can be readily applied by others. This collaborative "whole supply chain

process" approach will generate a higher degree of industry ownership. Intervention steps for implementing QA directives at each stage of production need to be planned
discussed to provide a way forward.

O-2-2: EFFECT OF AEROBIC FERMENTATION SUBSTRATE IN THE PRODUCTION OF *PLEUROTUS OSTREATUS* AND ITS RESISTANCE TO *TRICHODERMA VIRIDE*

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The cultivation of *Pleurotus* spp. on fermented substrates takes place at commercial level getting good yields and a low rate of contamination by antagonistic moulds. However, the experimental work on the factors involved in the production of a selective substrate for the growth of *Pleurotus* spp. still not enough. This research aims to obtain a suitable substrate for the cultivation of *Pleurotus ostreatus* through aerobic fermentation of barley straw added with coffee pulp (CP) or peat moss (PM). The effect of different times of substrate fermentation on the production of fruit bodies and resistance of *P. ostreatus* to the presence of *Trichoderma viride* were studied. The barley straw was cut into pieces 3 to 5 cm, hydrated and supplemented with 10% of CP or PM besides CaCO3 (1%). The substrate was placed into a container (2 m^3) for ease of ventilation and it was removed and mixed daily for 14 days, taking samples at 0, 3, 7 and 14 days of fermentation. After fermentation, substrate was pasteurized with steam for 6 hrs at 65 ° C and inoculated using two strains of *P. ostreatus*. The samples were incubated at 25 °C for 2 to 3 weeks. Later they were moved to a room with lighting, irrigation and artificial ventilation to assess the biological efficiency. Growth inhibition of *P. ostreatus* confronted with *T. viride* was also evaluated, using pasteurized substrate placed in Petri dishes which were inoculated with the mycelia of both antagonists. A portion of the pasteurized substrate was sterilized to eliminate the present bacterial communities and then inoculated with both antagonistic fungi in order to observe the inhibition produced by *T. viride*. The results showed higher biological efficiency between 3 and 7 days of fermentation. It was noted that the substrate fermentation, limits the growth of *T. viride* antagonist, especially when fermentation is done 7 or 14 days. Inhibition of *P. ostreatus* growth in the sterilized substrate was higher than other conditions this shows the importance of the participation of thermotolerant bacterial communities at the beginning of the mycelial growth of *P. ostreatus* in fermented barley straw.

O-2-3: RESPONSE OF *AGARICUS BRASILIENSIS* TO SUPPLEMENTATION AT SPAWNING

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Agaricus brasiliensis is a mushroom of medicinal value with both a low yield and slow production. Protein supplementation for *Agaricus bisporus* is routine practice used to increase production. With the objective of increasing production, two strains of *A. brasiliensis* were cultivated in compost used for production of *A. bisporus*. Compost was supplemented at spawning with Sylvan CS36 at 2 and 4% (w:w). After over two months of harvest, supplementation increased production by 35 and 48%, respectively for the 2 and 4% rates for strain 1 and 33% for both rates of strain 2.

O-2-4: CHARACTERIZATION OF BACTERIAL COMMUNITY CHANGES DURING OYSTER MUSHROOM SUBSTRATE PRODUCTION

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Oyster mushroom (*Pleurotus sp.*) is the second largest commercially produced mushroom in Hungary. Mushroom production is mainly dependent on the quality of the substrate. In Hungary two fundamentally different substrate technologies were developed by world famous scientists in the sixties and seventies: pasteurization of pre-wetted straw by steam and steaming dry straw on higher temperature. In our study we investigated pasteurized substrate. In spite of the long tradition in *Pleurotus* production, there is scanty of knowledge of the pasteurized substrate's microbiota. Substrate is produced in three phases: (1) composting the shredded, moistened straw, (2) pasteurization in tunnels, (3) conditioning under thermophilic conditions. The purpose of the procedure to eliminate all harmful organisms, and in the meantime to help the development of favourable micro-organisms for *Pleurotus*. Terminal restriction fragment length polymorphism (T-RFLP) was used to characterize the substrate considering the mushroom yield of the examined 12 production series. For identifying the dominant microbes in the community-fingerprints one clone library was constructed from each phase of a high mushroom-yielding production series. According to the T-RFLP results the three phases grouped well separately, but the second and the third phases were a bit more similar to each other. The first phase was dominated by Proteobacteria: *Sphingomonas* species, which occur typically in soils, *Pantoea* and *Pseudomonas* species, but also the members of Actinobacteria (*Saccharopolyspora spp.*) and Bacteriodetes (*Pedobacter spp.*) could be found. In the following two phases the dominance of thermophilic microbes were more characteristic. The most abundant bacteria in the second phase were the widespread *Flavobacterium spp.*, *Geobacillus debilis*, *Bacillus thermozeamaize*, *Sphingobacterium composta* and *Ureibacillus spp.* The third phase was dominated by *Microbispora bispora*, uncultured Chloroflexi clones, *Geobacillus thermodenitrificans*, *Thermobacillus xylanilyticus* and some uncultered clones from matured compost. There was no characteristic difference in the structure of the community among the different production series, which could explain the divergent mushroom-yields of each series. In conclusion, T-RFLP combined with cloning is suitable for characterizing the mushroom substrate production. For revealing the minor differences between the production series preparation of further clone libraries will be needed. *(This research was supported by the LASU1234-OMFB-00977/2005 grant.)*

O-2-5: THE IMPACT OF WASTE'S COMPOSITION, ON COMPOSTING AND CULTIVATION PROCEDURES OF MUSHROOMS

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Mushroom production is frequently based on utilization of "straw composts". These composts are made of broilers manure, as the main N source and wheat straw, as the C source. Analysis of straw and manure composition was made routinely during the years 2002-2007. The main trends in waste's composition were: (a) Seasonal fluctuations in humidity and N contact of the manure. (b)During the years 2004-2007 total N content in the manure decreased by 30% as compared to 2002-2003. (c) Wheat straw composition also changed during this period. Lignin content in the straw decreased. The stem length and width decreased and leaf content in the straw increased. These fluctuations in the compost raw materials led to necessary adjustments in composting and cultivation processes, including: a. Increase in manure content of the compost, and addition of other organic and mineral N sources, in order to maintain N and ammonium content at the optimal level, up to the limit when it causes the compost to become too greasy. b. Decrease in water content by 3%, during composting and water addition to the casing soil during mushroom cultivation, to avoid anaerobic conditions. Mushroom yields (quantity and quality) remained unharmed after these drastic reductions in water addition. Water evaporation, during the first week after

casing, decreased and energy invested in water evaporation process was saved. c. Machinery operations during composting and mushroom cultivation, were adjusted to avoid (as much as possible), grinding and compaction of the compost, and maintaining open structure and low volume weight.

O-2-6: EVALUATION OF SUBSTRATE COMPOSITION AND PRODUCTIVITY OF SHIITAKE LOGS TO ENHANCE MUSHROOM QUALITY

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The edible shiitake mushroom *Lentinula edodes* (Berk.) Sing., is the second most important mushroom in the world. Commercial cultivation of shiitake on sawdust and carbohydrate containing substrates is increasing worldwide and this has stimulated research intended to increase quality and quantity of the mushroom produced. Reports on many aspects of production have been published by researchers from Asia and North America. To increase the yield and to have a continuous commercial production, it is necessary to understand the effect of various physical and chemical factors, affecting fruiting and formation of shiitake mushrooms. Availability of productive logs in Europe is dependent on the use of readily accessible substrates. Regular evaluation of available raw materials for the production of Shiitake logs is necessary. Other factors, such as light, pH and gas concentration have been reported to have influence on different stages of shiitake growth. The present study was undertaken to assess the composition of raw materials for production of shiitake logs, the effects of light, temperature and other physical and chemical factors on the primordial stimulation and productivity of shiitake mushrooms. The raw materials were analysed for lignin, cellulose, hemicellulose, lipid, carbohydrate and inorganic components using wet chemistry techniques and instrumental methods such as thermogravimetry and near infrared spectroscopy. Comparative cropping trials of several formulations of logs were carried out and changes in the substrate composition of the test logs will be discussed.

O-2-7: YIELD AND MUSHROOM SOLIDS OF *AGARICUS BISPORUS* AS INFLUENCED BY MOISTURE CONTENT OF SUBSTRATES

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Yield, biological efficiency (BE), basidioma size, and mushroom solids were determined from mushrooms harvested from non-composted substrate (NCS) and a 1:1 mixture of NCS and spent mushroom compost (SMC) at three moisture contents (55%, 60%, and 65%). Substrate type and moisture content significantly influenced yield, BE, and mushroom solids. Moisture content also significantly influenced basidioma size. Mushroom yield (14.5 kg/m^2) was highest on a 1:1 mixture of NCS and SMC at 55% moisture, whereas BE (60.5%) was highest from Phase II compost (control). The largest mushrooms (23.8 g/mushroom) and highest mushroom solids (9.9%) were obtained from NCS at a moisture content of 60%. Optimum substrate moisture contents for yield and BE varied depending on substrate type.

III. GENETICS AND BREEDING

O-3-1: AN ASSESSMENT OF THE GENETIC DIVERSITY OF *GANODERMA* STRAINS CULTIVATED IN CHINA USING MOLECULAR METHODS

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Ganoderma lucidum (GL), called 'Lingzhi' in China, is a lamella-less basidiomycetous fungus belonging to the family Polyporaceae. The mushroom has played a role in Chinese folk medicine for more than 4,000 years, and has been used in China for the treatment of several different diseases. In recent years, many studies on the medicinal effects of *Ganoderma* spp have been undertaken worldwide, and have ascertained that components of these mushrooms possess various therapeutic functions including immunomodulating activity, tumor cell growth inhibition and radioprotective ability. The cultivation of *Ganoderma* has developed rapidly due to increased market potential, and germplasm resources have become more and more plentiful. However, for various reasons, the classification of *Ganoderma* isolates cultivated in China is presently in chaos. In order to classify strains of *Ganoderma* cultivated in China, genetic diversity among 150 strains of *Ganoderma* was studied using RAPD (Random Amplified Polymorphic DNA) and ERIC-PCR (Enterobacteria Repetitive Intergenic Consensus-PCR) techniques and inferred from nuclear ribosomal DNA ITS and partial β-tubulin gene sequences. Two dendrograms, derived from RAPD and ERIC-PCR data respectively, each indicated that the strains could be divided into four distinct groups, and the results obtained were in complete accordance with traditional classification. Compared to the RAPD method, ERIC-PCR is a cheaper and faster method for classifying *Ganoderma* strains. Genetic diversity among 34 *Ganoderma* isolates cultivated in China was also investigated using ITS and partial β-tubulin gene sequences. Five distinct groups were identified. In addition, ITS sequences were used to design primers capable of clearly distinguishing *G. sinense, G. tsugae, G. tenue, G. subamboinense and G. applantum* using PCR-based technology. The results emphasize the difficulties involved in identifying *Ganoderma* isolates using only morphological characteristics, and demonstrate that a more effective approach to resolving the taxomomy of *Ganoderma* is available through analysis of molecular data.

O-3-2: OUTCROSSING VIA THE BULLER PHENOMENON IN A COMPOST INOCULATED WITH SPORES AND MYCELIUM OF *AGARICUS BISPORUS,* A METHOD FOR BREEDING

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A novel outcrossing method proceeding through the Buller phenomenon was evaluated for its efficiency for producing hybrids with a useful variability in agronomics traits, and being considered of value in the early generations of selection. By simultaneously inoculating spores and the grain spawn of a homokaryon into a culture substrate, numerous sporophores are produced via outcrossing. Hybrid sporophores were produced in compost inoculated both with mycelium of an homocaryon of S608-2 (white hybrid parent) and with spores of a single spore print of a C9 sporophore (brown parent). With the conventional breeding method, half of the offspring can not be exploited due to the impossibility to growth the haploid mycelium bearing the *mat-x* allele of C9. Thanks to our novel outcrossing method, hybrids inheriting C9 *mat-x* are produced at a rate compatible with the expected Mendelian segregation in meiose spores. Mycelia isolated from tissue cultures of 42 of these sporophores were individually cultivated. Spawn was obtained from each hybrid and culture trials were performed under conventional conditions or with artificial contamination by the pathogen *Verticillium fungicola*. A significant correlation was observed between the high/low susceptibility to *V. fungicola* and *mat-x*/*mat-1* allele inherited from the C9 parent. The dispersion of the data for the susceptibility to the pathogen was

compared with those observed in a sample of wild strains and in a sample of hybrids obtained by conventional crossings. The variability obtained by outcrossing for this trait is significant and could be used to select interesting strains. The present outcrossing method used for hybridisation and selection allows the production of numerous different hybrids in one step, without the time consuming production of homocaryons and crossings, and trait segregation can be analysed.

O-3-3: MOLECULAR GENE TYPING OF COMMERCIAL STRAINS OF EDIBLE MUSHROOMS, *PLEUROTUS OSTREATUS* AND *AGARICUS BISPORUS*, CULTIVATED IN RUSSIA

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The oyster mushroom, *Pleurotus ostreatus,* and white button mushroom, *Agaricus bisporus* are commonly gathered and cultivated for consumption in Russia. Commercial cultivation of the oyster mushroom ranks next to the white button mushroom for annual production. To characterize mushrooms' commercial strains a complex approach was employed using allozyme loci, RAPD and mating compatibility tests. Eleven oyster mushroom strains and eighteen *A.bisporus* strains from the collection of mycelium production laboratory 'Zarechye LTD' were analysed. Groups of genetically similar and distant mushroom strains were discriminated within each species with RAPD and allozyme markers. Thirteen allozyme loci were analyzed (*Acp, Adh, Lap, Mdh, Idh, Pgm, Pgi, Sod, Gdh*, and *Est*) and a total of 32 alleles were resolved. *EST, LAP and PGM* allozyme spectra are shown to be highly specific for gene typing of the cultivated strains. Species- and cultivar-specific molecular markers were resolved to reliably identify genetic lines and to determine the degree of similarity between strains originated from different breeding sources. Three separate clusters of commercial *P.ostreatus* strains of different origin and two clusters of *A.bisporus* strains were clearly resolved. A majority of European strains of the both species were grouped within the separate clusters. Wild-collected oyster mushroom strains recently introduced into cultivation in Russia were placed distantly from the others. Among the commercial strains of *P.ostreatus*, the level of genetic variation was higher suggesting a broader genetic basis employed in breeding of this mushroom as compared with *A.bisporus*. The cultivars and hybrids of *A.bisporus* showed a higher level of homology. For screening mating ability of the oyster mushroom cultivars, mon-mon matings were performed against two sets of intercompatible monokaryotic tester strains. A majority of the commercial oyster mushroom strains of different origin were clearly assignable to an intersterile group II (*P.ostreatus*) reproductively isolated from the intersterile group I (*P.pulmonarius*). Sequence analysis of nuclear ribosomal ITS region (ITS1-5.8S-ITS2) was performed for some *Pleurotus* cultivars. No significant variation of the locus was revealed among the cultivars within the species. This study is of importance for mushrooms cultivation and preservation of native germ plasm.
The research was supported by 'Zarechye Ltd' and a RFBR grant.

O-3-4: CLONING AND SEQUENCE ANALYSIS OF THE *LENTINULA EDODES* OROTIDINE-5'-MONOPHOSPHATE DECARBOXYLASE GENE

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The *pyrG* gene of the edible fungi *Lentinula edodes*, encoding orotidine-5'-monophosphate decarboxylase (OMPDC) enzyme, was cloned by combining the methods of the nested degenerated PCR and the chromosome walking. The *L. edodes pyrG* gene has 946 bp and contains two introns of 67 bp and 51 bp. The deduced protein sequence of the *pyrG* gene has 275 amino acids and is highly similar to the OMPDC from other organisms. The sequence analyses on the 5'- and 3'- untranslated regions were also done. To our knowledge, this is first report of cloning the complete sequence of *pyrG* gene in the

cultivated edible fungi. The *pyrG* gene can be a safe selection marker for the transformation application of the edible fungi. The development of transformation system based on the auxotrophic marker of *pryG* gene is in process.

O-3-5: HETEROLOGOUS EXPRESSION IN ENOKI MUSHROOM *FLAMMULINA VELUTIPES*

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Heterologous expression in enoki mushroom *Flammulina velutipes*, using the glyceraldehyde-3-phosphate dehydrogenase (*gpd*) promoter and the transformation procedure based on electroporation of basidiospores or mycelial fragments, was reported. This method eliminated the problem of protoplast preparation, and the transformation efficiency were 30~150 transformants per □g DNA. Heterologous genes, including hygromycin resistant marker (*hpt*), enhanced green fluorescent protein (*egfp*), □-glucuronidase (*gus*), feed enzyme phytase (*appA*), and house dust mite allergen Der p 2 (*der p2*), have been shown to be faithfully expressed, and remained stable after multiple rounds of subculture. Using *gpd* promoter containing its first intron, *egfp* could be successfully expressed in *F. velutipes* at each stage of the life cycle, including primordia, mature fruiting bodies, basidiospores and monokaryons produced via meiosis. The promoter deletion analysis showed that the region of 336 bp upstream from the transcription start point was sufficient for the function of the *F. velutipes gpd* promoter. In addition to *F. velutipes*, this simple and reliable transformation procedure can be applied to other important edible mushrooms such as *Agaricus bisporus*, *Pleurotus ostreatus*, *Hypsizygus marmoreus,* and *Lentinula edodes* as well.

O-3-6: INHERITANCE PATTERNS OF A STRAIN-SPECIFIC SCAR MARKER FOR *LENTINULA EDODES* STRAIN 135

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A SCAR primer pair (amplifying a band of 601 base pairs) was designed to identify one strain of *L. edodes* (strain 135). The accuracy of the marker in identifying '135' strains was then verified by using the SCAR primer pair to correctly amplify the single unique fragment from DNA samples taken from a total of 164 *L. edodes* strains representing ten '135' strains and 154 'non-135' strains. The purpose of this study was to determine the distribution patterns of the SCAR marker among different types of monokaryons, and to establish if there is stable inheritance of the marker in offspring generated by crosses between these monokaryons. The data will provide a scientific basis for the rapid identification of offspring strains, alleviate confusion in the naming of strains, and also aid breeding programmes. Mycelium of *L. edodes* strain 135 were used to prepare protoplast monokaryons. Fifty-eight monokaryons were isolated from these sources and divided into two groups according to their phenotype, mating type and SCAR distribution patterns. The outcome was a regular distribution pattern that demonstrated the stable inheritance of the SCAR marker and which is fundamental to the feasibility of marker-assisted breeding. In addition, fruit body of 135 strain were used to prepare spore monokaryons. In this case, 294 spore monokaryons were isolated and their mating types determined. PCR amplification employing specific primers was used to establish the distribution of the '135' strain-specific SCAR marker among the spore monokaryons although no regular distribution of the marker relative to mating factors A and B was observed. Finally, experiments in which SCAR-positive protoplast or spore monokaryons were crossed with SCAR-negative dikaryons or SCAR-negative monokaryons revealed that all the dikaryotic offspring carried the marker. However, the marker was absent from all the offspring generated from crosses between monokaryons of SCAR-negative 135 strains and monokaryons of other SCAR-negative

strains. In conclusion, stable inheritance of the specific SCAR marker from monokaryons to dikaryotic offspring, and the feasibility of using the marker to identify offspring, have been demonstrated.

IV. INTEGRATED PEST MANAGEMENT

O-4-1: SPECIFIC PCR REVEALS THAT THE SUBSTRATE OF WILD GROWN *PLEUROTUS OSTREATUS* IS A POTENTIAL SOURCE OF GREEN MOULD AFFECTING OYSTER MUSHROOM PRODUCTION

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Behind champignon (*Agaricus bisporus*) and shiitake (*Lentinula edodes*), oyster mushroom (*Pleurotus ostreatus*) is the third most important commercially grown edible basidiomycete world wide. In the recent years severe green mould infections affecting the cultivation of *P. ostreatus* have been reported in South Korea, Italy, Hungary and Romania. The fungi responsible for the disease have proven to be different from *T. aggressivum* - which is well known as the causative agent of *Agaricus* green mould infections - according to their cultural, morphological and physiological characteristics, as well as molecular markers (sequences of the ITS1 and 2 regions, *tef1* and *rpb2* genes), therefore they have recently been described as the new species *Trichoderma pleurotum* and *T. pleuroticola.* The green mould disease of *P. ostreatus* is spreading fast world wide, therefore we developed a PCR-based technique in order to be able to detect the pathogens in substrate samples. Primers were designed according to the 4th large intron, the 5th exon and the 5th small intron of *tef1* gene, which are specific for both *Pleurotus* pathogenic *Trichoderma* species, as well as only for *T. pleurotum*. Based on our results, the two recently emerged *Pleurotus* pathogenic *Trichoderma* species can be detected rapidly without the need of cultivating the fungi and without ITS sequence analysis. This finding may help recognize and control green mould disease of *P. ostreatus* in its early phase. In order to study the occurrence of the two *Pleurotus* pathogenic *Trichoderma* species in the natural environment of oyster mushroom we involved *Trichoderma* strains isolated from association with wild grown *P. ostreatus* from two different samples in the study. We have found that exclusively *T. pleuroticola* is present in sample „A" (28 isolates), while in sample „B" (20 isolates) 70 percents of the isolates belong to the species *T. pleuroticola*, while the rest were identified as the members of the species duplet *Trichoderma longibrachiatum/Hypocrea orientalis.* Our results demonstrate that *T. pleuroticola* is highly accumulated in the natural environment of *P. ostreatus*, which might be a potential source of green mould infections in mushroom farms. However, the origin of *T. pleurotum* is still unclear.

O-4-2: *AGARICUS BISPORUS* INFECTION BY *VERTICILLIUM FUNGICOLA* AND INCIDENCE ON THE REGULATION OF GENES

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The button mushroom, *Agaricus bisporus* (Lange) Imbach is sensitive to various diseases, especially to dry bubble caused by *Verticillium fungicola.* The fungal pathogen developed increasing resistance to the available pesticides. A reduction in the registered fungicides to manage the disease has prompted a need to understand the interaction between *A. bisporus* and *V. fungicola* with the aim to breed for resistance. The disease is responsible for various symptoms on its host: bubbles (undifferentiated spherical masses), bent and/or split stipes (blowout) and spotty caps. DNA quantification by real-time PCR was used to study relationships between the type of symptoms and the level of tissue infection. Analyses performed on a cultivated strain revealed similar levels of infection in bubble and blowout stipe tissues, and showed

that *V. fungicola* was involved in bubble formation but did not seem to regulate its growth. The pathogen was also detected in healthy looking parts of mushrooms with spotty cap or stipe blowout. Quantification of *V. fungicola* was applied to tolerant and sensitive mushroom strains to detect a correlation between tissue infection level and resistance level. The second objective of this work was to assess the effect of infection on the disruption in gene transcription. A general approach by RT-AP PCR gave an overview on the incidence of infection on gene expression and showed that bubble is closer to cap tissue (without gills) than to undifferentiated healthy pinhead for the overall transcription. This study was completed by a target gene approach. Involvement of phenol oxydases (laccase, tyrosinase, Mn-peroxydase) and of *hspA*, a gene of the HSP70 family, in the response of a cultivar of *A. bisporus* to *V. fungicola* was investigated through mRNA and isozyme detection and quantification. *hspA* was the most regulated gene and could be associated to the strategy of defence in *A. bisporus*.

O-4-3: IMMUNOLOGICAL DETECTION OF DSRNAS IN WILD *AGARICUS* SPECIES AND IN VIRUS INFECTED CULTIVATED CHAMPIGNON

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Champignon (*Agaricus bisporus*) production accounts for a dominant portion of the Hungarian vegetable sector. Since champignons are susceptible to a large number of pests and infectious agents, it is important to have reliable methods for pathogen detection. Earlier the La France Isometric Virus caused significant yield losses. At present Mushroom Virus X (MVX) is the major virus causing losses. The unequivocal detection of virus diseases is problematic, because the symptoms are often similar to those caused by errors in cultivation. The detection of the MVX-infection presents an extreme challenge, because until now it has not been possible to identify which one of the 23 double-stranded RNA (dsRNA) species, occurring in variable number and size in MVX diseased mushrooms, is causally connected with disease development. This is why specific and sensitive polymerase chain reaction (PCR) based methods are still not available for reliable MVX detection. The aim of our experiments was to develop a sensitive, simple and reliable immunological method to detect all dsRNAs present in fungal nucleic acid extracts. The dsRNA-immunoblot method is based on the use of dsRNA-specific monoclonal antibodies. We tested more than a dozen samples of MVX-infected cultivated *A. bisporus*, and more than 50 wild *Agaricus* specimens from different species and from different areas of Hungary. In MVX diseased reference samples as well as in some "suspicious" samples we were able to detect dsRNAs not present in healthy mushrooms. In several of the wild *Agaricus* species dsRNAs of different sizes were found. In our hands dsRNA-immunoblotting was at least as sensitive as CF11 chromatography followed by agarose gel electrophoresis, and less than 3 g (fresh weight) mushroom was needed for analysis. Sufficiently early detection of viral disease by immunological methods may allow hygienic measures to be introduced and hence prevent the spreading of the virus and enable its elimination.

O-4-4: CONTROL OF YELLOW ROT ON REISHI MUSHROOM (*GANODERMA LUCIDUM* KARSTEN) USING A SHELF-CULTIVATION AND MODIFIED VINYL COVER METHOD

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Yellow rot on Reish mushroom (*Ganoderma lucidum*) has been the most destructive disease in the mushroom cultivation area in Korea. The causal pathogen was first reported as *Xylogone sphaerospora* (Anamorph *Sporendonema purpurascens*) and some effective fungicides were selected to control the disease. A cultural method, vinyl cover method (VCM), in which wood logs are wrapped in two layers of polyethylene film and sterilized before spawning, was also developed to control the disease by preventing

soil inoculum. However, the control methods were not so effective in the farm that severe yield losses have occurred in the mushroom farm in which the disease has occurred. In order to prevent air-borne inoculum as well as soil-borne inoculum we used the 3-floor cultivation shelf and VCM, in which whole film of the top of woods is removed in the commercial farm before fruit body formation. In the first year of the cultivation, none of the woods observed was infected with the pathogen. In the second year, almost of all the woods (>97%) were infected with the pathogen, which imply that the spores of the pathogen may be transmitted in the air. To reduce exposure to air-borne inoculum, only the polyethylene film near to the primordium was removed. In that treatment, 40% of the woods were infected until the second-year harvest.

O-4-5: ISOLATION OF FUNGAL VIRUSES, CAUSATIVE AGENTS OF MUSHROOM DISEASES, AND DEVELOPMENT OF THEIR DETECTION SYSTEMS

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Three double-stranded (ds) RNA viruses and a single-stranded (ss) RNA virus were isolated from the diseased fruiting bodies of the oyster mushroom, *Pleurotus ostreatus*. Infections of which principally cause a mushroom die-back disease, the symptoms of which include malformed fruiting bodies and retarded mycelial growth. Because early detection and removal of the viruses is crucial for the stable production of the mushroom, surface plasmon resonance (SPR) biosensor chips were developed as means for the rapid detection of the viruses, namely OMSV (ss), OMIV I, II, and III (ds). Monoclonal antibodies (mAb) against all 4 viruses were initially generated using purified viral particles. For the fabrication of the biosensor chip, the mAbs were layered onto an activated carboxymethyl-dextran (CM-Dex) gold thin film. Subsequently, the biosensor chip was applied to the detection of the viruses in the mushroom mycelial extract. It specifically detected the viruses in the extract in a concentration-dependent manner. The biosensor chip was also successfully employed for the detection of the viruses in the mushroom fruiting bodies collected from commercial farms. The SPR biosensor chip combined with mAb evidenced superior performance, particularly with regard to the prompt detection of the viral infection in *P. ostreatus*.

O-4-6: BIOLOGICAL CONTROL AGAINST *TRICHODERMA* SPECIES IN *AGARICUS* CULTIVATION

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The pathogens appear increasingly during mushroom growing, especially the different *Trichoderma* species may endanger the yield either in Hungary and in the world production.The plant protection against these pathogen is very limited, only a few growing technology opportunities can be used onto the elimination of their damaging. A few years ago intensive researches started to measure and characterize the different *Trichoderma* species and strains in Hungary, to develop a biocontrol product against the pathogens. Three different bacterium were selected in laboratory, which could hamper the growing of *Trichoderma* in vitro. Each antagonist belong to *Bacillus* genus. The aim of our experiments was to test these antagonist bacteria in vivo, during semi-industrial mushroom cultivation. Therefore we added the bacteria suspension to the second phase compost, and later we infected the bags with different *Trichoderma aggressivum* strains, which were earlier identified. Altogether 16 various treatment carried out in 3 repeat. During the cropping period, the healthy sporophores, the appearance of the symptoms and also the yield was measured. We found, that some bacterium can impede indeed the growing of harmful *Trichoderma*, and can increase the yield and safety production. The results promising a development of a

biocontrol product, but further examinations are necessary to decide: which bacteria and dose is good for prevention in industrial mushroom cultivation.

O-4-7: NOVEL MYCOVIRUSES IN *AGARICUS BISPORUS*

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Several mycoviruses, which cause malformation of the white mushroom, were isolated and characterized. Infestation of the viruses resulted in the abnormal basidium or even no basidium at all in severe cases. The novel viruses were isolated from the diseased fruiting bodies of *Agaricus bisporus* via polyethyleneglycol precipitation, differential centrifugation, and equilibrium centrifugation in 60% CsCl gradient. The biochemical and electron microscopic analyses on the fractions from the equilibrium centrifugation showed that the fractions contained at least 4 different virus-like particles. The bottom fraction contained a 32-34 nm spherical virus particles which encapsidated 8.0, 2.1, and 1.8 kb single-stranded (ss) RNAs by a coat polypeptide of 23 KDa. The top fraction contained three different spherical virus particles of 111 nm, 29 nm, and 18 nm in diameter. Further characterization of the latter three is under progress including the determination of their genomic RNA sequences.

O-4-8: GREEN MOLD DISEASE OF *PLEUROTUS OSTREATUS* IN HUNGARY AND ADVANCES IN ITS BIOCONTROL

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The main aim of our investigations was to isolate efficient antagonistic microbes against green mold fungi - strains of the genus *Trichoderma* - which cause significant losses in oyster mushroom cultivation both in Hungary and worldwide. Based on these strains it was planned to develop a compost supplement, which overcomes the green mold disease during mushroom cultivation. 85 *Trichoderma* strains were isolated from the samples of an oyster mushroom cultivating and substrate producing company. It was proved that the chemical fungicides carbendazim, benomyl and prochloraz are effective against the green mold strains. It was experienced that the aggressive *Trichoderma* strains produce much more chitinase as well as trypsin- and chymotrypsin-like proteases than the non-aggressive strains, suggesting that perhaps this phenomenon is responsible for the aggressivity against cultivated mushrooms. It was proved by specific PCR investigations that *Trichoderma aggressivum* is not present amongst the *Trichoderma* strains isolated from Hungarian oyster cultivating places. An important, new result of our investigations is that instead of *Trichoderma aggressivum*, a new *Trichoderma* species is responsible for the losses in oyster mushroom cultivation. We isolated *Bacillus* strains that are very effective in antagonizing *Trichoderma* but only moderately affecting the cultivated mushrooms. One of them is suitable for the development of biocontrol products, as it is very effective against oyster mushroom pathogenic *Trichoderma* strains *in vivo*, but not antagonistic against oyster mushroom, it is even elevating the amount of the crop.
This work was supported financially by a grant from the Hungarian government.

V. NUTRITIONAL & MEDICINAL ASPECTS

O-5-1: EVALUATION OF THE EFFICACY OF *CORIOLUS VERSICOLOR* SUPPLEMENTATION IN THE REGRESSION OF LOW-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS CAUSED BY HUMAN PAPILLOMA VIRUS

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There are 13 sub-types of human papilloma virus (HPV) that are considered "high risk" for cervical cancer, including HPV 16, 18, 31 and 45. Viral types 16 and 18 are thought to be responsible for 70% of clinical cases. HPV infection can result in a change in cervical epithelial (skin wall) cells from normal to one of two squamous cell types: high-grade squamous intraepithelial lesions (HSIL) or low-grade squamous intraepithelial lesions (LSIL). If a patient has persistent or frequent infections with any of the "high risk" types, they have a greater chance of developing pre-cancerous cervical cells or cervical cancer. The usual treatment for patients with cervical intraepithelial neoplasia (CIN-1: minimal or mild dysplasia) is laser vaporization or one of "wait and see". The prognosis of this condition is not as critical as in the case of patients with HSIL histology (CIN-2: moderate cervical dysplasia, CIN-3/CIS: severe cervical dysplasia/carcinoma *in situ*). In some cases of LSIL, especially among women below the age of 35, the immune system is capable of "clearing" or keeping the virus under control. However, in women (and their sexual partners) over the age of 35, especially those who take oral contraceptives and smoke, the immune system is often too compromised to clear the virus. Consequently, when diagnosed with CIN-1 (LSIL-HPV) infection, such patients may need adjunct supplementation to support their immune system against progressive HPV infection. In the present trial, 43 patients with HPV lesions (LSIL) were divided into two groups: a treatment group (22 patients) who each received *Coriolus versicolor* supplements for a period of one year (6 tablets/day i.e. 3g/day) and a control group (21 patients) who receive no treatment. Patients were initially examined with colposcopy, biopsy and HPV tipification (hybrid capture). Cervical cytology examinations (Pap smear tests) determined the patients' LSIL status and this was confirmed through colposcopy and biopsy. Four months after the first observations, colposcopy and cervical cytology was again carried out on all patients. At the same time, there was an evaluation of possible side effects from *Coriolus* supplementation. After one year, (at the end of the supplementation period), colposcopy, cervical cytology and HPV typing were carried out on all patients. The efficacy of *Coriolus* supplementation in LSIL patients was measured in terms of the evolution of HPV status from High Risk HPV^+ status to High Risk HPV^- status. High Risk HPV refers to infection with those strains of HPV (e.g. HPV 16, 18, 31 and 45) known to be associated with cervical cancer. The persistence of cervical lesions was also determined by colposcopy and cytology. *C. versicolor* supplementation over a period of one year substantially increased regression of the dysplasia (LSIL) and induced clearance of the high risk sub-types of the HPV virus responsible for cervical cancer. Supplementation resulted in a 72% regression rate in LSIL lesions compared to 47.5% without supplementation, and a 90% regression rate in the high risk HPV virus sub-types compared to 8.5% without supplementation. This study demonstrates proof-of-concept and provides supportive data that address the question of whether immuno-nutritional augmentation in the form of *C. versicolor* supplements can be successfully used to improve HPV status in patients.

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O-5-2: MUSHROOMS IN TRADITIONAL EUROPEAN FOLK MEDICINE

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In contrast to Eastern cultures, mushrooms have played only a limited role in European folk medicine. Traditionally, the Western cultures are mycophob and their interests have been more or less concentrated on a few number of edible species. Nevertheless, the names of some mushrooms undoubtedly indicate a specific medicinal use in the past. The most frequently and versatilely applicated species has obviously been *Laricifomes officinalis* (Vill ex. Fr.) Kotl. & Pouz. used as a laxative and generally against digestion problems, but also against asthma and other disaeses. *Fomes fomentarius* (L. ex Fr.) Fr. ("fungus chirurgorum") was applied for primary hemostasis, *Hirneola auricula-judae* (Bull. ex St. Amans) Berk. ("fungus sambucinus") against inflammations, especially those of the eyes. The aphrodisiac properties of truffles were also well-known: *Elaphomyces granulatus* Fr. was used in veterinary medicine, but oviously also available in pharmacies. The use of certain fungi or fungal products as pharmaceuticals (e.g. *Secale cornutum* as an abortifacient) is not definitively clarified.

O-5-3: IMMUNE STIMULATORY ACTIVITY OF GLIS: A BIOACTIVE PROTEOGLYCAN FROM *GANODERMA LUCIDUM*

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It is well known that complex carbohydrates can influence the immunoactivity. Traditional Chinese Medicine has long experiences to use fungal extracts, which contain high amount of carbohydrates, to improve the immunoactivity of tumor patients. In order to determinate the effective compounds and to understand the mechanisms of such fungal extracts a bioactive proteoglycan (GLIS) was isolated from the fruiting body of the fungus *Ganoderma lucidum* using successive chromatographic steps. The effect of this fraction on activation of lymphocytes, macrophages and NK-cells was investigated *in vitro* experiments. In this work we found that the proliferation of mice spleen lymphocytes and macrophages, tumor mice spleen lymphocytes and human peripheral blood lymphocytes were significantly induced by GLIS. Human peripheral blood lymphocytes secreted significantly IL-2 and TFN-α after stimulation by GLIS. After stimulation by GLIS, the percentage of B cells was increased in a several fold. The B cells were enlarged and the production of immunoglobulins by lymphocytes was increased significantly. In addition, GLIS stimulated remarkably the tumor killing activity of human peripheral blood NK cells. After stimulation the macrophages were spread, elongated, secreted IL-1 and TNF- α, and produced NO. The capacity of phagocytosis of macrophages was increased remarkably after stimulation by GLIS. These results indicate that GLIS, a proteoglycan of a Traditional Chinese Medicine *Ganoderma lucidum,* shows a marked capacity to stimulate both of the cellular and humoral immunoactivities, suggesting a therapeutic significance for tumor and infection diseases.
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O-5-4: ANTIOXIDANT PROPERTIES OF *GRIFOLA* GARGAL EXTRACTS

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The influence of solvents on the extraction yield, the antioxidant activity and polyphenolic compounds of Gargal mushroom extracts was investigated. Gargal mushrooms (*Grifola gargal* Singer) were gathered from the temperate primeval forest in the south of Chile. Solvents such as water, ethanol, acetone, ethyl acetate, n-hexane, and water – ethanol mixtures were used to produce the Gargal mushroom extracts. Antioxidant capacity was evaluated by four different assays, namely, 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, reducing power and chelating ability. The extraction yields were up to 20.9% w/w with ethanol and 19.6% w/w with water as solvent. A solvent mixture of ethanol and water (70:30, v/v) increased extraction yield until 24.1% w/w. The highest polyphenol (55.6 mg/100 g) and flavonoid (6.1 mg/100 g) contents in Gargal mushroom extracts were found using water as solvent. However, solvent mixtures of ethanol and water (70:30, v/v and 80:20, v/v) increased polyphenol contents until 63.3 – 63.8 mg/100 g. The Gargal mushroom extracts with acetone as solvent showed the highest free-radical scavenging activity of 96.0 mg ascorbic acid / L in the ABTS assay and 92.9% in the DPPH assay. On the other hand, the hydro-alcoholic extracts showed a strong reducing power (135.6 mg ascorbic acid / L), where the ethyl acetate extracts had a chelating ability of 19.8%. In this study, the extracts from Gargal mushrooms were found to have antioxidant activities (both ABTS free-radical scavenging and reducing power) which correlated well with the polyphenol and flavonoid contents ($R^2 \geq 0.90$). Finally, the specific antioxidant attributes of Gargal mushroom extracts were strongly dependent on the kind of solvents used during the extraction process.

O-5-5: CANCER PREVENTION AND MANAGEMENT BY MUSHROOM SUPPLEMENTED DIET

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When considering the effects of fungi their structure is important. Mushrooms are largely composed of glycoproteins and chitin. The carbohydrate consists of short, usually branched, chains of plain sugars (e g, glucose, galactose), amino sugars (sugars with an amino group, e g, N-acetylglucosamine) and acidic sugars (sugars with a carboxyl group, e g, sialic acid). The properties of the glycoproteins are very important. Sugars are very hydrophilic thanks to their many –OH groups. Their presence makes glycoproteins hydrophilic and are often essential for the proper folding of the protein into its tertiary structure. Most of the proteins exposed to the watery surroundings at the surface of cells are glycoproteins. This has implications for most structures in the body. Four amino acids can combine to form 24 different protein molecules; four monosaccharide (hexose) molecules can combine to produce 124,416 uniquely configured carbohydrate molecules. An explanation for this will be provided. Glycoproteins are necessary in neurotransmission. They are essential in protection against adhesion of bacteria, the protection against which is that the receptors on cells bind sugars which can block infection. Similarly viruses are attached through sialic acid residues and these can be blocked with glyconutrients. Both our cells and microorganisms have carbohydrates on their surface. Through the interactions of these surface carbohydrates, microorganisms can gain access to our body, start to multiply and cause disease. Various carbohydrates, such as mannose and other sugars, have been shown to interfere in this process. Metabolism of dietary sugars includes the pathways for metabolism of fructose and other sugars, which will be described. The relationship of these to Syndrome 'X' will also be explained. Glyconutritional supplements, proteoglycans from mushroom, contain various saccharides which can supply carbohydrates that assist in the maintenance of good health by interfering with the infection process. This may be

accomplished by at least two mechanisms: 1) hindering pathogen colonization or 2) stimulating immune cell function. Many cancers are caused by infectious agents. The relationship between a mushroom supplemented diet and cancer will be discussed

O-5-6: MYCOTHERAPY – TREATMENT WITH MEDICINAL MUSHROOMS

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Mushrooms belong to the oldest natural remedies of mankind. In Asia their application in medicine have been used for thousand of years but in the last years in Europe there has been also an enormous increasing interest in their application for preventive and curative purposes. Medicinal Mushrooms have already a strong scientifically based potential in immunotherapy, for example, as an alternative cancer treatment to complement or even to substitute chemotherapy or radiotherapy. A detailed overview of the following 10 most important medicinal mushrooms (*Ganoderma lucidum*, *Cordyceps sinensis*, *Agaricus blazei*, *Grifola frondosa*, *Hericium erinaceus*, *Coprinus comatus*, *Lentinula edodes*, *Auricularia judae*, *Coriolus versicolor* and *Polyporus umbellatus*) will be provided and their traditional application area will be outlined. Having regards to the available analysis results of the respective ingredients it will go into nutritional and pharmacological meaning of each medicinal mushroom. The results of the latest research and studies, concerning the application of individual medicinal mushrooms in numerous diseases and disturbances, will be compiled and evaluated with regard to the consequences for the practical and therapeutical action. The centre of interest will be the experiences in the treatment of Diabetes, hypertension, lipid metabolic disorder, overweight, allergies, psychosomatic disorder as well as the possibility of immune system stabilisation and the combat of various cancers. In this lecture will be discussed some methods of cultivation, harvesting and processing of medicinal mushrooms into the corresponding products. The correct application of medicinal mushrooms products will be explained in detail and the answers to questions about dosage, mixtures, administration forms and accompanying symptoms will play also an important role. The lecture will conclude with the incorporation of mycotherapy in other naturopathic treatments.

O-5-7: INHIBITORY EFFECT OF MOUSE SARCOMA 180 BY CRUDE B–D-GLUCAN EXTRACTED FROM *HERICIUM ERINACEUS*

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Hericium erinaceus, one of delicious edible and medicinal mushroom belonging to Hericiaceae, has been known to exhibit outstanding inhibitory effects against human and animal cancers. This study was initiated to evaluate the antitumor effects of crude β–D-glucan extracted from fruiting body of *H. erinaceus*. Neutral salt soluble (0.9% NaCl), hot water soluble and methanol soluble substances (hereinafter referred to Fr. NaCl, Fr. HW and Fr. MeOH, respectively) were isolated from the mushroom. *In vitro* cytotoxicity tests showed that Fr. HW does not have toxic effect against cancer cell lines such as Sarcoma 180, HepG2 and NIH 3T3. Intraperitoneal injection with Fr. HW showed antitumor activity with life prolongation effect of 54.1% in ICR mice previously inoculated with Sarcoma 180. Fr. MeOH increased numbers of spleen cell proliferation by 1.64~2.97-fold at the concentration of 200~1000 μg/ml. Fr. Na. showed immunopotentiating activity of B lymphocyte by increasing alkaline phosphatase activity by 5.77-fold at the concentration of 1000μg/ml. The weight of spleen in the test group was increased slightly compared with the control group. These experimental results suggested that the antitumor activity

of the crude β-D-glucan against Sarcoma 180 of ICR mice was likely due to immunopotentiating activity, but not direct cytotoxic effect.

O-5-8: BONE CELL STIMULATING EFFECTS OF EDIBLE MUSHROOMS - A NEW PERSPECTIVE FOR PREVENTION OF OSTEOPOROSIS

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Osteoporosis is widely recognized as a major public health problem. New possibilities for prevention and treatment are needed. We have established an in vitro test system using cultivated human bone cells, e.g. HOS58 cells and SaOS-2 cells, for the detection of possible bone-inductive properties of natural compounds and extracts. The protein content, the activity of alkaline phosphatase and mineralization are used as indicators for the vitality and maturation of osteoblastic, that means bone-building, cells. In the course of screening investigations of a broad spectrum of mushrooms we found that aqueous extracts of the fruiting bodies of *Grifola frondosa* (Maitake) and of *Lentinula edodes* (Shiitake) are able to stimulate the vitality and mineralization of the cells. The cultivation of HOS58 cells for 5 days in the presence of an extract of *G. frondosa* or of *L. edodes* resp. resulted in a significant elevation of alkaline phosphatase activity of the cells in comparison to untreated cells. SaOS-2 cells, incubated with one of the extracts for 21 days, show a nearly two times higher mineralization than cells cultured with the positive control ß-glycerophosphate alone. The obtained results clearly indicate that the named mushrooms have bone-inducing activities. Therefore we propose, that *G. frondosa* and *L. edodes* could be applied for prevention or supportively for treatment of osteoporosis and other bone disorders. The identification of the responsible compounds in the extracts is in progress. Besides, *in vivo* investigations are necessary.

O-5-9: A STUDY OF MUSHROOM NUTRIENTS AND CHEMICAL COMPOSITION ACCORDING TO TREATMENTS AND HARVEST

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Mushrooms contain sufficient nutrients and rich in chemical composition and aroma components. It is well known that mushrooms have both medicinal and nutritional importance for the human. Mushrooms can consume both fresh and also processed which possess some food treatments like canning and drying. Cultivated edible mushrooms are picked up according to flushes and harvest periods which are prepared in different compost substrate composition. The physical properties of mushrooms; texture and diameter evaluated in quality criteria. Flavor of mushrooms can be explained as aroma components or volatiles together with physical properties and their tastes. Mushroom flavors should be more distinguish and pleasant than lots of vegetables or plant products among the foods. Mushroom nutrients, chemical composition and volatile components were examined in a large perspective; macro and micro nutrients i.e. proximate composition; crude fiber, chitin, glucans which have the medicinal and nutritional importance. All of these components can be change or affected in different flushes and harvests. On the other hand, the food preservation techniques like blanching, dehydration, canning or sterilization can be applied mushrooms in the food industry. These treatments affect macro and micro nutrients and sensory properties of mushrooms. These preservation techniques of mushrooms are preferred for long shelf life and for the economical aspects. The nutritional levels of mushrooms are superior according to lots of vegetables. Some mushroom scientist claim that nutritional value of mushrooms are equal to milk. Mushrooms are functional properties for health. These treatments can affect their functional properties too. Mushrooms are good alternative foods because of biochemical composition and nutrients especially for developing countries. In this article, the changes of the all the mentioned components and properties

of cultivated edible mushrooms were summarized our own related research results and also together with international mushroom scientists' in the similar topics, in this article.

O-5-10: MUSHROOMS AS THE SOURCE OF NOVEL THERAPEUTIC STRATEGIES FOR THE TREATMENT OF NEURODEGENERATIVE CONDITIONS

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It is now widely recognized that a number of neurodegenerative pathologies, and cellular degeneration related to ageing, are associated with oxidative stress and cell damage induced by reactive oxygen species (ROS). Some of these adverse effects are offset by antioxidants, present either naturally or added as supplements in the diet. In view of recent findings showing that mushrooms are effective in the treatment of oxidative stress, we have determined the levels of various enzymes associated with the removal of ROS (superoxide dismutase, catalase, peroxidase, GSH-reductase, Se-dependent GSH peroxidase, NADPH-cytochrome C reductase, laccase) in a range of mushrooms (*Polyporus umbellatus*, *Agaricus blazei*, *Pleurotus ostreatus* and *Hericium erinaceus*) that form part of the product range of Mycology Research Laboratories Limited. Highest levels of superoxide dismutase (SOD) were recorded in *H. erinaceus* and *P. ostreatus* (421 and 589 U/g biomass, respectively), followed by *A. blazei* (263 U/g biomass) and *P. umbellatus* (53 U/g biomass). NADPH-cytochrome C reductase were also detected in all four mushroom species with *H. erinaceus* again exhibiting the highest activity (6.8 mU/g biomass), followed by *P. umbellatus* (5.59 mU/g biomass), *P. ostreatus* (4.8 mU/g biomass) and *A. blazei* (2.97 mU/g biomass). Readily detectable levels of tyrosinase were again observed in all four mushrooms, especially in *A. blazei*. Significant GSH reductase activity was present in *P. ostreatus*, lower levels were recorded in *H. erinaceus*, while no enzyme activity was detected either in *P. umbellatus* or *A. blazei*. Interestingly, we found high levels of Se-dependent GSH peroxidase in *H. erinaceus, P. ostreatus* and *P. umbellatus* but were unable to detect this enzyme in *A. blazei*. No laccase or peroxidase activity was detected in the biomass of all four mushrooms although, following cell lysis, significant levels of laccase and, to a lesser extent, peroxidase were recorded only in *H. erinaceus*. No catalase activity was found in any of the four species tested. In conclusion, our studies suggest that important antioxidant and cytoprotective enzymes are present in all the different fungi examined, suggesting considerable potential for therapeutic strategies based on nutritional interventions with medicinal mushrooms to limit and/or prevent the adverse consequences associated with free-radical induced damage in neurodegenerative disorders as well as other disease conditions including cancer and cardiovascular diseases.

O-5-11: FIBRINOLYTIC ACTIVITY OF SEVERAL COPRINOID MUSHROOMS

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Nowadays, medicine and food industry are in search for new natural sources of proteolytic enzymes (proteases). Extracellular fungal proteases are similar to proteases from plants and microorganisms and attack mainly casein and fibrin. Coprinoid mushrooms are characterized by a strong proteolytic (fibrinolytic, thrombolytic, caseinolytic) activity. It was shown that extracellular fibrinolytic enzymes of CMs actively secreted during submerged mycelial growth. *In vitro* fibrinolytic activities of 8 species and 9 strains of Coprinii from genera *Coprinus (C. comatus)*, *Coprinopsis (C. cinerea, C. strossmayeri)*, *Coprinellus (C. disseminatus* (str. 30 and C278), *C. domesticus*, *C. micaceus*, *C. radians)* and *Parasola (P. plicatilis)* were tested on fibrin clots received from human blood plasma. Cultural liquid (CL) and mycelial extract (ME) samples were obtained after submerged cultivation of mycelia in malt-extract

medium (pH=6.5) at 25 ^{0}C and agitation rate 200 rpm during 7 days. The test was carried out by adding different amounts of CL and ME on fibrin clots (1:1 and 1:2, v/v) and duration of fibrinolysis was estimated. Data was compared with control - the time of lyses of fibrin clot without mycelial samples. Tested CL and ME samples, except ME of *C. cinerea,* revealed different degrees of fibrinolytic activity compared with the control. However, activities of CL samples were higher than those of ME samples. Among all CL and ME samples, the highest activity was revealed in *C. comatus* which promoted fibrinolysis up to 25%, whereas the lowest activity (4%) showed CL of *C. cinerea*. Fibrinolysis took place faster in *C. domesticus,* then *C. strossmayeri*, *C. micaceus, C. disseminatus* (str. 30), *P. plicatilis, C. disseminatus* C278 and *C. radians*. Revealed dose-effect correlation was not significant. The results show that mycelia of tested mushrooms are producers of proteolytic/fibrinolytic extracellular enzymes which could be further used to obtain new thrombolytic biotech-products of mushroom origin.

VI. SAFETY, QUALITY CONTROL AND REGULATIONAL ASPECTS

O-6-1: MATHEMATICALLY CHARACTERIZING VARIATIONS OF SEC-HPLC PROFILE AMONG DIFFERENT *GANODERMA* POLYSACCHARIDES

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Medicinal mushroom polysaccharides exert multiple pharmacological and physiological actions, particularly on anti-cancer and stimulation of immunity. Size exclusive HPLC (SEC-HPLC) has been commonly used for analyzing the molecular weight distribution of mushroom polysaccharides and for their products quality control. However, the chromatogram obtained is difficult to be applied for characterizing the variations among different samples. In this research, we applied "vectorial angle cosine" and hierarchical cluster analysis method to analyze the chromatogram data, in order to set up a mathematical mean to finely quantify the sample similarity or dissimilarity. Twenty-three strains belonging to five Ganoderma species, 7 strains from *G. lucidum*, 3 from *G. oerstedii*, 6 from *G. resinaceum*, 2 from *G. subamboinens* and 5 from *G. tsugae* respectively were cultivated and their mycelial polysaccharides were extracted in a consistent condition. These samples were then analyzed by SEC-HPLC, and the chromatographic signal intensities at each time point were calculated to acquire the vectorial angle cosine value. The values were then processed by hierarchical clustering method for similarity comparison. The results revealed although samples from *G. tsugae* and *G. subamboinens* strains did not be clustered reasonably, the rest did. Six strains of *G. lucidum*, 2 strains of G. *oerstedii*, 5 strains of G. *resinaceum* could be grouped back to their original species when average distance between clusters was set as 1.5. The cluster accuracy was accounted as 87% for all strains from the three species. Similarity data also revealed that the samples among three different species had a further apart-from average distance, of which was 3.5. These results suggest that by processing SEC-HPLC data with "vectorial angle cosine" and hierarchical cluster analysis, the similarity of polysaccharide samples from a same species or dissimilarity of that from different species could be perceived and quantified. Therefore it could be potentially developed into a fingerprinting technique for mushroom polysaccharide quality analysis and quality control.

VII. MUSHROOM RESOURCE DEVELOPMENT

O-7-1: *GANODERMA PFEIFFERI* – A NEW MEDICINAL MUSHROOM

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In the frame of our biological and phytochemical investigations of European Ganoderma species we could identify *Ganoderma pfeifferi* as a promising species which has not been investigated before. In opposite to *Ganoderma lucidum, G. pfeifferi* occurs only in Europe. In nature it growth on deciduous trees like *Fagus,* but it can also be cultivated in the form of mycelium or fruiting bodies. *G. pfeifferi* contains a broad spectrum of bioactive compounds, among them triterpenoids, sesquiterpenoids, aromatic compounds, polysaccharides and essential fatty acids. Some of them are structurally new compounds, e.g. the antibacterial ganomycins and some triterpenoids; other substances, e.g. lucialdehyde B, occur in *G. pfeifferi* in much higher concentrations than in *G. lucidum*. Extracts of *G. pfeifferi* are of great interest for an application on the skin. They stimulate the vitality of cultivated human skin cells, support the regeneration of keratinocytes after UV radiation, increase the cellular protein content and prolonge the life span of human cells in a continuous cell culture. Besides, they exhibit strong antioxidative effects and have antibacterial and antiviral activities, especially against Herpes viruses. Preparation of so called Maresome™ from the biomass of this mushroom, alone or in combination with other natural materials, increases the efficiency. Therefore we assume that preparations with *G. pfeifferi* should find application in cosmetics and for dermatological purposes. Besides, an internal use seems to be possible.

O-7-2: INVESTIGATION OF THE POTENTIAL USE OF SPENT *PLEUROTUS OSTREATUS* (WILD TYPE, ORIGINATED FROM NEW MEXICO) SUBSTRATE IN RUMINANT FEED

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In this document test of the hypothesis of the use of spent mushroom substrate from P*leurotus ostreatus* mushrooms (wild type collected in Rio Grande river valley) grown on salt cedar wood is described. Investigation of the possibility for use spent substrate mixed with corn silage in animal feeding, is presented. We confirmed that waste salt cedar biomass can be used as a substrate for mycelia growth and fruiting of the edible mushroom *Pleurotus ostreatus*, and examined alterations of the chemical composition, mineral content and level of digestibility of the substrate during mushroom growth. The most significant modification found was in the nitrogen (N) content, which was reduced from 0.39% in the beginning of the life cycle of mushrooms to 0.19% at the end of the fruiting period. The content of Neutral Detergent Fiber (NDF) and hemicellulose (the more easily degradable lignocellulose complex) were lower at the end of growing cycle by 2.62 - 3.08%, consistent with the known activity of *P. ostreatus* enzyme complex (cellulase, hemicellulase, celobiase, ligninase etc). In contrast, the quantity of Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL) and cellulose (the less-easily degradable complex) was not changed significantly during the mushroom growing cycle. The digestibility of the substrate dry matter after the end of the mushroom growing cycle was lower (8.94%) than the digestibility immediately after inoculation of the substrate (10.84%). These data suggest only modest possibilities for utilizing spent *P. ostreatus* substrate produced by salt cedar sawdust directly in animal feeding. However, we have established the hypothesis for using spent mushroom substrate as a component of silage production together with ground corn grain. Experiment confirmed that possibility of using spent substrate like component of the silage exist.

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O-7-3: EASTERN MEDICINAL MUSHROOMS GO WEST

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After the hesitant opening of the PR China in the 1970s, the interest in TCM (Traditional Chinese Medicine) has distinctly increased. Globalization, the intense search of the pharmaceutical industries for new active compounds and a steadily growing demand for "health care"-products have contributed to the present situation that a broad range of "exotic", in the Western world formerly unknown natural remedies are available. A number of mushrooms and products from mushrooms with a long tradition in TCM and Eastern nutrition have found their way to Western markets and pharmacies. Shiitake and Maitake - unknown 40 years ago – are widespread and well-accepted fungal species. The current German compilation of dietary supplements lists about ten Shiitake and Reishi products, respectively, and six from Maitake; the number of products available via internet obtain a multiple.

O-7-4: MUSHROOMS AND SKIN TREATMENT – FROM TRADITIONAL USE TO MODERN APPLICATIONS

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In traditional Chinese medicine, several mushroom species have been successfully applied against different diseases of the human skin. These included e.g. the use of *Armillariella mellea* (Vahl. ex Fr.) Karst. against dermal dryness, *Auricularia auricula* (L. ex Hook.) Underw. against hemorrhoids, and *Bovista plumbea* Pers. to cure external wounds. The use of medicinal mushrooms against allergic skin reactions is also described: a more recent report on the application of *Ganoderma lucidum* (Leys. ex Fr.) Karst. recommends defined capsules from extracts of the fruiting bodies.
Up to now, there are only few products derived from mushrooms in Western medicine and cosmetics to cure skin problems and diseases. These include e.g. chitosan products from submerse fermentations or derivatives of beta-glucans against actinodermatitis.

O-7-5: ORGANIC PRODUCTION OF OYSTER MUSHROOM IN INDIA

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Oyster mushroom in India is commonly produced on cereal straw pasteurized in water containing effective doses of carbendazim and formaldehyde leading to the production of mushrooms containing some residual toxicity. Hot-water treatment as an alternative method of pasteurization also, has not been adopted by most growers due to high fuel-cost involved in the practice. In the present study, a Neem-oil formulation- azadirachtin 0.15% EC (1500 ppm) has been used to pasteurize chopped paddy straw ,as the substrate for growing Oyster mushroom organically. Results obtained both from in vitro and in vivo studies on the effect of low concentrations of Neem oil on the growth and yield of *Pleurotus flabellatus* (Berk & Broome) Sacc. is reported. Neem oil at 150, 15 and 1.5 ppm concentrations had no adverse effect on mycelial growth of *P. flabellatus* and a common laboratory contaminant, *Aspergillus niger*, but it restricted considerably the growth of a common mushroom parasite, *Trichoderma viride*, for more than a week. Results from an *in vivo* study on *P. flabellatus* indicated that Neem oil at 0.15 and 0.015 ppm

concentrations supported good mycelial growth, enhanced pinhead numbers and gave considerably higher mushroom yields than the chemically treated control beds and those soaked in plain water only, except that it extended the period of spawn-run by 2-3 days.

VIII. ENVIRONMENTAL IMPLICATIONS AND APPLICATIONS

O-8-1: OYSTER MUSHROOM *PLEUROTUS OSTREATUS* (JACQ.) P.KUMM. – ITS CULTIVATION AND UTILIZING IN SLOVAK FORESTRY

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The primary role of wood destroying fungi is to decompose wood, to humificate it and to restore it into an organic matter cycle. Effort to utilize those abilities rapidly increased when the techniques of artificial infection by spawn of some wood destroying fungi were developed. For forestry practice it was a primary interest to transform a waste wood in forest stands for useful organic matter in relatively short time. It is for instance the biodegradation of tree stumps after windstorms or cutting of full-grown trees. Its natural decomposition is relatively lengthy and it takes some decades. Decomposition of stumps artificially infected by *Pleurotus ostreatus* was from three- up to eight-times faster in comparison with natural conditions. The simplest and the least of all dangerous is growing of the oyster mushroom in wooden stumps. It commonly grows in wood of poplar, beech, birch, willow, hornbeam, lime, alder, apple, walnut, pear, plum and so on. Interesting method for the oyster mushroom growing in forestry practice is a decomposition of wood in soil reclamation of a deciduous forest. Thin broken branches are placed into a half metre drain and after infection by the oyster mushroom strain it is covered with thin soil layer. During a few years the mushroom is growing and producing fruitbodies depending on external, especially climatic conditions. Forest soil is enriched with new organic matter after the substrate is completely decomposed. Various techniques of oyster cultivation presented in actual topic are aimed to combination of their useful abilities with production of the quality fruitbodies. Presented examples were tested under conditions of Slovak deciduous forests. Growing of oyster mushroom does serve neither for speed nor for continual production of fruitbodies. But it could be a perspective fungus for growing in the forest management conditions. The result of oyster mushroom activity could be a faster decomposed wood-waste, faster input of organic matter to the forest soil, and also the tasty fruitbodies for healthful nutriment.

O-8-2: DEVELOPMENT OF SUBSTRATE FOR FRUITING OF *PLEUROTUS OSTREATUS* USING WASTE BED-LOG (*LENTINUS EDODES*)

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Waste Bed-log that is the *Lentinus edodes*'s waste after harvesting a mushroom was used as substrate for oyster mushroom cultivation. Duration of hyphal growth phase was shorter in the mixed substrate (waste bed-log sawdust: poplar sawdust: rice bran = 4:4:2) than in the poplar sawdust substrate. but Yield was higher in the mixed substrate(waste bed-log) than in the poplar sawdust substrate. Income can be increased 68won/kg with the mixed substrate with high yield and by lowing expense for the substrate since waste bed-log is cheaper than poplar sawdust substrate.

O-8-3: BIOTECHNOLOGY TO GROW EDIBLE AND MEDICINAL MUSHROOMS ON VINE AND WINERY WASTES

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The main aim of this research work was to find out the best way of recycling the vine and winery wastes by using them as a growing source for edible and medicinal mushrooms in order to extend the food chain in vineyard ecosystems. According to this purpose, three fungal species from Basidiomycetes, namely *Ganoderma lucidum* (Reishi), *Lentinus edodes* (Shiitake) and *Pleurotus ostreatus* (Oyster Mushroom) have been used to determine the effect of lignocellulosic vine and winery wastes used as culture composts on the production of mycelia and fruit bodies that could be processed and marketed as useful products such as, food and drugs. The experiments were achieved by growing these fungal species in special culture rooms, where all the culture parameters were kept at optimal levels in order to get the highest production of fruit bodies. During the experiments, the effects of culture compost composition (carbon, nitrogen and mineral sources) as well as other physical and chemical factors (such as: temperature, inoculum size, CO_2 and O_2 concentration, air humidity, watering, light intensity and incubation time, etc.) on mycelial net formation and especially, on fruit body induction were investigated. The registered data revealed that lignocellulosic vine wastes has to be used as substrates for mushroom growing only after some mechanical pre-treatments that could breakdown the whole lignocellulose structure in order to be more susceptible to the fungal enzyme action. All these pre-treated lignocellulosic wastes were disinfected by steam sterilization at 120^0C for 60 min. The final composition of culture composts was improved by adding only natural ingredients, such as: grain seeds and brains of wheat, rye and rice, as well as limestone powder, each kind of culture medium composition depending on the fungal species used to be grown. All the culture composts for mushroom growing were inoculated using liquid inoculum with the age of 5 – 7 days and the volume size ranging between 5 - 7% (v/w). The fruit body production of these three fungal species used in experiments ranged between 7 – 12 kg relative to 100 kg of compost, all the results of these experiments being presented in details in this paper.

IX. MYCORRHIZAL MUSHROOMS AND MYCORRHIZATION

O-9-1: MONITORING OF AN EXTENSIVE TRUFFLE ORCHARD IN HUNGARY

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Hypogeous fungi are living under the surface of the soil. Some of them develop carpophores with high economical value, having great respect in the gastronomy for centuries. Cultivation of these fungi called truffles started two hundred years ago in Europe. Different methods were applied since them to create a truffle orchard, including extensive techniques when in situ inoculated plants were planted or acorns covered with truffle suspension were sown. The biggest truffle plantation in Central Europe of 15 ha represents the above mentioned method, established periodically from the year 1999, sowing 20 pieces of truffle suspension covered acorns per meter and having 2 meters between the rows. The orchard is situated in a NW-SE oriented valley with a temporary watercourse in the bottom and a small lake. Chernozem, water affected meadow chernozem and heavy, highly carbonated chernozem–brown forest soils occur in the area. Around the orchard truffle producing habitats have been detected. Monitoring of the orchard has been carried out for three years from 2004. Plantation was divided to parcels based on the time of establishment, tree and truffle species and orientation. Soil samples have been taken and been analyzed in 2004 and in 2006 comparing them with natural truffle bed soil results. In both years root

sampling were carried out to follow mycorrhiza development of different truffle species on various hosts and parcels. Soil results showed mosaic structure and the orchard proved to be well suited concerning pH, salt content and lime. Level of organic matter and clay were also sufficient. Seedling developed variously due to different orientation and steepness of slopes and therefore water availability. Mycorrhiza levels were in harmony with the applied method, showing great variability. Mycorrhiza of *Tuber aestivum*, *Tuber macrosporum* and *Tuber brumale* were detected on various levels between 0-36,4%, 0-26,4% and 0-24%, respectively. Contaminant mycorrhizal fungi were also found on root samples reaching levels as far as 50,4%. In spite of the sometimes high contaminant mycorrhiza level truffle fructification and success of the orchard could not be excluded, proved by a recent founding of two *Tuber aestivum* ascocarps in one of the parcels.

O-9-2: APPEARANCE OF THE ECTOMYCORRHIZAL MUSHROOMS AT THE DISTURBED FOREST STANDS

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An occurrence of the ectomycorrhizal, saprophytic and parasitic fungi is a natural requirement for the existing of forest and the share of the number of fungi species from these ecological groups depends on the forest status, its health, age and growing conditions. Ectomycorrhizal symbiosis is inevitable for normal existence and optimal growth of the most of economically important tree species. Its optimal functioning is necessary for growing of the ectomycorrhizal fungi and the base for production of the number of their fruitbodies. Forest stands disturbed by various injurious agents (air pollution, wind, snow, insects, drought or clear cutting) are weakened and the spectrum of the fungal species is much modified. Actual research is oriented to register the changes in spectrum of macromycetes, especially to share of the ectomycorrhizal mushrooms, at the conditions of disturbed forest stands. The research is aimed to the most widespread tree species in Slovakia - beech. It was in the long run regarded as a stabile, not very endangered tree species with a good health status. Considerable rot of its health condition is a reason of research the various aspects of the beech growing at the last decades. Evaluation of the "mycorrhizal percentage" and the health condition parameters of trees confirmed the disturbed growing conditions and the assumption of a negative influence the imission pressure to ectomycorrhizal fungi. The preliminary results show the marked decline of the ectomycorrhizal fungi species in the disturbed forest areas.

O-9-3: ECOLOGICAL RESEARCH BASING SWEET TRUFFLE (*MATTIROLOMYCES TERFEZIOIDES* /MATTIR./ E. FISCHER) CULTIVATION

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Distribution of this species is limited mainly to Carpathian Basin and it follows the sandy areas deponated by the Danube. Attempts have already been made to produce mycorrhized seedlings, but no plantation has been established so far. Basic knowledge of ecological requirements of this species will considerably be favored by establishing and maintaining experimental plantations The species exclusively grows in Robinia-forests mostly on sandy soils with lime in traces, which are slightly alcalic, with high humus content, with various amount of phosphor and with medium or high potassium content. Fructifying season in Hungary lasts from August to November. Samples from sandy truffles habitats can hardly be ranged into groups during ordination. The 21 coenological descriptions can be divided into 5 associations. Most descriptios have been made in relation to *Bromo sterilis-Robinietum* association (13), *Urtico dioicae-Robinietum* (1), 4 descriptions belong to *Robinietum cultum* association planted out in the old site of *Scilla vindobonensis-Ulmetum* in middle part of Danube region, 2 descriptions concerned *Polygonato latifolio-Quercetum roboris* from Danube-Tisza region and one *Pinus sylvestris* cultum with *Robinia*

pseudo-acacia. In case of all habitats, phytoindication values (according to Borhidi) of *Mattirolomyces terfezioides* without correcting by abundance can be presented as follows: examining relative heat requirement (TB), species number of 7 values' species is the biggest one, according to thermophilous forest and woodland belt; examining relative soil water and soil humidity (WB), species number of 6 values' is the biggest one, according to plants of fresh soils; examining relative soil reaction (RB), species number of 7 values' is the biggest, according to basifrequent plants mostly on basic soils, never occuring on very acid sites; examining relative nitrogen requierement (NB), number species of 6 values species is the biggest one, according to plants of moderately nutrient rich habitats. This species is very honored in Hungarian gastronomy, since it can perfectly be „married" with desserts, sorbets and cookies due to its strong sweet taste. Meanwhile truffle production of natural truffières is varying to a great extent year after year. This problem can be solved by establishing plantations based on ecological demands of the sweet truffle.

O-9-4: NEW PHYTOINDICATION BASED PREDICTIONAL METHOD FOR BETTER SELECTION OF BURGUNDY TRUFFLE PLANTATION SITES AND HOST PLANTS

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Though truffle plantations were established in many thousands hectars fields all over Europe during last decades, these plantations could not meet hopes and expectations, they could not invert reducing of commercialized truffle amounts, even for the most part they did not fructify. Presumably non-suitable selection of plantations sites and of hostplants is partly a reason for that. A better knowledge of natural truffières' ecology can supply a solving of both problems, namely revealing of natural habitats' phytoindication which is a real subject of this paper in connection with *Tuber aestivum*. To the prepared plant coenological tables of 169 coenological descriptions from *Tuber aestivum* habitats of carpatho-pannon region have been attached relative ecological indicator values by Borhidi. Phytoindication values of *Tuber aestivum* without correction by abundance in case of all habitats can be presented as follows: examining relative heat requirement (TB), the species number of 5 values' species is the highest one, according to plants of montane mesophilous broad-leaved forest belt; examining relative soil water and soil humidity (WB), the species number of 5 values' species is also the highest one, according to plants of semihumid habitats, under intermediate conditions; examining relative soil reaction (RB), species number of 6 values' species is the biggest one, according to mostly on neutral soils but also in acid and basic ones, generally widely tolerant more or less indifferent plants; examining relative nitrogen requirement (NB), species number of 5 values' species is the biggest one, according to plants of mesotrophic habitats. By comparing phytoindication of herbs in Hungarian plantations with phytoindication of natural habitats presented above (reflecting ecological demands of *T. aestivum*), a plantations'rank can be set up with prediction. This prediction was confirmed by mycorrhization controls: mycorrhization was in fact higher in plantations more similar to natural habitats. Moreover, the rank set up according to phenologic and mycorrhization values of hostplants generally follows predictions made out by comparing values of phytoindication of the site's flora and of applied host plants. The above mentioned new approch is an expenditure sparing method by which establishing non-productive or scarcely productive plantations can be avoided.

X. ECONOMICS AND MARKETING

O-10-1: WILD MUSHROOMS AND SOCIOECONOMICS IN NORTHEAST THAILAND

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The objective of this study was to investigate the biodiversity of wild mushrooms as well as the socioeconomics of rural Northeast Thailand. The mushrooms were collected in cultural forests and markets along the roadside in 19 provinces of Northeast Thailand. Three plots were sampled in order to measure the quantity of mushrooms in the field. In total, there were 436 edible species. Of which, only 222 species were found sold in markets. The remaining edible species were not seen primarily because of their rarity. Examples include *Tuber separans*, *Cantharellus lutescens,* etc. There existed an estimated 62.6 kg of product per acre or 158.75 kg/hectare resulting in an estimated 28,342.15 tons per year for the whole area, the value of which was approximately $40,486,761US. The wild mushroom yield was highest during May to August with most of the edible species found in the Dipterocarp and Dry Evergreen Forests. Of all the mushroom collectors, 95% were women. This in-depth study included 1,114 households whose average yearly income totaled $990US. Of this, $133US came from the harvesting of mushrooms (approximately 13% of total yearly income). The highest selling price found was $7US/kg, which included *Amanita fulva* (Pers.) Fr., *Amanita priceps* Corner et Bas., *Amanita caesarea* (Fr.) Schw., and *Thermitomyces* sp. The lowest selling price found was $1.5US. Of those sold in markets, the top five species seen were *Amanita crocea* (Quil) Sing., *Termitomyces clypeatus* R. Heim, *Termitomyces fuliginosus* R.Heim, *Termitomyces perforans* R. Heim, and *Geastrum saccatum* (Fr.) Fischer.

Poster Session

PO-1: ANTIFUNGAL ACTIVITIES OF MINT AND THYME ESSENTIAL OILS AGAINST MYCOPATHOGEN *MYCOGONE PERNICIOSA*

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Wet bubble disease, caused by mycopathogen *Mycogone perniciosa*, is considered as one of the most important disease of cultivated mushroom *Agaricus bisporus*, wherever white button mushroom are produced commercially. Mushroom cultivation in Serbia is still less developed than in other countries, and *M. perniciosa* has a significant influence on quality and yield of mushrooms. Although WBD is routinely controlled using different fungicides *M. perniciosa* remains a constant threat. In addition, constant use of fungicides causes development of pathogen resistance. The antimicrobial property of volatile aromatic oils from medical as well as other edible plants has been recognized since antiquity. Essential oils of *Mentha piperita*, *M. spicata* var *crispa*, *Thymus vulgaris* and *T. tosevii* were assayed for inhibitory activity against *M. perniciosa* (5 isolates). In order to find alternative methods in treatment of fungal diseases we tested the antifungal activity of essential oils against *M. perniciosa* by microatmospheric method. The commercial antifungal agents, prochloraz-Mg was used as a control. Mint oils showed minimal inhibitory quantity (MIQ) of 4 µl/disc and minimal fungicidal quantity (MFQ) 5 µl/disc. Thyme oils inhibited all isolates with 0.2 µl/ disc. The values for thyme oils (MFQ) were 0.7 µl/ disc. Prochloraz-Mg showed fungicidal activity at 50 µl/disc and MIQ 5 µl/disc to all tested isolates. The mycelial growth and sporulation of isolates *M. perniciosa* were diferent. Isolates MPS and MPR, were the most sensitive strains in this experiment, while isolates MPH1 and MPH2 were the most resistant among the isolates tested. Mint and thyme essential oils may be used as an alternative for the synthetic chemicals in the treatment of fungal diseases. The essential oils give an ability for obtaining of antifungal agents which are not toxic. This study supports futher research of essential oils usage for the control of *M. perniciosa* in the mushroom farms.

PO-2: PHYLOGENETIC RELATIONSHIPS AMONG *PLEUROTUS ERYNGII* SPECIMENS FROM DIFFERENT ECO-GEOGRAPHIC SITES OF THE MEDITERRANEAN REGION

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The market and biotechnological relevance of *Pleurotus* genus is well known despite the fact that the phylogenetic relationships among various taxa are often masked by the lack of data on natural occurring molecular variation. This work focuses on clarifying the taxonomy and phylogeny of *Pleurotus eryngii* species-complex using the multigene approach analysis compared to the multitrait morpho-phenological classification. Thirty-seven target DNA sequences sampled on the base of geographic origin from a representative collection, have been analyzed by sequencing of the nuclear rRNA genes (ITS1-4), the elongation factor 1 alpha (EF-1α) single copy gene and also by evaluating the polymorphism of the minisatellite M13 fragments. Nucleotide sequence diversity data of ITS1-4 revealed 16 SNPs over a consensus sequence of 640bp. This variation classified consistently in two distinct taxa (*Pleurotus eryngii* and *P. nebrodensis)* the *P. eryngii* species-complex. In order to further clarify the within taxon grouping were used SNPs occurring over the elongation factor 1 alpha (EF-1α) and the M13 minisatellite variation. Morpho-phenologic data resulted less efficient than genomic one when used to reveal differences among taxa.

PO-3: LAYERING OF AGRICULTURAL WASTES FOR STRAW MUSHROOM (*VOLVARIELLA VOLVACEA*) PRODUCTION

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Agricultural wastes containing cellulose have a potential usage for mushroom cultivation. Paddy straw and cotton wastes commonly be used as substrate for straw mushroom (*Volvariella volvacea*) cultivation. In our experiment beside both wastes, sea grass and aren wastes as well as sawdust were composted with 5% $CaCO_3$ for four days, then mixed with 15% bran, and further composted for another three days. For paddy straw substrate the medium used was 25 cm hight, while for other waste we replaced 3 cm upper layer on top of the 22 cm high basic medium (paddy straw) with other substrates. Three beds were prepared for this experiment as replication. Each bed contained five agricultural waste treatments. The composts were pasteurized at 60°C for 6 hours and inoculated with straw mushroom spawn and incubated for growing the mycelia and developing the fruiting body. The best media for fruiting body production was paddy straw medium and it was as good as cotton and sea grass on top of paddy straw. The protein content of mushroom on sea grass was significantly higher than that on cotton was ($P<0.02$), while that on other substrates were not significantly different. Sea grass waste seemed to be a better choice for future use as substrate.

PO-4: IMAGE ANALYSIS OF MUSHROOMS STORED IN CONTROLLED ENVIRONMENTAL CONDITIONS

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Horticultural products are exposed to external mechanical forces during their post-harvest life, which are going to affect product quality. Loss of whiteness upon storage is particularly important in the mushroom industry. Rough handling and distribution, fruiting body senescence and bacterial infection may initiate browning. The objective of this work was to evaluate the efficacy of a simple computer imaging system to differentiate and monitor the browning of mushrooms. *Agaricus bisporus* mushrooms were grown under controlled conditions. Samples were harvested and transported with care to avoid unnecessary bruising. Mechanical damage of the mushrooms was induced by using a gyratory shaking table at controlled amplitude and time. Different shaking times led to different damage levels. Browning kinetics were monitored on undamaged (U) and damaged (D) mushrooms at controlled temperature and relative humidity. RGB images were taken periodically using commercial webcams and analysed using image processing software. The Grey Scale Value (GSV) and the difference in Grey Scale Value ($\Delta GSV = GSV_{t0} - GSV_t$) of each mushroom cap was modelled against time. Inter-mushroom variability was incorporated using a mixed effect model. Undamaged and damaged samples followed zero and first order kinetics respectively. Significant differences ($p<0.05$) was found for U/D imaging kinetics. Damaged samples could be identified at the lowest level of damage using classification rules based on GSV. Mushroom browning kinetics were correlated against reference measurements of Hunter colour (L,a,b), water activity (a_w), enzyme activity (PPO) and texture. This work found that browning kinetics due to mechanical damage may be monitored using webcams and correlated to off-line reference measurements.

PO-5: EFFICACY OF A SIMPLE COMPUTER IMAGING SYSTEM TO DIFFERENTIATE AND MONITOR THE BROWNING OF MUSHROOMS

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Horticultural products are exposed to external mechanical forces during their post-harvest life, which are going to affect product quality. Loss of whiteness upon storage is particularly important in the mushroom industry. Rough handling and distribution, fruiting body senescence and bacterial infection may initiate browning. The objective of this work was to evaluate the efficacy of a simple computer imaging system to differentiate and monitor the browning of mushrooms. *Agaricus bisporus* mushrooms were grown under controlled conditions. Samples were harvested and transported with care to avoid unnecessary bruising. Mechanical damage of the mushrooms was induced by using a gyratory shaking table at controlled amplitude and time. Different shaking times led to different damage levels. Browning kinetics were monitored on undamaged (U) and damaged (D) mushrooms at controlled temperature and relative humidity. RGB images were taken periodically using commercial webcams and analysed using image processing software. The Grey Scale Value (GSV) and the difference in Grey Scale Value ($\Delta GSV = GSV_{t0} - GSV_t$) of each mushroom cap was modelled against time. Inter-mushroom variability was incorporated using a mixed effect model. Undamaged and damaged samples followed zero and first order kinetics respectively. Significant differences ($p<0.05$) was found for U/D imaging kinetics. Damaged samples could be identified at the lowest level of damage using classification rules based on GSV. Mushroom browning kinetics were correlated against reference measurements of Hunter colour (L,a,b), water activity (a_w), enzyme activity (PPO) and texture. This work found that browning kinetics due to mechanical damage may be monitored using webcams and correlated to off-line reference measurements.

PO-6: ANALYSIS OF GENETIC CHARACTERISTICS AND DEVELOPMENT OF SUBSTRATE FOR CULTIVATION IN BROWN STRAINS OF *FLAMMULINA VELUTIPES*

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Analysis of genetic character of *Flammulina velutipes* showed that strains have a range of 85% in genetic distribution diagram. According to this result, we divided these strains into five groups. In experiment of determinating the optimum media and condition in cultivating *Flammulina velutipes*, we found the optimum temperature and pH range for hypha growth were 25 ^{O}C and 6.0 to 7.0, respectively. in addition, the best media for growth of that in plate was MCM(full name) which have a growth length from 68.4 to 83.4 mm. In vivo test, we observed that fast growth and good density of hyphae in mixture media of douglas fir sawdust, cotton seed meal and beet pulp (6:2:2). Also when we cultivated *F. velutipes* in this media, we harvested high yield of fruiting body.

PO-7: THE USE OF INFRA-RED SPECTROSCOPY COUPLED WITH CHEMOMETRIC TOOLS TO EVALUATE BETWEEN DAMAGED AND UNDAMAGED MUSHROOMS

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The term 'metabolome' has been used to describe the observable chemical profile or fingerprint of the metabolites in whole tissues. Fourier transform infra-red (FTIR) is an ideal candidate for high throughput metabolic fingerprinting. It is a physio-chemical method that measures predominantly the vibrations of bonds within functional groups to generate a spectrum that can be regarded as a biochemical 'fingerprint' of the sample. FTIR spectroscopy is an invaluable metabolic fingerprinting tool due to its ability to provide an analytical signal representing carbohydrates, amino acids, lipids and fatty acids, as well as proteins and polysaccharides simultaneously. The objective of this work was to determine the potential of infra-red spectroscopy coupled with chemometric tools to evaluate differences between damaged and undamaged mushroom tissues (caps, gills and stalks). *Agaricus bisporus* mushrooms were grown at controlled conditions. Samples were harvested and transported with care as to avoid damage. Mushrooms were then damaged mechanically using a gyratory shaking table at controlled amplitude and time. Ten samples were taken for each tissue each day and FTIR spectrra of the freeze dried tissues were taken. Over time some peaks became weaker particulary in the carbohydrate region (1200-800 cm^{-1}). Chemometric tools (PCA and recursive partition trees) were applied to the FTIR spectra to identify any further damage/undamaged characteristic peaks. This work found that FTIR could be used to distinguish damaged and undamaged mushrooms.

PO-7-A: STUDY OF MITOGENIC EFFECTS OF MYCELIA OF SEVERAL APHYLLOPHOROMYCETIDEAE MEDICINAL MUSHROOMS

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Mushrooms are producers of useful bioactive metabolites and enzymes with different therapeutic effects (immune-modulating, antifungal, thrombolytic, hypoglycemic, antiprotozoal, etc.). The regenerative and wound-healing properties of different wood-inhabiting mushrooms, such as *Ganoderma lucidum, Hypholoma fasciculare, Lentinula edodes* and *Pleurotus ostreatus* were reported, as well. It is known that terpenoid, phenolic and indolic compounds, as well as fungal lecti ns possess mitotic activities. However, the mechanism by which lectins stimulate mitosis hasn't been identified yet. We studied the mitogenic effects of cultural broth (CB) and mycelial extract (ME) samples obtained after submerged cultivation in liquid malt-extract medium (pH=6, at 25 ^{0}C and agitation rate 200 rpm) of 6 species and 18 strains of brown-rot (*Daedalea quercina, Laetiporus sulphureus*, *Piptoporus betulinus*) and white-rot (*Fomes fomentarius*, *Ganoderma applanatum*, *Ganoderma lucidum*) medicinal mushrooms (Aphyllophoromycetideae) on the growth of wheat and maize seeds. Mycelial samples of tested mushrooms, particularly ME samples of *Ganoderma* species, possess different levels of mitogenic effects which were strongly expressed on the growth of maize seeds, rather than on wheat seeds. The mitotic activities of ME and CB samples of *P. betulinus* and *D. quercina* on maize seed growth were characterized by a 2-3-fold increase. A weaker stimulatory effect on seed growth was revealed by *F. fomentarius* strains which was almost absent in the mycelium of *L. sulphureus*. Growth stimulatory and inhibitory effects of CB samples on epigeal and/or hypogeal parts of seeds were not observed, while significant growth stimulation of epigeal parts of maize seeds by tested ME samples was described. It was shown that mitotic activities of white-rot species were higher compared to these of brown-rot species. Revealed mitogenic effects of mycelia show that the biotechnological cultivation of tested mushrooms are promising for the development of new biotech-products that could be further used in the prevention and treatment of wounds, burns, ulcers and other regenerative processes.

PO-8: ENZYMATIC AND BIOLOGICAL ACTIVITY OF *PLEUROTUS OSTREATUS* A POPULAR EDIBLE MUSHROOM IN EGYPT

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In the recent years *Pleurotus ostreatus* has gained prominence as a type of edible mushroom in Egypt. *Pleurotus* species thrive over a wide range of subtropical climates and are representatives of white rot fungi which can degrade directly the lingo cellulosic organic wastes of nature. The production of cellulase complex enzymes by *P. ostreatus* mushroom in submerged culture supplement with rice straw was studied. The culture filtrate of *Pleurotus* exhibited relatively high activity of three cellulase complex (endoglucanase, exoglucanase and filter paper activity). The rice straw concentration 5% yielded the higher activity (18.5 U/ml) for endoglucanase While 6% concentration yielded higher activities of both exoglucanase and filter paper. Mushroom contains a number of bioactive compounds with beneficial effects on human health. The antibacterial and anti-tumor activity of *Pleurotus* isolate (polysaccharide) was studied and the polysaccharide showed strong inhibition activity against different bacterial laboratory strains (*Mycobacterium aurum, Staphylococcus aureus*, *Streptococcus sp.*, *Acinetobacter calcoaceticus* and *Klebsiella oxytoca*). Treatment with polysaccharide leads to increase in the mean survival time of tumored animals to 85 days compared to tumored controls (37 days) and a significant reduction of tumor growth rate.

PO-9: ISOLATION OF INHIBITORY MATERIAL AGAINST HARMFUL INTESTINAL BACTERIAL ENZYME FROM *GANODERMA LUCIDUM* KCTC 16806 MYCELIA

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In many food items, it has been considered that mushroom is good for health, and some of them have been used as medicine in Asia. Especially, *Ganoderma lucidum*(Fr) Karst is a well-known medicinal herb that has been used clinically in China, Japan and Korea as effective anti-cancer agent and so on. Therefore, in this study, we investigated to identify the inhibitory effects material of *Ganoderma lucidum* KCTC (Korean Collection for Type Cultures) 16806 mycelia against large colon cancer inducing harmful enzymes. The water extract of *Ganoderma lucidum* KCTC 16806 mycelia was fractionated by the basis of polarity; diethyl ether, ethyl acetate, n-butanol. And then, to examine the inhibitory effect against β-glucuronidase and tryptophanase, total microflora in rat feces or *Escherichia coli* KCCM (Korean Culture Center of Microorganisms) 40127 was inoculated and cultivated in GAM(general anaerobic medium) broth with each of its dried fractions for 24 hrs on 37℃. At that results, dried fraction from n-butanol exhibited the highest inhibitory effect had suppressed the β-glucuronidase (30 %) and tryptophanase activities (32 %) than in control not to be added. From this fraction, 9 kinds of substances were isolated by silica gel chromatography as its polarity. Among each substances, G-8 substance showed the highest inhibitory effect against β-glucuronidase and tryptophanase in 7.6 % and 5.3 %. The IC_{50} values of G-8 for β-glucuronidase and tryptophanase were 2 mg/ml and 6 mg/ml respectively. The G-8 substance was identified as uracil ribonucleoside derivatives from nucleic acid by the nuclear magnetic resonance.

PO-10: THE GROWTH PROPERTIES IN GINSENG FERMENTED BY *GANODERMA LUCIDUM*

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This research was conducted to investigated the growth properties of *Ganoderma lucidum* on ginseng. *Ganoderma lucidum* M1, M2 and M3 which were previously selected as the well-grown mycelia on ginseng were inoculated on finely chopped ginseng and observed their mycelia growing, β-glucosidase activity, mycelial contents, and ginsenosides composition by HPLC anaylsis for 8 weeks. In the results for 8 weeks, M2 was showed most rapid proliferation in mycelial length but mycelial content was highest in fermented ginseng by M1 because of its compact mycelial density. The crude saponin contents of mycelial fermented ginseng were 6.37～7.84 %. During the fermentating periods, ginsenoside Rd in ferments by each of M1, M2, and M3 was increased from 1.01 mg/g to 2.03～3.94 mg/g, whereas ginsenosides Rb1 was decreased from 5.21 mg/g to 1.65～3.80 mg/g.

PO-11: SOMATIC INCOMPATIBILITY IN *AGARICUS BITORQUIS* (QUEL.) SACC.

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The somatic incompatibility in *Agaricus bitorquis* was studied using ten wild strains. Heterokaryons from each isolate were all paired with the same unrelated heterokaryon and also paired together in all combinations. Two different types of somatic incompatible interaction were observed lightly or heavily pigmented lines developing between the two isolates. At SEM the widths of compatible and incompatible hyphae were measured. The width of the compatible hyphae is 2.15 mμ and the incompatible hyphae are 1.31mμ-1.55 mμ and 1.43mμ-1.45mμ. Also some of the elements were analyzed at SEM. Ca and Cu had the highest density. In both these elements the density values were measured as 36.68 c/s and 22.70 c/s consecutively.

PO-12: SUSTAINABLE TECHNIQUE FOR RURAL PRODUCTION OF OYSTER MUSHROOM *PLEUROTUS OSTREATUS*

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Rural development is one of the most perplexing issues for governments in the developing countries. In rural areas, the absence of such type of development leads to the displacement of villagers from the countryside to the cities. Therefore, involving local populations in the processes of development is very important; thus it is necessary to propose new activities other than traditional ones to ensure the success of development projects. As a case in point, we have proposed a simple technique for the cultivation of oyster mushroom by adopting practical processes at village level. The proposed technique is applicable by villagers, especially women, using indigenous biomass resources and utilizing neglected local places and tools. These neglected places are arranged in a way to produce oyster mushroom including the incubation period and fructification phase. This activity may be carried out in two seasons (autumn & spring) under natural conditions. Two other cycles are possible under artificial conditions. In sum, four cycles of oyster mushroom production may be done, and this will generate for each family an income estimated at about

1700 U$ per year. Fruit-bodies are packed and sold in the markets of near cities or for hotels that will directly improve the economic situation of the villagers especially women. In developing countries, the above mentioned activity may constitute, in developing countries, a simple and effective way for income generating, participating in local sustainable development projects, fighting poverty, producing food, maintaining rural populations in their villages, guaranteeing work for women in villages, preserving forests and recycling agricultural waste.

PO-13: SURGING INTEREST IN ANTIVIRAL ACTIVITY IN MUSHROOMS

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Drug resistance due to ongoing viral mutation precipitates the constant need for searching for effective and safe antivirals. Drug toxicity and side effects can also be a problem. Surging interest is evident in mushrooms as potential sources for desirable antivirals. We aim to 1) identify mushroom species score high on antiviral screening tests, 2) look for effective mushroom-derived extracts and mushroom-derived antiviral compounds, and 3) select target-viruses for antiviral testing in medicinal mushrooms. A variety of mushrooms, particularly polypores, have shown strong antiviral efficacy. Classic Chinese medicinal mushrooms, highly valued in Asia and beyond, including *Ganoderma lucidum*, *Coriolus (Trametes) versicolor*, *Polyporus umbrellatus* and *Wofia cocos*, along with popular exotic species, *Lentinula edodes*, *Pleurotus ostreatus*, *Grifola frondosa*, all have shown promising antiviral properties. Dr. Frank Piraino, University of Wisconsin, Medical School, has discovered a new antiviral, RC-183, from *Rozites caperata*, the gypsy mushroom, a mycorrhizal species associated with pines and Douglas firs. As early as 1970s, antiviral was found in *Psalliota xanthoderma.* Mushroom-derived antivirals comprise of a diverse group of chemical structures, ranging from low-molecular-weight compounds to high-molecular-weight polysaccharides and peptides. Certain highly-oxygenated triterpenes and ubiquitin-associated peptides are examples. Some of the viruses susceptible to mushroom antivirals are (HIV, herpes simplex, Pox including small pox, SARS, West Nile, flu viruses and H5N1, a bird virus.To benefit the health professionals, mushroom industry, and consumers, we examine a single virus or a single virus complex, such as HIV in relation to mushroom antivirals. We also approach from the oposite perspective in checking a single well-studied mushroom species, such as *G. lucidum* which has a wide spectrum of antiviral efficacy including HIV. A number of antiviral constituents have been detected from this species, for example, ganoderic acid a, b, ganoderiol-F, lucidmol and protein-bound polysaccharide. Current studies indicate the benefit of adjuvent use of mushroom antivirals in conjunction with prescribed antiviral in managing viral diseases. *G. lucidum*, mushroom of immortality, and *Inonotus obliquus*, chaga, are two unique mushrooms in containing both triterpenes and polysaccharides. Fungal strain, growth stage, and product processing all play a part in determining the quality of mushroom antivirals.

PO-14: *GANODERMA LUCIDUM* INTRASPECIES DIVERSITY IN PRODUCTION OF SELECTED LIGNINOLYTIC ENZYMES

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Ganoderma lucidum is an medicinal, white-rot, and widespread species with significant differences in cultural, biochemical, and molecular features. The aim of this study was to analyze the ability of selected *G. lucidum* strains to produce laccase (Lac), Mn-dependent peroxidase (MnP), and versatile peroxidase (VP) during solid-state fermentation of different plant raw materials. Three *G. lucidum* strains (HAI 246, HAI 447, and HAI 626 originated from USA, Israel, and Germany, respectively) were studied. Wheat straw, corn stem, oak and grapevine sawdust were the analyzed plant raw materials. The solid-state fermentation was carried out at 25 ^{0}C in the 100 ml flasks contained 2 g of selected plant raw material, as carbon source, and 10 ml of the synthetic NH_4NO_3-yeast extract medium (pH 5.0). Inoculum suspension of 3 ml was used per flask. Samples were harvested after 7 days of cultivation, extracellular enzymes were extracted with distilled water and their activity was determined spectrophotometricaly by ABTS for Lac and phenol red for MnP and VP. Three replications for each investigated strain and carbon sources were used. The best Lac producer was strain HAI 447 during wheat straw fermentation (93.0 U/l) and the lowest Lac activity levels were noted in strains HAI 246 and HAI 626 cultivated in grapevine sawdust (9.9 U/l and 10.2 U/l, respectively). Strain HAI 447 under conditions of wheat straw fermentation was the best, and strain HAI 626, in oak sawdust fermentation, the worst producer of MnP (102 U/l and 13.7 U/l, respectively). Production of VP by all analyzed strains, during cultivation in all selected plant raw materials, was significantly lower, especially in strain HAI 626 at grapevine sawdust (6.4 U/l).

PO-15: PELLET MORPHOLOGY AND BIOMASS FORMATION IN SEVERAL COPRINOID MUSHROOMS DURING SUBMERGED GROWTH

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Coprinoid mushrooms (CMs, species of former genus *Coprinus* Pers.) are regarded as sources of wide range of bioactive metabolites and enzymes. Cultivation of their mycelium has significant industrial potential. During submerged growth, fungi tend to form pellets or mixtures of dispersed mycelium and pellets. Pellets are formed as a result of hyphal agglomeration. The exact mechanism behind pellet formation is unknown. Agglomeration is determined by the properties of hyphal elements (length, branching pattern, growth rate, etc.) and environmental conditions (medium composition, pH, aeration, etc.). It varies from species to species and perhaps, from strain to strain. In some cases, specific morphology is required for optimum product yield which makes morphological characterization of the pellets necessary. We studied the morphology of pellets after four days of submerged growth of 15 species 25 strains of CMs from three genera *Coprinus* (*C. comatus*), *Coprinellus* (*C. angulatus, C. curtus, C. disseminatus, C. domesticus, C. ellisii, C. micaceus, C. radians, C. xanthothrix*) and *Coprinopsis* (*C. atramentaria, C. cinerea, C. radiata, C. scobicola, C. strossmayeri*), as well as *C. sp.* (related to *C. radians*) using malt-extract medium (pH 6.5) at 25 ^{0}C and agitation rate 200 rpm. All species/strains formed pellets with or without a dense core, smooth or filamentous surfaces, except *C. scobicola* which formed mixtures of dispersed mycelium and pellets. Not dense, smooth pellets were described in *C. comatus*, while dense filamentous pellets in *Coprinellus* species. Formation of smooth dense pellets was typical for *Coprinopsis* species. A correlation between pellets' density and biomass formation was found. A larger amount of biomass was formed by *Coprinellus* (2.5-6.3 g/L), compared to *C. comatus* and

Coprinopsis species (0.7-2.3 g/L). All species/strains, particularly from clades *Coprinopsis* (up to 3.8) and *Coprinellus* (up to 4.8) changed the medium pH into acidic, except *C. radians* which on the forth day of cultivation turned it into the alkali reaction (pH 7.5). Rare clamps (*C. disseminatus*) and oidia (*C. domesticus*, *C. comatus)*, apical hyphal enlargements *(C. strossmayeri*, *C. comatus, C. scobicola*, *C. micaceus)* were described. The revealed cultural characteristics will assist in the biotechnological cultivation of tested CMs.

PO-16: MACROMYCETES OF PROSISKO NATURAL RESERVE

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The Prosisko National Reserve is situated in the middle of the Slovak Republic nearby the town of Zvolen, within the area of Zvolenska Slatina village. The main reason for the declaration of that relatively small area as a reserve in 1998 was the protection of forest associations with concentrated presence of preglacial herbaceous species *Waldsteinia ternata ssp magicii.* The highest degree of protection is related to this area and no encroachment on the biotic or abiotic parts of the ecosystem is permitted. Fungi – the natural and inevitable part of nature, are very sensitive to changes in ecological conditions. There is an opportunity to observe the changes in the spectrum of fungal species in connection to both various growing conditions and changing forest community. The observed forest stand (surface area 20.54 ha, average age 100 years) is created firstly by oak *Quercus petraea* (85%), hornbeam *Carpinus betulus* (15%) and sporadically by maple, beech, lime, cherry, rowan, hazel, alder and hawthorn. The research was concentrated on four 50 x 50 metre monitoring plots (MP 1 – MP 4) situated in the characteristic places of the forest stand. There were inscrolled 154 species of macrofungi including 34 species of ectomycorrhizal, 44 species of terrestrial saprophytic, 66 species of wood saprophytic and 10 species of sapro-parasitic fungi. Initial results indicated, by means of the mycorrhizal percentage and through the ratio of mycorrhizal and wood-destroying fungi, considerable stage of disturbance of the ectotrophic stability of the forest stand. The presence of macromycetes will be compared to the progression of the health condition of trees during future years.

PO-17: RESPIRATION RATE (RR) & TRANSPIRATION RATE (TR) IN MUSHROOM (*AGARICUS BISPORUS*) UNDER ENVIRONMENTAL STORAGE CONDITIONS

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Stored or packaged mushrooms have a very high metabolic activity, consuming O_2, producing CO_2 and water as a result of their respiratory metabolism. A closed system methodology was employed to measure the O_2, CO_2 respiration rate (RR) and the transpiration rate (TR) of mushrooms. The effect of storage temperature and the change of those responses during storage time were studied. After careful examination of the data of RR & TR on the mushrooms during storage, a linear model (for the O_2 and CO_2 RR) and a Weibull model (for the TR) were proposed to describe the primary model. Both RR & TR were dependent on the temperature of storage. The O_2 and CO_2 RR were found to have a significant linear increase with storage time. This pointed to the need to adjust storage conditions to these dynamic changes. The variability of the RR was also dependent on temperature indicating that the use of low storage temperatures is more beneficial in terms of having an homogeneous product than to slow the metabolic activity. An optimal temperature for minimise weight losses by transpiration was found at 6.4°C.

PO-18: CULTIVATION OF *AGARICUS BITORQUIS* ON ENRICHED, HEAT-TREATED STRAW

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In Europe *Agaricus bitorquis* appeared in cultivation in the Netherlands and Belgium in the 60s. In Hungary the first trials were carried on in the coop farm Csepeli Duna in the 70s. Cultivation was motivated by its tolerance to virus diseases, good yield at higher temperature and palatability. Like *Agaricus bisporus* it can be grown on composted and heat-treated horse manure and recently, on a compost mixture of straw, horse manure and chicken manure. *A. bitorquis* has a characteristic round thick, flashy hat. Fruit bodies are blocky with good transportability and shelf-life. At harvest it is less sensitive to bruising and blotching than *A. bisporus*. In the Vegetable Research Crops Institute cultivation of *A. bisporus* and *A. bitorquis* began in the 80s on straw, heat-treated dry at 100°C. On natural straw yield was relatively low with 10-15% of the wet straw weight. Low yield was supposed to be caused by the low nitrogen level. Efforts were maid to increase the nitrogen content of the straw substrate by adding different enriching materials. The chopped straw was treated in two ways: heat-treated dry and heat-treated wet. Enriching agents consisted of alfalfa meal, wheat bran and ProMycell mixed into the substrate in 1, 2 and 3 weight per cent. Nitrogen content increased by 0,1-0,5% as compared to the control composed of heat-treated straw without enrichment. Grain spawn was mixed to the straw at a rate of 5 weight per cent. Straw was filled into plastic bags, incubated in climatised houses and harvested in a cellar. The date of the first harvest, yield and harvest duration were noted. Trials proved that *A. bitorquis* could be cultivated on both dry and wet heat-treated straw besides composted substrates. Enrichment increased yield in almost every case. The best results were obtained with 3 % ProMycell on both dry and wet heat-treated straw where yield was above 20-22%.

PO-19: MYCELIAL GROWTH RATE OF NEW HETEROKARYOTIC MYCELIUM OBTAINED FROM MATING WITH SINGLE SPORE ISOLATE OF *PLEUROTUS ERYNGII*

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Pleurotus eryngii is a commercially produced macrofungi. Spores obtained from wild basidiocarps of *P. eryngii* which were collected from different regions of Turkey were germinated in this study. Single spore isolates were crossed by each other in order to obtain new hybrid. Mycelial growth rate of new hybrids were determined by measuring diameter of colonies which incubated in PDA. The nine new hybrids which were determined active mycelial growth rate were improved at the end of this study. New hybrids can be used in the production of commercial spawn.

PO-20: NEW ANTIVIRAL NAPHTHALENE DERIVATIVES FROM AN UNIDENTIFIED FUNGUS ISOLATED FROM THE BALTIC SEA

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Balticols A-E, five new naphthalene derivatives were isolated from the EtOAc extract of the culture broth of fungal strain 222, isolated from driftwood collected in 2002 at the coast of the Greifswalder Bodden, Baltic Sea, Germany. All structures were elucidated on the basis of extensive one- and two-dimensional NMR spectroscopic data (1H, 13C, COSY, HMQC, HMBC, NOE difference spectra) and mass spectrometric analyses. The new compounds were found to exhibit inhibitory activity against influenza virus A and herpes simplex virus. The most potent activity was shown by Baltiocol E with an IC_{50} of 0.01 µg/mL against herpes simplex viruses.

PO-21: CLONING OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE PROMOTER AND HETEROLOGOUS EXPRESSION OF EGFP IN *PLEUROTUS OSTREATUS*

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Two glyceraldehyde-3-phosphate dehydrogenase genes of *Pleurotus ostreatus* were isolated. The promoter regions of these genes were fused to hygromycin B phosphotransferase gene (*hpt*) derived from *Escherichia coli* as a selection marker. Using the resulting constructions, *hpt* was efficiently transformed into *P. ostreatus* by basidiospores electroporation. No false positive antibiotic-resistant cultures were detected by PCR amplification and the hygromycin resistance trait remained stable during mitotic cell division for at least five months. Southern analysis of transformants indicated the integration of gene might occur by non-homologous recombination. Using *gpd* promoters containing their first intron, heterologous expression of enhanced green fluorescent protein (EGFP) within *P. ostreatus* was evidenced. *egfp* has been shown to be faithfully expressed, and maintained in a stable format despite multiple rounds of subculture without selection pressure. The percentage of EGFP in total soluble protein was 5.4×10^{-3} (0.5%) for the transformant with the highest EGFP expression level. This rapid and convenient electroporation procedure offers new prospects for the genetic manipulation of this important edible mushroom species.

PO-22: ANTIMICROBIAL ACTIVITY OF *AGARICUS BRASILIENSIS* POWDER

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The medicinal use of mushrooms has a very long tradition in the Asian countries, whereas their use in the Western hemisphere has been slightly increasing only since the last decades. A powdered extract of cultivated *Agaricus brasiliensis* containing primarily carbohydrates with minor amounts of protein and polyphenols was obtained from Innerlife B.V. (Venlo, The Netherlands). The powder was dissolved in water, ethyl acetate, methanol, ethanol and DMSO and all these solutions were subjected to NMR analysis. We made a pool of the solutions by mixing an equal volume of each of them. In the course of our investigations of natural substances which have antimicrobial potential we have tested the five solutions mentioned and the pool. The test was done by microdilution method against eight bacteria and eight fungi which are plant, animal and human pathogens. The watery solution showed the best antifungal activity against five microfungi, with MIC and MFC 1-2 mg/ml. The EtOH solution inhibited fungal growth of five fungi with MIC 1 mg/ml and depigmentation of three fungi was observed. The pool showed very strong activity against seven and depigmentation of four fungi. Bifonazole was not active against the microfungi tested in a concentration of 1-2 mg/ml. Water extract also showed the best antibacterial activity against five bacteria with MIC 1-4 mg/ml, MBC 4 mg/ml. DMSO extract possessed inhibitory effect against six bacteria with MIC 1-2 mg/ml. EtOAc extract showed MIC at 1-2 mg/ml and MBC 4 mg/ml against five bacteria. EtOH inhibited four bacteria (MIC 2 mg/ml), while MeOH exhibited

activity against all bacteria, MIC 1-2 mg/ml. The pool possessed higher antibacterial effect against six bacteria with MIC and MBC 1 mg/ml. Streptomycin reacted with the highest antibacterial activity NMR analysis showed the presence of polysaccharides in n-buthanol solution. Owing to the continuing development of microbial resistance in medicine and agriculture, discovery of new antimicrobial substances is an important research objective. In addition, the desire for safer agrochemicals with less environmental and mammalian toxicity is a major concern and these compounds isolated from medicinal mushrooms could possibly be a good alternative.

PO-23: MARESOME™ – A NEW APPLICATION FORM FOR FUNGI

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Formulation is an important parameter for the efficiency of drugs, cosmetics or food supplements. Micro- and nanoparticles, so called Maresome™, were prepared from the biomass of selected fungi by a special emulsion technique and represent a new formulation- and application form. In opposite to an extract they contain all components of the fungal biomass including lipids, proteins, polysaccharides, vitamins and so on in an encapsulated form. The preparation of Maresome™ was done according to PCT/DE 03/00747. A pre-suspension of the biomass and a surfactant-water mixture was produced by a stirring machine. Then, this pre-suspension was homogenized by a high pressure homogenizer. The particle size distribution was determined with a laser diffractometer at a wavelength of 633 nm. Depending on the components and preparation conditions Maresome™ can be used for different applications. Conversion of the well known yeast fungus *Saccharomyces cerevisiae* into Maresome™ leads to food additives. Maresome™ with different ratios of this fungal biomass and other components (clay minerals, Immunomin®) are used in various phases of pig breeding and fattening with very good results. Maresome™ with *Lentinula edodes*, *Auricularia auricula-judae or Ganoderma* spec. can be used as cosmetics for anti aging and sun protection. UV radiation destroys the normal bacterial flora on the skin. The treatment of the skin with a *Ganoderma pfeifferi*-Maresome™ or *Ganoderma resinaceum*-Maresome™ containing ointment prevents this undesired effect. We expect that this special formulation of fungal biomass (fruit bodies and/or mycelium; alone or in combination with other materials) can improve the application properties of several medicinal mushrooms.

PO-24: THE INFLUENCE OF DIFFERENT WHEAT STRAW PELLETS TREATMENT ON THE MYCELIUM GROWTH OF *P. OSTREATUS*, *C COMATUS* AND *L. EDODES* ON READY SUBSTRATES

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The fuel pellets appear to be a prospective source for the preparation of the substrates for the cultivation of edible mushrooms. The pellets were characterized from the point of view of the microbiology and chemical modifications within the pelletization of the material. Another characteristic was the evaluation of the penetration of selected mushrooms into the various modified pellets. The wheat straw pellets contained up to 4 digit places lower counts of fungi in comparison with the shredded straw. In the course of the straw pellets processing no significant C-substances changes took place, as proved by the measured IR spectra. The growth rate of *Pleurotus ostreatus* (Jacq.Fr.)Kumm, *Coprinus comatus* (Müll.:Fr.) S.F.Gray and *Lentinula edodes* (Berk.) Pegler was compared on substrates made of straw pellets dipped in temperate water (30–80 °C) and with water content of 65, 70 and 75%. At *L. edodes,* the highest mycelium growth rate was achieved with the dipping water temperature of 70 and 80 °C and water

content of substrate 75%. At *C. comatus*, no differences in mycelium growth rate were recorded at the temperature of dipping water of 50–80 °C and substrate water content range 65–75%. At *P. ostreatus,* the temperature of dipping water in range of 30–80 °C did not influence the mycelia growth rate. Generally, there wasn't noticed any relation of the temperature of dipping waters to the occurrence of contamination of the straw pellet substrate. The suggested method is suitable for growing the above mentioned mushrooms in 1–7 litres plastic sheet bags.
This work was supported by Agency NAZV QH 82173 and GACR J521183856

PO-25: TYROSINASE IN *AGARICUS BISPORUS* FRUIT BODIES FROM SUPPLEMENTED COMPOSTS

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Supplementation of compost is a common practice in commercial mushroom production to obtain yield increases. Supplements with high protein content have been used almost exclusively. However, recently the role of other nutrients present in supplements has been reevaluated and polysaccharides could play an important role for supplement efficacy. The significance of supplementation and the composition of supplement on the quality of mushrooms is an aspect that has not been evaluated. Tyrosinase is a polyphenoloxidase responsible of browning of fruits, vegetables and microorganism and it is one of the main factors causing quality loss in mushrooms. So in order to evaluate the role of nitrogen, lipids and polysaccharides in supplements, tyrosinase activity (EC 1.10.3.1) was determined in mushrooms produced on compost supplemented with 5 different types of materials, i.e. corn bran (S), corn bran and gluten (SG); corn bran and Soybean oil (SA); corn bran gluten and Soybean oil (SGA) and a commercial supplement made of cracked soy beans (Rp). Additionally, Non-supplemented compost (T) was used as control. Supplementation of compost (with polysaccharides, proteins and/or lipids) produced a decrease in tyrosinase specific activity in 1st flush mushrooms (0.244 U mg^{-1} protein) as compared with non supplemented composts. On the other hand, tyrosinase activity increased with progress of the crop, i.e. for the 2nd and 3rd mushrooms. With each treatment, the highest activity was obtained at the 3rd flush. From all supplements, S and SGA showed the highest activity, i.e. 0.471 an 0.424 U mg^{-1} protein, respectively. Solids content, another parameter directly related to mushroom quality, was also affected by supplementation and crop flush. Supplementation of compost produced in all cases a decrease in solids content. However, solids content increased as crop advanced with all types of treatments.

PO-26: *IN VIVO* IMMUNOPHENOTYING STUDY OF B-GLUCANS FRACTIONS ISOLATED FROM MUSHROOM SCLEROTIA

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β-Glucans, a non-starch polysaccharides isolated from mushrooms, are recognized as strong potent antitumor agents for decades. Their antitumor effects are mainly implemented by acting as biological response modifiers which boost up the host immune system. Our previous studies found that mushroom sclerotia (a dry compact biomass of fungal hyphae) namely, *Pleurotus tuber-regium* and *Polyporus rhinocerus* did not only possess substantial amount of β-glucans (> 80% on dry matter basis), but their hot water soluble β-glucans fractions (PTR-HWE and PR-HWE) also exhibited remarkable immunomodulatory and antitumor effects when either administered intraperitoneally on Sarcoma 180 (allogeneic solid tumor cell) bearing BALB/c mice or directly incubated with various mammalian cancer cell lines. Nevertheless, the underlying mechanisms of these effects are still unclear. In this *in vivo* immunophenotyping study, the type of effector immune cells involved, in response to the two aforesaid

sclerotial β-glucan fractions, were identified by using healthy BALB/c mice. In brief, after 10 days' intraperitoneal administration of individual β-glucan fractions, the spleens of each group of experimental mice were rapidly excised and weighed, while their splenic mononuclear cells were collected and stained for the cell surface antigens specific for different immune cell populations such as macrophages (Mac-1^+), B-cells ($CD45R/B220^+$) and T-cells ($CD4^+/CD8^+$ ratio) before further analysis using flow cytometry. Compared with the control group, apart from a significant increase (PTR-HWE: 145%; PR-HWE: 49.0%) in the spleen weight of the experimental mice, both PTR-HWE and PR-HWE also stimulated the splenic T-cells population with a $CD4^+/CD8^+$ ratio of 15.4:1 and 8.17:1, respectively. Nevertheless, proliferation (20.2%) of splenic macrophages population (Mac-1^+) was only observed in experimental mice pretreated with the PTR-HWE. These findings did not only indicate the involvement of splenic macrophages and/or T-cells populations in the immunomodulatory effects exerted by these two sclerotial β-glucans fractions, but they also implied the possible immunological activities occurred accordingly (such as phagocytosis and extracellular cytokines induction etc.). It is anticipated that by increasing knowledge of the interaction between the mushroom sclerotial β-glucans and the immune system, more effective utilization of these macromolecules as an immunomodulatory and antitumor agent can be made possible.

PO-27: DETECTION OF *VERTICILLIUM FUNGICOLA* IN SAMPLES FROM MUSHROOM FARMS USING MOLECULAR AND MICROBIOLOGICAL METHODS

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Verticillium fungicola (Pruess) Hassebrauk is the causative agent of Dry Bubble disease which is a pathogen of the cultivated mushroom *Agaricus bisporus* (Lange) Imbach. It is found worldwide wherever mushrooms are grown. *V. fungicola* can cause losses of yield of 10-20% or higher and sometimes outbreaks of disease can be so severe that cultivation may be terminated after the second flush. This study presents methods for the detection of *V. fungicola* in samples originating from mushroom farms. A PCR assay is being developed and optimised for the detection of *V. fungicola* in casing soil and other mushroom farm debris. Four different methods are being evaluated for the isolation of DNA from soil containing different concentrations of spores of *V. fungicola* including manual extraction and commercially available kits. A set of *Verticillium fungicola* specific primers has been developed that amplifies a 102bp sequence of the rRNA region. The primer set was tested against *V. fungicola* and *V. biguttatum* as well as *Aspergillus fumigatus, Cladobotryum mycophilum, Mycogone perniciosa, M. calospora, M. dervina, M. rosea, Pseudomonas* sp., *Scytalidium thermophilum, Trichoderma sp.* and *Zygorhinchus,* and only *V. fungicola* gave positive results. All methods succeeded in isolating DNA from soil and the PCR reaction gave a product specific for *V. fungicola* for soil containing 10^6 spores g^{-1} soil. A second methodology successfully detected 10^4 spores/g casing soil. Future work will optimize this reaction in order to allow consistent detection of conidia at low concentrations. Another method to detect *V. fungicola* is by a selective medium. A *Verticillium* selective medium already exists but the growth of *V. fungicola* is very slow due to the inhibitive nature of the ingredients on fungal growth. A modified selective medium is being developed to enable rapid and consistent detection of *V. fungicola* from contaminated soil and casing samples.

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PO-28: THE ANTI-CANCER ACTIVITY OF *AGROCYBE CHAXINGU* EXTRACTS

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Several edible mushrooms were reported to have anticancer effects. This study was carried to investigate the effect of *Agrocybe chaxingu* mushroom on the growth of cancer cell lines (HeLa, DU145, H1299 and HT29) and expression of tumor suppressor that regulate the cell cycle. The samples extracted from fruiting body of *A. chaxingu* inhibited to 40~60% for the proliferation of human carcinoma cell lines at a concentration of 1 mg/mL. Especially, the hot water extract of *A. chaxingu* showed strong inhibitory activity on the growth of HeLa, DU145, H1299 and HT29. The expression of p53 and p21 were increased by time-dependant manner when treated with 1mg/mL of hot water extract of fruiting body.

PO-29: ANTINEOPLASIC ACTIVITY OF *AGARICUS BRASILIENSIS* BASIDIOCARPS ON DIFFERENT MATURATION PHASES

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The fungus *Agaricus brasiliensis* is a Basidiomycete studied because of its immunomodulation and/or antitumor substances. The objective of this study was to verify the *A. brasiliensis* antineoplasic activity *in vivo* on different basidiocarp maturation phases on sarcoma 180 cells implanted in mice. Sarcoma cells were implanted in Swiss mice and after seven days mice were divided in three groups. The first group was treated with saline solution (control), the second group was treated with closed basidiocarp extract solution and the third group was treated with opened basidiocarp extract solution. After 30 days of being daily and orally treated with these three solutions all animals suffered euthanasia. It was determined the splenic index and tumour mass and volume. It was concluded that there is no significant differences of the tumour growth inhibition in function of the different basidiocarp maturation phases for the *A. brasiliensis* strain, being the *in vivo* basidiocarp antineoplasic activity average of 89.22%.

PO-30: CARBON-TO-NITROGEN RATIOS ON *AGARICUS BRASILIENSIS* AXENIC GROWTH ON AGRO-INDUSTRIAL BY-PRODUCTS

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This study aimed to verify the mycelia growth of *Agaricus brasiliensis* with different C:N ratios as substratum formulation using regional by-products (soybean and cassava fibres). Studied C:N ratios of substrate ranged from 12:1 to 249:1 with nitrogen concentrations ranged from 4.25% to 0.20%, respectively. It was conclude that substrate with only soybean fibre provide higher mycelial growth than any formulation with cassava fibre; the highest mycelial growth on substrate with C:N ratio of 12:1 (N = 4.25%); intermediate growth when C:N ratios range from 15:1 and 50:1 (N from 3.33% to 1.00%); and lower growth when the C:N ratios were 100:1 or higher ($N \leq 0.50\%$).

PO-31: CULTIVATION TECHNIQUE USING PLASTIC CONTAINER AND SELECTION THE SUPERIOR STRAIN OF MONKEY MUSHROOM (*HERICIUM ERINACEUS*)

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These experiments were conducted to find a superior strain of monkey mushroom (*Hericium erinaceus*), culture conditions for liquid inoculum, and the optimum media composition for culture using plastic containers. In general, the optimum medium for culturing the monkey mushroom strains was Hamada medium and, among five strains, the most energetic strain was MKACC51875. When culturing the liquid inoculum, medium acidity for most growing hypha was pH 4, and the temperature was 22.5 ^{O}C. The culture period was delayed with increased amounts of nutritient source, but the fruit body yield was increased. When cultivated at low temperature, the yield per container was increased and the quality of the fruit body was good. In conclusion, consider as culturing period, fruit body quality and yield, the most media composition rate for cultivate monkey mushroom (MKACC51875) was oak sawdust 80% + nutritient source 20%. Under these conditions, the yield per container was 140.6g.

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