

11th

INTERNATIONAL
Medicinal Mushroom
Conference **IMMC11**

SUPPORTED BY



МИНИСТАРСТВО ПРОСВЕТЕ,
НАУКЕ И ТЕХНОЛОШКОГ РАЗВОЈА

SEPTEMBER } CROWN PLAZA
27th-30th 2022 } Belgrade, Serbia

ELECTRONIC
ABSTRACT BOOK

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MESSAGE FROM THE ORGANIZERS



Dear Colleagues, distinguished Guests, Ladies and Gentlemen!

**It is a great honour and pleasure to host you in Belgrade
for the 11h International Medicinal Mushroom Conference.**

Here in Belgrade, in the first IMMC in the third decade of its' existence, we are proud to remind ourselves on the shared vision, strong belief and simultaneous efforts of the three global pioneers in our scientific field, late Prof. Takashi Mizuno, Prof. Shu-Ting Chang and Prof. Solomon Wasser at the turn of a century, which resulted in what we can now proudly call the largest and highest quality international scientific gathering in the Medicinal Mushrooms domain and which we consider our joint heritage.

In science, we receive new data and new results each day, constantly pushing our achievements towards the scientific community. This target remained unchanged for the whole, now more than two decades long history of our IMMC scientific gathering and all the activities of the International Society for medicinal mushrooms.

Here, in Belgrade, we hope to be able to create a floor for scientists from different continents, from different countries, and from different generations to meet, bring their knowledge and data on new scientific discoveries, and exchange on their methods and experience.

I would like to share with you our most relevant impressions on the preparatory process of the IMMC11. It was everything but easy, however, the stakes were high an NO was not an option.

As you all know we were in a position that we had to postpone this conference for a year. Still, IMMC11 Belgrade in September 2022, has been organized against many odds. From the beginning we struggled with the consequences of the COVID-19 global threat to the whole world, creating new future and changing our way of life. While being aware of very different and sometimes rigorous quarantine measures, we could not revise extreme flight transportation costs increase, especially from China, all of you clearly aware of the immense importance of China, its' researchers and businesspeople to our science and industrial production. We couldn't predict the currently ongoing war in Europe, energy crisis and global protracted financial crisis which, among other, created strikes at airports.

These were some of the reasons for the Organizing Committee to introduce hybrid conference for the IMMC11, with the intent to enable as many as possible colleagues and professionals to join and exploit benefits of this world class event in our scientific field.

Despite described serious obstacles, we felt that we are to invest as much energy as needed to retain the continuity of the IMMC congress, our society heritage and high quality and spirit of our gatherings. That's why we are immensely grateful to all of you who managed to join the congress and be here, in Belgrade, with us, to share your science, meet each other, exchange experience, and create new ideas and projects.

During many previous congresses culture of poster presentations was not promoted enough, hence, invested energy and work of authors might not be visible enough, so our approach has been to put more emphasis on this important aspect, refresh it and made sort of a restart.

We created and switched to electronic posters mode, visible throughout the conference. Six sessions with short oral communications in late afternoon hours are open for live presentation of authors, briefly presenting summary of their results for the audience and for discussions. (). Our expectations are that these sessions will create positive dynamics and intensify cooperation between the participants, and also contribute to future cooperation and projects.

In these times of hardship, the least we can do, is to sincerely thank our colleagues and partners, representatives of companies from medicinal mushrooms science, production, and industry, for their benevolent and substantial support that enabled IMMC11 to be organized on truly appropriate level.

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Here, on the IMMC11, besides scientific exchange, another important goal is to create opportunity for the broader international scientific community to converse in formal and informal settings, meet old friends and establish the new liaisons, as a legacy for the future, as well as to plan new scientific contacts and collaborations. The special emphasis on the IMMC11 is put on the young scientists – to be able to meet large number of renown international experts in our field.

Organized in Europe after five years, this conference has true importance for Serbian, regional and European scientists. It is our hope that this Conference, organized in Belgrade end September 2022, will inject a new energy, and become a strong push for the Europe scientific community of medicinal mushrooms.

Now the world center of medicinal mushrooms is here in Belgrade!

We would like to use this opportunity to welcome all of you and to extend my best wishes for a successful and enjoyable stay in Belgrade and in Serbia.

**Welcome!**



Miomir Nikšić

~~~~~  
PROF. DR. MIOMIR NIKŠIĆ

IMMC11 Organizing Committee, President
Institute of Food Technology and Biochemistry
Faculty of Agriculture, University of Belgrade



11th

INTERNATIONAL

Medicinal Mushroom

Conference **IMMC11**



**CONFERENCE
PROGRAM**



TUESDAY / SEPTEMBER 27th

CONFERENCE HALL 1 / Pacific and
CONFERENCE HALL 2 / Atlantic / *First floor*

8:30

REGISTRATION

9:30 - 10.00

OPENING CEREMONY - CONFERENCE HALL 1 / Pacific

Prof. Yu Li, Prof. Miomir Nikšić, Dr. Marina Soković

KEYNOTE SESSION

CONFERENCE HALL 1 / Pacific

~ ~ ~

Chairpersons: Prof. Miomir Nikšić, Prof. Guiseppe Venturella, Dr. John Holliday

10:00 - 10.30

Prof. Miomir Nikšić / Serbia, Prof. Solomon P. Wasser / Israel / Ukraine

THE HISTORY OF ORGANIZING IMMCs

10:30

11:15

KEYNOTE 1 / Prof. Shu-Ting Chang / China

~ ~ ~

Recent advances in the research of mushroom dietary fiber and bioactive polysaccharides

CONFERENCE HALL 1 / Pacific

11:15

12:00

KEYNOTE 2 / Dr. Marina Soković / Serbia

~ ~ ~

Mushrooms, food, or medicine? Or both?

CONFERENCE HALL 1 / Pacific

12:00

12:45

KEYNOTE 3 / Dr. Christopher Hobbs / USA

~ ~ ~

Mushroom Medicine: Latest News, Science, Clinical Uses, and Product Selection

CONFERENCE HALL 1 / Pacific

11:25-14:00

Lunch break / Poster Viewings / Exhibition

CONFERENCE HALL 1 / Pacific

CONFERENCE HALL 2 / Atlantic

14:00

18:30

SESSION 1

Biodiversity of medicinal mushrooms
(conservation, taxonomy
and ecological distribution)

~ Chairpersons: Prof. Georgios I. Zervakis,
Prof. Guiseppe Venturella

SESSION 2

Genetics and breeding of medicinal
mushrooms (including molecular biology);

~ ~ ~
Chairpersons: Dr. Boris Jakopović,
Dr. Ivana Sofrenić

14:00

-

14:30

14:30

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15:00

PLENARY LECTURE 1**Prof. Georgios I. Zervakis / Greece**

- Current overview of *Pleurotus species* taxonomy, Phylogenetic relationships and global distribution

PLENARY LECTURE 2**Prof. Guiseppa Venturella / Italy**

- Conservation, taxonomy, ecological distribution, and potential application of the culinary-medicinal mushroom *Leccinum scabrum*

} AV transmission of the Plenary lecture 1 from the Conference Hall 1 will be available in the Conference Hall 2.

PLENARY LECTURE 3**Dr. Boris Jakopović / Croatia**

- Proteomic research on the therapeutic properties of medicinal mushrooms

15:00-15:20

- **Prof. Paola Angelini / Italy**
- **Giancarlo Angeles Flores / Italy**

Diversity of *Pleurotus spp.* and their metabolites of nutraceutical and therapeutic importance

- **Dr. Ivana Sofrenić / Serbia**

Metabolomics study of cultivated fruiting bodies of medicinal mushroom *Fomitopsis betulina*

15:20-15:40

- **Dr. Maja Karaman / Serbia**

Diversity, chemistry and environmental contamination of wild growing medicinal mushrooms species as sources of biologically active substances (antioxidants, antidiabetics, AChE inhibitors and cosmeceuticals)

- **Dr. Georgios Koutrotsios / Greece**

Development of new hybrid strains for the cultivation of the endangered mushroom species *Pleurotus nebrodensis* on novel substrates

15:40-16:00

- **Dr. Mustafa Kemal Soylu / Turkey**

The collection and conservation of wild *Hericium* species in Türkiye

- **Prof. Dr. Lucía Ramírez et al. / Spain**

Strain degeneration in *Pleurotus ostreatus*: A genotype dependent oxidative stress process which triggers oxidative stress, cellular detoxifying and cell wall reshaping genes

16:00-16:20

- **Prof. Dr. Antonio Pissabaro et al. / Spain**

Process of detection and analysis of secondary metabolites and expression of genes involved in their production in the edible fungus *Pleurotus ostreatus* (Oyster Mushroom) as a model for the study in other basidiomycete

16:20-16:40

- **Dr. Yating Dong et al. / China**

Random mutagenesis and genetic diversity of white rot fungus (*Phellinus igniarius*) by physical mutagens

16:40-17:00

- **Abraham Nkumah et al. / Nigeria**

Molecular identification, cytotoxicity and invitro enzymatic activities of bioactive peptide rigido-sporous microporus (macro-fungus) as lead for antidiabetic agent

16:40-17:10

Coffee break

CONFERENCE HALL 1 / Pacific

CONFERENCE HALL 2 / Atlantic

17:10-17:50

**SHORT ORAL PRESENTATION
session NO 1**-
5 present. x 5 min – 25 min
+ 15 min discussion - 40 min~~~~~
**Chairpersons: Prof. Maja Karaman,
Prof. Guiseppe Venturella**

- **Ágnes Radnóti / Hungary**
- **Dr. Viktor Papp et al. / Hungary**
Taxonomic re-examination of *Macrolepiota olivascens*, a European taxon showing high similarity to parasol mushroom *M. procera* (*Agaricaceae, Basidiomycota*)

- **Ivan Dudaš / Serbia**
- **Eleonora Čapelja/ Serbia**
Online ethnomycological research in Serbia

17:10

-

17:50

- **Prof. Many Mboni Henry / DR of the Congo**
- **Prof. Dr. Caroline Stévigny / Belgium**
Identification and collection of medicinal mushrooms from DR Congo through an ethnomycological investigation

- **Assumpta Mukandera / DR of the Congo**
- **Prof. Dr. Caroline Stévigny / Belgium**
Ethnomycology, sensory analysis and nutritional composition of eight edible mushrooms consumed in the African Great Lakes Region

- **Prof. Alla Shnyreva et al. / Russia Federation**
Biodiversity of the tropical species of the genus *Ganoderma* in Veracruz, Mexico

**SHORT ORAL PRESENTATION
session NO 2**-
3 present. x 5 min – 15 min
+ 15 min discussion - 30 min~~~~~
**Chairpersons: : Prof. Bojana Vidović,
Dr. Ivan Jablonský**

- **Dr. Ivan Jablonský / Czech Republic**
Differences in metabolome of selected strains of *Hericium erinaceus*

- **Prof. Milana Rakić et al. / Serbia**
Variations in ITS region of medicin. fungal species *Cerioporus varius* and its phylogeographic analysis

- **Xiaotong Yang / China**
A polysaccharide-protein complex isolated from Cultivated Chinese Cordyceps drives M1 phenotype polarization in macrophages

18:00 - 19:00

WELCOME RECEPTION

WEDNESDAY / SEPTEMBER 28thCONFERENCE HALL 1 / Pacific and
CONFERENCE HALL 2 / Atlantic / *First floor*

9:00

9:45

KEYNOTE 4 / Prof. Marin Berovič / Slovenia

~ ~ ~

Engineering aspects in cultivation of medicinal mushroom biomass in solid state and submerged bioreactors

CONFERENCE HALL 1 / Pacific

9:45

10:30

KEYNOTE 5 / Prof. Lillian Barros / Portugal

~ ~ ~

Mushrooms as a source of bio-based ingredients: food and cosmetic applications

CONFERENCE HALL 1 / Pacific

10:30

-

13:50

SESSION 3 – A

New trends in the cultivation, industrial production and fermentation of medicinal mushrooms

~ ~ ~

Chairpersons: Prof. Marin Berovič,
Prof. Angela Amazonas**SESSION 7 – A**

Nutritional and medicinal value of mushroom products;

~ ~ ~

Chairpersons: Prof. Lillian Barros,
Prof. Milena Pantić

10:30

11:00

PLENARY LECTURE 4

Dr. John Holliday / USA

Future of mushroom farming

PLENARY LECTURE 5

Dr. Jovana Petrović / Serbia

Mushrooms as a source of functional food ingredients

11:00-11:30

Coffee break

11:30-11:50

• Prof. Angela Amazonas / Brazil

Industrialization of medicinal mushrooms products in Brazil

• Changhai Wu, et al. / China

Senolytic effect of triterpenoid complex NTexttracted from *Ganoderma lucidum* on senescence liver cancer cells

11:50-12:10

• Dr. Elizabeth Morrison / USA

Newer and more economically viable methods of mushroom cultivation

• Prof. Milena Pantić / Serbia

Potential application of selenium-enriched mushrooms in the food and pharmaceutical industry

12:10-12:30

• Dr. Idan Pereman / Israel

The correlation between cultivation and nutritional value in the medicinal mushroom *Pleurotus eryngii*

• Dr. Jovana Vunduk / Serbia

Give me my dose-A researcher's and consumer's dive into the mushroom-based nutraceutical industry

12:30-12:50

• Dr. Valeria Ferraro / Italy

Preliminary results on the cultivation and the clinical use against human pathogens of *Pleurotus eryngii* var. thapsiae, a culinary-medicinal mushroom from Sicily

• Dr. Roman Bleha / Czech Republic

Isolation and structural analysis of polysaccharides from various medicinal mushrooms

12:50-13:10

- **Rebeca Lavega González / Spain**

Characterization of the composition, structure, enzymatic activity and bacterial metataxonomy of the sterile substrate used during *Lentinula edodes* growth and development.

13:10-13:30

- **Dr. Jaime Carrasco / Spain**

Mechanism of biostimulation to prevent biotic disorders during mushroom cultivation

13:30-13:50

- **Saed Harsini / Iran**

How mushroom can help us to achieve SDG 2 (Zero Hunger) and Optimal method of producing edible & Medicinal Mushrooms in hot and dry areas

13:50-15:00

Lunch break / Poster Viewings / Exhibition

CONFERENCE HALL 1 / Pacific

SESSION 3 – B

New trends in the cultivation, industrial production and fermentation of medicinal mushrooms

Chairpersons: **Prof. Marin Berović,**
Prof. Manuela Rollini

15:00

-

17:10

CONFERENCE HALL 2 / Atlantic

SESSION 7 – B

Nutritional and medicinal value of mushroom products;

~ ~ ~

Chairpersons: **Prof. Lillian Barros,**
Prof. Milena Pantić

15:00

-

15:30

AV transmission of the Plenary lecture 6 from the Conference Hall 2 will be available in the Conference Hall 1.

PLENARY LECTURE 6

Professor Segula Masaphy / Israel

Aspects in more mushrooms qualities and health promoting activities

15:30-15:50

- **Prof. Manuela Rollini / Italy**

Growth of medicinal mushrooms on maize cobs with different pigmentation: NovEl biotechnological approaches to valorize Maize by-Products (NETMAP)

- **Prof. Saša Despotović / Serbia**

Investigation of the effect of beer with *Ganoderma lucidum* on blood pressure and heart rate in young men

15:50-16:10

- **Dr. Sandra Montoya / Colombia**

Solid culture parameters for the production of fruiting bodies of the turkey tail fungus (*Trametes versicolor*) in tropical weathers under controlled conditions

- **Prof. Mi Kyeong Lee / Republic of Korea**

Lion's mane mushroom (*Hericium erinaceus*) as good source of bioactive constituents

16:10-16:30

- **Dr. Mustafa Kemal Soylu / Turkey**

The obtaining of the pure culture of some edible ectomycorrhizal mushrooms

- **Asma Husein Zaki / China**

- **Haiying Bao / China**

Effect of isolated and purified chemical constituents from the culinary-medicinal mushroom *Leucocolocybe mongolica* fruiting body on mammary epithelial cells proliferation (In vitro study)

16:30-16:50

- **Dr. Iryna Bandura / Ukraine**

Microbiota in mushroom fruiting houses and the effect of isolated organisms on *P. ostreatus mycelia* growth and development in vitro

16:50-17:10

- **Dr. Wan Abd Al Qadr Imad Wan-Mohtar / Malaysia**

Bioreactor-produced *Ganoderma lucidum* Eats Wastewater

17:10-17:40

Coffee break

17:40-17:50 **BOOK PRESENTATION:**

Edible Fungi - Chemical Composition, nutrition and health effects / Prof. Lillian Barros, Dr. Dejan Stojković

CONFERENCE HALL 1 / Pacific

CONFERENCE HALL 2 / Atlantic

17:50-19:00

SHORT ORAL PRESENTATION session NO 3

11 present. x 5 min – 55 min
+ 25 min discussion - 80 min

Chairpersons: **Dr. Jovana Vunduk**
Dr. Agnieszka Jasinska

SHORT ORAL PRESENTATION session NO 4

10 present. x 5 min – 50 min
+ 30 min discussion - 80 min

Chairpersons: **Prof. Jadwiga Turlo,**
Dr. Ana Ćirić

- **Dr. Jaime Carrasco / Spain**
BIOSCHAMP: Design of biostimulant alternative casing for the mushroom industry

- **Nefeli Sofia Sotiropoulou / Greece**
- **Vassileios Daskalopoulos et.al. / Greece**
Volatile organic compounds in *Tuber magnatum* specimens from Greece detected by SPME-GC-MS

17:50

-

19:00

- **Lydia Olagunju /**
- **Prof.Omoanghe Isikhuemhnen / USA**
Effects of *Pleurotus ostreatus* fermentation on the chemical composition of milo

- **Prof. Martin Pavlik / Slovakia**
Identification of triterpenes in *Ganoderma lingzhi* and *Ganoderma lucidum* and dynamics of fungal respiration during growth

- **Peter A. Delle /**
- **Prof.Omoanghe Isikhuemhnen / USA**
Effect of *Pleurotus ostreatus* treated Sorghum bicolor on in vitro gas production, dry matter digestibility, and volatile fatty acid concentration

- **Nevena Petrović / Serbia**
- **Prof. Marijana Kosanić / Serbia**
Chemical characterization and bioactive properties of the honey fungus *Armillaria mellea* (*Agaricomycetes*) from Serbia

- **Dr. Ivan Jablonský / Czech Republic**

- **Lukáš Praus / Czech Republic**

Supplementation of medicinal mushroom substrates with trace elements Se and Zn

- **Dr. Agnieszka Jasinska / Norway**

Bioactive compounds in *Agaricus subrufescens* grown in closed loop cultivation system – VegWaMus CirCrop

- **Dr. Agnieszka Jasinska / Norway**

Edible and medicinal mushroom production in an integrated food to waste to food biosystem (VegWaMusCirCrop)

- **Prof. Martin Pavlik / Slovakia**

Evaluation of antioxidant and proteolytic activity of mycelial biomass formed during growth of the fungi *Ophiocordyceps sinensis* and *Paecilomyces hepiali* on rice substrates

- **Dr. Ayman Turk / Lebanon**

Boosting cordycepin production in solid-state cultivation of *Cordyceps militaris* by adding oleic acid

- **Dr. Andrej Gregori / Slovenia**

Culture degeneration and the role of cordycepin/pentostatin synthesis in the entomopathogen *Cordyceps militaris*

- **Dr. Marina Kostić / Serbia**

Chemical composition and biological activity of two *Lactarius* species from Serbia

- **Dr. Viktor Papp / Hungary**

Secondary metabolites from *Buglossoporus quercinus* (*Fomitopsidaceae*, *Basidiomycota*), a threatened brown-rot polypore species

- **Dr. Jovana Vunduk / Serbia**

Evaluation of ligninolytic enzymes activity of spent *Pleurotus ostreatus* substrate

- **Vesna Lazić / Serbia**

Antibacterial and antioxidative activity of chitosan and chitosan hydrochloride isolated from mushrooms

- **Prof. Bojana Vidović / Serbia**

Comparative analysis of beta-glucan content and antioxidant activities of some edible wild-growing Serbian mushrooms

17:50

-

19:00

20:00 - 24:00

CONFERENCE DINNER

THURSDAY / SEPTEMBER 29thCONFERENCE HALL 1 / Pacific and
CONFERENCE HALL 2 / Atlantic / *First floor*

10:00

-
10:45**KEYNOTE 6 / Prof. Zhi-Bin Lin / China**~ ~ ~
Multi-target antitumor effect of *Ganoderma lucidum* and its clinical application

CONFERENCE HALL 1 / Pacific

CONFERENCE HALL 1 / Pacific

CONFERENCE HALL 2 / Atlantic

10:45

-

13:25

SESSION 4

Biochemistry and pharmacology of medicinal mushrooms active compounds;

~ ~ ~

Chairpersons: **Prof. Anita Klaus,**
Prof. Ulrike Lindequist**SESSION 6 – A**

Medicinal mushrooms in clinical practice; antiviral and antimicrobial compounds;

~ ~ ~

Chairpersons: **Dr. Vladimir Laudanović,**
Dr. Jasmina Glamoclija

10.45

-
11.15**PLENARY LECTURE 7****Prof. Ulrike Lindequist / Germany**

Interactions between medicinal mushroom components and conventional drugs - molecular basis and practical consequences

PLENARY LECTURE 8**Dr. Mikheil Asatiani / Georgia**Antifungal and antioxidant potential of *Schizophyllum commune*

11:15-11:45

Coffee break

11:45-12:05

• **Prof. Anita Klaus / Serbia**Pink oyster mushroom *Pleurotus flabellatus* mycelium is a valuable source of biologically active components• **Dr. Vladimir Laudanović / Andorra**

Role of heteropolysaccharides derived from medicinal mushrooms in chemoprevention and carcinogenesis

12:05-12:25

• **Dr. Ralph Schmidt / Norway**

Immunomodulatory and anti-inflammatory activity of the mushroom extract AndoSan

• **Soumaya Boudagga / Tunisia**Using biolog OmniLog system to determine nutritional phenome and antibacterial activity of Lion's Mane mushroom (*Hericium erinaceus*)

12:25-12:45

• **Prof. Maja Kozarski / Serbia**

Mushroom polyphenols as immune system balancers: What's the mechanism behind it and possible interactions with dietary fibers?

• **Prof. Haiying Bao / China**

Research on antitumor activity and mechanism of mycomedicine

12:45-13:05

• **Nathan Scott / United Kingdom**Antidiabetic effects of *Ganoderma lucidum* fruiting body polysaccharide extracts obtained through novel purification procedures• **Dr. Siddhart Pramod Dubhashi / India**

Role of Cordyceps in mild to moderate COVID infection

13:05-13:25

• Sébastien Sinaeve / Belgium

Nephroprotective effect of a methanolic extract of two *Ganoderma* species and its association in an in vitro model of cisplatin induced tubulotoxicity

13:25-15:00

Lunch break / Poster Viewings / Exhibition

15:00

-

17:00

SESSION 8

CONFERENCE HALL 1 / Pacific

Industrialization of medicinal mushrooms products (including management, marketing, laws and regulations, standardization, ecotourism and mushroom hunting);

~ ~ ~

Chairpersons: Dr. Jasmina Glamočlija, Peter Petros

15:00-15:20

• Prof. Elena Savino / Italy

Selection of wood decay fungal strains with medicinal properties useful for development of myco-materials

15:20-15:50

• Hana Vašatko / Austria • Prof. Milena Stavrić / Austria

Mycelium-based composites in the architectural scale

15:50-16:10

• Peter Petros / Finland

Validation of Large-scale forest-fungi inoculation and Chaga cultivation network in the Nordics: A circular bioeconomic strategy for sustainable forest management

16:10-16:40

• Dr. Gabriele Beltrame et al. / Finland

Effect of sea buckthorn press cake on the cultivation of *Inonotus obliquus mycekium* and its polysaccharides

16:40-17:00

• Amazing Grace Heath Products

Natural Phellinus Mushroom - New Visions of a Modern Elixir of Life

17:00-17:30

Coffee break

CONFERENCE HALL 1 / Pacific

CONFERENCE HALL 2 / Atlantic

17:30-19:00

SHORT ORAL PRESENTATION
session NO 5 & 6

-

12 present. x 5 min – 60 min
+ 30 min discussion - 90 min

Chairpersons: Prof. Mirjana Stajić,
Dr. Ewa Zapora

SHORT ORAL PRESENTATION
session NO 7

-

10 present. x 5 min – 50 min
+ 40 min discussion - 90 min

Chairpersons: Prof. Milena Pantić,
Dr. Marina De Bonis

17:30

-

19:00

- **Prof. Natalija Velić / Croatia**

Waste or resource: biosorption potential of the inactive biomass of *Fomitopsis pinicola* mushroom for the removal of the synthetic dye Congo red from water

- **Halyna Dubova / Ukraine**

Use of Mushrooms (*Pleurotus Osteratus*) to Inhibit Enzymatic Browning of Potatoes

- **Urszula Waszczuk / Poland**

- **Dr. Ewa Zapora / Poland**

Wood-decaying fungi as plant protection agents against *Fusarium oxysporum*

- **Prof. Halina Car / Poland**

Anticancer potential of *Laetiporus sulphureus*

- **Alexander Areesanan / Switzerland**

Impact of the therapeutic properties of European mushrooms on human corneal epithelial cells and T Lymphocytes to treat eye diseases

- **Dr. Anna Sadowska / Poland**

Heterobasidion annosum activity on colorectal cell lines

- **Dr. Józef Kaczor / Poland**

Evaluation of mushrooms and fermented wheat grain for activity against colorectal cancer cells

- **Dr. Tamara Teplyakova / Russian Federation**

The Activity of components of true tinder mushroom, *Chaga inonotus obliquus* (Fr.) Pil. against coronavirus SARS CoV-2

- **Vanessa Grifoll Garcia / Spain**

Effect of vitamin D2, selenium and glucans on anti-cancer activity in *Portobello* mushroom

- **Vanessa Grifoll Garcia / Spain**

Assessment of the *in vitro* antioxidant and anti-inflammatory potential of ethanol extracts of cultivable mushrooms

- **Dr. Marina de Bonis / Italy**

Effect of LED illumination on yield, quality and vitamin D2 content in *Pleurotus Ostreatus*

- **Dr. Lorenzo Goppa / Italy**

Enhancing "longevity vitamin" L-Ergothioneine extraction from mushrooms collected in Italy: a preliminary study

- **Dr. Dejan Stojković / Serbia**

Nutritional properties, chemical composition and biological properties of *Craterellus cornucopioides*

- **Prof. Sandra Konstatinović / Serbia**

Antioxidative and antimicrobial activity of ethanolic extracts from *Reishi* and *Reishi/Propolis*

- **Urszula Waszczuk /**

- **Dr. Ewa Zapora/ Poland**

Fungi Extract Bank – from innovation to application

- **Dr. Marija Gregori / Slovenia**

A newly developed *Hericium erinaceus* food supplement with standardized erinacine A content

- **Dr. Nina Vedenicheva / Ukraine**

Antitumor activity of Cytokinin-containing extracts from mycelium of medicinal mushrooms *Ganoderma lucidum* and *Lentinula edodes* in vitro

- **Erik Bird / Slovenia**

- **Dr. Marija Gregori / Slovenia**

CoPro Project: development of a first standardized food supplement from *Cordyceps*

- **Milica M. Galić / Serbia**

- **Prof. Mirjana Stajić / Serbia**

Anticandidal and anti-*Aspergillus* effects of *Pleurotus ostreatus* and *Laetiporus sulphureus* extracts

17:30

-

19:00

- **Katja Bezek /**

- **Dr. Andrej Gregori / Slovenia**

Effect of erinacine A enriched *Hericium erinaceus* supplementation on cognition – A Pilot Double-Blind Placebo-Controlled Study

- **Mateja Zotler / Slovenia**

- **Dr. Andrej Gregori / Slovenia**

Extraction of cordycepin from *Cordyceps militaris* and its characterization by HPLC and potentiometric titrations

- **Matija Bajželj /**

- **Dr. Andrej Gregori / Slovenia**

Cordycepin as a potential pharmacological inhibitor of pro-fibrotic pathways in systemic sclerosis

FRIDAY / SEPTEMBER 30th

EXHIBITION HALL / *Ground floor*

9:00

9:45

KEYNOTE 7 / Prof. Omon S. Isikhuemhen / USA/Nigeria

~ ~ ~

Psychedelic mushrooms: research, opportunities and future prospects

CONFERENCE HALL 3
Exhibition hall

9:00

-

11:15

SESSION 9

Psychedelic mushrooms: research, opportunities and the future for Psilocybe in medicine

~ ~ ~

Chairpersons: Prof. Omon S. Isikhuemhen, Dr. John Holliday

CONFERENCE HALL 3 / Exhibition hall

9:45

-

10:15

PLENARY LECTURE 9

Dr. John Holliday / USA

-

Psilocybin - A Microscope into the mind and telescope into consciousness

CONFERENCE HALL 3 / Exhibition hall

10:15-10:35

• Dr. Pawlik Lucas / *Liechtenstein*

Psilocybin & movement - Game changer for the health of society?

10:35-10:55

• Dr. Alexis Kaiser / *Liechtenstein*

Microdosing with Magic Mushrooms – Current and future perspectives as of 2022

10:55-11:15

• Dr. Dirk Proeckl / *Liechtenstein*

Psychosomatic solutions for the Global Health Crises

11:15-12:00

Coffee break

12:00

-

13:10

SESSION 5

Medicinal mushrooms in veterinary and agriculture;

~ ~ ~

Chairpersons: Prof. Paolla Rossi, Dr. Ewa Zapora

CONFERENCE HALL 3
Exhibition hall

12:00

-

12:30

PLENARY LECTURE 10

Dr. Ewa Zapora / Poland

-

Tyromyces fissilis in the prevention and treatment of American foulbrood in honeybees

CONFERENCE HALL 3 / Exhibition hall

12:30-12:50

• Prof. Paola Rossi / *Italy*

Hericium erinaceus neuroprotection of choroid plexus and blood brain barrier in wild-type frail mice during aging

IMMC12 PRESENTATION

Prof. Guiseppa Venturella / *Italy*

CONFERENCE HALL 3
Exhibition hall

13.00 - 13.30

CLOSING CEREMONY

14:00 - 17:00

Visit to the Exhibition of Wild mushrooms of Serbia at Botanical Garden Jevremovac



11th

INTERNATIONAL

Medicinal Mushroom

Conference **IMMC11**



**CONFERENCE
ABSTRACTS**



THE HISTORY OF ORGANIZING INTERNATIONAL MEDICINAL MUSHROOM CONFERENCES (IMMCS)

Prof. Miomir Nikšić¹, Prof. Solomon P. Wasser²

1 Faculty of Agriculture University of Belgrade, Department of Industrial Microbiology, Serbia

2 University of Haifa, Haifa , Israel

In science, each day we receive new data and new results, constantly pushing our achievements towards the scientific community. This target remained unchanged for the whole, now more than two decades long history of our IMMC scientific gathering and all the activities of the International Society for medicinal mushrooms.

Shared vision, great belief and simultaneous efforts of the three global pioneers in our scientific field, late Prof. Takashi Mizuno, Prof. Shu-Ting Chang and Prof. Solomon Wasser at the turn of a century, resulted in what we can now proudly call the largest and highest quality international scientific gathering in the Medicinal Mushrooms domain and which we consider our joint heritage.

Here, in Belgrade, we hope to be able to create a floor for scientists from different Continents, from different Countries, and from different generations to meet, bring their knowledge and data on new scientific discoveries, and exchange on their methods and experience.



Before the first International Medicinal Mushroom Conferences (IMMC) were organized, the inaugural issue of International Journal of Medicinal Mushroom (JMM) was published in January 1999 by Begell House. On the initiative and with the great effort of late Prof. Takashi Mizuno from Japan, who was the world's guru and pioneer in the study of medicinal mushrooms, with Prof. Shu-Ting Chang, a world guru in the field of mushroom biotechnology and Prof. Solomon Wasser, famous fungal taxonomist, in September 2001, the 1st Conference was held on a high scientific and organizational levels in Kiev, Ukraine. The Chairman of this conference was Prof. Tetsuro Ikekawa from Japan, one of the developers of lentinan, together with Professor Giro Chihara, a developer of other medicinal mushroom products. At the Kiev Conference 350 scientists came together from 40 countries and it was decided to organize IMMCS every 2 years. Organizing International Medicinal Mushroom Conferences (IMMCS) was important to unite scientists in different fields of medicinal mushrooms from all over the world.

THE HISTORY OF ORGANIZING INTERNATIONAL MEDICINAL MUSHROOM CONFERENCES (IMMCS)

The initiative sustained, the dream became reality and the new tradition has been created. The next Conferences followed, and, in July 2003 the 2nd IMMC was held in a beautiful place near Pathaya, Thailand under the Royal Family's patronage. At the Thailand conference, approximately 1000 scientists participated, mainly from Asian countries.

Dr Paul Stamets, founder, and President of Fungi Perfecti Co., than organized a very productive and successful 3rd IMMC in the State of Washington in the USA in October 2005, in beautiful Port Townsend, a historical city on the Pacific Coast but also a region with fantastic diversity of wild mushrooms. Approximately 300 scientists attended from 35 countries.

Professor Franz Pohleven and Prof. Marin Berovic from Slovenia organized the 4th IMMC in Ljubljana, in 2007. and this conference was very successful, too.

In 2009 in Nantoong, China, under the patronage of Prof S.T. Chang and Prof. YU LI, and Head of China Chamber of Commerce of Foodstuffs and Native Produce, Mr. Zi-Qiang Liu, based on Alphay Bio-Technological Company, organized the 5th IMMC Conference that was held on a very high scientific, organizational, and cultural levels, as well as gourmet delights. At Nantong IMMC 1200 participants were present.

In 2011 in Zagreb, Croatia Dr Jakopovich President of Dr. Myko San Medicinal Mushroom Company together with his family, organized the 6th IMMC, a very productive, effective, and successful conference.

In August 2013 in Beijing the group of Chinese Colleagues, again under the patronage of Professor S.T. Chang, Prof LI, and Mr. Liu, organized a universally successful 7th IMMC medicinal mushroom conference.

In Beijing, the Society received an invitation from colleagues based in South America, Dr. Carmenza Jaramillo from Colombia and Dr. Angela Amazonas from Brazil to hold the 8th IMMC in Manizales in 2015. The beautiful venue "Termales el Otoño Hotel" near Manizales, where very productive meeting has been organized, become event that will always be remembered.

Professor Giuseppe Venturella organized the 9th Medicinal Mushroom conference in Palermo in 2017. We were delighted to attend in Sicily, Palermo near the volcano Etna, a very famous historical place.

THE HISTORY OF ORGANIZING INTERNATIONAL MEDICINAL MUSHROOM CONFERENCES (IMMCS)

Society accepted the invitation of Chinese colleagues to organize huge, 10th, jubilar huge International Medicinal Mushroom Conference in 2019 in China based on Alphay Biotechnological Company in Nantong. It was then, in 2019, in China, when we received an invitation to organize the next 11th IMMC conference in Belgrade, Serbia in 2021, however, to be postponed to this year due to the Covid-19 pandemics.

Here, on the IMMC11 besides scientific exchange, another important goal is to create opportunity for the broader international scientific community to converse in formal and informal settings, meet old friends and establish the new ones, as a legacy for the future, as well as to plan new scientific contacts and collaborations. The special emphasis on the IMMC11 is put on the young scientists – to be able to meet large number of renown international experts in our field.

Organized in Europe after five years, this conference has true importance for Serbian, regional and European scientists.

It is our hope that this Conference, organized in Belgrade end September 2022, will inject a new energy and become a strong push for the Europe scientific community of medicinal mushrooms.

Now the world center of medicinal mushrooms is here in Belgrade!

Welcome!

KEYNOTE

LECTURE **RECENT ADVANCES IN THE RESEARCH OF MUSHROOM**
01 OF DIETARY FIBER AND BIOACTIVE POLYSACCHARIDES

Peter C.K. Cheung

School of Life Sciences, The Chinese University of Hong Kong, Hong Kong, China

Mushroom cell wall is a rich source of non-digestible polysaccharides that are regarded as dietary fiber with multifunctional health benefits. The developmental stages of different mushroom species including spores, mycelia, fruit bodies and sclerotia provide various types of polysaccharides with different chemical structure and physical properties that have significant impact to their bioactivities.

Traditional mushroom dietary fiber is well-known for its immunomodulatory and anticancer effects, hypoglycemic and hypocholesterolemic as well as antioxidant activities. Emerging evidence has revealed that the mechanisms by which mushroom dietary fiber can impart its specific health effects are mediated by gut microbiota and the prebiotic properties of mushroom polysaccharides. In particular, recent research findings have shown that the preferential utilization of mushroom beta-glucans by gut bacteria is associated with the newly identified polysaccharide utilization loci (PULs). Mushroom beta-glucans are shown to be a potential high molecular weight prebiotic with longer fermentation time in the colon and bifidogenic property.

Current findings have indicated the unique role of mushroom beta-glucans to be used as a natural, safe and biocompatible nanomaterials to act as nanocarrier of drugs. Because of its specific recognition by immune cells such as macrophages via cell surface receptors, mushroom beta-glucans can targeted deliver the therapeutic agents that it carries to specific cell types. However, there remains several challenges for the applications of mushroom dietary fiber/polysaccharides in the functional food industry. Moreover, future research on mushroom polysaccharides needs to be focused on the structure-function relationship as well as clinical studies using structurally well-characterized samples.

MUSHROOMS, FOOD, OR MEDICINE? OR BOTH?

KEYNOTE

LECTURE 02

Marina Sokovic¹

¹ Institute for Biological Research „Siniša Stanković“, National Institute of Republic of Serbia, University of Belgrade, Bulevar despota Stefana 142, 11040 Belgrade, Serbia

Mushrooms are rich sources of bioactive compounds. The potential health benefits associated with mushroom intake are well-known. The study of the role of mushroom bioactive compounds to fight diseases, including cancer, is of major interest for our society. In the present study we have focused on the Portobello variety of *Agaricus bisporus* mushrooms. The aim is to find out whether agronomic intervention during mushroom cultivation may increase the content of certain bioactive compounds in the mushrooms and ultimately increase its anticancer activity.

The present study evaluated the effects of vitamin D2, selenium and glucans on anticancer activity. For this purpose, several Portobello crops were grown on commercial phase II compost following standard cultivation practice to correlate the increase in vitamin D2 and the anticancer activity. For this purpose, ultraviolet radiation (UVA, UVB and UVC) was used to increase vitamin D2. UV-lamps were placed at 25 cm from the casing layer in the mushroom beds and treated with 6-18 J/cm² for periods ranging from 15 to 45 min. UVA and UVB were applied during cultivation while UVC radiation was applied to freshly harvested mushrooms. Mushroom samples were extracted by saponification process and the levels of vitamin D2 were determined by HPLC. A significant increase in vitamin D2 was detected when mushrooms were irradiated with UVB but was not found in UVA-irradiated specimens.

This increase in vitamin D2 was not followed by a significant improvement on the anti-cancer activity, indicating that the antitumor activity was not due to vitamin D2 contents.

In addition, different crop trials were performed to evaluate the influence of selenium treatment during cultivation on the anti-cancer activity of cultivated Portobello. Sodium selenate was incorporated with irrigation water to raise the selenium contents of the mushrooms. Five separate treatments were performed through drench applications onto the casing layer prior to primordial formation, with water containing.

MUSHROOM MEDICINE: LATEST NEWS, SCIENCE, PRODUCT CONTROVERSIES, AND THERAPEUTIC USES

KEYNOTE

LECTURE **03**

Dr. Christopher Hobbs Ph.D.

chrisrhobbs@gmail.com

Author of the ground-breaking text, *Medicinal Mushrooms* (1989, 1995) and the recently released *Medicinal Mushrooms, the Essential Guide* (2021), Dr. Hobbs will discuss the historical and modern uses of fungi for medicine, and their place in modern integrative health care and medicinal practices.

Review the complex cellular and molecular signaling pathways activated by exposure to cell wall constituents like beta-glucans, and the resulting immune changes that are the well-studied mechanisms of action enhancing immune vigilance against viruses and other pathogens.

Other important constituent groups like di- and triterpenes, and phenolic compounds and their pharmacological effects will be discussed in light of their therapeutic actions and benefits.

Mushrooms are among the highest fiber foods, and recent research on their health benefits as a source of prebiotics for helping to increase microbial diversity in the human microbiome, and associated health benefits will be reviewed. Practical considerations for making mushroom powder concentrates for addition to prepared foods like soups and smoothies will be detailed with samples to try.

Finally, a special emphasis will be placed on how to collect or purchase fruiting bodies and make the best extracts and preparations, as well as choose the best commercial products including a discussion about recent controversies such as mycelium vs. fruiting body, and testing for starch content in mycelium products.

ENGINEERING ASPECTS IN CULTIVATION OF MEDICINAL MUSHROOM BIOMASS IN SOLID STATE AND SUBMERGED BIOREACTORS

KEYNOTE

LECTURE 04

Prof.Dr. Marin Berović

Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

Production of fungal fruit bodies using farming technology could hardly meet the demand of World market. Great interest for large scale production of various medicinal mushroom pharmaceutically active compounds requests the development of new comprehensive technologies. Research in physiology, basic and applied studies in fungal metabolism, process engineering and (pre)clinical studies in last two decades represent large contribution to the development of submerged and solid state cultivation of medicinal fungi biomass in bioreactors.

In last three decades the fundamental aspects of solid state cultivation of various kinds of medicinal mushroom mycelia in various types of bioreactors was established. Solid state cultivation of various medicinal mushroom biomass is very close to fungal natural growth. Solid state cultivation in bioreactors is well controlled comprehensive technology suitable for medium scale production especially for recycling organic waste materials of different types. As the substrates various secondary wastes from wood, agriculture and fruit industry are successfully used. No fungal fruit boddies are produced. Final product represents delignolized, wooden material overgrown by medicinal fungi biomass enreach with proteins and various pharmaceutically products. Dried and pulverized it could be directly used as a special veterinary remedies in a veterinary need.

Development of comprehensive submerged technologies using stirred tank and air lift bioreactors are the most promising technologies for fast and large scale cultivation of medicinal fungi biomass and its pharmaceutically active products for human need. Pilot scale studies in this types of bioreactors represent the bridge and the balance between the gap of laboratory and industrial scale. In that sence it is not a surprise that most of pilot scale results and experiences remain uncovered industrial secrets. Comprehensive submerged cultivation represents fastest and the most suitable technology for a large scale production of medicinal mushroom biomass and its pharmaceutically active compounds for human use.

This presentation is an overview of the engineering achievements in submerged and solid state cultivation in bioreactors.

MUSHROOMS AS A SOURCE OF BIO-BASED INGREDIENTS: FOOD AND COSMETIC APPLICATIONS

KEYNOTE

LECTURE 05

Lillian Barros

- 1 Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal;
- 2 Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
lillian@ipb.pt

The growing world population and consumers' awareness about what they eat and how it can affect their health, have boosted the interest in the consumption of mushrooms, and the investment of the food and pharmaceutical industries in the development of healthier products with bioactive assets. For centuries, mushrooms have been collected and consumed worldwide due to their valuable nutritional and medicinal properties and unique aroma, flavor, and texture. Their nutritional profile, mainly composed by carbohydrates, proteins, fibers, vitamins (B and C complexes), and minerals, makes them an excellent food product to be included in a daily diet.

Besides, mushrooms are seen as functional ingredients and/or bases of nutraceuticals, given the presence of physiologically and biologically active substances, mostly in their fruiting bodies, mycelium and spores, such as polysaccharides, proteoglycans, terpenes, phenolic compounds, and others. These are responsible for several bioactive properties occurring in mushrooms, namely anti-proliferative, antioxidant, cholesterol reducing, and several others.

Although mushroom extracts are widely studied for their bioactive value, little is known about their potential benefits in cosmetic products. Mushroom-derived metabolites can be sustainably used in the development of nutricosmetic and cosmeceutical formulations to suppress the severity of inflammatory skin diseases, to offer photoprotection to the skin, correct hyperpigmentation, among others. However, the use of mushroom extracts and their associated metabolites in these industries may be a challenge, since it includes several steps from extraction optimization, estimation of efficacy and safety, the use of micro and nano carriers, and the pros and cons associated with the use of extracts vs individual compounds.

Also, and according with literature, mushrooms and their residues are a rich source of mycosterols, specially ergosterol (precursor of vitamin D2) and other steroids, bioactive molecules found in the nature that can exert different bioactive properties, being the only non-animal food source of vitamin D2, which is also formed during UV exposure, and whose deficiency can cause serious health problems.

MUSHROOMS AS A SOURCE OF BIO-BASED INGREDIENTS: FOOD AND COSMETIC APPLICATIONS

KEYNOTE

LECTURE 05

For that, the fully investigation of these mushroom biomolecules and their incorporation into dairy, bakery and other types of food products represents a valuable opportunity to the food industry to provide functional foods to the world's growing population.

Therefore, the exploitation of mushrooms as a source of bioactive molecules has open new horizons making it possible to develop new bio-based food and cosmetic products.

Aknowledgements:

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021). L. Barros is also grateful to FCT, for her contract, through the institutional scientific employment program-contract.

**ANTITUMOR EFFECT OF GANODERMA (LINGZHI)
MEDIATED BY IMMUNOLOGICAL MECHANISM
AND ITS CLINICAL APPLICATION**

KEYNOTE

LECTURE **06**

Zhi-Bin Lin

Department of Pharmacology, Peking University Health Science Center, Beijing, China

The anti-tumor effect of Ganoderma(Lingzhi) is closely related to immunoregulation. Based on our research and other references this article discussed the antitumor effect of Ganoderma mediated by immunological mechanism including promoting the function of mononuclear- macro phages and natural killers promoting maturation and differentiation of dendritic cells increasing its antigen presentation activating lymphocytes and increasing cytotoxicity of cytotoxin T lymphocyte promoting production of cytokines inhibiting tumor escape from immune surveillance. Also, clinical studies with immunological indexes were reviewed.

Key words: Ganoderma; Lingzhi; polysaccharides; triterpens; immune; tumor

PSYCHEDELIC MUSHROOMS: RESEARCH, OPPORTUNITIES, AND THE FUTURE FOR PSILOCYBE IN MEDICINE

KEYNOTE

LECTURE 07

Omoanghe S. Isikhuemhen¹ and John C. Holliday

1 North Carolina A&T State University, Greensboro, NC 27411, USA.

2 Ayla Bioscience Inc., 6646 Sierra Vista Lane, Carson City, NV 89702, USA.

The genus *Psilocybe* contains unique mushroom-forming species of fungi renowned for their hallucinogenic properties, exploited for neurotropic use, especially in many sacred religious ceremonies worldwide. There are about 300 *Psilocybe* species distributed worldwide in unique habitats like stems, leaves, seeds, earth, dung, sawdust, straw, dead wood, etc. Their hallucinogenic and psychoactive properties have earned them different common names like magic mushrooms, shrooms, goody-goody, psychedelics, etc. *Psilocybe* mushrooms are the principal source of naturally occurring psychedelics.

The association of psychedelic use of *Psilocybe* with the hippie movement in the early 20th century and its classification as a Schedule 1 drug by the US government in 1970 brought scientific research on them to a stop and left lingering negative stigmatization until today. The compound psilocybin, commonly found in *Psilocybe* mushrooms and implicated in the psychedelic effects experienced when ingested, is biologically inactive. Upon ingestion, psilocybin is dephosphorylated to its active metabolite psilocin (4-hydroxy-N,N-dimethyltryptamine), which has mind-altering effects like euphoria, and visual and mental hallucinations, including changes in perception and perceived spiritual experiences.

However, the prohibition on psychedelic drug research significantly delayed advances in medical knowledge on the therapeutic uses of agents such as psilocybin. A 2004 study on the use of psilocybin on advanced stage cancer patients gave results that reignited interest and significantly renewed efforts in psilocybin research and their exploration for psychedelic therapy. Results from clinical trials have shown significant reductions in depression and anxiety in cases of addiction, depression, and end-of-life mood disorders. Studies have also shown that psilocybin may reduce depression and anxiety associated with psychological crises due to a terminal diagnosis of advanced-stage cancer. Microdosing has become a term in everyday use. Users' supposed positive experiences are the biggest driver of effort to change the negative popular opinion about psychedelic mushrooms and the laws prohibiting their use for research and therapy.

Microdosing consists of three components: A) the use of a low dose below the perceptual threshold that does not impair 'normal' functioning of an individual. B) a procedure that includes multiple dosing sessions. C) The intention to improve well-being and enhance cognitive and/or emotional processes.

PSYCHEDELIC MUSHROOMS: RESEARCH, OPPORTUNITIES, AND THE FUTURE FOR PSILOCYBE IN MEDICINE

KEYNOTE

LECTURE 07

Nevertheless, psilocybin cultivation, sale, and processing for medicinal use remain illegal worldwide, except in a few countries, including Jamaica and some South American countries. Countries in the west have started to decriminalize and legalize bits and pieces of cultivation, possession, recreational use, and medical research. Canada is taking the lead among Western countries on decriminalizing their use in medicine.

In January 2022, Canada legalized psilocybin for prescription use, the first Western country to do so. However, magic mushrooms are prohibited in commerce in most of Europe and Asia. In general, laws and restrictions are still a significant obstacle for scientists seeking research grants to advance Psilocybe mushrooms' research from cultivation to clinical applications. However, most countries where they are legal currently see enormous investments flowing from developed countries.

Companies are trouping to these few countries to establish research from cultivation to clinical trials. They aim to perfect products and their use in the clinical treatment of various disorders to take financial advantage of the inevitable decriminalization and full-scale clinical use in western countries.

Keywords: Hallucinogenic mushrooms, health benefits, psilocybe, psilocybin, psychedelics

PLENARY

LECTURE 01 CURRENT OVERVIEW OF PLEUROTUS SPECIES TAXONOMY, PHYLOGENETIC RELATIONSHIPS AND GLOBAL DISTRIBUTION

Georgios I. Zervakis

Agricultural University of Athens, Laboratory of General and Agricultural Microbiology,
 Iera Odos 75, 11855 Athens, Greece (zervakis@aua.gr)

The genus *Pleurotus* (Fr.) P. Kumm. includes taxa and species-complexes with a cosmopolitan distribution many of which are of high economic interest. In fact, commercial production of *Pleurotus* ("oyster") mushrooms represents ca. 20% of the respective global production, and their cultivation is widespread throughout the world on a large variety of agricultural, agro-industrial and forestry residues.

However, their study and subsequent exploitation was repeatedly hindered by problems related to ambiguous initial identifications (largely due to environmentally-influenced morphological characters) unclear and obsolete descriptions, erroneous use of taxonomic names, and conclusions based on fragmentary and/or not robust information. In addition, for many species, phylogenetic data are missing or are incomplete. Currently (June 2022), the number of *Pleurotus* taxa included in major databases is several hundred, e.g., 769 in Index Fungorum and 830 in Mycobank; this is indicative of the perplexed status of taxonomy in the genus, and highlights the need to elucidate phylogenetic relationships among *Pleurotus* species, understand evolution issues and correctly assess the genus diversity.

Although a substantial amount of work has been performed on certain groups, e.g., within the *P. eryngii* complex with members that are closely associated with plants of the family Apiaceae and are known to occur in the Old World only, or for coremia-forming *Pleurotus* (i.e., *P. cystidiosus* and allied taxa originating from various continents), species boundaries are not clear in several other groups. Such cases exist among the monomitic *P. ostreatus*, *P. pulmonarius*, *P. floridanus*, *P. abieticola*, *P. eous*, *P. placentodes* and *P. populinus*, or the dimitic *P. cornucopiae*, *P. citrinopileatus* and *P. euosmus*.

Moreover, available data suggest that there is a large (yet untapped) diversity within tropical and subtropical species, e.g., the *P. djamor* complex (which includes several morphotaxa), *P. giganteus sensu lato* and *P. tuber-regium*. Last, limited phylogenetic information is available on species reported from the Neotropics, e.g., *P. albidus*, *P. rickii* and *P. levis*. In addition, this overview discusses indicative cases of uncertain/ambiguous taxonomic identity, and the erroneous use of names which are frequently met in scientific literature.

**CONSERVATION, TAXONOMY, ECOLOGICAL DISTRIBUTION,
AND POTENTIAL APPLICATION OF THE CULINARY-
MEDICINAL MUSHROOM LECCINUM SCABRUM**

PLENARY

LECTURE **02**

**Giuseppe Venturella^{1,2}, Maria Maddalena Cavalluzzi³, Giovanni Lentini³, Antonio Rosato³,
Alexia Barbarossa³, Valeria Ferraro¹, Fortunato Cirlincione¹, Giulia Mirabile¹, Ewa Zapora⁴,
Marek Wolkowycski⁴, Marcin Stocki⁴, Pasqualina Colasuonno², Maria Letizia Gargano^{2,5}**

- 1** Department of Agricultural, Food and Forest Sciences, University of Palermo, Palermo, Italy;
- 2** Italian Society of Medicinal Mushrooms, Pisa, Italy;
- 3** Department of Pharmacy - Pharmaceutical Sciences, University of Bari "Aldo Moro",
via E. Orabona, 4 70125 Bari (Italy);
- 4** Institute of Forest Sciences, Bialystok University of Technology, Poland;
- 5** Department of Agricultural and Environmental Science, University of Bari "Aldo Moro",
Via Amendola 165/A, I-70126 Bari, Italy.

E-mail: giuseppe.venturella@unipa.it

Leccinum scabrum (Bull.) Gray, is a member of the family Boletaceae Chevall. It is an edible, mycorrhizal mushroom of potential application interest for both food and medicinal properties. Described in 1783 by French naturalist J.B.F. Bulliard under the name *Boletus scaber*, in 1821 it was included by S.F. Gray in the genus *Leccinum*. *L. scabrum* is mainly collected under birch trees, it prefers deciduous woods and is also found under *Fagus sylvatica* L. Fructification period extends from early summer to autumn, in grassy areas or with the presence of low bushes, in open spaces or at the edge of the woods. The cap is up to 15 cm width, when unripe, hemispheric then convex and finally flat. The surface of the cuticle is rather smooth, generally without depressions, fairly velvety, with a color ranging from off-white to light brown, to hazel, with yellowish tones, and is dry with dry weather, becoming somewhat viscous when it moistens.

Sometimes, the cap have a fairly regular margin with small lighter or darker spots. Hymenium with white tubules then darker and gray-green in the ripe basidiomata. The pores are small with a rounded shape with a color, from whitish to gray and then brownish. Stipe 8-15 cm, 2-4 cm in diameter, firm and gradually fibrous, off-white, covered by dark small scales. Flesh off-white, unchanging when touched, cut or cooked. The flesh is tender but firm in the unripe specimens, while it tends to become soft when ripe. Pleasant light smell, taste sweetish. Basidiospores yellow-cinnamon, elongated shape, smooth and guttulate, 13-21 x 4-6 µm. *L. scabrum* is one of the most sought after and appreciated boletes. The characteristic of the meat that does not blacken, makes it particularly appreciated by many people.

**CONSERVATION, TAXONOMY, ECOLOGICAL DISTRIBUTION,
AND POTENTIAL APPLICATION OF THE CULINARY-
MEDICINAL MUSHROOM *LECCINUM SCABRUM***

PLENARY

LECTURE 02

The firm consistency of the meat also contributes to its good edibility.

Basidiomata of *L. scabrum* were collected within the Białowieża Forest (Poland), an ancient virgin forest (87,600 ha) with a unique biodiversity of fungi in Europe. Białowieża Forest is the best preserved forest ecosystem and the last low-land deciduous and mixed old-growth forest in Europe. Specimens of fungi of the genus *Leccinum* were identified on the basis of specialist literature using classical methods of taxonomic mycology. Evidence collections were made from the collected fruiting bodies and deposited at the Fungarium of the Institute of Forest Sciences (collection acronym in the Index Herbariorum - BLS).

Results obtained from analyses performed on mushrooms powder obtained by drying of fresh basidiomata, show a relevant percentage of vitamins and minerals, among which vitamin D3, B2 and among minerals, sodium, potassium, iron and calcium. Remarkable is the content of carbohydrates, proteins and dietary fiber and the content of total sugars and total free amino acids. Polyunsaturated fatty acids represent the most marked value while saturated fatty acids represent the lowest value. Significant contents were found in vitamin D3 and B2 as well as sodium, potassium, iron, and calcium. Centesimal analysis shows a higher quantity of carbohydrates, proteins, dietary fiber and ashes compared to other foods. Furthermore, chemical composition of *L. scabrum* powder methanolic extract was also analyzed using gas chromatography with mass spectrometry. The main components of the extract were carbohydrates (79.61%), as well as fatty acids and their esters (6.59%).

The extract also contained ergosterols (2.65%), polyalcohols (1.67%) and, amino acids (1.25%). In the extract was found presence of biologically active compounds belonging to hydroxy acids (e.g. malic acid, lactic acid, glyceric acid), dicarboxylic acids (e.g. succinic acid, fumaric acid, glutaric acid) and aromatic acids (i.e. benzenoacetic acid, 4-hydroxybenzenoacetic acid, benzoic acid).

A preliminary study on its possible antibacterial effect was carried out by testing some extracts obtained under microwave irradiation against a panel of *Staphylococcus aureus* (ATCC and clinical isolates). Four different solvents with increasing polarity were used to extract the bioactive compounds of *L. scabrum* and the observed antibacterial activity was expressed as Minimal Inhibitory Concentration (MIC), assessed by microdilution method. The results obtained open the way for further investigation and for the activation of appropriate conservation strategies.

Keywords: Biodiversity, Boletaceae, Taxonomy, Ecology, Conservation, Białowieża Forest, Poland

PROTEOMIC RESEARCH ON THE THERAPEUTIC PROPERTIES OF MEDICINAL MUSHROOMS

PLENARY

LECTURE 03

Boris Jakopović, PhD

Dr Myco San – Zdravlje iz gljiva d.o.o. Croatia

Medicinal mushrooms are increasingly being recognized as an important therapeutic modality in complementary oncology. Until now, more than 800 mushroom species have been known to possess significant pharmacological properties, of which antitumor and immunomodulatory properties have been the most researched. Besides a number of medicinal mushroom preparations being used as dietary supplements and nutraceuticals, several isolates from mushrooms have been used as official antitumor drugs in clinical settings for several decades. Proteomics is a large scale study of proteins, which is characterized by a hypothesis-free and comprehensive approach to studying novel mechanisms of potential therapeutics. Specifically, differential proteomics, also known as comparative or functional proteomics, studies the changes in proteome in different physiological or pathological states between two or more samples. Cancer proteomics encompasses the identification and quantitative analysis of healthy tissue from neoplasia and can be used to identify markers for cancer diagnosis and treatment (biomarkers), monitoring disease progression, and identifying therapeutic targets serendipitously.

Despite its complexity, proteomics is necessary for accurate characterization of pharmacological action. The complexity of cancer, which includes various pathways of its ontogeny and progression, tumor microenvironment, and therapeutic resistance mechanisms, poses demanding challenges that require a systems biology approach that is now increasingly beginning to re-examine the historical reductionist approach. In this regard, the previous research on cancer as well as on cancer therapeutics might be regarded as preliminary or partial and in need of a more in-depth study.

However, various challenges remain. Besides the complexity and variability of biological material, i.e., medicinal mushrooms, the variability in the results may also be influenced by the model, either in vitro or in vivo, where the timing of the treatment (early vs. late models of disease; immunocompetent vs. nude mice xenografts), as well as the tumor model (orthotopic vs. heterotopic), can produce different results.

The results are also highly dependent on the proteomic methods, which also differ in their sensitivity. However, these obstacles must be overcome in order to study complex diseases such as cancer. This review is focused on the current state of proteomic research into antitumor mechanisms of some of the most researched medicinal mushroom species, including *Pheellinus linteus*, *Ganoderma lucidum*, *Auricularia auricula*, *Agrocybe aegerita*, *Grifola frondosa*, and *Lentinus edodes*, as whole body extracts or various isolates, as well as of complex extract mixtures.

FUTURE OF MUSHROOM FARMING

PLENARY

LECTURE 04

John Holliday

Ayla Bioscience Inc, USA

The last 20 or 30 years has brought great change to the field of mushroom cultivation.

Advances in equipment, chemistry, strains, and technology are driving ever increasing yields at lower input costs than ever before. By designing and building our farms using today's knowledge, materials and techniques, mushroom farming is one of the most profitable agricultural endeavors known.

To best understand these changes and look at how mushroom farms of today and tomorrow are evolving less as agricultural endeavors but more as Biological Manufacturing Processes, we need to first look at the past. How the mushroom industry first developed and has advanced up until now.

The earliest recorded cultivation of mushrooms started around the year 800 AD, with growing wood ears (*Auricularia*) on natural logs in China. By the year 1200, shiitake was widely cultivated this way as well. First the bark was split lengthwise and peeled back from the wood. A hole was made with a hollow cylindrical tool attached to a handle, resulting in a tool that looks like a hollow round hammer. This tool driven into the wood, removing a round plug about 12mm in dia. A fresh mushroom fruitbody was then squashed into the hole, and the bark allowed to settle back down over this inoculation to maintain moisture. Amazing that a technique developed a thousand years ago is still in use today. Similar to the old Chinese methods for cultivating wood decomposing mushrooms, some still practice natural log culture. This is an unreliable method that is unlikely to produce economically viable farms in most of the world. This is mostly due to the fact it is not possible to control the timing or the duration of the crop in natural log cultivation. The mushrooms fruit when the conditions are right, and the logs sit idle the rest of the year. This makes it impossible to market a year-round crop.

While Asia developed methods to cultivate wood decomposing mushrooms, in Europe the secondary decomposers like *Agaricus* (button mushrooms) were more popularly gathered and eaten. The mushroom collectors knew that the mushrooms would reappear in the same fields year after year. Around the turn of the 19th century in France, it became popular to go to these fields, and take a shovel full of dirt where the mushrooms were fruiting. This was taken back to the farm, and applied to some substrate, usually in the barnyard. It didn't take long to figure out that when this "soil spawn" was tossed into the corner of the barn onto the old, partially decomposed straw and hay from the horses, that was where the most mushrooms would grow. From this observation, creative farmers began to purposely compost their used horse bedding and using this material to create mushroom beds. These beds were inoculated with this wild soil spawn, and the button mushroom industry was born.

FUTURE OF MUSHROOM FARMING

PLENARY

LECTURE 04

The harvesting of mushroom "soil spawn", known then as Brick Spawn, became an industry, and by the turn of the 20th century this spawn was being exported from France to the rest of Europe and the United States, where a new and popular industry developed into large scale button mushroom farming. While this soil spawn represented a rather hit-or-miss approach to cultivation, it was the best available method at the time.

As the understanding of microbiology advanced, and the microscope came into more widespread use, researchers saw that what the soil contained was the mycelium of the mushrooms. The mycelium from this soil was easy to isolate and grow on different media, which by 1930 led to the development of Grain Spawn. This was one of the first real breakthrough in creating sustainable, reliable, and consistent mushroom cultivation. Sterile tissue culture came into widespread use by the end of World War II, and the grain spawn became more reliable and more widely available, with many spawn suppliers coming into the market and the number of mushroom farms exploding. By 1980, there were approximately 1000 button mushroom farms in America.

Through the late 1980's and the 1990's, large companies such as Campbells took over much of the mushroom industry. The smaller mom-and-pop farms grew less and less viable, since the larger farms could produce mushrooms so much more economically. By the early 2000's, the American mushroom industry was down to just a few large companies. In my 45 years in the mushroom industry, I have seen farm after farm go under, due to competition from the large companies. Any button mushroom farm in America today that produces less than about 500,000 pounds per month has a hard time surviving in today's competitive marketplace. The button mushroom industry by 2022 has become stagnant, with not many new *Agaricus* farms being built.

So, what is the future of mushroom farming?

The more recent methods of cultivation for these wood decomposing species is to sterilize small bags of sawdust with steam, and grow the mushrooms in controlled conditions to allow for a reliable year-round harvest. This is a well proven technology that works. But it is also a technology that is being replaced by a newer method. The reason it is being replaced by newer methods is the cost of equipment and energy continues to go up as time goes on. This makes mushroom cultivation less profitable, and the construction of new farms following this method more expensive every year, and therefore less attractive.

To understand the future potential for mushroom farming with these newer methods, we need to unwrap the cultivation steps a bit. First and foremost, we need to understand that growing mushrooms to the exclusion of all other organisms is a very unnatural process. Yet we need to grow our mushrooms in a true monoculture system to have reliable yields.

FUTURE OF MUSHROOM FARMING

PLENARY

LECTURE 04

This requires two things: Sterilize the substrate to kill off all the potential competitors prior to inoculation with our target species and maintain sterility to the best of our ability throughout the entire process.

Consider sterilization: To be “sterile” means all organisms are killed, nothing is left alive.

In the early days of sterilization, circa 1850, it was found that exposing a material to steam under. But a lot of things have changed since 1850, including the development of many different processes to sterilize material. Like the use of microwaves, cold plasma, ionizing radiation, and chemical agents. In the end, sterile is sterile, and it makes no difference how this sterility is achieved.

While any of these methods could work to sterilize substrate, most of them are even more expensive than using steam for sterilization. The exception is chemical sterilization. The use of chemicals to sterilize the substrate is termed the Cold System of Mushroom Cultivation. This is the primary type of mushroom farm being built today because the use of cheap and available chemicals is more profitable and less capital intensive than the use of steam.

I predict the future of smaller scale, and probably larger scale mushroom farms, are purpose designed, HEPA filtered sterile “Biological Manufacturing” facilities, using a cold sterilization process.

We have come a long way from throwing a shovel full of dirt into the back corner of the barn. Today's and tomorrow's farms are designed using an end-to-end systems approach of sterile manufacturing. For a mushroom farmer to try to compete in today's market and with today's ever-increasing costs of supplies and materials using yesterday's technology doesn't work very well. Tomorrow's farms are more like a space station than a barn. Clean Rooms containing no wood, sterility as the number one operational feature, and a well-trained staff. This leads to a reliable and consistent yield, and an easy path to market and profitability.

MUSHROOMS AS SOURCE OF FUNCTIONAL FOOD INGREDIENTS

PLENARY

LECTURE 05

Jovana Petrović

Institute for Biological Research "Siniša Stanković", University of Belgrade,
National Institute of the Republic of Serbia, Bulevar despota Stefana 142, 11040 Belgrade

Ever since the origin of the first man, food has been used not only to satisfy hunger, but to provide nutrition. However, it seems as thoughtful eating, with enjoying in food for nourishment is currently in the background, and only hedonic experience of food in mind. Medicine and science revealed that the malnutrition on one side, and overeating on the other, are responsible for various diet related issues, such as: vitamins deficiency, scurvy, pellagra (in case poor diet) or coronary heart disease, high blood pressure, atherosclerosis, type 2 diabetes etc. (in case of excessive food intake).

Nevertheless, the awareness on how eating habits influence health status has been set out as a trend only recently, opening up new avenues for the food industry development. This has been largely influenced by the increasing demand for healthy, nutritious and tasteful food of sustainable origin, which aside from fulfilling basic nutritional needs, also have health beneficial properties, thus fitting into the concept of functional food.

Mushrooms emerged as rather versatile food which blend equally well with both sweet and savory ingredients, with nutritional richness, low caloric value, taste, and nutraceutical properties; their intake satisfies need for both nutrient and non-nutrient compounds that benefit human health in several aspects making them excellent candidates for functional food. Thus, regular consumption of mushrooms will provide recommended amount of fiber and other carbohydrates, proteins and contribute to daily intake of vitamins (B1, B2, B12, C, D, E), as well as polyunsaturated fatty acids. Along with this, it will supply with minerals (iron, copper, manganese, zinc) as well as terpenoids, phenolic and flavonoid compounds, which do not contribute to nutritive features of mushrooms, but play an important role in proper functioning of different metabolic pathways and/or have bioactive properties. Recent fundamental and clinical research highlighted potential of mushrooms as source of compounds with wide ranging bioactivities, including antimicrobial, antitumor, antiinflammatory, hepatoprotective, neuroprotective, cardioprotective, immunostimulatory etc.

Palatability, versatility and bioactivity of wild growing and cultivated mushrooms with high demand of consumers for novel, tasteful and nutritive products, put them in the spotlight of the contemporary food industry, which led to the development of innovative designing mushroom-based food and beverages with functional food properties. Furthermore, extractability of bioactive/nutritive compounds from mushrooms enables their availability in form of pills/capsules which allows consumption in populations with limited accessibility to these highly valued ingredients. After all, since cultivation of mushrooms increases the amount of this underutilized food products and permits circular economy, it is expected that mushrooms and/or mushroom based food ingredients will soon be accessible to everyone.

ASPECTS IN MOREL MUSHROOMS QUALITIES AND HEALTH PROMOTING ACTIVITIES

PLENARY

LECTURE 06

Segula Masaphy^{1,2}

1 Applied Microbiology and Mycology Department, MIGAL, P.O. Box 831, 11016 Kiryat Shmona, Israel
2 Tel Hai College, 12210 Upper Galilee, Israel

Morels (*Morchella* spp., Pezizales, Ascomycota) are edible mushrooms appreciated worldwide mainly for their taste and aroma. However, they are also rich in health promoting activities. These mushrooms have been in use in traditional medicine for centuries, especially in Southeast Asian countries.

More recently, mushrooms of this genus became one of the most hunting targeted mushrooms by citizen in western countries as well. In nature, in most cases, they appear once at site, in unexpected manner. For many years, morels were difficult to cultivate due to lack of understanding of the conditions regulating their fruiting. In recent years, with the increasing of the knowledge on morels behavior in nature, there are increasing cases of successful cultivation practices of different species of morels, in outdoor or indoor cultivation systems. Yet, in most countries, cultivation of morels is still unstable, and the mushrooms are mostly harvested from the wild or their mycelium is grown in fermented culture, for consumption as a functional food and for food-flavoring.

Morel species demonstrate high phenotypic as well as ecotyping diversities, resulting in different bioactivities. For example, the flavor's molecules composition of morels was related to their ecotyping and phenotypic diversities. Similarly, the mushrooms in this genus have been found to contain high anti-oxidative activities, related to their phenotypic diversity. Moreover, the fruiting bodies were reported to harbor range of bacterial populations. This mushroom's microbiome also affects the mushrooms metabolites production and bioactivities.

Morels mushrooms demonstrate range of health promoting bioactivities as anti-inflammatory bioactivities, immunostimulatory and anti-tumor properties. These health benefits were attributed mainly to polysaccharides as the active compounds, and to various phytochemicals, mainly phenolic compounds, tocopherols, ascorbic acid, and vitamin D. Morel's nutritional composition was reported, including sugar, amino acid, fatty and organic acid, and mineral profile. The increasing in controlled cultivations systems might increase the control on the mushrooms qualities in all aspects, including their bioactive compounds production.

INTERACTIONS BETWEEN MEDICINAL MUSHROOM COMPONENTS AND CONVENTIONAL DRUGS – MOLECULAR BASIS AND PRACTICAL CONSEQUENCES

PLENARY

LECTURE **07**

Ulrike Lindequist

Institute of Pharmacy, Pharmaceutical Biology, University of Greifswald, D-17487 Greifswald, Germany;
Email: lindequi@uni-greifswald.de

Drug-drug interactions are a major issue in the application of drugs. Pharmacodynamic interactions take place when the pharmacological effect of one drug is altered by that of another drug or food component (Niu et al. 2019). Pharmacokinetic interactions occur when one drug interacts with another at the level of pharmacokinetics, i.e., absorption, metabolism, or excretion.

Since the detection of the so-called grapefruit-effect and of interactions between St. John's wort and conventional drugs some decades before we know that also interactions between herbal drugs and/or food and medicines can be relevant for the efficacy and/or safety of drugs, especially for those with a small therapeutic index.

One underlying mechanism for pharmacokinetic interactions is the induction of cytochrome enzymes. Cytochrome P450 isoenzymes, especially CYP3A4, are responsible for the biotransformation of most xenobiotics including many drugs in clinical use. The other important mechanism is the influence on drug transporters. Uptake (members of SLC family) or efflux (members of ABC family, e.g., P-glycoprotein, ABCF1 = MDR1 and ABCC2 = MRP2) transporters facilitate the transport of drugs and influence drug exposure. In both mechanisms, the PXR receptor plays an important role. It is a member of the family of nuclear receptors and is involved in the regulation of metabolic processes in response to xenobiotics (Nicolussi 2019).

There is some evidence of possible pharmacokinetic interactions caused by components of medicinal mushrooms. Examples include the influence of polysaccharides from *Ganoderma lucidum* on the activities of cytochrome P-450 in rat hepatic microsomes (Wang et al. 2007) and of *Agaricus* on this metabolizing enzyme also in vitro (Engdal and Nilson 2009). The relevance of these in vitro results for in vivo conditions remains unclear. A short time clinical study about the influence of *Trametes versicolor* on cytochrome P450 (Nicandro et al. 2007) did not reveal clinically relevant effects.

The study of Toh et al. (2013) did show an effect of *Lentinula edodes* with a high content of ergothioneine on the renal clearance of gabapentine but did not estimate this to be clinically relevant.

INTERACTIONS BETWEEN MEDICINAL MUSHROOM COMPONENTS AND CONVENTIONAL DRUGS – MOLECULAR BASIS AND PRACTICAL CONSEQUENCES

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Pharmacodynamic interactions can be derived from the pharmacological profile of the respective partners. Possible examples are interactions between antidiabetics and hypoglycemic mushrooms or between anticoagulants and *Auricularia auricula judae*.

The lecture explains the underlying mechanisms of interactions, gives an overview of the current state of knowledge on those originating from medicinal mushrooms, and provides information on possible consequences for their practical use.

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Key words: medicinal mushroom, pharmacokinetic interactions, pharmacodynamic interactions

**ANTIFUNGAL AND ANTIOXIDANT POTENTIAL
OF SCHIZOPHYLLUM COMMUNE**

PLENARY

LECTURE 08

Mikheil Asatiani*, Violeta Berikashvili, Tamar Khardziani, Aza Kobakhidze, Eka Metreveli,
Eva Kachlishvili, Vladimir Elisashvili

The Institute of Microbial Biotechnology, Agricultural University of Georgia, Tbilisi, Georgia

Corresponding author: m.asatiani@agrni.edu.ge

Keywords: antifungal potential, higher basidiomycetes, plant pathogenic fungi, submerged fermentation, antioxidant activity.

In the present research, twenty-three basidiomycetes belonging to different taxonomic groups were screened for their antifungal properties against plant pathogenic fungi, such as *Aspergillus niger*, *Botrytis cinerea*, *Fusarium oxysporum*, and *Guignardia bidwellii*. In the frame of the screening program several promising species with antifungal properties were detected. Among them *Schizophyllum commune* in submerged cultivation on glucose contained medium revealed the best antifungal potential (36%–66%). After optimization of the nutrient medium it was established that among different lignocellulosic substrates, the presence of 4% mandarin juice production waste (MJPW), caused the increase of antifungal activity (growth inhibition: *A. niger* – 65%, *B. cinerea* – 18%, *F. oxysporum* – 57%, *G. bidwellii* – 85%). Beside this, it was detected that 6% of peptone was a most appropriate nitrogen source to enhance antifungal properties of *Sch. commune*.

Moreover, hot water, ethanol (80%), and ethyl acetate extracts obtained from submerged mycelium and culture liquid of *Sch. commune* contained considerable amount of bioactive substances such as, total phenolics, flavonoids and ascorbic acid. It is noteworthy that, ethyl acetate extracts obtained from the biomass and culture liquid of *Sch. commune* which was cultivated in presence of MJPW (6%) and peptone (6%) in nutrient medium, demonstrate highest content of total phenols, 120 GAE/g and 291 GAE/g, respectively. High content of ascorbic acid was observed in the same extracts (17 mg/g and 15 mg/g, respectively).

In addition, the flavonoid content was considerable low to compare to the ethyl acetate extracts obtained from biomass and culture liquid after cultivation of *Sch. commune* on glucose contained nutrient medium which demonstrate 21 QE/g and 27 QE/g. In contrary, water and ethanol extracts revealed relatively lower content of total phenols, flavonoids and ascorbic acid. Beside this, it was demonstrated that DPPH free radical scavenging activities of tested extracts were dose dependent and increased according to concentration.

ANTIFUNGAL AND ANTIOXIDANT POTENTIAL OF SCHIZOPHYLLUM COMMUNE

PLENARY

LECTURE 08

Highest radical scavenging activities were showed by water and ethanol extracts and varied in 74-89 % range at 15 mg/ml, which was close to the data of standard antioxidants (BHA, TBHQ, and α -tocopherol) used as positive control (94%, 83%, 91%) at same concentrations. The results obtained show that the antifungal activity of mushrooms depends on physiological factors of growth and the use of lignocellulosic substrates in submerged fermentation is a favorable approach.

Moreover, *Sch. commune* can be considered as a promising source of antifungal and antioxidant bioactive substances.

Acknowledgements: The work was implemented with financial support of the project FR-19-3719 by the Shota Rustaveli National Science Foundation of Georgia.

PSILOCYBIN – A MICROSCOPE INTO THE MIND AND TELESCOPE INTO CONSCIOUSNESS

PLENARY

LECTURE 09

Johh Holliday

Ayla Bioscience Inc, USA

The 1950's and 1960's were fertile times for research into consciousness, mental health and psychiatric treatment options. The discovery of LSD and the re-discovery in the west of Psilocybin in the mid 20th century lead directly to the discovery of neurotransmitters. This discovery opened a whole new realm of mental health treatment possibilities.

Could various mental health issues be in fact, chemical imbalances? And if so, could other chemicals work to adapt our minds, perhaps leading to improvements or even complete recovery for some mental illnesses? While a tremendous amount of research was done between 1955 and 1970 into psilocybin therapy, the social disorder caused by the "Counterculture Movement" of the 1960's doomed further research for many years. Nearly all psychedelic compounds were outlawed as Schedule 1 drugs, and research into psychedelic therapy shut down entirely until the late 1990's.

Over the last twenty years though, there has been a great resurgence into research on the use of psilocybin for treating many mental disorders, including depression, PTSD, bipolar disorder, and addictions. The value of psilocybin in mainstream psychiatry has now become well accepted. Public perception of psilocybin and other psychedelics has changed greatly in the last two decades.

The legal prohibitions are loosening across the world today, with psilocybin currently legal in 10 countries, and legislation occurring to further legalize it in many more. Many new companies have formed in the last few years to produce psilocybin for pharmaceutical use, and huge amount of investment capital is going into this field. This article looks at the current situation in the psilocybin industry, one of the fastest developing sectors of the mushroom industry, and one which shows every sign of booming in the coming years.

TYROMYCES FISSILIS IN THE PREVENTION AND TREATMENT OF AMERICAN FOULBROOD IN HONEYBEES

PLENARY

LECTURE 10

Stawomir Bakier, Marek Wołkowycki, Ewa Zapora

Institute of Forest Sciences, Białystok University of Technology, Poland

American foulbrood (AFB) is a fatal bacterial disease of honey bee caused by the bacteria *Paenibacillus* larvae, which produce endospores with high resistance.

In most countries, the fight against AFB consists in destroying infected bee colonies by sulfating and burning the bees together with the accompanying beekeeping equipment. Preventive protection of bee colonies against AFB is particularly troublesome. Currently, there are no pharmacological agents approved for the prophylaxis and treatment of bee colonies suffering from AFB that could replace antibiotics and polysulfamides banned for use in the EU.

Wood decay fungi (mainly Polypores), especially those found in the Białowieża Primeval Forest, are the object of our special interest. The innovative research project we created - Fungi Extract Bank (FEB, <https://fungiextractbank.com/en/>) was the basis for planning and carrying out this research. The research allowed to determine the activity of selected fungal extracts against *P. larvae* and their direct effect on bees.

In the first stage, the qualitative verification of the antibacterial activity of the fungal preparations was carried out against *P. larvae* bacteria representing two genotypes, ie Eric I and Eric II (two reference strains and four wild strains). Antibacterial screening tests were carried out in which the activity of 125 extracts collected in the Fungi Extract Bank was verified. The result of this step was the identification of 6 species of fungi that showed a particularly strong bactericidal effect against *P. larvae*.

In the second stage, both the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of selected extracts against *P. larvae* were determined. It turned out that the composition prepared on the basis of *Tyromyces fissilis* extract showed the highest activity: MIC = 1.99 µg/ml and MBC = 4.99 µg/ml.

The third research stage was carried out at National Veterinary Research Institute in Puławy. The in vivo verification of *Tyromyces fissilis* extract with a concentration above MBC was carried out against larvae and adult bees. The bactericidal activity of *Tyromyces fissilis* extract was clearly confirmed in groups of larvae infected with a lethal dose of *P. larvae* bacteria of the Eric II strain. Progression of the infection was inhibited in 33% of the larvae fed with the preparation with the concentration of the extract of 0.025%, while in the group of larvae fed with the preparation containing 0.05% of the extract, the pupal stage was reached by 50% of infected larvae. In the group infected with Eric I, 3% of infected larvae survived. It should be emphasized that it is the Eric II bacterial strains that are commonly considered to be "more virulent".

Natural origin composition based on the *Tyromyces fissilis* extract shows a strong activity against *P. larvae* bacteria and does not contain any toxic substances for bees or humans. Therefore, *T. fissilis* extract can be used in the prevention and treatment of AFB. The research results were the basis for the European patent application (WO 2021/235954 A1).

SESSION 01

LECTURE 01 DIVERSITY OF PLEUROTUS SPP. AND THEIR METABOLITES OF NUTRACEUTICAL AND THERAPEUTIC IMPORTANCE

Giancarlo Angeles Flores¹, Carolina Elena Girometta², Gaia Cusumano³, Roberto Maria Pellegrino³, Salvatore Silviani⁴, Giancarlo Bistocchi⁵, Andrea Arcangeli⁵, Federica Ianni⁶, Francesca Blasi⁶, Lina Cossignani⁶⁻⁷, Andrea Rubini⁸, Bruno Tirillini⁹, Carla Emiliani³, Luigi Menghini¹, Claudio Ferrante¹, Giuseppe Venturella¹⁰, Pasqualina Colosuonno¹⁰, Fortunato Cirlincione¹⁰, Maria Letizia Gargano¹¹, Roberto Venanzoni³, Paola Angelini³

1 Department of Pharmacy, Botanic Garden "Giardino dei Semplici", "Gabriele d'Annunzio" University, via dei Vestini 31, 66100 Chieti, Italy

2 Department of Earth and Environmental Science (DSTA), University of Pavia, Pavia, Italy;

3 Department of Chemistry, Biology and Biotechnology, University of Perugia, 06122 Perugia, Italy

4 Circolo Micologico Giovanni Carini di Brescia, AMB Trento, Italy

5 Circolo Micologico Naturalistico Perugino, 06123 Perugia, Italy

6 Department of Pharmaceutical Sciences, University of Perugia, 06126 Perugia, Italy

7 Center for Perinatal and Reproductive Medicine, Santa Maria della Misericordia University Hospital, University of Perugia, Sant'Andrea delle Fratte, 06132 Perugia, Italy

8 National Research Council, Institute of Biosciences and Bioresources, 06128 Perugia (PG), Italy

9 Department of Biomolecular Sciences, University of Urbino, 61029 Urbino, Italy.

10 Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze, Bldg. 5, I-90128 Palermo, Italy

11 Department of Agricultural and Environmental Science, University of Bari Aldo Moro, Via Amendola 165/A, I-70126 Bari, Italy

The diversity within *Pleurotus* has been creating a range of new potential applications with a profound biological and economical value. *Pleurotus* spp. are, in fact, becoming increasingly attractive for the development of functional food, nutraceuticals and new drugs, due to their potential antioxidant, antimicrobial, antiproliferative, immunomodulatory, anti-inflammatory and antihypertensive properties. About 200 species are currently accepted in this genus, but only a few of them such as *P. florida*, *P. sajor-caju* and *P. ostreatus* are available in the market.

The genetic and metabolic diversity within *Pleurotus* is of main concern for many researchers. Most of the researches were conducted from a taxonomic point of view in order to clarify and disentangle and biochemical markers have been significantly contributing to these issues.

SESSION 01

LECTURE 01 DIVERSITY OF PLEUROTUS SPP. AND THEIR METABOLITES
OF NUTRACEUTICAL AND THERAPEUTIC IMPORTANCE

In this work, *Pleurotus columbinus*, *Pleurotus ostreatus* and *Pleurotus eryngii* species complex cultivated as mycelium in vitro were studied to determine the effect of different media on the production of secondary metabolites, antimicrobial and antioxidant activity. The metabolomic profile of *Pleurotus* spp. was evaluated through ultra-performance liquid chromatography mass spectrometry (UHPLC)-QTOF method. The results highlighted that the differential metabolites among M1-M14 *Pleurotus* samples covered a total of 58 pathways. Comparisons were made between the metabolic profiles of *Pleurotus* spp. mycelia grown in solid substrates containing PDA and straw, with respect to substrate containing only PDA taken as reference.

As major outcome, this study demonstrates that the metabolic pathways are strongly influenced by the chemical composition of the growth substrate. All aqueous extracts from *Pleurotus* mycelia displayed antimicrobial activity in the concentration range of 1.56 to 200 µg/mL. The antibacterial effects were particularly evident against *Escherichia coli* (ATCC 10536) (MIC<2.47mg/mL), whereas the dermatophytes *Arthroderma curreyi* (CCF 5207) and *Trichophyton rubrum* (CCF 4933) resulted to be the most sensitive with MIC range between 31.49 and 158.74 µg/mL to the mushroom extracts.

Further investigations are needed to evaluate whether and how the growth substrate influences the antimicrobial and antioxidant properties of *Pleurotus* spp.

Keywords: *Pleurotus*, inter- and intraspecific diversity, Italy, metabolic pathways, Untargeted LC-MS/MS-Based Metabolomics

**DIVERSITY, CHEMISTRY AND ENVIRONMENTAL
 CONTAMINATION OF WILD GROWING MEDICINAL
 MUSHROOM SPECIES AS SOURCES OF BIOLOGICALLY
 ACTIVE SUBSTANCES (ANTIOXIDANTS, ANTIDIABETICS,
 ACHE INHIBITORS AND COSMECEUTICALS)**

SESSION 01

LECTURE 02

Maja Karaman, Eleonora Čapelja, Milena Rašeta, Milana Rakić

1 Department of Biology and Ecology, Faculty of Sciences, Trg D. Obradovića 2,
 University of Novi Sad, 21000 Novi Sad, SERBIA; FAX: +381-21-450-620

2 Department of Chemistry, Biochemistry and Environmental Protection, Trg D. Obradovića 3,
 Faculty of Sciences, University of Novi Sad, 21000 Novi Sad, SERBIA FAX: +381-21-454-065

maja.karaman@dbe.uns.ac.rs, maja.karaman@gmail.com

Mushrooms have been the subject of interest and appreciation of human civilization since ancient times. It is estimated that only 50 % of the total known species of macrofungi are edible, while almost 2,000 species exhibit healing properties. In Europe around 650 macrofungal species possess medicinal properties (Gründemann et al. 2019). In the last few decades, mushrooms have become a significant help in solving the problems such as food shortages, pollution of the environment and impaired immune system incurred as a consequence of the accelerated growth of the human population and a stressful lifestyle. Some of them are edible species (*L. sulphureus*, *F. velutipes*, *G. frondosa*, *H. erinaceus*, *L. edodes*, *Pleurotus* spp. etc.) but majority belongs to the non-edible perennial species with a woody or leathery consistency (*Ganoderma* spp., *F. fomentarius*, *P. betulinus*, *Sch. commune*, *T. versicolor* etc).

Here we aimed to introduce the main problems in investigation of wild-growing medicinal mushroom species by presenting investigations from the period 2005 to 2020, with special emphasis on autochthonous species of Serbia and the Balcan region (Croatia, Bosnia and Herzegovina, North Macedonia, Slovenia and Montenegro). Four major problems can be stressed: 1) problematic identification of the species, 2) their biodiversity, 3) chemical characterization and 4) environmental contamination since they represent great sources of bioactive compounds with various activities: antioxidative, antimicrobial, antidiabetic and anti-AChE inhibition.

It has been recognized that use of mushrooms mostly varies between Eastern and Western Europe where Western Europeans are leaning towards mycophobia while Eastern Europeans (e.g., in Russia and Poland) tend to be more mycophilic and they use mushrooms not only as food but also for health purposes. Since we have studied the interest and knowledge of Balkan people through their scientific investigations and scarce ethno mycological data of medicinal mushrooms during the last 15 years we can underline that Balkans are mycophilic due to the fact that majority of species analyzed for food and medicinal properties represent more than 70 species.

DIVERSITY, CHEMISTRY AND ENVIRONMENTAL CONTAMINATION OF WILD GROWING MEDICINAL MUSHROOM SPECIES AS SOURCES OF BIOLOGICALLY ACTIVE SUBSTANCES (ANTIOXIDANTS, ANTIDIABETICS, ACHE INHIBITORS AND COSMECEUTICALS)

SESSION 01

LECTURE 02

One of the basic problems of the utmost importance is a proper taxonomic identification as a first step in a further investigation. The problem that comes to the fore is an exact definition of the species which is often problematic due to similarity of morphological characteristics, especially in species complexes like *Pleurotus* and *Ganoderma*. Molecular identification through multi-gene phylogenetic analysis helped to resolve some of these issues while full genome sequencing enabled annotation of genes as it was done with *Sch. commune* and *H. erinaceus*.

Chemical characterization of secondary bioactive compounds mostly confirmed the existence of terpenoids, phenols and sterols, while polysaccharides and immunomodulatory proteins including polysaccharide-peptide complexes have been mostly identified recently.

Although wild fungal strains represent powerful sources of medicinal substances they can also pose a potential risk to human health through (hyper)accumulation of toxic elements (e.g. Hg, Pb, Cd, Ni, ²³⁸U, ¹³⁷Cs) from different substrates not only in the urban polluted environments, but also in protected natural areas. Their use should be well reasoned and controlled next to their conservation and protection.

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**DIVERSITY, CHEMISTRY AND ENVIRONMENTAL
CONTAMINATION OF WILD GROWING MEDICINAL
MUSHROOM SPECIES AS SOURCES OF BIOLOGICALLY
ACTIVE SUBSTANCES (ANTIOXIDANTS, ANTIDIABETICS,
ACHE INHIBITORS AND COSMECEUTICALS)**

SESSION **01**

LECTURE **02**

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Acknowledgment

This investigation is the result of research within the project financially supported by the Ministry of Science Education, and Technological Development of Republic of Serbia (Grant No. 451-03-68/2022-14/200125).

THE COLLECTION AND CONSERVATION OF WILD HERICIIUM SPECIES IN TÜRKIYE

SESSION 01

LECTURE 03

Mustafa Kemal SOYLU^{1*}

¹ Atatürk Central Horticultural Research Institute, Yalova, Turkey

* Corresponding author: mksoylu@hotmail.com

Hericium species are called lion's mane, monkey head, bear's head, pom pom or bearded tooth, medicinal and edible mushrooms and include more than 10 species in taxonomic class in Europe, Asia and America. Hericium erinaceus (Bull.) Pers. is the most common cultivated and consumed fresh and used as a medicinal food supplement especially in Far East Countries.

Hericium erinaceus contain neuroactive compounds enhancing memory function and polysaccharides which have anti-tumor effects. However climate change, habitat destruction, forest fire, local population collection effect the declining population of Hericium species. Hericium erinaceus and H. rajchenbergii species are IUCN (International Union for Conservation of Nature) red list of threatened species thus it is important to collect and conserve them in situ and ex situ. Aslan yelesi, dede sakalı, suğora, tülübüzük are Turkish local name of Hericium species and collected and consumed fresh by local population and mushroom hunters.

Hericium erinaceus (Bull.) Pers. has been started to produced and evaluated medically recently in Turkey. H. erinaceus (Bull.) Pers., H. coralloides (Scop.) Pers., H. abietis (Weir ex Hubert) K.A. Harrison and H. cirrhatum (Pers.) Nikol were found by the previous study in Türkiye. Two species, Hericium erinaceus and H. coralloides were collected in various locations (Bursa, Yalova, Sakarya, Bolu, Artvin, Kastomunu, Osmaniye) of Türkiye. Total 21 wild Hericium strains were collected and isolated in PDA (Potato Dextrose Agar) medium. Wild genotypes were saprophytic or weak parasitic to hard wood trees especially oak (Quercus), beech (Fagus), fir (Abies) and spruce (Picea) and emerge fruit body especially at old stumps from September to December. Hericium species are rare in the wild habitat in Turkey too. Genetic resources of Hericium were conserved both in tubes in refrigerator and in cryopreservation to use further research (breeding program, molecular identification and extraction bioactive compounds etc.) in National Mushroom Gen bank of Mushroom Research Center of Atatürk Central Horticulture Research Institute in Türkiye.

Key Words: Hericium, wild, genetic resource, collection, conservation, Türkiye

SHORT ORAL PRESENTATION / session NO 1

LECTURE
01 TAXONOMIC RE-EXAMINATION OF MACROLEPIOTA OLIVASCENS,
A EUROPEAN TAXON SHOWING HIGH SIMILARITY TO PARASOL
MUSHROOM *M. PROCERA* (AGARICACEAE, BASIDIOMYCOTA)

Ágnes Radnóti¹, György Vrba², Bálint Dima³ & Viktor Papp^{1*}

1 Department of Botany, Hungarian University of Agriculture and Life Sciences, Hungary

2 Erdei Gombász Tanoda Bt., Moravcsik Street 4, H-2800 Tatabánya, Hungary

3 Department of Plant Anatomy, Institute of Biology, Eötvös Loránd University,
Pázmány Péter sétány 1/C, H-1117 Budapest, Hungary

The saprotrophic fungal genus *Macrolepiota* Singer contains ca. 40 species worldwide, characterized by large agaricoid basidiomata with a loose ring and squamules covering the pileus. The type species of the genus is *M. procera* (Scop.) Singer, a popular edible mushroom due to its delicious taste and faint nutty aroma of the cap. It also has a good medicinal value, as it contains proteins, minerals, vitamins, carbohydrates, and high amounts of dietary fibres.

However, *M. procera* taxonomically belongs to a difficult species complex, which includes taxa previously separated as species on morphological ground (e.g., *M. permixta* (Barla) Pacioni, *M. fuliginosa* (Barla) Bon). *Macrolepiota olivascens* Singer & M.M. Moser also shares similar micro- and macromorphological characteristics like *M. procera*, but its distinguishing feature that the pileus and stem shows a greenish discoloration. *Macrolepiota olivascens* was described from Austria and considered as a rare species throughout Europe.

An examination of recent *Macrolepiota* collections from Hungary has failed to show reliable micromorphological differences between specimens identified as both *M. olivascens* and *M. procera*. As far as we know, the separation of *M. olivascens* at the species level has not been studied and confirmed by molecular genetic studies. For this purpose, examination of the nrDNA ITS regions of Hungarian and Danish *M. olivascens* materials were carried out in this study. Based on our results the ITS sequences do not separate this taxon from *M. procera* on species level. Therefore, our preliminary phylogenetic study suggests that the two species are conspecific, with *M. olivascens* being only a synonym or a form of the earlier described *M. procera*. In order to support this preliminary result and clarify the taxonomic position, ecological and evolutionary background of the *M. procera* species complex, it is necessary to study additional samples (incl. type materials). In addition, we are planning further investigations to chemically explore and identify the compounds responsible for the greenish discoloration, which was previously considered a taxonomically relevant feature.

Keywords: edible mushroom, taxonomy, phylogeny

ONLINE ETHNOMYCOLOGICAL RESEARCH IN SERBIA

SHORT
 ORAL
 PRES

01

LECTURE 02

Ivan Dudaš^{1*}, Eleonora Čapelja¹

¹ University of Novi Sad, Faculty of Sciences, Department for Biology and Ecology,
 Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia

e-mail: ivan.dudas@dbe.uns.ac.rs

Keywords: ethnomycology, edible fungi, medicinal mushrooms, online research

Ethnomycology is a part of ethnobiology that study the use of fungi by people and the influence of fungi on people's day-to-day life. This paper presents ethnomycological research conducted through an anonymous online questionnaire, which consisted of 23 questions related to the use of fungi by respondents from the Republic of Serbia. The questionnaire was filled out by 87 respondents, of which 62% were female and 38% were male. The majority (64.4%) of respondents are not members of any mycological association, while of those who are, the majority are members of the Mycological Society of Novi Sad (27.6%). It was recorded that respondents use a total of 134 fungal species in their diet. *Agaricus bisporus* is the edible species used by most respondents (95.4%). This is followed by *Pleurotus ostreatus* (85.1%), *Cantharellus cibarius* (80.1%), *Boletus edulis* (66.7%), *Laetiporus sulphureus* (61%), and *Macrolepiota procera* (61%). On average, one respondent use 23 species of edible fungi, with the majority (more than 50%) of respondents using only up to 9 species of edible mushrooms. The 31 species of fungi is used for medicinal purposes with *Ganoderma lucidum* used by the greatest number of respondents (31%). This is followed by *Trametes versicolor* (21%), *Fomitopsis betulina* (17.2%),

Fomes fomentarius (10.3%), and *Auricularia auricula-judae* (9.2%). On average, one respondent use 1.5 species of medicinal fungi. Of the recorded use of 31 medicinal fungal species, 24 species (77.4%) are used by less than 5 respondents. At the same time, the majority (95%) of the respondents would choose the use of medicinal mushrooms and their preparations for the purpose of treating the disease. Respondents use medicinal fungi mostly for boosting immunity (69.4%), and to a much lesser extent for: lowering cholesterol levels (8.3%), treating diabetes and cancer (5.6%), suppressing pain, treating arthritis, hypertension and improving cognitive abilities (2.8%). The results of this research indicate that the inhabitants of Serbia use fungi more for food than for medicinal purposes (4.3 times) and that, on average, they use a relatively small number of edible and, especially, medicinal species.

Also, respondents showed a desire to increase their knowledge about medicinal species and their use. The conducted research contributes to the development of ethnomycology in Serbia and the region and indicates the necessity of promoting mushrooms and their use among the population.

IDENTIFICATION AND COLLECTION OF MEDICINAL MUSHROOMS FROM DR CONGO THROUGH AN ETHNOMYCOLOGICAL INVESTIGATION

SHORT
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LECTURE 03

Henry Many Mboni^{1,2}, Hérítier Milenge Kamalebo³, Bill Kasongo⁴, Simon Dibaluka⁵,
Jean-Claude Rizinde^{6,7}, André De Kesel⁸, Jérôme Degreef^{8,9}, Caroline Stévigny¹

1 Unité de Pharmacognosie, Bioanalyse et Médicaments, Faculté de Pharmacie,
Université Libre de Bruxelles, Belgium

2 Unité de Chimie Thérapeutique, Faculté des Sciences Pharmaceutiques,
Université de Lubumbashi, Lubumbashi, D. R. Congo

3 Département de Biologie, Institut Supérieur Pédagogique (ISP) de Bukavu, D.R. Congo

4 Faculty of Agronomy, University of Lubumbashi, Lubumbashi, D.R. Congo

5 Faculty of Sciences, University of Kinshasa, Kinshasa, D.R. Congo

6 University of Goma, D.R. Congo

7 Catholic University of Louvain-la-Neuve, Belgium

8 Meise Botanic Garden, Meise, Belgium

9 Fédération Wallonie-Bruxelles, Service Général de l'Enseignement supérieur
et de la Recherche scientifique, Brussels, Belgium

Corresponding author at : Unité de Chimie Thérapeutique, Faculté des Sciences Pharmaceutiques, Université de Lubumbashi, Lubumbashi, D. R. Congo.

Tel.: +243851695009.

E-mail addresses: henry.manya.mboni@ulb.be; mbonim@unilu.ac.cd

Ethnopharmacological relevance

This study aimed to collect and gather information on medicinal mushrooms used to treat various pathologies in DRC.

Material and methods

Structured and semi-structured surveys allowed collecting ethnomycological data among Congolese most popular traditional healers of five provinces throughout D. R: Congo (Haut-Katanga, Kinshasa, Kongo-Central, Nord-Kivu and Tshopo). Vernacular names, main illnesses or symptoms treated, methods of preparation and administration of remedies, and treatment methods were recorded. Fungal specimens were harvested in the presence of the informant and were identified with the help of mycologists present in the five provinces of DRC and at Meise Botanic Garden (Belgium).

IDENTIFICATION AND COLLECTION OF MEDICINAL MUSHROOMS FROM DR CONGO THROUGH AN ETHNOMYCOLOGICAL INVESTIGATION

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Results

A total of 20 medicinal mushroom species belonging to 15 genera and 13 families were identified in the different studied areas, all of these mushrooms were also reported to be wild, some of them edible. The study has highlighted that the most represented families are Auriculariaceae and Polyporaceae both with 4 species (20 %), followed by Hypoxylaceae with 2 species (10 %). The species *Daldinia eschscholtzii* (Ehrenb.) Rehm (Hypoxylaceae) and *Pycnoporus sanguineus* (L.) Murrill (Polyporaceae) were the most frequently cited. *Schizophyllum commune* Fr. (Schizophyllaceae) is known as a medicinal mushroom in Haut-Katanga, Nord-Kivu and Tshopo. All these fungi species are collected in the wild and some of them are also used as food. In addition, each mushroom has minimum one of the local names of 5 provinces studied. Our investigation revealed that the 20 recorded fungal species are used to treat 27 symptoms or pathologies; sexual impotence being the pathology treated by at least 3 fungal species. In most cases, medicinal mushroom remedies are prepared using the entire mushroom (90 %), by cooking (26.9 %) and grinding the raw mushroom (23.1 %) and administering it orally (46.2 %).

Conclusion

In all of the 5 surveyed provinces of the DRC, the populations use mushrooms to treat various pathologies and symptoms. The most cited mushrooms should be investigated in more detail, i.e. for their nutrient composition and active pharmacological components.

Keywords: Survey, Medicinal mushrooms, Ethnomycology, RD Congo.

ETHNOMYCOLOGY, SENSORY ANALYSIS AND NUTRITIONAL COMPOSITION OF EIGHT EDIBLE MUSHROOMS CONSUMED IN THE AFRICAN GREAT LAKES REGION

SHORT
ORAL
PRES

01

LECTURE 04

Assumpta Mukandera^{1,2}, Eric Dulière³, Jérôme Degreef⁴, Claire Beaufay³, Cony Decock⁵, Stéphanie Fraselle¹, Jean Claude Rizinde^{5,6}, Charles Karangwa^{7,8}, Caroline Stévigny¹

- 1 RD3-Pharmacognosy, Bioanalysis and Drug Discovery Unit, Faculty of Pharmacy, Université libre de Bruxelles, Brussels, Belgium
- 2 University of Rwanda College of Education (URCE), Kayonza, P.O Box: 55 Rwamagana, Rwanda.
- 3 Bromatologie/Laboratoire d'analyses des denrées alimentaires, Haute Ecole Léonard de Vinci, Campus Woluwe-Paul Lambin 41-43, Clos Chapelle-aux-Champs, 1200 Bruxelles
- 4 Botanic Garden Meise, Nieuwelaan, 38, BE-1860 Meise (Belgium)
- 5 Mycothèque de l'Université Catholique de Louvain (BCCM/MUCL), Croix du Sud 2 bte L7.05.06 1348, Louvain-la-Neuve, Belgium
- 6 Université de Goma, Faculté des Sciences Agronomiques, BP 204 Goma, République Démocratique du Congo
- 7 Rwanda Forensic Laboratory, KN 8 Ave, Gasabo, Kacyiru, P.O Box 979 Kigali, Rwanda
- 8 University of Rwanda, College of Medicine and Health Sciences (UR-CMHS), Avenue de l'Université, 37, PO Box 117 Butare

The Great Lakes Region is well known for its astonishing biodiversity, but also for its economic and food shortage. The protein sources are extremely low for local populations. The mushroom resources found in the biodiversity of the mountain forests are recognized for their interesting nutritional qualities, in particular their presumed high protein content. The studies on mushroom nutritional values have been carried out in various African countries, including the Miombo woodlands of the R.D.

Congo but, until now, no complete nutritional analysis of edible mushrooms of the mountain forests has been achieved. The overall objective of this study is to assess the nutritional quality of edible mushrooms consumed in the Great Lakes Region. Their quality as food is also linked to food surveys carried out locally to assess the interest in mushroom consumption in the local diet.

The carpophores of eight edible mushrooms *Termitomyces microcarpus* (Berk. & Broome) R. Heim, *Termitomyces schimperi* (Pat.) R. Heim, *Termitomyces robustus* (Beeli) R. Heim, *Auricularia delicata* (Mont. ex Fr.) Henn., *Schizophyllum commune* Fr., *Hypholoma subviride* (Berk. & M.A. Curtis) Dennis, *Pleurotus ostreatus* (Jacq.) P. Kumm. and *Agaricus bisporus* (J.E. Lange) Imbach were collected, subjected to ethnomycological surveys, sensory analysis, and further dried, powdered and analysed for macronutrients and mineral elements compositions.

**ETHNOMYCOLOGY, SENSORY ANALYSIS AND NUTRITIONAL
COMPOSITION OF EIGHT EDIBLE MUSHROOMS CONSUMED
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For sensory analysis, the fresh carpophores were cooked, and a nine-point hedonic scale method was used to establish food preferences. For proximate and mineral elements composition, data were calculated as % on a dry matter basis.

The results showed that all mushroom samples contained significant amounts of essential nutrients. The ash, crude protein, total lipids, and crude fiber were in the ranges of 3.40-10.85%, 9.38-28.77%, 1.13-2.62%, and 10.29-44.99%, respectively. Among the eight studied mushrooms, *Termitomyces* genus was the one containing the highest protein amounts. This specific property coincides with their use to treat children suffering from kwashiorkor as reported by the interviewed local populations.

Other mushroom species reported for traditional medicine are *Schizophyllum commune* Fr used for relieving joint pain, and *Auricularia delicata* (Mont. ex Fr.) Henn. used for enhancing sexual potency in men.

Further analyses on the responsible active compounds are needed for scientific confirmation. By considering the reference mineral intake values established by the European Food Safety Authority, the analysed samples contained appreciable amounts of K, Mg, Fe, and Cu. The mushroom species subjected to sensory analysis were highly appreciated as they scored 8 on the nine-point hedonic scale.

**BIODIVERSITY OF THE TROPICAL SPECIES OF THE GENUS
GANODERMA IN VERACRUZ, MEXICO**SHORT
ORAL
PRES

01

LECTURE 05

Alla V. Shnyreva¹, Victoria Espinosa-García², Ángel Trigos²**1** Department Mycology and Algology, Faculty of Biology, Moscow Lomonosov State University, Moscow, Russia;**2** Center of Applied Mycology, University Veracruzana, Xalapa-Veracruz, Mexico

The genus *Ganoderma* has more than 200 species, which are mainly found in regions with warm temperate, subtropical and tropical climates. Phylogenetic analysis was performed with fourteen *Ganoderma* specimens collected from stumps, live trees, and dead trees in the central-south area of Veracruz Province, Mexico. Morphological identification of the *Ganoderma* specimens was performed by using taxonomic keys of G. Guzmán and L. Ryvarden. Molecular identification of *Ganoderma* specimens was confirmed by sequencing an internal transcribed spacer (ITS) region of a ribosomal RNA gene cluster (ITS1–5.8S rRNA–ITS2). Seven *Ganoderma* species were identified in this study: six 'laccate' species (with a shiny, varnished-looking surface) - *G. tuberculosis*, *G. lobatum*, *G. multiplicatum*, *G. weberianum*, *G. curtisii*, *G. martinicense*; and one non-laccate species with a "matte crust" - *G. tornatum* which is known to be quite common in tropical and subtropical zones.

One of the strains collected from a live tree (*Acacia pennatula*) was identified as *G. curtisii* that is considered to be conspecific to *G. meredithae* with the affinity of the latter to colonize and decay pines. The 'multipileum' clade combined two closely related species, *G. multiplicatum* and *G. martinicense*. Interestingly, two strains of *G. multiplicatum* were found on live *Pinus* tree, whereas *G. martinicense* was collected from dead stump. *G. martinicense* was first found in Martinique and was related to Asian species *G. multipileum*. *G. multiplicatum* was originally collected in French Guyana and was considered as a neotropical species that is widely distributed in South America. Although originally described from South America, *G. multiplicatum* was subsequently found in Africa and Asia, including India and Taiwan.

Our phylogenetic analysis demonstrated that the *G. multiplicatum* – *G. martinicense* clade is a sister one to the *G. curtisii* clade. Three strains of *G. weberianum* collected on hardwood host substrates (*Quercus* sp., and *A. pennatula*) were placed in the same group that was clustered with the *G. tuberculosis* strains. *G. tuberculosis* is known to be restricted solely to tropical locations. Interestingly, all four *G. tuberculosis* strains were collected on different substrates - on a live *Ficus* tree, on the tree trunk of *A. pennatula*, and on a dead tree. Thus, Mexican strains of *G. tuberculosis* appear to be both saprotrophic and tree pathogeni (or primary decay) fungi.

Thus, the ITS region of the rDNA is proved to be a quite reliable diagnostic tool for most *Ganoderma* species and can be used as molecular barcode for identifying and taxonomically verifying natural specimens. However, in some cases, further analysis is needed to discriminate between *Ganoderma* specimens of different origin. This study was undertaken with the aim to further analyze medicinal potential - anti-proliferative, antioxidant, and antibacterial activity of Mexican *Ganoderma* strains.

Key words: *Ganoderma*, complex species, ITS region, phylogeny

SESSION 02

LECTURE 01 METABOLOMICS STUDY OF CULTIVATED FRUITING BODIES OF MEDICINAL MUSHROOM FOMITOPSIS BETULINA

Ivana Sofrenić¹, Aleksandar Knežević², Boban Anđelković¹, Dejan Gođevac³, Ljubodrag Vujisić¹, Slobodan Milosavljević^{1,4} and Vele Tešević¹

1 University of Belgrade, Faculty of Chemistry, Studentski trg 12-16, 11000, Belgrade, Serbia

2 University of Belgrade, Faculty of Biology, Takovska 43, 11000, Belgrade, Serbia

3 University of Belgrade, Institute of Chemistry, Technology and Metallurgy, National Institute of the Republic of Serbia, Studentski trg 12-16, 11000, Belgrade, Serbia

4 Serbian Academy of Sciences and Arts, Knez Mihailova 35, 11000, Belgrade, Serbia

Fomitopsis betulina (Bull.) commonly known as the birch polypore, birch bracket, or razor strop is a necrotrophic parasite on weakened birches. The oldest evidence from paleobotanical studies shows that Ötzi the Iceman 5300 years ago carried a piece of *F. betulina* with him probably due to its curative properties. Error! Reference source not found. Recent publications have shown that this fungus contains quite a few biologically active compounds, primarily polysaccharides and lanostane type triterpenes. Error! Reference source not found., Error! Reference source not found.

In order to preserve natural habitats, biodiversity and to obtain larger quantities of fruiting bodies of standardized quality, the cultivation of this fungus was performed in experimental conditions. Error! Reference source not found. Cultivation experiments were parallelly performed on alternative substrates that were recognized as industrial waste with high antioxidant potential. The changes in chemical composition of fruiting bodies during development as well as cultivation on different substrates, monitored using infrared (IR) and nuclear magnetic resonance (NMR) spectroscopic techniques combined with a multivariate analysis revealed metabolomics models. IR spectroscopy was chosen to monitor differences in the structure of cell wall macromolecules, while high resolution magic angle spinning (HR MAS) ¹H NMR experiments (cpmg and 1d-noesy) were performed on small molecules.

For the first time *F. betulina* fruiting bodies have been cultivated in laboratory conditions on substrates made of inner shell of hazelnuts, *Cotinus coggygria* and *Alnus glutinosa* sawdust. Substrat from *Betula alba* sawdust have been used as a control.

Ground dried fruiting bodies were analyzed with IR and HR MAS ¹H NMR techniques to obtain statistical PCA (Principal Component Analysis) and OPLS-DA (Orthogonal Projections to Latent Structures Discriminant Analysis) models. The established models enabled monitoring the stage of maturation, determination of potential biomarkers of aging as well as the influence of the type of medium on cultivation.

SESSION 02

LECTURE **01** **METABOLOMICS STUDY OF CULTIVATED FRUITING BODIES OF MEDICINAL MUSHROOM FOMITOPSIS BETULINA**

It is well known that the chemical composition, and thus, the medicinal potential of the fungus is directly related to the maturation phase. Error! Reference source not found.

Metabolomics opens the possibility of detecting changes in chemical composition due to cultivation on different substrates or analysis of fruiting bodies in different phase of maturation. This can lead to changes in medicinal potential of the fungi and open a possibility for discovering potentially new therapeutics.

DEVELOPMENT OF NEW HYBRID STRAINS FOR THE CULTIVATION OF THE ENDANGERED MUSHROOM SPECIES *PLEUROTUS NEBRODENSIS* ON NOVEL SUBSTRATES

SESSION 02

LECTURE 02

Georgios Koutrotsios¹, Dimitra Tagkouli², Georgios Bekiaris¹,
Konstantinos C. Mountzouris³, Nick Kalogeropoulos² and Georgios I. Zervakis¹

- 1 Agricultural University of Athens, Laboratory of General and Agricultural Microbiology, Iera Odos 75, 1855 Athens, Greece
- 2 Harokopio University, Department of Nutrition and Dietetics, Laboratory of Chemistry, Biochemistry and Physical Chemistry of Foods, El. Venizelou 70, 17671 Kallithea, Greece
- 3 Agricultural University of Athens, Department of Nutritional Physiology and Feeding, Iera Odos 75, 11855 Athens, Greece

Pleurotus nebrodensis is an endemic species of the alpine zone of Sicily and southern Greece which grow on root and stem residues of *Prangos ferulacea* (Apiaceae). It was the first mushroom placed in the Red List of Threatened Species by the International Union for Conservation of Nature (IUCN). Few individuals reach maturity each year, and although its successful cultivation is considered of major importance for reducing the human pressure on wild populations due to harvesting, large-scale mushroom production is practically non-existent. In the frame of this work, strains of diverse geographic origin were initially evaluated in terms of their crop performance, and then matings among selected homokaryons were performed to generate several new hybrid dikaryons. Among them, the hybrid strain 'PnbH17' demonstrated significantly better performance in respect to all cultivation parameters, and was hence qualified for further research. Thus, the effect of various substrates based on locally available lignocellulosic residues and the use of a casing layer were studied as regards both the production and quality parameters of mushrooms.

Results revealed that the total yield was high in substrates consisting of olive leaves or of wheat straw and grape marc (299 and 303 g/kg of substrate, respectively) but relatively low in wheat straw or beech sawdust which are commonly used in *Pleurotus* commercial-scale cultivation. Furthermore, the addition of a casing layer delayed the appearance of mushrooms by up to 6 days, but it increased biological efficiency in all substrates from 2 to 23%. As a result of using various substrates, significant differences were recorded both in the crude composition and in the content of selected bioactive compounds including β -glucans, amino acids, phenolic compounds and ergosterol. In conclusion, the new hybrid 'PnbH17' exhibits satisfactory mushroom crop performance and organoleptic properties, as well as high content in proteins and bioactive compounds, and could be further exploited for the large-scale cultivation of *P. nebrodensis* contributing to the conservation of this endangered species.

Acknowledgments: This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project Reinforcement of Postdoctoral Researchers – 2nd Cycle” (MIS-5033021), implemented by the State Scholarships Foundation (IKY)

STRAIN DEGENERATION IN PLEUROTUS OSTREATUS: A GENOTYPE DEPENDENT OXIDATIVE STRESS PROCESS WHICH TRIGGERS OXIDATIVE STRESS, CELLULAR DETOXIFYING, AND CELL WALL RESHAPING GENES

SESSION 02

LECTURE 03

Lucía Ramírez¹, Gumer Pérez¹, Federico Lopez-Moya², Emilia Chuina¹, María Ibañez-Vea¹,
Edurne Garde¹, Luis V. López-Llorca², and Antonio G. Pisabarro¹

1 Genetics, Genomics and Microbiology Research Group, Institute for Multidisciplinary Research
in Applied Biology (IMAB), Public University of Navarre (UPNA), 31006 Pamplona, Spain

2 Laboratory of Plant Pathology, Department of Marine Sciences and Applied Biology,
University of Alicante, 03690 Alicante, Spain

Strain degeneration of cultivated mushrooms can be defined as a decrease or loss in the yield of relevant commercial traits resulting from subsequent culture, which ultimately leads to Reactive Oxygen Species (ROS) production. *Pleurotus ostreatus* is a white rot (lignin-degrader) fungus with different applications, including its use as food and medicinal mushroom, the production of enzymes and secondary metabolites of interest, and as pest controller due to its nematophagous activity. Mycelia for mushroom production are usually maintained in subsequent culture in solid media and frequently show symptoms of strain degeneration.

We are interested in studying the molecular bases of strain degeneration. For this purpose, the dikaryotic strain *P. ostreatus* (DkN001) has been used in our lab as a model organism. This dikaryotic strain contains two nuclei that were separated and led to the obtention of two monokaryotic strains (mkPC9 and mkPC159) whose genomes were sequenced. In this framework, we developed different tools to uncover this fungus's genetic and molecular processes associated with strain degeneration. In the present work, we study strain degeneration in a full-sib monokaryotic progeny of the dikaryotic strain DkN001. This progeny contained fast (F) and slow (S) growing individuals. The monokaryons were analyzed using different experimental approaches, including light microscopy, determination of the malondialdehyde levels, whole-genome transcriptome analysis, and chitosan's effect on monokaryotic mycelia.

The results obtained showed that: (i) strain degeneration in *P. ostreatus* is linked to oxidative stress, (ii) the oxidative stress response in monokaryons is genotype dependent, (iii) stress and detoxifying genes are highly expressed in S monokaryons with symptoms of strain degeneration, (iv) chitosan addition to F and S monokaryons uncovered the constitutive expression of both oxidative stress and cellular detoxifying genes in S monokaryon strains which suggest their adaptation to oxidative stress, and (v) the overexpression of the cell wall genes, *Uap1* and *Cda1*, in S monokaryons with strain degeneration phenotype indicates cell wall reshaping and the activation of High Osmolarity Glycerol (HOG) and Cell Wall Integrity (CWI) pathways. These results could constitute a hallmark for mushroom producers to distinguish strain degeneration in commercial mushrooms.

PROCESS OF DETECTION AND ANALYSIS OF SECONDARY METABOLITES AND EXPRESSION OF GENES INVOLVED IN THEIR PRODUCTION IN THE EDIBLE FUNGUS *PLEUROTUS OSTREATUS* (OYSTER MUSHROOM) AS A MODEL FOR THE STUDY IN OTHER BASIDIOMYCETES

SESSION 02

LECTURE 04

Antonio G Pisabarro¹, Karen Portocarrero¹, Manuel Alfaro¹, Edurne Garde¹,
 María Isabel Calvo² and Lucía Ramírez¹

1 Genetics, Genomics and Microbiology Research Group, Institute for Multidisciplinary Research in Applied Biology (IMAB), Public University of Navarre (UPNA), 31006 Pamplona, Spain

2 Department of Technology and Pharmaceutical Chemistry, University of Navarra, 31009 Pamplona, Spain

This work aims to explore the possibilities of producing secondary metabolites of pharmacological interest by basidiomycete fungi. *Pleurotus ostreatus* has been used as a model system for the search for secondary metabolites, analyzing mycelia of the same grown in different liquid culture media. In our laboratory, we work on the development of genetic, genomic and molecular tools for studying this fungus, its agricultural and industrial production, and the production of some of its enzymes of biotechnological interest. Our model system is the dikaryon dkN001, whose two constituting nuclei (established as separate monokaryotic lines called protoclones) have been individually sequenced (mkPC9 and mkPC15). We have studied the exudates produced by the mycelia under different cultural conditions and analyzed their preliminary characteristics.

We have applied an approach that combines thin layer chromatography with mass spectrometry for the analyses. This technique permits the individual analysis of chromatographic bands that can be visualized using different reagents, simultaneously revealing the chemical and functional properties of the compound. Using this approach, we have identified many compounds chemically related to terpenes, five being studied in more detail in the exudates produced under different culture conditions.

The genome analysis of *P. ostreatus* reveals the presence of 10 clusters of terpene cyclase genes. To study the expression of these genes under different conditions, we analyzed the transcriptomes of the *P. ostreatus* dikaryotic strain dkN001, the protoclones mkPC9 and mkPC15, 18 descendants of dkN001, and different dikaryons obtained through crosses. The results showed that the 10 terpene cyclase genes in the genome of *P. ostreatus* show variable expression in the different media and culture conditions.

Finally, the presence of genes related to secondary metabolites was studied in the genomes of the Basidiomycetes available in the MycoCosm database of the Joint Genome Institute were analyzed. The bioinformatic analysis revealed more than 12,000 clusters of genes involved in synthesizing secondary metabolites. More than 4,500 genes coded for terpene cyclases, and more than 6,000 coded for polyketide synthases and similar proteins. This genetic wealth opens a large field of research focused on identifying and expressing proteins involved in specific synthetic steps that can enlarge the production of molecules with pharmaceutical or biotechnological interest.

**RANDOM MUTAGENESIS OF PHELLINUS
IGNIARIUS RANDOM MUTAGENESIS AND GENETIC
DIVERSITY OF WHITE ROT FUNGUS
(PHELLINUS IGNIARIUS) BY PHYSICAL MUTAGENS**

SESSION 02

LECTURE 05

Yating Dong^{1,2}, Haile Ma², Xiaofei Ye³, Bingcheng Gan¹

- 1 Institute of Urban Agriculture, Chinese Academy of Agricultural Sciences, National Agricultural Science & Technology Center (NASC), 9 Hupan West Road, Tianfu New Area, Chengdu, 610000 China;
- 2 School of Food and Biological Engineering, Institute of Food Physical Processing, International Joint Research Center for Food Physical Processing, Jiangsu University, No.301 Xuefu Road, Zhenjiang 212013, China;
- 3 Department of Biosystems Engineering and Soil Science, University of Tennessee, Knoxville 37996, USA

Phellinus igniarius is species of the genus *Phellinus* that induces white-rot owing to the unique ability to break down lignin, which has been widely exploited for laccase synthesis and offer a great potential to accomplish several bioprocesses. Pulsed light and pulsed magnetic field techniques were applied to develop *Ph. igniarius* mutants with high biomass, flavonoid and laccase activity. The pulsed light treatment yielded QB72, while the pulsed magnetic field treatment yielded 13c-j and 7c-a.

All the three mutants showed a higher growth rate on the plate. Higher fermentation was occurred in the mutant QB72 (23.997 ± 1.916)g/L. In the mutant 3T-7c-a, on day 6, laccase activity increased by 48.170% compared to that of the wild strain. Analysis of esterase isoenzyme and laccase genes showed occurrence of polymorphisms in strains obtained by physical treatment. The pulsed light and pulsed magnetic field could be a mutagenesis method applied to treat the *Ph. igniarius* protoplast with the goal of obtaining strains with higher growth rate and highly active laccase.

KEY WORDS: *Phellinus igniarius*; pulsed light; pulsed magnet field; mutagenesis; TRAP-PCR; isoenzyme electrophoresis.

**MOLECULAR IDENTIFICATION, CYTOTOXICITY AND INVITRO
ENZYMATIC ACTIVITIES OF BIOACTIVE PEPTIDE
RIGIDOSPOROUS MICROPORUS (MACRO-FUNGUS)
AS LEAD FOR ANTIDIABETIC AGENT**

SESSION 02

LECTURE 06

Abraham O. Nkumah, Joy O. Imosemi, Omonike O. Ogbole

Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria.

Corresponding Author: Email: nikeoa@yahoo.com, Tel: 08056434577

From time immemorial, mushrooms served as sources of food, nutrient and medicine. Lack of proper identity has resulted in misidentification often leading to poisoning from toxic species. The scarce data on the identity and medicinal properties of many wild Nigeria mushrooms have restricted their utilization. Bioactive peptides are known for health-promoting properties due to several pharmacological effects they modulate in physiological systems. This study was carried out to identify and authenticate this mushroom using molecular tools and investigate the bioactivities of the crude peptide extract.

Taxonomic guides were employed in morphological identifying the mushroom, while molecular tools were employed to authenticate the identity, using internal transcribed spacers (ITS) sequence analysis, the sequence generated were compared using the basic local alignment search tool (BLAST) at NCBI Gen-Bank for the authentication of the mushroom. The partially purified peptide-rich fraction (P-PPf) was obtained by reverse-phase solid-phase extraction and the presence of cyclic peptides was detected by a modified thin layer chromatography TLC chemical detection method. The peptide-rich fraction (P-PPf) and the aqueous-rich (crude peptide) were screened for their bioactivities, using brine shrimp lethality assay for cytotoxicity assay, in vitro α -amylase and α -glucosidase enzyme inhibitory assay.

Data were analyzed using GraphPad prism 6.0.

The mushroom was morphologically identified as *Ganoderma applanatum*. Blast analysis revealed the mushroom (accession number, KJ559465.1) to show 99.82% identity to be *Rigidosporus microporus*. The crude peptide (aqueous-rich fraction) was more toxic to brine shrimp with (LC50 = 131.1 $\mu\text{g}/\text{mL}$) than peptide-rich fraction (P-PPf) with (LC50 = 165.3 $\mu\text{g}/\text{mL}$) as compare to cyclophosphamide standard drug with (LC50 = 63.82 $\mu\text{g}/\text{mL}$). The in vitro α -amylase and α -glucosidase enzyme inhibitory assay established that the crude peptide and peptide-enrich fraction displayed a good enzyme inhibitory activity, with the peptide-rich fraction (P-PPf) showing more inhibitory activities with α -glucosidase (IC50 = 45.6 $\mu\text{g}/\text{mL}$) than α -amylase (IC50 = 75.6 $\mu\text{g}/\text{mL}$). The crude peptide fraction displayed a better activity with the α -amylase (IC50 = 56.9 $\mu\text{g}/\text{mL}$) than in α -glucosidase (IC50 = 66.5 $\mu\text{g}/\text{mL}$) as compared to the standard drug acarbose with IC50 = 68.9 $\mu\text{g}/\text{mL}$ and 43.52 $\mu\text{g}/\text{mL}$, α -amylase and α -glucosidase respectively.

**MOLECULAR IDENTIFICATION, CYTOTOXICITY AND INVITRO
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The result from this study shows that the crude peptide and a peptide-enrich fraction (P-PPf) are moderately cytotoxic and they also possess potent inhibitory activities against α -amylase and α -glucosidase, therefore this bioactive peptide from this mushroom could serve as lead for an antidiabetic agent.

Keywords: Mushroom, Molecular identification, Cytotoxicity, α -amylase, α -glucosidase
Word Count: 386

SHORT ORAL PRESENTATION / session N02

LECTURE 01 DIFFERENCES IN METABOLOME OF SELECTED STRAINS OF HERICIUM ERINACEUS

Matejka Jaroslav, Marsik Petr, Stastny Jan, Jablonsky Ivan, Kloucek Pavel

Czech University of Life Sciences Prague

From time immemorial, *Hericium erinaceus* has been known and used in traditional Chinese medicine for its positive effects on human health. It has been used to treat a wide range of health problems, such as the prevention of various diseases, especially diseases of the digestive tract. Today, biologically active substances that are responsible for these positive effects are being thoroughly researched.

The metabolites contained in *H. erinaceus* have been found to be potential medicine for the treatment of serious human diseases due to their anticancer effects, neuroprotective properties, antidepressant, antihyperglycemic, antimicrobial and antioxidant effects. It is used to treat Alzheimer's and Parkinson's disease, ischemic stroke or stomach problems and Crohn's disease.

Its specific metabolome is responsible for these effects. *H. erinaceus* mushrooms contain a wide range of substances with positive bioactive effects. These substances include, for example: erinacins, erinacerins, hericenones, herierins, coralloxins, erinaceolactones, erinaroles, glucans or ergothionein.

The aim of our work was to determine the differences in the metabolome of selected strains of *H. erinaceus* mushrooms. Six different *H. erinaceus* strains from Asia, USA and Europe (including the Czech Republic) were used for the analysis. The hypothesis of the work was that different strains will show different profiles of bioactive compounds. After extraction of metabolites with 80% methanol, the organic compounds in crude extract were determined by UHPLC-tandem high resolution mass spectrometry type Q-TOF. Subsequently, a multidimensional statistical analysis was performed, which provided information of the differences of various bioactive compounds in the metabolic profile of these mushrooms.

The differences in metabolites content among selected mushrooms of the species *Hericium erinaceus* were tentatively evaluated. The data were filtered using the XCMS software and based on evaluation using the multidimensional statistical method PCA. The number of potential candidates (features) was reduced to 206. From the reduced set of features, the most important ones were compared with the data from literature and there were selected 13 of them, which were tentatively identified based on MS/MS spectra and extracted ion chromatograms. A statistical comparison of individual mushroom samples performed using multivariate PCA statistical method and cluster analysis confirmed, that there are evident differences among the strains. The hypothesis of differences among *H. erinaceus* strains was therefore confirmed. Substances with significant biological activity hericerin A (anticancer effects) and hericenone J (neurotrophic activity) were significantly more represented in all strains of Czech origin compared to other strains. In one of them (KZ92), hericerin A was even dominant among the selected 13 observed compounds.

**VARIATIONS IN ITS REGION OF MEDICINAL FUNGAL SPECIES
CERIOPORUS VARIUS AND ITS PHYLOGEOGRAPHIC ANALYSIS**SHORT
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LECTURE 02

Milana Rakić¹, Vladislava Galović², Miroslav Marković², Eleonora Čapelja¹, Maja Karaman¹**1 University of Novi Sad, Faculty of Sciences, Serbia****2 University of Novi Sad, Institute of Lowland Forestry and Environment, Serbia**

Genus *Polyporus* and allied genera within family Polyporaceae contain many medicinal mushroom species. Novel studies reveal that *Cerioporus varius* (syn. *Polyporus varius*) is among them, demonstrating antioxidant and antimicrobial activity. Contrary to the well investigated morphological diversity of this species, studies of its genetic diversity are still scarce.

The objective of this study was to investigate variability within the ITS rDNA region of *C. varius* originating from several mountain forest areas in Serbia, as well as to determine their phylogeographic relationships with samples of the same species from other parts of the world. Fruiting bodies sampled from Mt. Tara, Kopaonik and Vidlič were used for analysis. As a result of DNA isolation, ITS amplification and sequencing, a total of 7 sequences were obtained and their length was in the following range: 560–638 bp. BLAST examination yielded results that were consistent with the morphological identification of the fungal samples. After their alignment with other *C. varius* sequences available in GenBank and Unite databases, the "Neighbor - joining" method was used to construct a phylogenetic tree in order to determine specific evolutionary relationships between different geographical groups. The resulting grouping was supported by appropriate average genetic distances (GD) and in accordance with observed polymorphic nucleotide positions.

In this research, *C. varius* exhibited an ITS region variability of 7%. Sequences obtained from four Vidlič samples (KX533917, KX524509, KX533919, 529RH05), two samples from Tara (KX533920, KX524508) and one from Kopaonik (529RH05) were used for construction of phylogenetic tree, as well as 17 available sequences taken from GenBank and Unite databases. As a result, 4 main clades were formed: clade A (includes most of the Serbian sequences generated in this study, together with one German sequence), clade B (Eurasian sequences and one Serbian sequence KX533920 from Tara), clade C (North American and Chinese sequences) and clade D (two Chinese sequences, evolutionarily separated from the previous three clades).

These results indicate the existence of clearly defined geographical groups within the *C. varius* complex. In forest habitats on all three studied mountains in Serbia, a unique genotype is represented that differs from other available sequences of *C. varius* in the world (GD: 0.007 - 0.013).

VARIATIONS IN ITS REGION OF MEDICINAL FUNGAL SPECIES CERIOPORUS VARIUS AND ITS PHYLOGEOGRAPHIC ANALYSIS

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The sequence KX533920 from Tara, which is phylogenetically distant from all the other Serbian sequences (GD = 0.007), is almost twice as close to Eurasian sequences from clade B (GD = 0.004). Analysis of *C. varius* sequence alignment revealed 38 polymorphic positions.

Clade A has unique set of 5 specific polymorphic nucleotides which are not present in sequences from any other geographic region, while Serbian sequence KX533920 and other European sequences in clade B share unique set of 6 polymorphic sites and 5 nucleotides in common with the North American and Asian sequences.

This study indicates that Serbian *C. varius* strains have specifically diverged in relation to other world populations of this species and thus may represent an important genetic resource that should be examined on the basis of other DNA regions with potential biomedical and pharmacological importance.

Key words: *Cerionorus varius*, diversity, ITS, phylogeography

A POLYSACCHARIDE-PROTEIN COMPLEX ISOLATED FROM CULTIVATED CHINESE CORDYCEPS DRIVES M1 PHENOTYPE POLARIZATION IN MACROPHAGES

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LECTURE 03

Xiaotong Yang,^{1*} Linyu Ai,¹ Huiqin Feng,¹ Jun Wu,¹ Wenjia Li,² Zhengming Qian²

1 Institute of Microbiology and Immunology, College of Life Sciences, Shanghai Normal University, China. 2: Dongguan HEC Cordyceps R&D Co., Ltd. Dongguan, Guangdong Province, China

* Address all correspondence to Xiaotong Yang, Institute of Microbiology and Immunology, College of Life Sciences, Shanghai Normal University, 100 Guilin Road, Shanghai 200234, China. Tel: +86-21-6432-2895; xtyang@shnu.edu.cn

Macrophages are highly plastic immune cells. In different physiological and pathological environments, macrophages can be polarized into two subsets with distinct phenotypes and functions: classically activated (M1) and alternatively activated macrophages (M2). M1 macrophages are pro-inflammatory and regarded as anti-tumor, while M2 macrophages are associated with tumorigenic responses. Driving macrophages to undergo M1 polarization or to transform M2 to M1 is believed as one of the potentially effective tumor immunotherapy strategies, and some mushroom polysaccharides are known to be attractive macrophage stimuli.

Chinese Cordyceps is a well-known traditional medicine. In recent years, it has been successfully cultivated on an industrial scale by HEC Company. The immune-enhancing effects of Chinese Cordyceps are attributed to their polysaccharides, but little is known about their polysaccharide-protein complexes. Using anion exchange, a strongly adsorbed fraction (Fn4) was isolated from alkaline-soluble extracts. Fn4 is a polysaccharide-protein complex analyzed with the western blotting method. The polysaccharide and protein content ratio was 1.89 to 1.00, and the estimated molecular mass was 40.6 KDa. Its polysaccharide contained glucose, mannose, galactose, galacturonic acid, fructose, and n-acetyl glucosamine. The nanoliter liquid chromatography-quadrupole orbitrap mass spectrometry matched three majority proteins annotated as T5ACM3, linoleate 8R-lipoxygenase, and T5A562 in MS database of *Ophiocordyceps sinensis*. Sevag deproteinization deprived 80% of Fn4 mass and demolished its stimulative activities on macrophages, implying the complex structure of protein and polysaccharide was crucial for the activity.

Fn4 significantly stimulated TNF- α and nitric oxide productions in human THP-1-derived macrophages, with 2- to 5-fold higher activity compared to the other three fractions in alkaline extracts. Fn4 also significantly upregulated pro-inflammatory cytokine gene expressions of Tnfa, Il1b and Il12, and elevated Cd80 gene expression, a marker of M1-polarization. In contrast, Fn4 did not affect Il4, Il10, Ccl18, Ccl22, and Cd206 gene expressions, all considered as M2-polarization phenotypes.

**A POLYSACCHARIDE-PROTEIN COMPLEX ISOLATED
FROM CULTIVATED CHINESE CORDYCEPS DRIVES
M1 PHENOTYPE POLARIZATION IN MACROPHAGES**

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Fn4 showed no effect against human colon cancer cell line HT-29 cells, but its conditioned macrophages culture media did, suggesting that Fn4 inhibits cancer cells by activating macrophages.

Taken together, the Fn4 fraction isolated from the alkaline extract of Chinese Cordyceps is a polysaccharide-protein complex that can activate macrophages by driving M1 polarization.

KEYWORDS: Cultivated Chinese Cordyceps, *Ophiocordyceps sinensis*, polysaccharide-protein complex, macrophage, polarization

NEWER AND MORE ECONOMICALLY VIABLE METHODS OF MUSHROOM CULTIVATION

SESSION 03

LECTURE 02

Elizabeth Morrison

Ayla Bioscience Inc

Over the last century mushroom cultivation evolved from backyard farming into a huge industrial-based agricultural businesses. Mushroom cultivation is involving many more people today, but also cheaper and more efficient methods are becoming available, which opens the door for small farmers and less developed areas around the world. Up until recently large scale technology in the mushroom world has been unavailable or too expensive to utilize. This is not true anymore. The purpose of this presentation is to introduce a low-cost cold process method, which can be applied to both small- and large-scale farms. In the United States, Doctor John Holliday has developed a cold sterilization process for mushroom cultivation that requires low investment. Ultimately sterile is sterile, and it does not matter the process used to achieve this, whether it be heat, plasma or chemicals.

The yield seen are extremely high and doubling the harvest of even the most advanced conventional farm technologies that involve heat sterilization system are commonly observed. These cold system farms require the least amount of training and one of the smallest capital investments required to implement a commercially viable mushroom operation, while providing the easiest method to grow locally acclimatized mushroom strains for a large and valued mushroom crop in the shortest possible time with minimal financial risk for farmers. The best candidates for this method have been the oyster mushroom of the Genus *Pleurotus*. There are other mushroom species that have also proven to grow well with this method. The mushroom species chosen for this system are primarily the cellulous decomposers that can grow on almost any plant material substrate, including banana waste, coffee residue, cereal straw, paper, cardboard, river grass, sawdust, and in fact, virtually all other agricultural waste. These raw materials are currently being disposed of as waste products from conventional agriculture. We have taken these wastes, the residual farm waste substrates, and using sterile tissue culture are bioconverting from the non-nutritional cellulosic raw material into edible fungal high protein content. The final spent substrate from this process can be utilized as a nutrient rich feed additive for cattle, poultry, sheep, goats, as well as raw material for synthetic meats for humans. This diversified agricultural approach is true circular farming, resulting in three successive cash crops from any agricultural operation: the initial primary crop, the secondary crop of edible mushrooms and the tertiary crop of animal feed. This new method of mushroom cultivation is a true paradigm shift in the mushroom cultivation world. Using these methods, we are turning our ag waste into food, just as nature intended. Using this economically viable method, not only are we being farmers and feeding the world, but we are unleashing our fairy magic, turning waste into artificial logs made from cellulose waste, producing mushrooms to eat, cattle feed, food security for the developing world and economic stability for impoverished nations.

THE CORRELATION BETWEEN CULTIVATION AND NUTRITIONAL VALUE IN THE MEDICINAL MUSHROOM PLEUROTUS ERYNGII

SESSION 03

LECTURE 03

Adir Amiram, Nirit Ezov, Dan Levanon, Ofer Danay and Idan Pereman.

Olive Mill Solid Waste (OMSW) is a by-product originating from the olive oil industry and represents an environmental problem in Mediterranean countries. The use of OMSW as a main component in mushroom cultivation substrates was suggested as a potential solution for this issue. Subjecting agriculture wastes for mushroom cultivation makes beneficial use of their lignocellulose degradation metabolic activity. Moreover, in addition to the benefit gained by harnessing fungi's unique ability to decompose otherwise un-degradable substrates, the resulting cultivated mushrooms make a good source for complete protein and functional metabolites such as α - and β glucans. In the current study we tested *Pleurotus eryngii* cultivation efficiency on substrates which were added with varying concentrations of OMSW (0%, 33%, 60% and 80%). In addition to *Pleurotus eryngii* cultivation protocols optimization, we wanted to further determine total metabolic activity and glucan synthase activity specifically, in correlation with OMSW concentrations. Gene expression analysis via real time PCR of α - and β glucan synthase has demonstrated transcription up regulation in 60% vs 0% OMSW substrate in ratios of 2.17 and 2.0 respectively. Direct measurement of α - and β glucan has indicated a positive correlation between OMSW concentration and glucan levels. In contrast, Total protein concentration exhibited a negative correlation with OMSW concentration.

Furthermore, total gene expression via RNA Seq in 80% Vs. 0% OMSW substrate has indicated substantial reduction in the transcription of genes involved in ribosomal activity in contrast to up regulation of genes involved in secondary metabolite and polysaccharide synthesis. These observations were further supported by LC-MS analysis which has determined differential increase/decrease of secondary metabolites concentrations which correlated with altering percentages of OMSW.

Overall, our results demonstrate the pivotal role of substrate composition and its dominant impact on the transcriptomic-metabolic profile of cultivated mushrooms.

**PRELIMINARY RESULTS ON THE CULTIVATION
AND THE CLINICAL USE AGAINST HUMAN PATHOGENS
OF PLEUROTUS ERYNGII VAR. THAPSIAE,
A CULINARY-MEDICINAL MUSHROOM FROM SICILY**

SESSION 03

LECTURE 04

**Valeria Ferraro¹, Fortunato Cirlincione¹, Giulia Mirabile¹, Teresa Fasciana²,
Anna Giammanco², Maria Rita Tricoli², Laura Di Paola², Maria Letizia Gargano³,
Raimondo Pardi³, Emilio Di Gristina¹, Giuseppe Venturella¹, Pasqualina Colasuonno³**

1 Department of Agricultural, Food and Forest Sciences, University of Palermo,
Viale delle Scienze, Bldg.5, I-90128 Palermo (Italy);

2 Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties,
University of Palermo, Piazza delle Cliniche, 2, I-90127 Palermo (Italy);

3 Department of Agricultural and Environmental Science, University of Bari Aldo Moro,
Via Amendola 165/A, I-70126 Bari, Italy; marialetizia.gargano@uniba.it

The genus *Pleurotus* (Fr.) P. Kumm. includes economically important mushrooms, cultivated around the world, widely used in human nutrition and recognized as medicinal mushrooms. Within this genus, the *P. eryngii* species-complex is undoubtedly worth mentioning. It is now known to be a complex of different, closely related varieties belonging to *P. eryngii*, with different ecological and morphological characters. The *P. eryngii* species complex includes saprotrophs or facultative biotrophs which grows in association with plants of the Apiaceae family [1].

Among the members of the *P. eryngii* species-complex, our attention focused on *P. eryngii* var. *thapsiae* Venturella, Zervakis & Saitta, whose basidiomes grow in association with *Thapsia garganica* L. This mushroom has been reported so far only in Sicily (Italy), where it has a rather limited geographical distribution, at altitude ranging from 0 to 1500 m [2]. Cultivation was carried out to assess its productive efficiency and qualitative properties, in the perspective of a potential large-scale cultivation of wild mushroom species and consequent enhancement of some territories. The cultivation was carried out on two different substrates, one based on wheat straw and another consisting of a mix of wheat straw and *Aegilops geniculata* Roth., a wheat weed. *P. eryngii* var. *thapsiae* grew on both substrates, exhibiting rather good production performance. Besides, the collected basidiomes were subjected to drying, then reduced to powder and a chemical analysis was then performed on this powder. One share of the mushroom powder was used to obtain aqueous extracts using two different techniques, conventional solvent and ultrasound-assisted extraction, in order to evaluate and compare their activity against ATCC Gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*), and Gram-negative (*Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*) strains.

**PRELIMINARY RESULTS ON THE CULTIVATION
AND THE CLINICAL USE AGAINST HUMAN PATHOGENS
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The best activity was obtained against *S. aureus*, with a reduction of about 60% of biofilm biomass, by means of both ultrasonic extracts. Evaluations of clinical strains are in progress together with the potential application of *P. eryngii* var. *thapsiae* for large-scale cultivation, aimed at the marketing of a local, quality product with high nutritional and organoleptic properties.

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CHARACTERIZATION OF THE COMPOSITION, STRUCTURE, ENZYMIC ACTIVITY AND BACTERIAL METATAXONOMY OF THE STERILE SUBSTRATE USED DURING LENTINULA EDODES GROWTH AND DEVELOPMENT

SESSION 03

LECTURE 05

Lavega R.^{1*}; Carrasco J.^{1,2}; Rascón E.¹; García-Delgado C.³; Eymar E.⁴; Soler-Rivas C.⁵; Pérez M.¹

1 Centro Tecnológico de Investigación del Champiñón de La Rioja (CTICH);

2 Department of Plant Sciences, University of Oxford, Oxford OX1 3RB, UK;

3 Department of Geology and Geochemistry, Faculty of Sciences, Universidad Autónoma de Madrid, Cantoblanco, 28049, Madrid, Spain;

4 Department of Agricultural Chemistry and Food Sciences, Faculty of Sciences, Universidad Autónoma de Madrid, Cantoblanco, 28049, Madrid, Spain;

5 Department of Production and Characterization of Novel Foods, Institute of Food Science Research – CIAL (UAM+CSIC), Universidad Autónoma de Madrid, Cantoblanco, 28049, Madrid, Spain.

* Corresponding author: r.lavega@ctich.com

Shiitake mushroom (*Lentinula edodes*), which presents relevant medicinal properties and interesting nutritional value, ranks first in the global mushroom market. For the commercial production of this edible fungus, a sawdust-based substrate subjected to sterilization or pasteurization is commonly used. A mixture of lignin-rich ingredients that provides broad heterogeneity in terms of chemical composition, physical and bromatological characteristics in the harvested basidiomes. The selection of raw material varies depending on the long-term regional availability.

This work aims to characterize the variation of the chemical composition, structure, enzymatic activity and bacterial population during the growth of *Lentinula edodes* in the sterile substrate produced at CTICH, La Rioja, Spain. Substrate samples were subsequently taken during 9 weeks, from day 0 (sterile substrate without mushroom spawn) to day 63 (spent mushroom substrate). The samples collected were stored and managed as required for later analysis. Firstly, physical and chemical analysis of the samples was carried out. Moisture content, ash, nitrogen content, pH, electrical conductivity, organic matter content, fiber content and the C/N ratio were measured.

Physicochemical differences were noted depending on the moment of sampling throughout the shiitake crop cycle. Moreover, the element composition was measured by ICP-MS after microwaves acid digestion, showing relevant changes in the relative concentration of important elements, such as Mg, Fe, Cu, Si, Li, Cs and B, during mushroom development. Besides, the activity of a variety of enzymes, both cellulolytic and ligninolytic, were measured by spectrophotometry. As expected, a correlation has been noted between the observed mushroom enzymatic activity and the process of biodegradation in the substrates.

CHARACTERIZATION OF THE COMPOSITION, STRUCTURE, ENZYMATIC ACTIVITY AND BACTERIAL METATAXONOMY OF THE STERILE SUBSTRATE USED DURING LENTINULA EDODES GROWTH AND DEVELOPMENT

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LECTURE 05

Higher activity of some of them, such as peroxidases, was noted during the mycelial colonization of the substrate whereas other enzymes, such as laccases and proteases, have their maximum activity during the fructification of the shiitake mushroom. Suggesting different enzymatic patterns depending on the stage of the crop cycle.

Ultimately, the structure and evolution of the bacteria microbiome present in each step of the cycle was analyzed through metataxonomy of 16S rRNA library sequencing in Illumina MiSeq and the corresponding bioinformatics analysis through Qiime 2. The composition of bacterial microbiome in shiitake substrate was determined and assigned at level of phylum. Colonization of compost by *Lentinula* mycelium and mushroom fructification resulted were the steps that showed important changes in bacterial communities. The variety of techniques employed can provide relevant knowledge and a holistic picture of the crop cycle for the grower to optimize the cultivation process. This research was financed by the Department of Economic Development and Innovation of La Rioja. Res. n° 1168/2018.

MECHANISM OF BIOSTIMULATION TO PREVENT BIOTIC DISORDERS DURING MUSHROOM CULTIVATION

SESSION 03

LECTURE 06

Carrasco, J.^{1,2*}; Kay, W.¹; Kusari, S.³; Preston, G.M.¹

1 Department of Plant Sciences, University of Oxford, South Parks Rd, Oxford, OX1 3RB, UK

2 Centro Tecnológico de Investigación del Champiñón de La Rioja (CTICH), Autol, Spain

3 Center for Mass Spectrometry (CMS), Department of Chemistry and Chemical Biology, Technische Universität Dortmund, Otto-Hahn-Str. 6, 44227 Dortmund, Germany

* Presenting author: jaime.carrasco@plants.ox.ac.uk; j.carrasco@ctich.com

Edible and medicinal mushrooms are susceptible to different biotic disorders, mostly pathogenic bacteria and fungi, which can provoke substantial yield losses.

The environmental niche where mushrooms grow and fructify is cohabited by a number of different macro- and microbial species that interact positively and negatively with the crop. Some inhabitants of the casing material produce secondary metabolites with antifungal and antibiotic properties that may inhibit the germination of pathogenic spores such as *Cladobotryum* spp. (cobweb disease) or *Trichoderma* spp. (green mould) or may prevent the proliferation of harmful bacteria such as pathogenic

Pseudomonas. However, their natural presence in the substrate used for mushroom cultivation does not show detrimental impact on basidiomes.

The cultivable microbiome obtained from the casing material employed in the cultivation of button mushroom and healthy microbiome has been identified. Among a panel of 150 strains identified, several strains showed antifungal activity when co-cultured with pathogenic and competitor fungi such as *Lecanicillium fungicola* 150/1; *Cladobotryum mycophilum* CM3900 or *Trichoderma atroviride* Tav1. Genome sequencing showed that three of these strains, *Bacillus velezensis* CM5, CM19, CM35 are phylogenetically very closely related with only some single-nucleotide polymorphism (SNP) among them. Genome mining using the antiSMASH tool for the analysis of secondary metabolite biosynthetic gene clusters in bacterial genomes that have been published earlier showed that these strains may express antibiotics (e.g. bacilysin, bacillomycin, iturin A, surfactin or fengycin), siderophores, lytic enzymes (e.g. β -1,3-glucanase, chitinase, protease, lipase or amylase) or polyketides (e.g. bacillaene, diffidin or macrolactin).

Bioinformatics-guided identification and structural elucidation of secondary metabolites that are strongly linked to mycoparasite suppression using high-performance liquid chromatography-high-resolution tandem mass spectrometry (HPLC-HRMSn) has been employed and has identified cyclic and non-cyclic fengycins as the most prominent group of antimicrobial secondary metabolites produced by these strains.

MECHANISM OF BIOSTIMULATION TO PREVENT BIOTIC DISORDERS DURING MUSHROOM CULTIVATION

SESSION **03**

LECTURE **06**

The project leading to this report has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie GA No. 742966 and the project BIOSCHAMP GA n° 101000651. These strains have been patented with a publication number WO/2021/255181, International Application No - PCT/EP2021/066441.

HOW MUSHROOM CAN HELP US TO ACHIEVE SDG 2 (ZERO HUNGER) AND OPTIMAL METHOD OF PRODUCING EDIBLE & MEDICINAL MUSHROOMS IN HOT AND DRY AREAS

SESSION 03

LECTURE 07

Saed Harsini¹, Madjid Soltani², Mohammad Hatefi³, Saman Harsini⁴

1 Post Doc in Global SME Management - FHM University Germany & IMI Iran -
Winner of SDG 2 EUTECH 2021

2 Associate Prof. Department of Electrical & Computer University of Waterloo ON/Canada.

3 MSc Student in Mechanical Engineering at K.N.Toosi University of Technology Tehran/Iran

4 BSc Student of Plant Pathology at Azad University Alborz/Iran

Edible and medicinal mushrooms have received a lot of attention in recent decades. The nutritional value of edible mushrooms competes with other plant and animal products and in some respects is significantly superior to them. Mushrooms are the source of completely organic foods and the amount of water used to produce them is much less than agricultural and livestock products. They contain compounds that strengthen the immune system, anti-diabetic properties, reduce fat and have a lot of fiber, and despite a variety of probiotics and prebiotics, provide us with healthy food.

Mushroom can be classified into three basic ecological groups; mycorrhiza, parasites, and saprobes. Most of the gourmet mushrooms are saprobes, wood-decomposing fungi. Saprobic fungi are the premier recyclers on the plant. As decomposers, they can be separated into three key groups. Primary decomposers are typically fast-growing and quickly attach to decompose plant tissue. Most of the decomposers degrade wood. The majority of these are woodland species, such as Oyster mushrooms, Shiitake, and King Stropharia.

Secondary decomposers typically grow at composted materials, a classic example is the Button Mushroom, *Agaricus brunnescens*, the most commonly cultivated mushroom.

Tertiary decomposers are typically soil dwellers. Primary and secondary decomposers afford the most opportunities for cultivation. To select the best species for cultivation, several variables must be carefully matched, climate, available raw materials, the mushroom strain and indirect sunlight.

Using a wind catcher in hot and dry areas has been a common practice among desert residents. Modification and adaptation of this method to the growing conditions of edible and medicinal mushrooms can be used for economic production of these species in hot and dry areas that have little access to fresh water. The substrate used in the production of mushrooms is prepared by cold sterilization of any agricultural waste, especially straw, sawdust, and wood waste, and addition of lime, compounds which are very cheap and abundant everywhere. After harvesting the mushroom, the remaining substrate is used as animal feed and no residue remains.

HOW MUSHROOM CAN HELP US TO ACHIEVE SDG 2 (ZERO HUNGER) AND OPTIMAL METHOD OF PRODUCING EDIBLE & MEDICINAL MUSHROOMS IN HOT AND DRY AREAS

SESSION 03

LECTURE 07

Advantages of this method of production:

Wind energy instead of electricity to create cooling and humidification.

Salt water (sea water) instead of fresh water.

Significant amount of edible mushrooms and livestock feed, especially for light livestock such as goats.

Produce a significant amount of fresh water and economic production of sea salt for general use.

Soil enrichment and improving dry land.

Keywords: Mushroom, Wind catcher, Livestock feed, Agricultural waste, Healthy food, Soil enrichment

GROWTH OF MEDICINAL MUSHROOMS (MMS) ON MAIZE COBS WITH DIFFERENT PIGMENTATION: NOVEL BIOTECHNOLOGICAL APPROACHES TO VALORIZE MAIZE BY-PRODUCTS (NETMAP)

SESSION 03

LECTURE 08

Manuela Rollini¹, Carola Cappa¹, Daniela Erba¹, Maria Cristina Casiraghi¹,
Gabriella Consonni², Giulia Castorina², Alessandra Marti¹, Daniele Cavicchioli³,
Noemi Negrini²

1 Department of Food, Environmental and Nutritional Sciences (DeFENS),
Università degli Studi di Milano, Via Celoria 2, 20133 Milano (Italy)

2 Department of Agricultural and Environmental Sciences - Production, Territory, Agroenergy (DiSAA),
Università degli Studi di Milano, Via Celoria 2, 20133 Milano (Italy)

3 Department of Environmental Science and Policy (ESP), Via Celoria 2, 20133 Milano (Italy)

Background:

Medicinal mushrooms (MMs) are gaining attention for their content of bioactive compounds whose effects are relevant for human health. The possibility to exploit cultural residues for the fungi cultivation represents a challenge to enhance the commercial use of MMs. Moreover, these different growth substrates may have positive impact on the fungi development with effects on their activities and final composition. Maize landraces are an important source of genetic variants for adaptation to specific environment and have high nutritional value. They are rich in secondary compounds and often show characteristic pigmentations of kernels and cobs. Nevertheless, their cultivation in small-scale farming does not allow an efficient recycling of their residues, like corncobs that could instead represent an interesting material for alternative uses.

Objectives:

The research aims at developing a high value-added ingredient with interesting nutritional properties, obtained by growing MMs on corncobs of traditional Lombard maize varieties. Investigation will highlight whether and to what extent MMs bioactivities are maintained up to the production of the functional ingredient.

Methods:

Local maize varieties with different cob pigmentation (Spinato di Gandino, Rostrato Rosso di Rovetta, Spinoso Nero Valle Camonica, Fiorine di Clusone) and the B73 inbred line were field grown. The harvested cobs to be used as growth substrate have been characterized for fiber and a few secondary metabolites contents both before and after the fungi growth and used as growth substrate. Solid State Fermentations (SSFs) were set-up employing the MMs *Pleurotus ostreatus*, *Ganoderma annularis*, *Flammulina velutipes* and *Lentinula edodes*: trials were first carried out in small jars using sterile chopped corncobs; the best performing strains were subsequently grown in boxes equipped with a semipermeable gas membrane.

**GROWTH OF MEDICINAL MUSHROOMS (MMS) ON MAIZE
COBS WITH DIFFERENT PIGMENTATION:
NOVEL BIOTECHNOLOGICAL APPROACHES
TO VALORIZE MAIZE BY-PRODUCTS (NETMAP)**

SESSION 03

LECTURE 08

Results:

among corncobs, Spinoso Nero Valle Camonica showed the highest content of lignin, total monomeric antocyanins as well as the highest presence of free phenols with high antioxidant activity while Rostrato Rosso di Rovetta has the highest content of phenolic compounds, mainly present in a bound form. Among MMs, Pleurotus and Ganoderma had the best growing performances as indicated by the Image Analysis that proved a successful tool to monitor and characterize the mycelial growth on SSF. Ganoderma was found to grow immediately after the inoculum (no lag phase), while Pleurotus showed a lag phase, possibly required to activate inducible lignocellulose-degrading enzymatic activities, as suggested also by its slow and reduced growth on corncobs of Spinoso Nero Valle Camonica landrace.

Conclusions:

results will provide new insights on the role of fungal biomass in enhancing the nutritional and functional properties of food, considering the potential impact of processing conditions and/or the interactions with other maize components, on the functional and technological properties of the final product. The assessment on whether and to what extent the new product will be accepted by consumers will also represent an innovative NETMAP feature.

SOLID CULTURE PARAMETERS FOR THE PRODUCTION OF FRUITING BODIES OF THE TURKEY TAIL FUNGUS (*TRAMETES VERSICOLOR*) IN TROPICAL CLIMATE UNDER CONTROLLED CONDITIONS

SESSION 03

LECTURE 09

Sandra Montoya¹, Juan Pablo Ortiz², Yenny Leandra Valencia¹

1 Technological Development Center - Bioprocesses and Agroindustry Plant, Universidad de Caldas, Manizales Colombia

2 Core Research Group, Universidad de Boyacá, Tunja, Colombia

The turkey tail macromycete (*Trametes versicolor*), with important medicinal attributes, has a short history of cultivation for the production of its fruiting bodies, particularly in tropical climates. So far, little effort has been devoted to improving culture conditions for commercial production of the fungus, let alone using wild strains. The purpose of this research was to evaluate the production of basidiomas from two wild strains of the fungus *T. versicolor*, coded as 22.1 and 21.63, collected from the forests of the department of Boyacá (Colombia). These fungi were cultivated in the Macromycetes Unit of the TDC - Bioprocesses and Agroindustry Plant. The seed of the two strains of the study fungus was produced on wheat grains hydrated up to 38% humidity in units of 1 kg, sterilized at 121°C and incubated at 25°C for 10 days.

Four substrates were formulated from Colombian agro-industrial by-products of lignocellulosic origin, three with cane bagasse, tuza and corn bran (F1, F2, F3) and one with oak sawdust and corn bran (F4), for the production of the fruiting bodies of the two study strains; The substrates were hydrated to 60% humidity, packed in 2 kg bags, sterilized at 121°C and taken to experimental culture rooms with a capacity of 200 kg. The total cycle of the culture of each strain and on each substrate formulation presented variations between 105 days and 120 days; frequently, controlled environmental conditions were developed for basidiome production at the stages of incubation (25°C, 60% RH, two air changes of two hours each day, penumbra(?), 32 days), induction of primordia (18°C, RH greater than 85%, three air changes of two hours per day, cuts to the bags to allow the formation of basidiomas, 500 – 1,000 luxes, 10 to 30 days), production of fruiting bodies (18°C, RH greater than 85%, three air changes of two hours per day, cuts to the bags to allow the formation of basidiomas, 800 - 1,000 lux, 50 to 65 days).

With the four formulations, fruiting bodies of the two study strains were obtained. However, the highest biological efficiencies (BE) were obtained with strain 22.1 and formulation F4, 26.9%, followed by formulation F2 with strain 22.1, 19.97% EB. Likewise, the quality of the fruiting bodies obtained with bromatological analysis was determined, finding slight differences in the percentage of ashes, fat, potassium, magnesium, and calcium content as an effect of the culture medium. Additionally, some quality patterns referring to color, size and shape were defined, finding that the 22.1 strain grown in the F1 and F4 substrates of the first harvest showed the best quality parameters.

SOLID CULTURE PARAMETERS FOR THE PRODUCTION OF FRUITING BODIES OF THE TURKEY TAIL FUNGUS (*TRAMETES VERSICOLOR*) IN TROPICAL CLIMATE UNDER CONTROLLED CONDITIONS

SESSION 03

LECTURE 09

The results achieved in this research contribute to the knowledge about the solid culture of the fungus *T. versicolor*, and compare favorably with previous works in several countries with respect to BE, quality of the fungus and cycle time of the culture obtained.

Keywords: *Trametes versicolor*, solid culture in bags, development parameters, environmental conditions.

THE OBTAINING OF THE PURE CULTURE OF SOME EDIBLE ECTOMYCORRHIZAL MUSHROOMS

SESSION 03

LECTURE 10

Mustafa Kemal SOYLU¹ Burak BAYBAŞ¹ Selime Semra CANDAR EROL²

1 Ataturk Central Horticultural Research Institute, Yalova, Turkey

2 Düzce University Traditional and Complementary Medicine Practice and Research Center,
Düzce-Turkey

There are almost 2500 species of edible mushroom in the world. Half of these species are ectomycorrhizal mushrooms (ECMM). Türkiye has very rich potential for the production of most of these species. Most of Türkiye's production is exported to other countries, primarily in Southern Europe. During the last 30 years or so, conditions attributable to global warming have severely impacted Turkey's production of ectomycorrhizal mushrooms, as well as the world's, generally. In addition, premature harvesting, abuse of forest lands and, most importantly serious devastation of the mushrooms' host plants have brought about this decreased production.

This Project was proposed to collect and obtain pure cultures of *Boletus edulis* Rostk., *Boletus aestivalis* (Paulet) Fr., *Leccinum scabrum* (Bull.) Gray, *Suillus granulatus* (L.) Roussel, *Tricholoma terrum* (Schaeff.) Quel., *Laccaria laccata* (Scop.) Fr., *Sarcodon imbricatus* (L.) P. Karst., *Cantharellus cibarius* Fr., *Lactarius deliciosus* (L.) Gray, *Lactarius sanguifluus* (Paulet) Fr., *Lactarius semisanguifluus* R. Heim and Leclair, *Terfezia boudieri* Chatin, and *Amanita caesarea* (Scop.) Pers. which have high economic value in Türkiye and in the World, all ectomycorrhizal mushroom species, from their natural habitats. Mushrooms were identified according to the macro and micro morphological data and habitat information.

Pure cultures were obtained by tissue culture from the inside of the fresh mushroom caps. Different PDA, Hagem, MMN, BAF, MEA, PGA, MFM, Fontana agar media were used according to the mushroom species. Bolets and *Amanita caesarea* cultures obtained on MNN, *Lactarius* species and were obtained on BAF, *Cantharellus cibarius* on MFM. Morphological features of the cultures of ECMM were determined. ECMM cultures were usually colorful and have pigments besides *A. caesarea* and *T. boudieri* cultures were whitish in that study. Obtained cultures of the various ectomycorrhizal mushroom species were conserve in Mushroom Gene bank of Atatürk Central Horticulture Research Institute in Yalova, Türkiye. The cultures could be used for further research.

Keywords: Edible, ectomycorrhizal mushroom, pure culture, *Lactarius*, *Boletus*

MICROBIOTA IN MUSHROOM FRUITING HOUSES AND THE EFFECT OF ISOLATED ORGANISMS ON *P. OSTREATUS* MYCELIA GROWTH AND DEVELOPMENT IN VITRO

SESSION 03

LECTURE 11

Iryna Bandura^{1*}, Omoanghe S. Isikhuemhen², Alina Kulyk¹, Nina Bisko³, Serhii Makohon¹

1 Tavria State Agrotechnological University, 18 B. Khmelnytsky Ave., Melitopol, Ukraine.

2 Mushroom Biology & Fungal Biotechnology Laboratory, North Carolina A&T State University, 1601 East Market St., Greensboro, NC 27411, USA.

3 M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, #2 Tereshchenkivska st., 01004, Kyiv, Ukraine.

* *Presenter*

Mushroom fruiting houses are critical infrastructure in commercial mushroom production. The interactions of environmental factors and the mushrooms cultivated affect the microbiota in fruiting houses. Also, many pathogenic organisms that cause severe diseases and crop losses in mushroom production are known to produce airborne spores. Airspora in nine commercial fruiting houses (Seven above-ground and two below-ground bunkers) were sampled for microbial load using the settle plate method. Isolates were identified by morphology and 16s and ITS-based DNA sequences of bacteria and fungi isolates, respectively.

The data obtained were analyzed to determine the presence and abundance of different culturable microorganisms. Isolated organisms from the airspora studies were tested against *P. ostreatus* mycelia in co-culture interactions experiments. *Penicillium* (60%), bacteria (30%), *Aspergillus* (5%), *Alternaria* (4%) species, and other molds such as *Trichoderma* and actinomycetes (1%) represented the existence and abundance of organisms in the airspora studied. Results after each cultivation cycle indicated the increase in airspora composition by a factor of 3.6 for above and 4.1 to 5.8 for below-ground fruiting houses. Pure cultures from airspora interaction studies with *Postreatus* mycelia indicated 1. No competition (no visual decrease in the medium's colonization rate by *P. ostreatus*, which can grow over the mold), 2. Competition (a pronounced zone of suppression or complete cessation of *P. ostreatus* mycelia development) and 3. Antagonism (the mold colony suppresses, overgrows, and sometimes kills *P. ostreatus* mycelia).

Representative organisms of the 'No competition' group were *Aspergillus* spp, *Coniothyrium pyrinum* (Sacc.), which is better known as *Phyllosticta pirina* Sacc. and *Alternaria alternate* (Fr.) Keissl., those from the competition group were *Penicillium roqueforti* and *Fusarium oxysporum*, while antagonistic were *Cladobotryum mycophilum*, *Trichoderma pleuroticola*, *T. harzianum*, and *T. atroviride*. The results reveal the dynamics of common microbial organisms in oyster mushroom fruiting houses, which could inform the strategies to mitigate their presence and control in mushroom fruiting houses before they cause economic loss in commercial oyster mushroom production. Further work to determine measures and practices that can reduce contaminating organisms in the airspora in mushroom houses is recommended.

**BIOREACTOR-PRODUCED GANODERMA LUCIDUM
EATS WASTEWATER**

SESSION 03

LECTURE 12

Wan Abd Al Qadr Imad Wan Mohtar

Functional omics and bioprocess development laboratory, Institute of Biological Sciences,
Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.

Population growth, rapid urbanisation, and industrialization aggravate pollution-related issues. The impact of soil and water pollution on human health and the environment is significant. Therefore, it is essential to develop strategies to combat this escalating issue. Mycoremediation, the use of fungi or their derivatives for the remediation of environmental pollutants, is a relatively inexpensive, environmentally friendly, and efficient technique. It has benefits over conventional and bioremediation techniques. In this study, the renowned medicinal mushroom *Ganoderma lucidum* (GL) is introduced as a myco-alternative to existing conventional microbial-based wastewater treatment as a feasible alternative treatment.

Mycoremediation is highly tenacious in its ability to oxidatively degrade pollution substrates, and its therapeutic effects are being studied. Using the non-hazardous features and high degrading performance of GL, this study seeks to optimise the mycoremediation treatment design for chemical oxygen demand (COD) and ammonia-nitrogen (N) removal in residential wastewater, as well as its decolorization capacity. Under regulated conditions, GL was fully grown in a customised stirred-tank bioreactor called Air-L-Shaped Bioreactor (ALSB) to generate specialised mycelial pellets (GLMP) and then treated against effluent. Thus, the performance of GLMP was evaluated on four different wastewaters under different conditions of initial pH (pH 4, 5, and 7) and COD to nitrogen (COD/N) ratio of 3.6:1, 7.1:1, 14.2:1, and 17.8:1 (C3.6N1, C7.1N1, C14.2N1, and C17.8N1). The COD/N ratios with a constant concentration of AN were chosen on the basis of the urban domestic wastewater characteristics sampled at the inlet basin of a sewage treatment plant (STP).

The parameters of pH, COD, and AN were measured periodically during the experiment. The GLMP efficiently removed the pollutants from the sewage. The COD/N ratio of C17.8N1 wastewater had the best COD and AN removal, as compared to the lower COD/N ratio, and the shortest treatment time was obtained in an acidic environment at pH 4. The highest percentage for COD and AN removal achieved was 96.0% and 93.2%, respectively. In addition, GLMP successfully decolorizes 90% of waste dyes at a culture ratio of 50% and killed pre-added *Escherichia coli* spoilage bacterium. These data supported the utilisation of GLMP as a tertiary treatment in a sewage treatment plant. Mushroom culture usage in wastewater treatment is paving the way for the future. The ability to produce in bulk, safe, cheap, environmentally friendly, and most importantly found to be consistently produced in high quality are the obvious advantages of mushroom cultures, imposing themselves as attractive alternatives to bacteria and algae. We are adamant that mushroom support and are consistent with the UN's Sustainable Development Goals. Goal #6, "Clean water and sanitation.

SHORT ORAL PRESENTATION / session NO 3

LECTURE 01 BIOSCHAMP: DESIGN OF BIOSTIMULANT ALTERNATIVE CASING FOR THE MUSHROOM INDUSTRY

Jaime Carrasco^{1,2*}; Jan van der Wolf³; Pablo Martínez¹; Nancy Pyck⁴;
 Ivanka Milenkovic^{5*}; Gail Preston²

- 1 Centro Tecnológico de Investigación del Champiñón de La Rioja (CTICH), Autol, Spain.
- 2 Department of Plant Sciences, University of Oxford, S Parks Rd, Oxford, OX1 3RB, UK.
- 3 Biointeractions and Plant Health, Wageningen University and Research, Wageningen, Netherlands;
- 4 INAGRO, Ieperseweg 87, 8800 Rumbeke-Beitem, Belgium;
- 5 Ekofungi Ltd., Belgrade, Serbia.

* Presenting authors: j.carrasco@ctich.com; ekofungi@gmail.com

Consumers are increasingly aware of the importance of health care through a balanced diet. In this regards, mushrooms are a reliable food supply with high protein content and multiple micronutrients such as oligo elements (i.a. Se), vitamins (i.a. vit D2) or polysaccharides (i.a. β -glucans). Commercial cultivation of edible and medicinal varieties enables availability of mushrooms to be consumed throughout the entire year with stable production beyond climate events unfavourable for traditional agriculture.

Different cultivated species within the Agaricales (white button mushroom, Portobello or sun mushroom) require the application of peat-based casing layers of top of the compost colonized by the crop mycelium to generate a profitable crop. The casing material employed, require specific physicochemical and micro-biological properties to induce the fructification of the crop. However, the main component of the casing layer is black peat extracted from peatlands, a non-renewable fossil material. Black peat is becoming scarce and expensive since their extraction provokes harmful damage in singular environments that act as natural carbon sinks.

Besides, most of the cultivated mushrooms species are sensitive to biotic disorders such as fungal diseases (i.a. cobweb or green mould). Very few active substances to cope with harmful mycoparasites are available for the farmers with multiple evidences of resistance strains to the chemicals currently approved. Due to the size of the mushroom industry and the required selectivity among fungicides to apply in the crop it is unlikely to expect new efficient pesticides in the near future.

BIOSCHAMP is an H2020 funded project generating knowledge for the production of low-peat alternative materials that present efficient agronomical properties and maximise crop yield. The alternative casing material incorporates selected biostimulants in order to prevent fungal disorders, and reduce the dependence upon chemical pesticides. The European mushroom industry is well represented in our consortium while including researchers, substrate makers, casing suppliers and mushroom growers for the scaling and validation at commercial size.

SHORT ORAL PRESENTATION / session NO 3

LECTURE **BIOSCHAMP: DESIGN OF BIOSTIMULANT ALTERNATIVE**
01 CASING FOR THE MUSHROOM INDUSTRY

The diverse international consortium including 5 Research Technological Centres, 3 large companies and 4 SMEs from six European countries will design, on the basis of evidences collected from previous projects, a sustainable alternative casing layer with enhanced biostimulant activity for the commercial cultivation of edible and medicinal mushrooms.

The project BIOSCHAMP is funded by the H2020 programme of the European Commission under the Grant Agreement n° 101000651 (<https://cordis.europa.eu/project/id/101000651>). Webpage: www.bioschamp.eu.

EFFECTS OF PLEUROTUS OSTREATUS FERMENTATION ON THE CHEMICAL COMPOSITION OF MILO

SHORT
ORAL
PRES

03

LECTURE 02

Lydia Olagunju¹, Omoanghe S. Isikhuemhen^{2*}, Felicia N. Anike², Xavier Crumel¹,
Tylah Robinson¹, Uchenna Anele¹

1 Department of Animal Sciences, North Carolina Agricultural & Technical State University,
Greensboro NC 27411, USA.

2 Department of Environmental Design, North Carolina Agricultural & Technical State University,
Greensboro NC 27411, USA.

* *Presenter*

Fungi, especially macromycetes have been reported to be very efficient in the biosynthesis of bioactive compounds, some of which exist as components of their cell wall in the fruiting bodies they produce for sporulation. Mycelia of these fungi also have similar bioactive compounds found in their fruiting bodies. Hence, mycelia, which take a short time to produce, have been explored as a potential alternative source of fungal bioactive compounds instead of fruiting bodies. In the present study, we evaluated the effects of mycelia selected from nine strains of white-rot fungi (*Pleurotus ostreatus*) on the chemical composition of milo (*Sorghum bicolor*).

The milo samples were inoculated in triplicates per treatment and incubated at 25°C for 4, 6, and 8 weeks. Samples from each treatment were dried and analyzed for crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), and hemicellulose. Strain x time interactions ($P < 0.05$) were noted for all the analyzed chemical constituents except for EE. Fermentation with test *P. ostreatus* strains increased the CP of some of the samples from 4.6 to 147.1%. The 147% increase noted for strain 3 after eight weeks of incubation was approximately 2.5 times the CP value (87 g/kg DM) of the control sample (non-inoculated milo).

Except for strains 4 (with a value of 78 g/kg DM for EE) and 6 (857 g/kg DM for NDF), eight out of the nine strains of *P. ostreatus* reduced EE and NDF contents of milo, with values ranging from 72 g/kg DM (control) to 34 g/kg DM after six weeks for EE and from 847 g/kg DM (control) to 520 g/kg DM after eight weeks for NDF. Six of the *P. ostreatus* isolates tested significantly ($P < 0.001$) reduced ADF (isolates 2, 3, 5, 7, 8, and 9) and hemicellulose (isolates 1, 2, 4, 7, 8, and 9) contents of treated samples. Our results indicate that the CP content was improved at different fermentation periods by all the strains during the solid-state fermentation of an underutilized grain (milo). These results demonstrate the potential for edible and medicinal mushroom-forming fungi in solid-state fermentation to upgrade the nutritional values of commonly used grains in animal feed.

Keywords: chemical composition, milo, mycelia, white-rot fungi

EFFECT OF PLEUROTUS OSTREATUS TREATED SORGHUM BICOLOR ON IN VITRO GAS PRODUCTION, DRY MATTER DIGESTIBILITY, AND VOLATILE FATTY ACID CONCENTRATIONSHORT
ORAL
PRES

03

LECTURE 03

Peter A. Dele¹, Omoanghe Isikhuemhen^{2*}, Felicia Anike², Tylah Robinson¹,
Lydia K. Olagunju¹, Xavier L. Crumel¹, Uchenna Y. Anele¹

1 Department of Animal Sciences, North Carolina Agricultural & Technical State University, Greensboro NC 27411, USA.

2 Department of Natural Resources and Environmental Design, North Carolina Agricultural & Technical State University, Greensboro NC 27411, USA.

* *Presenter*

Mushroom fruiting bodies have been used for centuries for food and medicinal purposes, but it takes time to produce and process into needed bio-products, hence the recent attention on the use of mycelia-based biomaterials. The presence of bioactive components in mushroom mycelium makes it an attractive ingredient that is now being used to improve the quality and feeding value of non-conventional cereal grains. The present study investigated the effects of nine strains of *Pleurotus ostreatus* fermented milo (*Sorghum bicolor*) on total gas production, in vitro dry matter digestibility, and short-chain fatty acid (SCFA) concentrations.

Milo grains were inoculated and incubated for four, six, and eight weeks at 25°C. For the in vitro study, approximately 0.5 ± 0.55 g of the treated milo samples were weighed into Ankom F57 filter bags and sealed with an impulse heat sealer. Once sealed, the bags were placed into respective 100 ml serum bottles. Artificial saliva preparation was based on McDougall's recipe and maintained in a water bath at 39°C until dispensed into the serum bottles. Ruminant fluid was collected after morning feeding from two ruminally cannulated dairy cows. Their diets consisted of 18% protein, grain, corn silage, and alfalfa hay daily. The batch culture media was dispensed into the 100 ml glass serum bottles. Each serum bottle received 45 ml of artificial saliva and 15 ml of rumen fluid anaerobically by flushing with carbon dioxide. Bottles were capped with a 14-mm rubber stopper and crimped with an aluminum seal cap.

Bottles were incubated on an orbital shaker at 39°C at 125 rpm for 24 h. Strains versus fermentation time interaction were significant for dry matter digestibility ($P = 0.013$) and short-chain fatty acids ($P < 0.001$). The result showed that gas production differed and ranged between 93.4 and 174 ml/g DM across the strains and fermentation times. The *P. ostreatus* strain 7 (PO7) fermented for four weeks produced the highest gas volume, which is 50% more than the control, and also had the highest dry matter digestibility across the three fermentation periods. Also, PO7 had a higher SCFA concentration (51% higher) than the control group and other *P. ostreatus* isolates tested. Furthermore, the results showed that five of the *P. ostreatus* strains fermented for four weeks improved dry matter digestibility and total short-chain fatty acid concentration of milo. Therefore, fermentation for four weeks is the optimum time to improve the parameters monitored in the experiments.

Keywords: dry matter digestibility, *Pleurotus ostreatus*, *Sorghum bicolor*, volatile fatty acid

**SUPPLEMENTATION OF MEDICINAL MUSHROOM
SUBSTRATES WITH TRACE ELEMENTS SE AND ZN**SHORT
ORAL
PRES

03

LECTURE 04

Ivan Jablonský, Lukáš Praus

Czech University of Life Sciences Prague

Selenium (Se) and zinc (Zn) are among the trace elements having an irreplaceable role in the metabolism of the human body. It is therefore desirable to ensure their regular income. One way to naturally supply these microelements to the body is through dietary intake. The aim of the work was to determine the differences in the accumulation of Se and Zn between individual species, fungal strains and the possible influence of the composition of substrates on the accumulation of the studied microelements.

Gradually increasing doses of selenium Se in substrates in the form of Na_2SeO_3 and zinc (Zn) in the form of ZnSO_4 in cultivated fungi were studied. The fungi that are used also in the form of dietary supplements were compared, namely, *Pleurotus ostreatus* (Jacq. Ex Fr.) P. Kumm., *Ganoderma lingzhi* (Sheng H. Wu, Y. Cao & Y.C. Dai), *G. adspersum* (Schulz.) Donk., *Lentinula edodes* (Berk.) Pegler, and *Hericium erinaceus* (Bull.) Persoon, and the accumulation of Se and Zn in fruiting bodies and mycelium was monitored. To increase the content of Se and Zn in oyster mushroom fruiting bodies, the substrates were enriched with Na_2SeO_3 at a concentration of 2, 6, and 18 mg/kg of substrate and ZnSO_4 at a concentration of 10, 20, and 40 mg/kg, respectively. The substrate consisted of hardwood sawdust enriched with 20% wheat bran. The liquid broth contained 2% malt extract. The content of individual elements in the samples of fruiting bodies was determined by inductively coupled plasma mass spectrometry (ICP-MS) after mineralization of the samples in a microwave digestion system.

As the doses in the substrate increased, so did its content in the fruiting bodies. In general, in the experiments, the Se content in the fruiting bodies increased significantly, especially at the dose of 18 mg Na_2SeO_3 . Of the *P. ostreatus* strains, strain HK35 showed the highest intake of Se. The growth rate of *P. ostreatus* mycelia in the liquid medium decreased with Se concentration. The substrate used (*Miscanthus*) also had an effect on the accumulation of Se in the fruiting bodies. The content of Se and Zn in mushroom caps was significantly higher than in stipes.

The highest accumulation of Se in the fruiting bodies was achieved at a concentration of 18 mg Se/kg substrate. Zinc Zn enrichment of the substrate had a negligible effect. At increased doses, zinc Zn content in *P. ostreatus* fruiting bodies was only slightly increased.

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When comparing the intake of different species of *Ganoderma*, it was found that selenium Se concentrations in the fruiting bodies of *G. adspersum*, *G. lingzhi* and *G. lucidum* increased as the concentration of Na_2SeO_3 in the substrate increased. The highest intake in fruiting bodies reached a maximum intake *G. lingzhi*, and *L. edodes* was compared. In the 18 mg / kg enriched substrate, *L. edodes* mycelium contained 140 μg / dry matter of biomass compared to 75 μg in *G. lingzhi* and 53 μg in *G. adspersum*. After enrichment of *L. edodes* Se substrate with Se, differences in Se content in stipes (85 μg) and caps (160 μg) were recorded.

To sum up, analyses showed that Se uptake increased in all fungi in proportion to increasing Na_2SeO_3 dose. On the contrary, increasing doses of Zn did not lead to accumulation in fruiting bodies even at higher concentrations. When comparing the Se content in the fruiting bodies, a higher Se content was found in the caps than in the stipes.

We have shown that all studied edible and medicinal mushrooms can be used as a source of selenium. Tests on the bioavailability of selenium Se will follow. Increasing selenium Se doses per yield will also be monitored.

BIOACTIVE COMPOUNDS IN AGARICUS SUBRUFESCENS GROWN IN CLOSED LOOP CULTIVATION SYSTEM – VEGWAMUS CIRCROP

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PRES

03

LECTURE 05

Agnieszka Jasinska

Agaricus subrufescens, also known as the almond mushroom or the sun mushroom was mostly cultivated in warmer regions of South America however, since discovery of its medical properties its cultivation spread all over the Globe. The VegWaMus CirCrop is a closed loop mushroom- vegetables cultivation system reusing digestate after anaerobic digestion of combined dairy manure food waste feedstock as the mushroom growing compost. The spent mushroom substrate is afterwards reused in vegetable greenhouse cultivation. In this research the content of bioactive compounds including phenolic compounds and organic acids has been examined in fruiting bodies of *A. subrufescens* as well as in the cultivation substrate and spent mushroom substrate. The objective of this experiment was to compare the composition of components of substrates used in cultivation, content in the mushroom fruiting body and characterize SMC material to elucidate their potential utility as a compost ingredient and soil amendment.

The profile of organic acids showed that seven acids were detected in initial substrate for investigated *Agaricus* mushrooms. The content of determined acids decreased as follows: malic (407 $\mu\text{g g}^{-1}$), fumaric (167 $\mu\text{g g}^{-1}$), acetic (125 $\mu\text{g g}^{-1}$), lactic (103 $\mu\text{g g}^{-1}$), quinic (21 $\mu\text{g g}^{-1}$), oxalic (14 $\mu\text{g g}^{-1}$), and malonic (3.18 $\mu\text{g g}^{-1}$). In SMC after *A. subrufescens* cultivation only quinic (584 $\mu\text{g g}^{-1}$), lactic (253 $\mu\text{g g}^{-1}$), acetic (139 $\mu\text{g g}^{-1}$), malic (58 $\mu\text{g g}^{-1}$), oxalic (6.34 $\mu\text{g g}^{-1}$), and malonic (4.10 $\mu\text{g g}^{-1}$) acid was detected. The investigation showed that 13 phenolic acids were detected in analysed substrates. Rutin and catechin were the only two flavonoids detected.

The content of the phenolic compounds decreased in following order: rutin (172 $\mu\text{g g}^{-1}$), 2,5-dihydroxybenzoic (171 $\mu\text{g g}^{-1}$), syringic (93 $\mu\text{g g}^{-1}$), chlorogenic (75 $\mu\text{g g}^{-1}$), 4-hydroxybenzoic (40 $\mu\text{g g}^{-1}$), gallic (30 $\mu\text{g g}^{-1}$), ferulic (27 $\mu\text{g g}^{-1}$), protocatechuic (26 $\mu\text{g g}^{-1}$), caffeic (21 $\mu\text{g g}^{-1}$), sinapic (14 $\mu\text{g g}^{-1}$), catechin (12 $\mu\text{g g}^{-1}$), vanillic (8.38 $\mu\text{g g}^{-1}$), p-coumaric (3.29 $\mu\text{g g}^{-1}$) and t-cinnamic (2.19 $\mu\text{g g}^{-1}$). None of the phenolic compounds were detected in spent mushroom compost after cultivation of *Agaricus subrufescens*. The profile of organic acids showed that 7 organic acids and 12 phenolic compounds has been detected in investigated *A. subrufescens*. Content of organic acids detected in fruiting bodies from all three harvested flushes was as follows: acetic (285 mg kg⁻¹), succinic (27 mg kg⁻¹), lactic (15 mg kg⁻¹), quinic (7527 $\mu\text{g g}^{-1}$), malic (6761 $\mu\text{g g}^{-1}$), oxalic (5435 $\mu\text{g g}^{-1}$), and malonic (3294 $\mu\text{g g}^{-1}$).

The investigation showed that 6 phenolic compounds were detected in the fruiting bodies in all three flushes: 4-hydroxybenzoic (15 mg kg⁻¹), gallic (7934 $\mu\text{g g}^{-1}$), protocatechuic (2309 $\mu\text{g g}^{-1}$), syringic (1466 $\mu\text{g g}^{-1}$), catechin (843 $\mu\text{g g}^{-1}$) and vanillic (770 $\mu\text{g g}^{-1}$).

**BIOACTIVE COMPOUNDS IN AGARICUS SUBRUFESCENS
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While 2,5-dihydroxybenzoic (504 $\mu\text{g g}^{-1}$), p-coumaric (365 $\mu\text{g g}^{-1}$), t-cinnamic (356 $\mu\text{g g}^{-1}$), and caffeic (204 $\mu\text{g g}^{-1}$). Chlorogenic (222 $\mu\text{g g}^{-1}$) and ferulic (187 $\mu\text{g g}^{-1}$). acids were in only present in second flush. Rutin was not detected in any of harvested mushroom crops of analysed mushroom species.

This investigation shows the value of *Agaricus subrufescens* as a source of bioactive compounds including phenolic compounds and organic acids. What is more the organic acids and phenolic compounds are present in the initial substrate, however only organic acids remain in the spent mushroom substrate, which may imply that mushrooms could absorb them from the cultivation substrate.

EDIBLE AND MEDICINAL MUSHROOM PRODUCTION IN AN INTEGRATED FOOD TO WASTE TO FOOD BIOSYSTEM (VEGWAMUSCIRCROP)

SHORT
 ORAL
 PRES

03

LECTURE 06

Agnieszka Jasinska^{1,2}, Ketil Stoknes¹, Michal Roszak³

1 Lindum AS, Lerpeveien 155, 3036 Drammen, Norway;

2 Poznan University of Life Sciences, Department of Vegetable Crops, ul. J.H. Dabrowskiego 159, 60-594 Poznan, Poland;

3 Soppas, Nedre Keisemark 1E, 3183 Horten

VegWaMus CirCrop aim to develop commercial edible and medicinal mushroom, and vegetables production in an integrated food to waste to food biosystem. The aim of the project was to close the loop between combined dairy manure and food waste-based biogas production, mushroom, and vegetable cultivation and to create the zero-waste cultivation system. Mushroom and plant crop cultivation is usually performed through energy- and resource-intensive process (based on fossil or mineral resources), which generate wastes and the high CO₂ footprint. So far main emphasis has been on plant crops, but integrated mushroom cultivation has been found to be highly interesting from circular approach in which organic wastes are utilized for both energy (renewable biogas) and crop production in a closed system. Foodwaste treated in anaerobic digestion (AD) can be considered as a multicomponent organic fertiliser and byproduct for plant and mushroom cultivation substrate. Sustainable horticulture should reuse organic waste.

Several mushroom species ie. *Agaricus brasiliensis*, *Agaricus bisporus*, brown and white strains, *Pleurotus djamor*, *Pleurotus citrinopileatus*, *Pleurotus ostreatus* has been indicated and were grown commercially on substrate based on digestate and vegetable plant such as *Solanum lycopersicum*, *Lactuca sativa*, *Cucumis sativus*, herbs: *Ocimum basilicum*, were grown rousing spent mushroom substrate (after cultivation). Combined cultivation was incorporated in sustainable greenhouse environment in food to waste to food biosystem based on crop and substrate circulation. We are presenting here new approach for no waste strategy. We investigate and demonstrate full utilization of output CO₂, heat and organic residue (digestate) from the AD. The project can realize a 98% reduction of CO₂ footprint from Nordic greenhouse tomatoes. In this pilot there are researchers investigating microbiology of the root zone, climate control in a closed atmosphere with utilization of CO₂ from biogas, life cycle analysis, and growth of different mushroom species and various plant crops, nutrient and elemental recirculation from the substrate into mushrooms and vegetable crops, together with and biologically active compounds formulation within the crops, even pedagogical aspects of the green change and circular economy, and many more. This is the circular and future way of food production.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 751052

EVALUATION OF ANTIOXIDANT AND PROTEOLYTIC ACTIVITY OF MYCELIAL BIOMASS FORMED DURING GROWTH OF THE FUNGI *OPHIOCORDYCEPS SINENSIS* AND *PAECILOMYCES HEPIALI* ON RICE SUBSTRATES

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PRES

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LECTURE 07

Martin Pavlík¹, Anna Uhrinová², Lucia Ungvarská Maľučká²

- 1 Technical University in Zvolen, Faculty of Forestry, Department of Integrated Forest and Landscape Protection, Zvolen, Slovak Republic;
- 2 University of Veterinary Medicine and Pharmacy in Košice, Department of Chemistry, Biochemistry and Biophysics, Institute of Pharmaceutical Chemistry, Košice, Slovak Republic;

The properties of the fungus *Ophiocordyceps sinensis* (Berk.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora 2007 have been utilized for over 500 years for the treatment of many human diseases and general enhancement of strength and vitality of the human organism. The species *Paecilomyces hepiali* Q.T. Chen & R.Q. Dai 1989 has a comparable amount of biologically significant chemical compounds with interesting pharmacological activity. The goal of our research (realised within the projects of the KEGA Grant Agency No. 002TU Z-4/2022 and the VEGA Grant Agency No. 1/0071/21) was to compare the properties of the fungi *P. hepiali* with *O. sinensis* cultivated by method of solid fermentation on various substrates.

Since rice (*Oryza sativa* L.) comprises the nutritional basis for billions of people and cultivated varieties of rice are among the most important cereal crops worldwide. Rice is grown in the form of two main subspecies: the sticky, short-grained variety *japonica* or *sinica* and the non-sticky, long-grained variety *indica*. Our main goal was to evaluate the antioxidant and proteolytic activity of metabolites isolated from mycelial biomass formed by overgrowing of the species *O. sinensis* and *P. hepiali* on two rice varieties.

Proteolysis is the breakdown of proteins into amino acids via digestive enzymes. Proteolytic activity is important in preventing the accumulation of abnormal proteins in cells. Antioxidants play an important role in protecting human's health and lowering the risk of chronic diseases, including cancer and heart disease.

Isolation of metabolites was carried out utilizing reflux, ultrasound and microwave extraction, where reflux provided the highest extraction amount. The highest proteolytic activity was found in *O. sinensis* cultivated on *Oryza sativa* var. *indica*. The greatest ability to retain the stable DPPH radical scavenging activity was recorded in *P. hepiali* extract cultivated on *Oryza sativa* var. *japonica*. The chemical structure of alcohol extracts was assessed using NMR and IR spectroscopy. Based on the detailed analysis of 1D and 2D NMR spectra, Z-oleic acid, linoleic acid and D-mannitol were identified as major compounds in the methanol extracts.

Keywords: antioxidant activity, *Ophiocordyceps sinensis*, *Paecilomyces hepiali*, NMR and IR spectroscopy, *Oryza sativa*

**BOOSTING CORDYCEPIN PRODUCTION IN SOLID-STATE
CULTIVATION OF CORDYCEPS MILITARIS BY ADDING
OLEIC ACID**SHORT
ORAL
PRES

03

LECTURE 08

Ayman Turk^a, Mohamed A. A. Abdelhamid^b, Sang Won Yeon^a, Se Hwan Ryu^a, Sung Min Ko^{a,c},
Beom Seok Kim^c, Seung Pil Park^b, Bang Yeon Hwang^a, Mi Kyeong Lee^{a,*}

a College of Pharmacy, Chungbuk National University, Cheongju, 28160, Republic of Korea

b Department of Biotechnology and Bioinformatics, Korea University, Sejong 30019, Republic of Korea

c C&G Agricultural Association, Sejong 30067, Republic of Korea

Cordyceps militaris, an entomopathogenic fungus of the Ascomycetes class that forms a fungal fruit body on an insect larva, has long been used as a folk medicine in East Asia, including Korea, China, and Japan. Cordycepin, a nucleoside analogue (3'-deoxyadenosine) with a wide variety of biological effects, is primarily extracted from natural *C. militaris* in conventional procedures. Due to the low yield of cordycepin in natural *C. militaris*, natural *C. militaris* is not sufficient to meet market needs. Up to date, various fermentation techniques, mainly solid and liquid fermentation, have been established to boost cordycepin biosynthesis in *C. militaris*. According to a recent report, when *C. militaris* was cultivated on insect hosts rather than artificial media, the biosynthesis of cordycepin became more effective. Additionally, carbon and nitrogen sources have a critical impact on the growth of *C. militaris*.

In this study, six edible insect species were used as a medium for *C. militaris* fruiting body development. Among the samples investigated in our study; *Allomyrina dichotoma*, *Protaetia brevitarsis*, and *Caelifera* bearing the highest yield of cordycepin were found to have oleic acid as a major fatty acid. The cordycepin production increased by 51.4% after adding oleic acid and decreased by 13.2% after adding linoleic acid relative to the control. The addition of oleic acid up-regulated the expression of the genes involved in the cordycepin biosynthesis pathway, *cns1*, and *cns2*, during the cultivation of *C. militaris* as determined by qRT-PCR and enzyme activity analysis. In this study, the strategy of using insects with a high level of oleic acid in the solid-state culture of *C. militaris* was provided for efficient cordycepin production.

Keywords: *Cordyceps militaris*, cordycepin, oleic acid, linoleic acid, qRT-PCR, *cns1*, *cns2*

CULTURE DEGENERATION AND THE ROLE OF CORDYCEPIN/PENTOSTATIN SYNTHESIS IN THE ENTOMOPATHOGEN *CORDYCEPS MILITARIS*

SHORT
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03

LECTURE 09

Peter A. D. Wellham^{1,2,3}, Abdul Hafeez^{1,2}, Andrej Gregori⁴, Matthias Brock³, Dong-Hyun Kim², David Chandler⁵, and Cornelia H. de Moor¹

- 1 Gene Regulation and RNA Biology Laboratory, Division of Molecular Therapeutics and Formulation, School of Pharmacy, University of Nottingham, UK
- 2 Centre for Analytical Biosciences, Division of Advanced Materials and Healthcare Technology, School of Pharmacy, University of Nottingham, UK
- 3 Fungal Genetics and Biology Group, School of Life Sciences, University of Nottingham, UK
- 4 Mycomedica d.o.o., Podkoren, Slovenia
- 5 Warwick Crop Centre, School of Life Sciences, University of Warwick, UK

Cordyceps militaris (CM) is a cosmopolitan fungal pathogenic species of caterpillars and other insect hosts. The fungus produces cordycepin (3'-deoxyadenosine), a secondary metabolite known for its anti-inflammatory properties. Less however is known about cordycepin and associated protector compound pentostatin in the context the entomopathogenic growth of the fungus.

In this study, an isolate of CM was successively subcultivated; with this, decreasing levels of cordycepin production occurred, in a process named degeneration. Lower cordycepin production was shown to coincide with the reduction of expression of genes related to sexual development, as well as levels of other metabolites. This was apparent in comparisons between degenerated and control strains of the CM isolate. Cordycepin was also shown to inhibit the immune response of haemocytes in CM-infected *Galleria mellonella* caterpillars. As such, the degenerated strain was less pathogenic, having less cordycepin. These findings indicate a link between cordycepin production, sexual development, and pathogenicity in CM – which may have implications for the potential biological control of insect crop pests.

Repeated subcultivation of the CM isolate in PDA resulted in a degenerated strain (DS), which was compared to the parental control strain (PCS). Using LC-MS analysis of compounds extracted using methanol as the solvent, cordycepin and pentostatin production were confirmed to be lower in DS than PCS. Accompanied were decreased expression levels of cordycepin-pentostatin synthesis cluster genes Cns 1, 3, and 4 – assessed using RT-qPCR. Other data from LC-MS analyses showed reduction of relevant metabolites from purine metabolism, consistent with the previously-proposed cordycepin synthesis pathway with which it relates.

As well as these changes, the degenerated strain showed a decline in the expression of genes relating to sexual development, indicating a shift towards the vegetative state, away from the teleomorph. The genes in question are velvet genes VeA and VosA, which are conserved in the wider ascomycetes.

CULTURE DEGENERATION AND THE ROLE OF CORDYCEPIN/PENTOSTATIN SYNTHESIS IN THE ENTOMOPATHOGEN *CORDYCEPS MILITARIS*

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Master regulator gene *LaeA*, encoding a methyltransferase involved in regulation of velvet genes, was also downregulated in DS compared with PCS. Furthermore, *SteA* and *Ste7*, genes acting downstream of the velvet proteins as a transcription factor and MAP kinase respectively, were downregulated. We suggest that epigenetic changes, brought on by the subcultivation in PDA, are responsible for these changes in sexual development-related gene expression.

The degenerated strain also exhibited differences in infection assays using *Galleria mellonella* caterpillars, which were injected with spores. In these assays, immune response of the caterpillars in the form of melanisation, as well as responsiveness of caterpillars, and fungal hyphal emergence were assessed. The degenerated strain-injected caterpillars showed significantly-lower proportions of melanisation and hyphal emergence, than those injected with PCS spores. This indicates a reduced pathogenic response in the host caterpillars to the degenerated strain.

It was possible that cordycepin and pentostatin production (reduced in the degenerated strain) played a significant role in the reduced pathogenic ability of the degenerated strain. To test this, additional cordycepin and pentostatin were added to injection serums with spores in another infection assay. Spores of the degeneration strain supplemented with by cordycepin and pentostatin were shown to result in greater proportions of fungal hyphal emergence in the caterpillars, compared to those injected with spores alone. This indicates that cordycepin and pentostatin confer a pathogenic advantage to the fungus, supporting previous suggestions that cordycepin may play a role in host infection.

In this study, degeneration of a CM isolate, has been assessed with regard to declines in cordycepin and in a host. Additionally, evidence has been gathered to indicate the biological purpose of cordycepin and pentostatin synthesis by the fungus to improve host infection, which leads to sexual development and the formation of fruiting bodies in this species.

SESSION 04

LECTURE 01 **PINK OYSTER MUSHROOM PLEUROTUS FLABELLATUS MYCELIUM IS A VALUABLE SOURCE OF BIOLOGICALLY ACTIVE COMPONENTS**

Anita Klaus

Institute for Food Technology and Biochemistry, University of Belgrade - Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia

Pleurotus flabellatus, also known as Pink Oyster mushroom, is one of the most desirable species of the genus *Pleurotus* due to its pleasant organoleptic properties as well as extremely beneficial health effects. Favorable nutritional composition and abundance of naturally occurring compounds capable of preventing many health disorders make this mushroom a functional food and a significant source of nutraceuticals. Since it is not possible to harvest as much *P. flabellatus* from nature as the world's population needs today, and traditional solid fermentation can only produce limited quantities of fruitbodies, controlled submerged liquid cultivation that is able to provide a significant amount of mycelium is currently an acceptable solution.

This investigation aimed to: a) produce *P. flabellatus* mycelium using airlift bioreactor (ALB) submerged fermentation; b) prepare four types of mycelial extracts - exopolysaccharide (EX), endopolysaccharide (EN), hot water (WE), and hot alkali (AE) extracts; c) study their active components and biological potential.

Cultivation in 5-L ALB submerged fermentation system revealed average concentrations of between 0.015 g/L of EX and 0.45 g/L EN in 10 days. Using hot water and hot alkali solutions as extractants, the yields of WE and AE were 12.39 and 4.56% of mycelium dry weight, respectively. EN has been shown to be most effective in inhibition of lipid peroxidation ($EC_{50} = 1.71 \pm 0.02$ mg/mL) and in Cupric ion reducing antioxidant capacity (CUPRAC) assay ($EC_{50} = 2.91 \pm 0.01$ mg TE/g). AE exhibited most pronounced ability to chelate ferrous ions ($EC_{50} = 4.96 \pm 0.08$ mg/mL) and to scavenge ABTS radicals ($EC_{50} = 3.36 \pm 0.03$ mg TE/g). β -glucans and total phenols contributed most to the chelating ability and quenching of ABTS radicals. Inhibition of lipid peroxidation correlated best with total glucans, total proteins and β -glucans. Total proteins contributed most to CUPRAC antioxidant capacity. *P. flabellatus* mycelial extracts showed a bacteriostatic effect towards to almost all tested bacteria (MIC: 2.5 - 20 mg/mL).

A bactericidal ability against clinical strain of *Pseudomonas aeruginosa* was observed in WE and AE (MBC: 20.0 mg/mL), while this potential WE, EN and EX expressed against clinical strain of *Escherichia coli* (MBC: 20.0 mg/mL).

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LECTURE
01 **PINK OYSTER MUSHROOM PLEUROTUS FLABELLATUS
MYCELIUM IS A VALUABLE SOURCE OF BIOLOGICALLY
ACTIVE COMPONENTS**

Antifungal effect was determined against *Candida albicans* ATCC 10231 (MIC: 0.019–0.625 mg/mL; MFC: 0.039–2.5 mg/mL), and towards *C. albicans* clinical isolate (MIC and MFC: 10.0–20.0 mg/mL). Antiadhesion activity against clinical isolates was confirmed in all four extracts; however, WE and EX exhibited more than 50% antiadhesion potential against *Enterococcus faecalis*. A similar trend was observed in the antibiofilm potential of the extracts, and WE most intensively prevented the formation of *E. faecalis* biofilm. Comparison of cytotoxicity against colorectal carcinoma HCT 116 cells (IC₅₀: 1.8 ± 0.3–24.6 ± 4.2 mg/mL) and normal lung MRC-5 fibroblasts (IC₅₀: 17.0 ± 4.2–42.1 ± 6.1 mg/mL) showed that EN, and especially AE possess selective anticancer activity.

The exceptional biological activity of four types of *P. flabellatus* mycelium extracts indicates their pro-health properties as well as their possible use as a functional food. Also, antiadhesion and antibiofilm properties of these extracts suggest their potential application in the food industry.

Key words: *Pleurotus flabellatus*, submerged liquid fermentation, biological activity

IMMUNOMODULATORY AND ANTI-INFLAMMATORY ACTIVITY OF THE MUSHROOM EXTRACT ANDOSAN

SESSION 04

LECTURE 02

Bernd L. Fiebich¹, Joerg Gruenwald², Henning Fjøs³, Ralf Schmidt^{3*}

1 VivaCell Biotechnology GmbH, Ferdinand-Porsche-Str. 5, D-79211 Denzlingen, Germany

2 Analyze & realize GmbH, Waldseeweg 6, D-13467 Berlin, Germany

3 Mycotech Pharma, P.O. Box 52 Lilleaker, NO-0216 Oslo, Norway

Since the 1980s the medical effect of *Agaricus blazei* Murill (AbM), *Heridium erinaceus* (HE), and *Grifola frondose* (GF) have been documented in scientific studies. The commercial product AndoSan, developed and marketed by Immunopharma AS, combines these mushrooms in a unique way (82% AbM, 15% HE, and 3% GF). The unique combination and processing of these mushrooms leads to an extract which has been studied in five human pilot studies ranging from 20 to 40 patients.

These human trials have investigated AndoSan's anti-allergic, anti-tumorigenic and anti-inflammatory showing promising results. Immunopharma together with its partners (Helmholtz/ VivaCell Biotechnology GmbH) has now initiated a structured effort to investigate in the mechanism of action (MOA) of the beneficial effects of AndoSan more detail. Chemically analysing the components in AndoSan and mapping them towards a pharmacological data base and applying extensive in vitro research will be combined with the human clinical observations. This will result in a holistic understanding of the different MOA involved and potential may even result in product optimization in respect of indication to be targeted. To confirm the clinical findings showing immune boosting and anti-inflammatory activities of AndoSan, we studied the effects of AndoSan on important inflammatory parameters in primary human monocytes.

The treatment of the monocytes by AndoSan alone led to a very slight increase of the pro-inflammatory cytokines interleukin (IL)-6, IL-1beta, and tumor necrosis factor (TNF)alpha, but to a more prominent increase of G-CSF. Two chemokines, IL-8 and MIP1beta, and prostaglandin (PG)F2alpha (8-Isoprostane) were potently increased by the AndoSan batches indicating an immune boosting effect also in vitro. In line with the clinical observations, AndoSan also showed potent anti-inflammatory effects, if added to an inflammatory set up such as the induction of inflammation in human monocytes by lipopolysaccharide (LPS).

with a 10% dose of AndoSan several inflammatory parameters induced by LPS were potently decreased, especially the proinflammatory cytokines IL-1beta, TNFalpha, IL-6, and G-CSF as well as the prostanoid PGE2.

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The two chemokines IL-8 and MIP1beta and PGF2 alpha were also decreased but not as prominent as the other parameters. Further studies are needed to reveal the molecular mechanisms by which AndoSan reveals its anti-inflammatory activity.

In summary, our studies support previous scientific data, including clinical trials, suggesting that AndoSan is an immune activator and booster in the healthy situation but a strong anti-inflammatory agent in diseases with an inflammatory background.

MUSHROOM POLYPHENOLS AS IMMUNE SYSTEM BALANCERS: WHAT'S THE MECHANISM BEHIND IT AND POSSIBLE INTERACTIONS WITH DIETARY FIBERS?

SESSION 04

LECTURE 03

Maja Kozarski^{1*}, Milena Pantic¹, Vesna Lazic¹, Anita Klaus¹, Jovana Vunduk²,
Leo van Griensven³, Miomir Niksic¹

1 University of Belgrade, Faculty of Agriculture, Institute of Food Technology and Biochemistry,
Nemanjina 6, 11080 Belgrade, Serbia;

2 University of Belgrade, Institute of General and Physical
Chemistry, Studentski trg 12/V, 11158 Belgrade, Serbia;

3 Plant Research International, Wageningen

University and Research Centre, Droevendaalsesteeg 1, Wageningen 6700 AA, The Netherlands

Corresponding author address: maja@agrif.bg.ac.rs (M. Kozarski)

Mushroom polyphenols have been proposed as a new therapy that can improve the immune system. Different immune cells express multiple types of polyphenol receptors that recognize and allow cellular uptake of polyphenols, which subsequently activate signaling pathways to initiate immune responses. Polyphenols can be useful for immunocompromised patients with malignancies, viral or bacterial infections and chronic disorders of organ systems, as well as for those whose immunity is naturally weakened. Cosmetic formulations rich in mushroom polyphenols have the potential to regulate inflammatory skin disorders, as well as eczema or atopic dermatitis and photocarcinogenesis.

As primary antioxidants, polyphenols have an important role in preserving immune cells in a reduced environment and in protecting them against oxidative damages and immunosuppression as well as maintaining their suitable function. As signaling pathway modulators, mushroom polyphenols can affect immune cell regulation, cytokine synthesis, and gene expression, in both innate and adaptive immune responses. There are different pathways in the integrated immunomodulatory polyphenol response, such as: 1) the nuclear NF- κ B signaling pathway leading to suppression of various inflammatory cytokines expression, and enzymes such as COX-2; 2) MAPK signaling pathways that play a key role in many fundamental cellular processes such as cell growth, proliferation, death and differentiation, and also regulate gene transcription and the activity of transcription factors involved in inflammation; 3) arachidonic acid signaling pathway leading to a decrease in the release of inflammatory mediators; and 4) the Nrf2 / ARE signaling pathway involved in the activation of genes encoding cytoprotective and antioxidant enzymes.

Given that polyphenols represent great potential in the design of immune-boosting formulations in line with their widespread structural diversity, it should be noted that some functionality issues require further clarification. For instance different observations and conclusions were reported by the scientists in absence of enzymes involved in their biosynthesis steps.

MUSHROOM POLYPHENOLS AS IMMUNE SYSTEM BALANCERS: WHAT'S THE MECHANISM BEHIND IT AND POSSIBLE INTERACTIONS WITH DIETARY FIBERS?

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Concerning the dietary application of mushroom polyphenols, it is necessary to mention that after oral consumption they are recognized by the human body as xenobiotics and often a small amount is absorbed in the intestine. Likewise, there are significant differences between the activities of the metabolic form of phenols and their form in the mushroom nutraceutical matrix. For an example, dihydroferulic acid, a metabolite of the ferulic acid, expresses anti-inflammatory activity, opposite to metabolites derived after sulfation and glucuronidation. The scientific focus is on improving and increasing polyphenols bioavailability by designing colloidal systems and using nanosystems. Moreover many studies have found that polyphenols can interact with macromolecules like dietary fibers, i.e. chitin and β -glucans. It has been shown that these interactions can affect the bioaccessibility of polyphenols in a food matrix as well as in nutraceutical formulations. Therefore, chitin and β -glucans have an impact on polyphenols' immunomodulating activities if they were applied together in commercial formulations. Another disadvantage in the commercialization of polyphenol-based formulations is quantitative and qualitative variations in their content among different mushroom species. As secondary metabolites with a protective role e.g. phenols, their content in mushrooms depends on the locality and environmental conditions of growth. Using extracts or preparations that are based on mycelia grown under controlled conditions may be the solution for the reduction of the natural variability in polyphenol composition.

Integrated information on all aspects of functionality will confirm the use of polyphenol-rich mushroom formulations as effective enhancers of immunity.

Keywords: mushrooms, polyphenols, immunomodulators, nutraceuticals, natural cosmetics

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Acknowledgment

This investigation is the result of research within the "Agreement on the implementation and financing of scientific research work in 2022 between the Faculty of Agriculture in Belgrade and the Ministry of Education, Science and Technological Development of the Republic of Serbia", contract record number: 451-03-68/2022-14/200116, and supported by the Science Fund of the Republic of Serbia, #Grant No: 7748088, "Composite clays as advanced materials in animal nutrition and biomedicine-AniNutBiomedCLAYs".

**ANTIDIABETIC EFFECTS OF GANODERMA LUCIDUM
FRUITING BODY POLYSACCHARIDE EXTRACTS
OBTAINED THROUGH NOVEL PURIFICATION PROCEDURES**

SESSION 04

LECTURE 04

Nathan Scott, Neil Tanday, Keith Thomas, Victor Gault Coleraïne

Ulster University, UK

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia and has become a leading cause of morbidity with prevalence rising at an alarming rate. *Ganoderma lucidum* (*G. lucidum*) polysaccharides (GLPs) have been utilized by traditional Chinese medicine over thousands of years for various conditions. Recently, numerous studies reported pharmacological activities including antidiabetic effects. Novel extraction and purification procedures have led to the discovery of many bioactive compounds within the mushroom.

The aims of the present study were to isolate GLPs from a fruiting body water extract using an ionic liquid (GLP-IL) and fractional precipitation using ethanol (GLP-FR) and investigate the antidiabetic effects *in vitro* and *in vivo*. Extracts GLP-IL and GLP-FR were quantified for their α and β -glucan content using the Megazyme β -Glucan (Yeast & Mushroom) Assay Kit and protein content with Bradford reagent. *In vitro* effects of extracts were assessed using rodent derived BRIN BD11 insulin secreting pancreatic beta cells.

Cell viability was measured using MTT assay, effects on starch digestion were determined, and acute insulin release quantified using radioimmunoassay. *In vivo* effects were recorded in non-diabetic lean and induced diabetic C57BL/6 mice using a high fat fed (HFF) / low dose streptozotocin (STZ) compromised beta-cell model. Acute glucose tolerance tests (GTT) were carried out and long-term effects on kidney and liver toxicity, body weight, food intake and blood glucose were monitored over 28 days, and GTTs and insulin tolerance (ITT) measured at the end of the study. The novel extract purification procedures are suggested to produce proteoglycans containing 55.39% β -glucan and 1.56% α -glucan content with 18% protein present (GLP-IL) and 56.4% β -glucan and 1.66% α -glucan content with 14.5% protein present (GLP-FR). No significant reduction in *in vitro* cell viability (in the range of 1-50 $\mu\text{g/ml}$) was observed with both extracts. Both extracts caused significant dose-dependent reductions in starch digestion (0.05-50 $\mu\text{g/ml}$) causing a 66% (GLP-FR) and 24% (GLP-IL) reduction at 50 $\mu\text{g/ml}$ compared to control. In hyperglycaemic conditions (16.7mM glucose), only GLP-FR caused a significant increase in insulin release by 85% compared to glucose alone with no effects on insulin release at hypoglycaemic or basal conditions. Both extracts (100mg/kg, *i.p.*) significantly reduced blood glucose after 15 minutes in non-diabetic mice compared to saline control in acute GTTs. Following 28 days treatment no toxicity was observed with no significant change in urea and alanine aminotransferase (ALT) compared to pre-treatment.

**ANTIDIABETIC EFFECTS OF GANODERMA LUCIDUM
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No significant change in body weight and food intake was recorded and a modest reduction in blood glucose was observed. Terminal GTTs significantly reduced blood glucose only at 15 minutes after

No changes to insulin tolerance were recorded after treatment. This study suggests extracts may act in reducing blood glucose through slowing starch digestion and increasing insulin release. These findings indicate that both extracts possess antidiabetic potential in vitro and in vivo which may serve as promising therapies for clinical application of medicinal mushroom extracts in the management of Type 2 diabetes.

Key words: Ganoderma lucidum, proteoglycan, diabetes, ionic liquid, insulin release

Key topic 4: Biochemistry and pharmacology of medicinal mushrooms active compounds

NEPHROPROTECTIVE EFFECT OF A METHANOLIC EXTRACT OF TWO GANODERMA SPECIES AND ITS ASSOCIATION IN AN IN VITRO MODEL OF CISPLATIN-INDUCED TUBULOTOXICITY

SESSION 04

LECTURE 05

Sébastien Sinaeve¹, Cécile Husson², Marie-Hélène Antoine², Stéphane Welti³,
Cory Decock⁴, Cedric Delporte^{1,5}, Caroline Stévigny¹, Joëlle Nortier²

1 RD3-Pharmacognosy, Bioanalysis and Drug Discovery Unit, Faculty of Pharmacy, Université libre de Bruxelles, Brussels, Belgium

2 Laboratory of Experimental Nephrology, Faculty of Medicine, Université libre de Bruxelles, Brussels, Belgium

3 Laboratoire des Sciences Végétales et Fongiques, UFR Pharmacie, Université Lille 2, Lille, France

4 Mycothèque de l'Université Catholique de Louvain (BCCM/MUCL), Louvain-la-Neuve, Belgium

5 Analytical Platform of the Faculty of Pharmacy (APFP), Faculty of Pharmacy, Université libre de Bruxelles, Boulevard du Triomphe, 1050 Brussels, Belgium

Cisplatin is currently used as a first-line cancer treatment, such as for testicular, ovarian or pulmonary cancers. Their nephrotoxicity remains a real problem. Acute kidney injury induced by cisplatin is located on proximal tubular cells, causing necrosis and possibly subsequent interstitial fibrosis and chronic dysfunction. These severe side effects can lead to a cessation of the patient's treatment. Currently, there is no effective prophylactic action to reduce cisplatin nephrotoxicity, besides hyperhydration of the patient.

The aim of the present work is therefore to identify new prophylactic therapy. For this, natural products can be studied, in this case, the interest of potential new medicinal mushroom extracts. Among 13 mushroom extracts, the methanolic extracts of *Ganoderma parvigibbosum* Welti & Courtecuisse, *Ganoderma tuberculosis* Murrill and their association were selected to study the effects on human proximal tubular cells (HK-2) intoxicated with cisplatin.

HK-2 cells were grown in 75cm² sterile flasks using DMEM low glucose (1mg/mL), supplemented with FBS (10%), L-Glutamin and a mix of Penicillin/Streptomycin. Dried mushrooms were grounded and extracted 3 times by methanol, and evaporated extracts are stored at -20°C. A viability assay allowed to determine the work concentration of extracts range has been done. After that, tests were performed after a pretreatment of 1h with the extracts before adding cisplatin at a concentration of 20 µM. Viability assays (CCK-8) and antioxidant activity (DPPH) were done in 96-well. The intracellular concentration of β-catenin and calcium, Caspase-3, p53, cytochrome C, IL-6, NFκB, the membranal expression of KIM-1, and finally the ROS production (H2DCFDA) were studied by flow cytometry.

**NEPHROPROTECTIVE EFFECT OF A METHANOLIC
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Tests have shown that methanolic extracts of *G. parvigibbosum* and *G. tuberculosum* (10 µg/mL) and their association (5 + 5 µg/mL) prevented the loss of viability after a 24h incubation. They also have prevented apoptosis and the induction pathway after 24h. *G. parvigibbosum* and the association of the two mushroom extracts have also prevented the increase of caspase-3 and intracellular β-catenin. Finally, *G. parvigibbosum* was the only one to prevent ROS overproduction and having a scavenger activity at work concentration. None of them showed prevention in the increase of IL-6 and NFκB or the membrane expression of KIM-1.

G. parvigibbosum appears to be therefore more beneficial than *G. tuberculosum* and the association of the two mushroom extracts by acting also on the ROS overproduction. In conclusion, in this study, the extracts have shown a significant activity in the prevention of the pro-apoptosis pathway rather than pro-inflammatory prevention. Further investigation about metabolomic analysis is undergoing and will be presented to identify the precise activity and chemical content of these extracts.

SHORT ORAL PRESENTATION / session NO 4

LECTURE **VOLATILE ORGANIC COMPOUNDS IN TUBER MAGNATUM**
01 SPECIMENS FROM GREECE DETECTED BY SPME-GC-MS

Nefeli Sofia Sotiropoulou¹, Vassileios Daskalopoulos², Elias Polemis²,
Georgios I. Zervakis², Petros Tarantilis¹

1 Laboratory of General Chemistry, Agricultural University of Athens, Iera Odos 75,
11855 Athens, Greece

2 Laboratory of General and Agricultural Microbiology, Agricultural University of Athens, Iera Odos 75,
11855 Athens, Greece

Several species of the genus *Tuber* ("true truffles") are widely recognized for their excellent organoleptic characteristics. Among them, the most popular and highly prized species is *Tuber magnatum* Picco, often referred to as "white truffle" or "Piedmont truffle". Until recently, *T. magnatum* was thought to occur in Northern Italy only, but during the past 20-25 years it became evident that its distribution also extends to the Balkan Peninsula; since 2013 it was confirmed to exist in Greece as well, with Mt Olympus being its southernmost limit in the Balkans.

Nowadays, significant quantities of high-quality white truffles are collected and traded in Greece; therefore, this particular activity is of commercial importance. The aim of this work is to detect the major volatile organic compounds (VOCs) that contribute to the characteristic aroma of *T. magnatum* specimens collected from various regions of Greece. The VOCs of 14 fresh samples of *T. magnatum* were trapped by solid phase microextraction (SPME) using SPME fiber assembly Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS). Qualitative and quantitative (%) analysis of VOCs was accomplished by gas chromatography combined with mass spectrometry (GC-MS). According to our results, 13 VOCs were identified in total; they belong to the groups of sulfur compounds, ketones, alcohols, aldehydes, and hydrocarbons. The maximum and minimum number of compounds detected in truffle samples were ten and four, respectively. The most abundant VOC existing in all *T. magnatum* samples was bis (methylthio) methane, ranging from 70.9% to 87.7% (average 80,1%); this sulfur compound with alliaceous (garlic) flavor is widely prescribed as the key component of white truffles aroma, and contributes substantially to their unique gastronomic value. .

sulfide (sulfury, onion, corn and cabbage flavors) and 2-acetyl-5-methylfuran (nutty and hay-coumarin flavors) were also detected in all truffle samples, but in lower relative percentages, i.e., 4.63-17.7% and 0.34-3.56%, respectively.

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The other 10 compounds were not present in all samples, and in most cases, they were detected at low concentrations. These VOCs are considered as the main (or key) aroma compounds of white truffles, and were also found in specimens of *T. magnatum* originating from other Balkan countries and Italy.

Acknowledgements. The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "2nd Call for H.F.R.I. Research Projects to support Post-Doctoral Researchers" (Project Number: 1057).

IDENTIFICATION OF TRITERPENES IN GANODERMA LINGZHI AND GANODERMA LUCIDUM AND DYNAMICS OF FUNGAL RESPIRATION DURING GROWTH

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LECTURE 02

Martin Pavlík¹, Zhou Shuai², Feng Na², Zhang Jingsong², Peter Fleischer¹,
Peter Fleischer jr.¹, Slavomir Rell³

1 Technical University in Zvolen, Department of Integrated Forest and Landscape Protection, Zvolen 96001, Slovak Republic;

2 National Engineering Research Centre of Edible Fungi, Key Laboratory of Applied Mycological Resources and Utilisation, Ministry of Agriculture, Institute of Edible Fungi, Shanghai Academy of Agriculture Sciences, Shanghai 201403, People's Republic of China;

3 National Forest Centre, Forest Protection Service Centre, 96901 Banska Stiavnica, Slovak Republic

Intense research focused on the identification of biologically active compounds in fungi of the genus *Ganoderma* has already produced many important results. Mainly the species *Ganoderma lingzhi* and *Ganoderma lucidum* have a long history of recognition and surely great potential for further utilization. The natural occurrence of these fungi and their fruiting bodies in the forests of Slovakia and China is the basis of joint research of the Technical University in Zvolen (Slovakia) and the Shanghai Academy of Agricultural Sciences (China).

The current contribution provides the newest results of research into triterpenes in chosen *Ganoderma lingzhi* Sheng H. Wu, Y. Cao & Y.C. Dai 2012 and *Ganoderma lucidum* (Curtis) P. Karst. 1881 strains along with new knowledge on the physiology of these fungi in relation with growth conditions in the process of cultivation. Using UPLC-MS/MS method, significant amounts of triterpenes with moderate polarity (Ganoderic acid, Ganoderenic acid, Lucidenic acid, etc.) in strains of *G. lingzhi* and triterpenes with low polarity (Ganoderol, Ganoderiol, Lucidone, Ganoderal, Ganolactone, Ganoderone, Ganoderate, etc.) in strains of *G. lucidum*, were identified. It is known that triterpenes are compounds effective in the treatment of various forms of cancer, liver and kidney disease, prostate treatment, pain reduction, and their antioxidant properties protect cells from aging and damage. The highest proportion of triterpenes was recorded in young fruiting bodies compared with their content in primordia and mature fruiting bodies in both fungal species.

During our research, the maximum values in total triterpenoid content was obtained in the production strain *G. lingzhi*: 21.92±3.82 mg/g in a young fruiting body, 19.55±0.44 mg/g in a mature fruiting body and 1.27±0.13 in primordium. In the species *G. lucidum*, these values were 6.69±0.54 mg/g in a young fruiting body, 3.92±0.11 mg/g in a mature fruiting body and 0.96±0.50 mg/g in primordium.

**IDENTIFICATION OF TRITERPENES IN GANODERMA
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The physiological activity of fungi in the process of growth on oak and beech chip substrates in various climatic conditions was evaluated based on measurement of fungal respiration. Measurement of CO₂ production was carried out over a period of 160 days using a method based on absorption of infrared radiation by CO₂ molecules in a closed chamber by infrared gasometric probes (IRGA). Respiration since inoculation is balanced or moderately decreasing (values 0.75 to 0.40g CO₂ kg⁻¹ h⁻¹), and increases markedly with the development of primordia (max. 1.6g CO₂ kg⁻¹ h⁻¹). Fruiting body production is connected with a decrease in respiration (under 0.9g CO₂ kg⁻¹ h⁻¹).

Our research (within the project KEGA 002TU Z-4/2022 and PERT G2022003) will bring new knowledge about the optimal conditions for fungal growth. It is very important in terms of cultivating sufficient amounts of fruiting bodies containing desired bioactive compounds.

KEY WORDS: Ganoderma lingzhi, Ganoderma lucidum, triterpene, UPLC-MS/MS, CO₂ production

CHEMICAL CHARACTERIZATION AND BIOACTIVE PROPERTIES OF THE EDIBLE AND MEDICINAL HONEY MUSHROOM ARMILLARIA MELLEA (AGARICOMYCETES) FROM SERBIASHORT
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LECTURE 03

SHORT TITLE: THE BIOACTIVITY OF ARMILLARIA MELLEA**Nevena Petrović,^{a*} Marijana Kosanić,^a Tomislav Tosti,^b Ivana Srbljak,^c Ana Đurić,^c****a** Faculty of Science, Department of Biology and Ecology, University of Kragujevac, Radoja Domanovića 12, 34 000, Kragujevac, Serbia;**b** Faculty of Chemistry, University of Belgrade, 11158 Belgrade, Serbia;**c** Institute of Oncology and Radiology of Serbia, 11000 Belgrade, Serbia

** Address all correspondence to: Nevena Petrović, Faculty of Science, Department of Biology and Ecology, University of Kragujevac, Radoja Domanovića 12, 34 000, Kragujevac, Serbia; Tel.: +381 34336223; Fax: +381 34335040; E-mail: nevena.n.petrovic@pmf.kg.ac.rs*

Honey mushroom, *Armillaria mellea*, is an edible and medicinal lignicolous basidiomycete. In this study, we investigated the chemical composition and bioactive properties of its methanolic and acetonetic extracts. The chemical characterization of extracts was done with HPLC-DAD-MS/MS method. The results showed that potassium was the most abundant mineral; chlorogenic acid the most abundant polyphenol; malic acid the most abundant organic acid, and among carbohydrates, the most abundant were sorbitol, glucose, fructose and saccharose. Antioxidative activity was assessed by DPPH (IC₅₀ of the methanolic extract was 608.32 µg/mL and of the acetonetic extract 595.71 µg/mL) and reducing power assays (the results ranged from 0.034-0.102 µg/mL).

Total phenolic content was determined as gallic acid equivalent (methanolic extract - 4.74 mg GAE/g; acetonetic extract - 5.68 mg GAE/g). The microdilution assay was used to evaluate the antimicrobial activity of the extracts and the results ranged from 1.25 to 20 mg/mL. The antidiabetic effect of extracts was tested by the α-amylase (the results ranged from 34.90 to 41.98%) and α-glucosidase assays (the results were in the range 0.55-2.79%).

The neuroprotective activity was explored by the acetylcholinesterase inhibition assay (the results were in the range 1.94-7.76%). The microtetrazolium assay was used to investigate the cytotoxic activity of extracts (the IC₅₀ values ranged from 212.06 to >400 µg/mL). Although some results suggest that some activities of the extracts are relatively moderate, the honey mushroom can still be considered an excellent food source and a natural source of bioactive compounds with medicinal value.

CHEMICAL CHARACTERIZATION AND BIOACTIVE PROPERTIES OF THE EDIBLE AND MEDICINAL HONEY MUSHROOM ARMILLARIA MELLEA (AGARICOMYCETES) FROM SERBIA

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SHORT TITLE: **THE BIOACTIVITY OF ARMILLARIA MELLEA**

KEYWORDS: antidiabetic, antimicrobial, antioxidative, Armillaria mellea, bioactivity, chemical characterization, cytotoxic, methanolic and acetonc extracts, neuroprotective

ABBREVIATIONS: ATCC, American Type Culture Collection; DAD, diode array detector; DMSO, dimethyl sulfoxide; DPPH, 1,1-diphenyl-2-picryl-hydrazil; ICP-OES, inductively coupled plasma optical emission spectrometry; MIC, minimum inhibitory concentration; MTT, microculture tetrazolium test.

**CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY
OF TWO LACTARIUS SPECIES FROM SERBIA**SHORT
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Marina Kostić,¹ Marija Ivanov,¹ Ana Ćirić,¹ Cristina Caleja,² Eliana Pereira,² Lillian Barros,² Dejan Stojković,¹ Marina Soković,¹ Jasmina Glamočlija¹

1 Department of Plant Physiology, Institute for Biological Research "SinišaStanković" - National Institute of Republic of Serbia, University of Belgrade, BulevarDespotaStefana 142, 11000 Belgrade, Serbia

2 Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

Mushrooms are highly appreciated for their nutritional value and therapeutic effects. They have found wide application in pharmacy, medicine and as nutraceuticals, mostly due to the synergistic activity of their bioactive compounds. This study aimed to investigate the chemical composition and antioxidant, antibacterial, antibiofilm, and cytotoxic potential of two *Lactarius* species, *L. piperatus* and *L. vellereus*, wild - growing mushrooms from Serbia.

Mannitol and trehalose were the most abundant free sugars and malic and citric acids were the major organic acids detected. The four tocopherol isoforms were found in *L. piperatus*, and the β -tocopherol was the only tocopherol isoform found in the *L. vellereus* fruiting body. Regarding phenolic compounds, cinnamic and protocatechinic acids were identified in the prepared methanolic and ethanolic extracts, which displayed antioxidant activity through the inhibition of thiobarbituric acid reactive substances (TBARS) formation (IC₅₀ 0.30-1.39 mg/mL).

Antibacterial activity of *Lactarius* ethanolic and methanolic extracts was investigated against bacteria isolated from tonsils from the patient with chronic tonsillitis. All tested extracts demonstrated promising results regarding antibacterial and antibiofilm activity. In both extracts, the most promising activity was observed for *Streptococcus* spp. (0.40–6.25 mg/mL as the minimal inhibitory concentration). Ethanolic extracts of tested mushrooms showed a stronger potential to inhibit the formation of *Staphylococcus aureus* biofilm than methanolic. *L. vellereus* ethanolic extract showed highest inhibition activity (86%) against *S. aureus* biofilm formation. Cytotoxic potential of *L. piperatus* and *L. vellereus* extracts were examined against four tumor cell lines (HeLa, MCF-7, HepG2, NCI-H460) and porcine liver primary culture - PLP2. The most promising cytotoxic effect was caused by the *L. vellereus* ethanolic extract on cervical carcinoma cell line (HeLa). However, extensive screening of biological activities is needed in future studies that will allow the exploitation of these mushrooms at an industrial level, taking advantage of its benefits for human health.

CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF TWO LACTARIUS SPECIES FROM SERBIA

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This research is funded by the Serbian Ministry of Education, Science and Technological Development [Contract No. 451-03-68/2022-14/ 200007].The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Programme PT2020 for financial support to CIMO (UIDB/00690/2020); national funding by FCT, P.I., through the institutional scientific employment program-contract for L. Barros's contract though the celebration of program-contract foreseen in No. 4, 5 and 6 of article 23° of Decree-Law No. 57/2016, of 29th August, amended by Law No. 57/2017, of 19th July;research contract of C. Caleja (Project AllNatt, POCI- 01-0145-FEDER-030463)and of E. Pereira (Project Mobilizador Norte-01-0247-FEDER-024479: ValorNatural®)

**SECONDARY METABOLITES FROM BUGLOSSOPORUS
QUERCINUS (FOMITOPSIDACEAE, BASIDIOMYCOTA),
A THREATENED BROWN-ROT POLYPORE SPECIES**SHORT
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LECTURE 05

Viktor Papp¹, Kristóf Felegyi², Zsófia Garádi^{2,3}, Imre Boldizsár², Gabriella Spengler⁴, Szabolcs Béni² & Attila Ványolós²**1** Department of Botany, Hungarian University of Agriculture and Life Sciences, Hungary**2** Department of Pharmacognosy, Semmelweis University, Budapest, Hungary**3** Directorate of Drug Substance Development, Egis Pharmaceuticals Plc, Hungary**4** Department of Medical Microbiology and Immunobiology, University of Szeged, Hungary

The anatrodia clade (Basidiomycota, Polyporales) includes wood-inhabiting fungi that produce a brown-rot type of decay. Amongst of these many are known as significant and well-studied medicinal mushroom species, such as *Fomitopsis betulina*, *F. pinicola*, *Laetiporus sulphureus*, *Laricifomes officinalis* or *Wolfiporia hoelen*. The *Buglossoporus* genus also belongs to this clade, but according to our literature review, there is no available study on its chemical composition and/or biological activity. *Buglossoporus quercinus* is the only known representative of the genus in Europe, and considered to be a rare and threatened species associated with old oak trees (*Quercus* spp.). Therefore we aimed to examine the chemical profile of fruiting bodies of *B. quercinus*, to isolate the most important secondary metabolites and determine the potential pharmacological activities.

The examined basidiocarps were collected from *Quercus petraea* logs in Hungary and identified based on morphological features. The freeze-dried *B. quercinus* samples were ground and extracted with methanol on room temperature. The dried extract was dissolved in aqueous methanol, then subjected to solvent-solvent partition using n-hexane, chloroform and ethyl-acetate. The chloroform and n-hexane fractions were then separated in multiple steps by flash chromatography on normal and reversed phases, using n-hexane-acetone and water-methanol eluent systems, respectively. Final purification was performed by reversed phase HPCL using water-methanol or water-acetonitrile as eluent systems. The purification process was monitored by TLC and UHPLC-MS. The structure of compounds was established by extensive spectroscopic analyses, including 1D and 2D NMR and UHPLC-MS/MS techniques.

In-depth chemical analysis led to the isolation of eight fungal metabolites, all belong to the vast group of triterpenes. Among them two constituents are new natural products, while the remaining six are identified for the first time in this species. The current study demonstrated that *B. quercinus* is a valuable source of fungal triterpene steroids. Further biological studies are planned to examine the potential pharmacological properties of the isolated compounds and to highlight the structure-activity relationships.

Keywords: Polyporales, triterpenes, steroids

**EVALUATION OF LIGNINOLYTIC ENZYMES ACTIVITY
OF SPENT PLEUROTUS OSTREATUS SUBSTRATE**SHORT
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LECTURE 06

Jovana Vunduk^{1*}, Sonja Veljović¹, Saša Đurović¹, Zorica Svirčev²**1** Institute of General and Physical Chemistry, Belgrade**2** University of Novi Sad, Faculty of Science, Novi Sad**Corresponding author: vunduk.jovana@hotmail.com*

Nowadays, mushroom cultivation is very intensive in order to produce sufficient quantity for the growing global market. Consequently, a large vast of the spent mushroom substrate (SMS) is generated, which presents increasing ecological and environmental issues. After the harvest is done SMS is still "alive", rich in fungal enzymes, which offers the additional multi-production stream possibility and adds to the economic efficiency of mushroom cultivation. Ligninolytic enzymes have an important role in the degradation of lignin and thus have a wide industrial application in many areas of industries (food, biofuel etc.).

One of the top three most popular and most produced mushrooms is *Pleurotus ostreatus* or oyster mushroom. To broaden the applicability of *P. ostreatus* SMS, this material was investigated as a source of ligninolytic enzymes (lignin peroxidase, manganese peroxidase and laccase). The different extraction conditions (solvent, pH and time of extraction) were applied for enzyme isolation, citrate buffer (pH 4.5), distilled water (pH 7.0) and phosphate buffer (pH 8.0) for 6, 24, and 30 hours at room temperature.

The protein content of crude extracts was determined by Lowry's method. The manganese peroxidase activity was determined by the oxidation of phenol red, while lignin peroxidase activity was measured by oxidation of Azure B. Guaiacol was used for the determination of laccase activity.

The protein content was in the range of 439.63 to 669.26 mg/L. The increasing extraction time had a different effect on the protein extraction efficiencies depending on the solvents. Alkali and neutral conditions were favorable for the total protein content. In general, the lignin peroxidase activity was lower than 1U/L, whereas the highest activity was detected in samples obtained from the SMS exposed 24 h to the acidic and neutral solution (0.92 U/L). Manganese peroxidase activity was from 0.011 to 3.139 U/L, while laccase activity ranged from 0.842 to 32.015 U/L.

EVALUATION OF LIGNINOLYTIC ENZYMES ACTIVITY OF SPENT PLEUROTUS OSTREATUS SUBSTRATE

SHORT
ORAL
PRES

04

LECTURE 06

This study was performed to optimize the isolation of ligninolytic enzymes from SMS by using different extraction parameters (extraction time and solvent). Analysed *P. ostreatus* spent substrate appeared to be a more plentiful source of laccase and manganese peroxidase than of lignin peroxidase. The results showed that the utilization of SMS as a source of ligninolytic enzymes provides viable use for this waste as a low-cost and readily available substrate.

Acknowledgment: This research was supported by the Science Fund of the Republic of Serbia, #7726976, Integrated Strategy for Rehabilitation of Disturbed Land Surfaces and Control of Air Pollution – RECAP, within Program IDEJE.

ANTIBACTERIAL AND ANTIOXIDATIVE ACTIVITY OF CHITOSAN AND CHITOSAN HYDROCHLORIDE ISOLATED FROM MUSHROOMS

SHORT
ORAL
PRES

04

LECTURE 07

Vesna Lazić, Aleksandra Ilić, Marina Dunčević, Maja Kozarski, Miomir Nikšić, Milena Pantić

University of Belgrade, Faculty of Agriculture, Institute of Food Technology and Biochemistry, Belgrade, Serbia

Chitosan is a linear polysaccharide formed from β -D-glucosamine randomly distributed chains and N-acetyl-D-glucosamine, which is obtained in the process of deacetylation of chitin. Only a small number of mushrooms have chitosan as one of the components in their cell wall. It is assumed to exhibit antimicrobial, antifungal and antiviral activity. The task of this research was to test the antioxidant and antibacterial activity of commercial products chitosan (Aldrich, Germany) and chitosan hydrochloride (Glenthams Life Sciences, UK). The degree of deacetylation of both products was about 85%. Commercially, *Agaricus bisporus* is mostly used for chitosan production.

The antibacterial potential was tested by the microdilution method (2.5-0.019 mg/mL) using one Gram-positive bacteria *Enterococcus faecalis* ATCC 29219 and one Gram-negative *Escherichia coli* ATCC 25922 and the reduction of the number of microorganisms was monitored using the highest tested concentrations of 2.5 mg/mL samples. Chitosan and chitosan hydrochloride showed a significant effect on the tested microorganisms. The minimal inhibitory (MIC) concentration of chitosan hydrochloride against *E. coli* was 1.25 mg/mL, while a concentration of 2.5 mg/mL was the minimal bactericidal concentration (MBC) for the same bacteria, while the MIC against *E. faecalis* was a concentration of 2.5 mg/mL. There was also a complete reduction in the number of *E. coli*. Commercial chitosan had a stronger effect on *E. faecalis* (MIC 1.25 and MBC 2.5 mg/mL), than on *E. coli* where a significant growth inhibition was also observed (MIC 2.5 mg/mL, MBC was not detected for the tested concentrations).

The function of antioxidants is to remove reactive oxygen species and to prevent the occurrence and spread of oxidative stress. By examining the antioxidant activity, the absence of DPPH radical scavenging ability was determined for the tested samples with a concentration of 5mg/ml, while the ability to chelate Fe^{2+} was low and amounted to 27.22 % for chitosan and 41.56 % for chitosan hydrochloride. In further research, it is necessary to find the best way to extract chitosan from mushrooms in order to obtain samples with pronounced biological activity.

Keywords: Biological activity, chitosan, mushrooms

Acknowledgment: This research was supported by the Science Fund of the Republic of Serbia, #Grant No: 7748088, "Composite clays as advanced materials in animal nutrition and biomedicine- AniNutBiomedCLAYS".

**COMPARATIVE ANALYSIS OF BETA-GLUCAN CONTENT
AND ANTIOXIDANT ACTIVITIES OF SOME EDIBLE
WILD-GROWING SERBIAN MUSHROOMS**SHORT
ORAL
PRES

04

LECTURE 08

Bojana Vidović^{1*}, Margarita Dodevska², Milica Zrnić Ćirić¹, Vanja Todorović¹, Slađana Šobajić¹**1** University of Belgrade-Faculty of Pharmacy, Department of Bromatology, Belgrade, Serbia**2** Institute of Public Health of Serbia "Dr Milan Jovanović Batut", Center for Hygiene and Human Ecology, Belgrade, Serbia* email: bojana.vidovic@pharmacy.bg.ac.rs

In addition to their nutritional value, wild-growing mushrooms have been gaining attention as sources of biologically active compounds with health-promoting effects. The objective of this study was to analyze the β -glucan, total phenolic contents, and antioxidant activities of seven wild mushrooms (*Boletus edulis* Bull., *Cantharellus cibarius* Fr., *Craterellus cornucopioides* (L.) Pers., *Fistulina hepatica* (Schaeff) With., *Hydnum repandum* L., *Lactarius deliciosus* (L.) Gray, and *Morchella esculenta* (L.) Pers.) collected in Serbia.

The contents of β -glucans were determined by the K-YBGL β -glucan Assay Kit (Megazyme, Bray, Ireland), while total phenolic contents of mushroom fruiting bodies were assessed using the Folin-Ciocalteu assay. The aqueous extracts of the fruit bodies of wild mushrooms, were evaluated for ferric reducing (FRAP) and radical scavenging (DPPH, ABTS) activities. The β -glucan content among analyzed mushrooms varied from 21.8-28.4 g/100 g dry weight. The highest total phenolic content was found in *Boletus edulis*, followed by *Morchella esculenta*. Although differences were observed among species, all analyzed mushroom extracts showed reducing and radical scavenging activity. Overall, further studies are needed to explore their nutritional properties and nutraceutical potential.

Keywords: wild mushrooms, beta-glucan, polyphenols, antioxidant activities, nutraceutical

SESSION 05

LECTURE **HERICIUM ERINACEUS NEUROPROTECTION OF CHOROID PLEXUS AND BLOOD BRAIN BARRIER IN WILD-TYPE FRAIL MICE DURING AGING**

01

Rossi Paola¹, Priori Erica Cecilia¹, Ratto Daniela¹, Anthea Desiderio², Lorenzo Goppa², Savino Elena², Bottone Maria Grazia¹, Roda Elisa^{1,3}

1 Department of Biology and Biotechnology "L. Spallanzani", University of Pavia, 27100 Pavia, Italy.

2 Department of Earth and Environmental Sciences, University of Pavia, 27100 Pavia, Italy.

3 Laboratory of Clinical & Experimental Toxicology, Pavia Poison Centre, National Toxicology Information Centre, Toxicology Unit, Istituti Clinici Scientifici Maugeri IRCCS, 27100 Pavia, Italy.

Aging is a natural process that affects all living organisms, and it is characterized by a deterioration in physiological processes (Lopez-Otin et al., 2013). The decrease in physiological functions has different rates from individual to individual, making aging a heterogeneous process. Among elderly, frailty is a geriatric syndrome implicated in poor quality of life and adverse health consequences and it is associated with cognitive decline. Thus, frailty is related to aging, but it does not reflect chronologic age, showing the heterogeneity of the aging course between different subjects (Piggott and Tuddenham, 2020). *Hericium erinaceus* is a medicinal mushroom that enhances longevity (Li et al., 2019; Roda et al., 2021) and displays several health promoting properties, particularly on Central Nervous System (CNS) (Ratto et al., 2019).

In this study, we investigated the effect of oral supplementation with an Italian strain of *H. erinaceus* (He1) on frail elderly wild-type mice. In particular, we supplemented frail mice for two months with a He1 blend of mycelium and sporophore, at a content of 1 mg/die, thus mimicking human's supplementation.

Firstly, by employing HPLC-UV-ESI/MS and specific standards, we measured the amount of nootropic (erinacine A, hericenones C and D) and antioxidant (L-ergothioneine) metabolites in He1 extracts. Next, by using spontaneous behavioral tests (Emergence and Novel Object Recognition Tasks), we demonstrated that He1 supplementation significantly improved recognition memory in frail elderly mice. In order to study the possible mechanism involved, we investigated the blood brain barrier (BBB) and the choroid plexus structure, integrity, and permeability. The BBB is composed of basal lamina, astrocytic end feet processes, pericytes, and endothelial cells that interact with each other to control the flow of different molecules from the arteriosus blood circulation to the brain arteriosus circulation. The choroid plexus epithelial (CP) barrier is involved in the passage of molecules from the cerebral circulation to the blood-cerebrospinal fluid.

SESSION 05

LECTURE 01 **HERICIUM ERINACEUS NEUROPROTECTION OF CHOROID PLEXUS AND BLOOD BRAIN BARRIER IN WILD-TYPE FRAIL MICE DURING AGING**

The BBB and the CP permeability increase during aging due to the impairment of tight junctions' structure and function (Costea et al., 2019, Serot et al., 2003) and the presence of those barriers that become "leaky" is related to cognitive decline, allowing the translocation of proinflammatory molecules from the blood to the brain. Two useful markers of tight junctions' integrity are zonulin-1 (ZO-1) and occludin (Gawdi et al., 2021).

Compared to untreated mice, we found that He1 oral supplementation significantly increased the ZO-1 and occludin expression in ependymal cells, choroid plexus, cortical, hippocampal, and hypothalamic blood vessels, suggesting that He1 preserves the integrity and the function of these structures during aging. In parallel, we also assessed the expression of GluT1 on the same brain areas, because glucose is the principal energy source of neurons and GluT1 is the uniporter responsible for its passage across the BBB and the CP (Gawdi et al., 2021). We found an increase in GluT1 expression in the choroid plexus and cortical and hippocampal blood vessels in He1 treated mice compared to untreated animals. All together, these results suggest that He1 treatment improves cognitive performances, preserves BBB and CP.

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SESSION 05

LECTURE **01** **HERICIUM ERINACEUS NEUROPROTECTION OF CHOROID PLEXUS AND BLOOD BRAIN BARRIER IN WILD-TYPE FRAIL MICE DURING AGING**

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SHORT ORAL PRESENTATION / session NO 5

LECTURE
01 **WASTE OR RESOURCE: BIOSORPTION POTENTIAL
OF THE INACTIVE BIOMASS OF FOMITOPSIS PINICOLA
MUSHROOM FOR THE REMOVAL OF THE SYNTHETIC DYE
CONGO RED FROM WATER**

Natalija Velić^{1*}, Ana-Marija Drempetić¹, Janez Gorenšek², Marija Stjepanović¹,
Indira Kosović¹, Darko Velić¹, Hrvoje Pavlović¹

1 University Osijek, Faculty of Food Technology Osijek, Franje Kuhača 18, 31000 Osijek, Croatia

2 IAMB-Institute for Applied Mycology and Biotechnology, Vegova 26, Celje, Slovenia

The market for medicinal mushrooms is steadily increasing as they are a source of valuable biologically active compounds, which are extracted from the fruiting bodies and mycelial biomass of the mushrooms using suitable solvents. After extraction, the inactive extracted fungal biomass remains in the form of a filter cake that has not yet been properly valorised. Promoting the use of by-products and waste materials as a resource is one of the basic ideas of the circular economy model that has been implemented in the development strategies of many countries.

The potential of microbial biomass, both living and inactive, as adsorbents (biosorbents) for the removal of a variety of pollutants (e.g., heavy metals, synthetic dyes, pharmaceuticals, etc.) has been intensively studied. The objective of this study was to investigate the biosorption potential of the extracted inactive biomass of the medicinal mushroom *Fomitopsis pinicola* for the removal of the synthetic dye Congo red (CR) from a model dye solution. The effect of various factors affecting the adsorption process was studied in a batch procedure, namely the concentration of biosorbent (0.4 - 10 g/L), contact time (5 - 360 min), initial CR concentration (10 - 150 mg/L), pH (4 - 10), and temperature (15 - 35 °C).

The obtained data were analyzed using adsorption equilibrium models (Freundlich and Langmuir) and kinetic models (pseudo-first order model and pseudo second order model). The percentage of CR removal increased with increasing biosorbent concentration and contact time between biosorbent and CR. Increasing the initial CR concentration resulted in a decrease in the percentage of its removal, but also increased the adsorption capacity. The biosorption efficiency was highest at pH=4. Analysis of the experimentally obtained data using equilibrium and kinetic adsorption models revealed that the Freundlich model and the pseudo-second-order model better described the process of CR biosorption on inactive *F. pinicola* biomass. The results suggest that the extracted inactive biomass of the medicinal mushroom *F. pinicola* has potential for remediation of wastewater contaminated with synthetic dyes.

**USE OF MUSHROOMS (PLEUROTUS OSTERATUS)
TO INHIBIT ENZYMATIC BROWNING OF POTATOES**SHORT
ORAL
PRES **05****LECTURE 02****Dubova H.Ye, Poyedinok N. L., Galkin O.Yu**

National Technical University of Ukraine Igor Sikorsky Kyiv Polytechnic Institute, Kyiv, Ukraine

Enzymatic browning of potatoes and juice from it may be due to the action of the enzymes phenolase, phenol oxidase, polyphenol oxidase, catecholase, cresolase and tyrosinase. The common structural unit of these enzymes is the copper-copper active site. There are several ways to eliminate enzymatic browning – dehydration, freezing, heat treatment, as well as packaging products in a controlled environment, glazing tissues (especially frozen ones) with sugar syrup or covering with an edible film to limit oxygen access, sulfitation.

The darkening of the tuber surface is slowed down by sodium bisulfite. When it is split, sulfur dioxide is released, which reduces the activity of polyphenol oxidase and forms colorless or barely colored compounds with dyes. The disadvantage of this method is the harmful effects on the body of sulfur dioxide. The purpose of the work is to use the properties of mushrooms *Pl. Osteratus* to prevent darkening of fresh potato juice. Chitosan is a promising reagent that forms complexes with natural substrates and thereby reduces the access of enzymes to them. Mushrooms contain chitin and/or chitosan as structural cell wall polysaccharides, so this approach has potential for application.

The positive effect of the chitosan coating on the control of the browning of mushrooms by inhibiting the activity of enzymes has been proven. Chitosan coating maintains the quality of harvested fruits and vegetables (Liand Yu 2000; Su et al. 2001) and slows down the browning of lychee and longan fruit (Jiang and Li 2001). In addition, chitosan, a high molecular weight cationic polysaccharide obtained by deacetylation of chitin, has been shown to be a dietary fiber analogue with many health benefits and thus safe compared to sulfites (Van Der Lubben et al. 2001). In addition to chitosan, an important factor in stopping browning is the peroxidase activity of mushrooms *Pl. Osteratus*. The reaction of enzymatic browning is due to the oxidation of the amino acid tyrosine by atmospheric oxygen and proceeds due to the catalytic action of the tyrosinase enzyme. In the process of oxidation from tyrosine, the red pigment galachrome is formed, which then turns into a dark pigment related to natural melanin.

Peroxidase always delays the oxidation of tyrosine by tyrosinase. The above facts prove the feasibility of using mushrooms *Pl. Osteratus* to prevent enzymatic browning of potato juice. To compare the effectiveness of preventing enzymatic darkening by the proposed method, the light transmission coefficient of the samples was measured on a KFK-2 photocolormeter at a wavelength of 670 nm. A control sample of potato juice without the use of browning inhibitors has a light transmission coefficient of 1.65-1.70, which is about 3 times higher than in samples using mushrooms *Pl. Osteratus*.

SESSION 06

LECTURE **01** **ROLE OF HETEROPOLYSACCHARIDES DERIVED FROM MEDICINAL MUSHROOMS IN CHEMOPREVENTION AND CARCINOGENESIS**

Dr. Vladimir Laudanović

Moj imunitet / Belgrade, Serbia

Our aging, heredity, lifestyle, race, regular consumption of over processed foods grown on farmland soil depleted of minerals and soil microorganisms rich in nitrate fertilizers and pesticides, irradiated food, industrial toxins (fluoridated drinking water), smoking, viral infections, etc, are directly connected to DNA destabilization by breakage of hydrogen bonds between the base pairs of DNA causing abnormal accelerated replication of open strands to the development and expression of many negative and even fatal processes, such as carcinogenesis. The consequence of so much toxicity is humankind's real and dramatic increase in rates of cancer, autoimmune diseases, toxic metal syndrome and much more.

During the last two decades there has been an increasing recognition of the importance of the human immune system for maintaining good health. Carcinogenesis is process which normally takes several years during which progressive genetic changes occur leading to malignant transformation. With recent advances in technology, biotechnology, information technology a new class of pharmacologically active water soluble heteropolysaccharide immunomodulators emerged from selected medicinal mushrooms found in the fruiting bodies, cultured mycelia, and culture broth. Very few of immunologically active polysaccharides from medicinal mushrooms are pure (1-3)- (1-6)- beta-D-glucans, most are heteroglucans containing other sugar molecules, such as galactose, xylose or mannose. Scientists of world's premier cancer centers are recognizing medicinal mushrooms for their anticancer effects:

The University of Texas MD Anderson Cancer Center gave scientific review of *Coriolus Versicolor* (PSK and PSP) as promising candidate for chemoprevention due to the multiple effects on the malignant process by inhibiting adhesion, invasion, motility, and metastatic growth of tumor cells.

Cancer Research UK- wrote : Mushroom-derived polysaccharides have shown anti tumor activities in both pre-clinical models and in clinical trials.

Memorial Sloan Kettering Cancer Center — the world's oldest and largest private cancer center wrote: Lentinan, a polysaccharide, derived from the mycelium of the shiitake mushroom is classified as an antineoplastic polysaccharide and is available for clinical use.

SESSION 06

LECTURE **01** **ROLE OF HETEROPOLYSACCHARIDES DERIVED FROM MEDICINAL MUSHROOMS IN CHEMOPREVENTION AND CARCINOGENESIS**

Heteroglucans from mushrooms produce their anti-tumor effects in two ways, by activating many kinds of immune cells of innate and acquired immunity that are important for the maintenance of homeostasis, (such as macrophages, monocytes, neutrophils, natural killer cells, dendritic cells) and chemical messengers (cytokines such as interleukins, interferon, colony stimulating factors), triggering complement and by direct cytotoxic effect by triggering apoptosis in mitochondria of cancer cells (Betulinic acid) or interfering with DNA replication in tumor and virally infected cells like Cordycepin.

From clinical practice in different clinical trials in colorectal carcinoma PSK extended 5-year and 8-year survival after surgery and chemotherapy. In lung cancer stages I-III, PSK extended 5-year survival 2-4 times. In patients with advanced stomach cancer and metastasis PSK given alongside surgery and chemotherapy at 3-6g/day doubled 2-year and 5-year survival and extended 15-year survival. In stage III of cervical and uterine cancer in combination with radiotherapy PSK given at (3-6g/day) enhanced survival and increased sensitivity of the cancers to radiotherapy.

In different clinical trials in colorectal carcinoma PSK extended 5-year and 8-year survival after surgery and chemotherapy. Analyzing the efficacy of Coriolus extracts on survival of cancer patients from 13 randomized, placebo-controlled clinical trials, Coriolus was found to result in a significant survival advantage compared with standard conventional anti-cancer treatment alone. Many of the mushroom polysaccharide compounds have proceeded through to Phases I, II and III clinical trials.

USING BIOLOG OMNILOG SYSTEM TO DETERMINE NUTRITIONAL PHENOME AND ANTIBACTERIAL ACTIVITY OF LION'S MANE (*HERICIUM ERINACEUS*)

SESSION 06

LECTURE 02

Soumaya Boudagga

Higher Institute of Applied Biological Sciences of Tunis, University of Tunis El Manar

Hericum erinaceus, a medicinal mushroom, has been broadly cultivated and widely studied for its nutraceutical properties, which include anti-oxidant, anti-tumor, anti-aging, hypolipidemic, and gastric mucosal protection effects. The main objective of this study was to apply the Biolog Omnilog system to determine the nutritional phenome and the antibacterial activity of fruit bodies and/or mycelial culture extracts of *H. erinaceus*.

Biolog Phenotype MicroArrays (PMs) provide a high-throughput technology for the global analysis of microbial growth phenotypes. Using a colorimetric reaction that is indicative of cell respiration (NADH production). In this study, Biolog methodology was used for the first time to elucidate a comprehensive nutritional utilization profile of *H. erinaceus* isolate. In total, 190 substrate utilization assays for carbon sources (PM1-PM2), 190 substrate utilization assays for nitrogen sources (PM3-PM8), 59 nutrient utilization assays for phosphorus sources, 35 nutrient utilization assays for sulphur sources (PM4) and 95 assays for nutritional supplements (PM5) were performed. On the other hand, the antibacterial activity of crude extracts obtained from the fermented mycelium and cultivated fruiting bodies of *H. erinaceus* was studied by a metabolic growth assay based on the Biolog Omnilog system. Traditional hole-plate method was used as a control assay.

H. erinaceus showed relatively restricted carbon utilization. Only 22 of the 190 carbon sources tested were used, principally simple sugars. Several complex carbohydrates, such as D-Cellobiose, D-Xylose, and Arbutin, may also promote growth at levels comparable to simple sugars. *H. erinaceus* grew efficiently on many of the simple nitrogenous compounds tested, including ammonia and nitrate, and especially well on urea and D-glucosamine. *H. erinaceus* was capable of at least poor growth on all of P-sources with two exceptions being pyrophosphate and tripolyphosphate. Moreover the assays suggested that *H. erinaceus* uses both inorganic and organic compounds as S-sources. No stimulation by nutrients was noticed; however, there was evidence of partial inhibition by some compounds.

All extracts obtained from fruit body and mycelial culture of *H. erinaceus* isolate presented antibacterial activity against one or more of the target microorganisms. Antibacterial activity was higher for fruiting body extracts than mycelium extracts. Our data suggest that *H. erinaceus* extracts may have potential applications in the treatment and prevention of infections caused by gram-positive and gram-negative bacteria.

**USING BIOLOG OMNILOG SYSTEM TO DETERMINE
NUTRITIONAL PHENOME AND ANTIBACTERIAL
ACTIVITY OF LION'S MANE (HERICIUM ERINACEUS)**

SESSION **06**

LECTURE **02**

In summary, our results present the most comprehensive analysis of the metabolic capabilities of *H. erinaceus* strain in different conditions, providing information on the global phenotypes and specific nutrient utilization profiles of the fungus. This nutritional phenome can be used to develop new or modified cultivation media to enhance *H. erinaceus* fungal growth for large scale production.

Moreover, we demonstrate that the Biolog metabolic assay could be successfully used as an effective and timesaving, alternative method for evaluation of the antibacterial activity of mushroom extracts comparing to traditional hole-plate approach.

**RESEARCH ON ANTITUMOR ACTIVITY
AND MECHANISM OF MYCOMEDICINE**

SESSION 06

LECTURE 03

BAO Hai-ying

(College of Traditional Chinese Medicinal Material, Jilin Agricultural University, Changchun 130118, China)

Mycomedicine is a kind of natural medicine with therapeutic, preventive and health functions, which comes from the specific parts of medicinal fungi and is processed by harvesting, processing or fermentation according to the prescribed process. Up to now, there are more than 800 kinds of mycomedicines in China, including the kinds of fungal medicines accumulated in the herbs of all dynasties since ancient times, the kinds collected from folk surveys and the kinds discovered in modern pharmacy research. Mycomedicine resources derived from fungi, Oomycota and Myxomycetes. Among them, 98% of edible and medicinal mushroom showed obviously antitumor activity for many kinds of cancer.

They are including powder of fruiting body and sclerotium, fermented product, extract, fraction and compounds. The chemical composition of mycomedicine include polysaccharides, proteins, peptides, volatile oil, fatty acid, terpenoids, sterols, polyphenols, alkaloid, flavone, sphingolipids and so on. Among the extracts of different polarities, the water extract and Petroleum ether extract showed better antitumor activity. The water extract can significantly activate the immune function of macrophages and lymphocytes and regulate the immune system of the body and the petroleum ether extract can via endogenous mitochondrial apoptosis pathway, endogenous endoplasmic reticulum pathway, and death receptor pathway to play the role of antitumor. In the different species, there are both common antitumor components and their own special antitumor components. The common antitumor components are polysaccharides and sterols which are the most important antitumor compounds and must pay the attention to them.

The antitumor mechanism of polysaccharides can significantly enhance the immunity of the body and exert anti-tumor activity through the immune system. Sterols can significantly activate the mitochondrial apoptosis pathway to exert anti-tumor activity in vivo. The antitumor effects and mechanism of polysaccharides, proteins, and small molecules active substances from medicinal fungi by collecting, studying and analyzing literature and data. There are great diversity and different structures in antitumor active ingredient of medicinal fungi. The research and development of antitumor medicinal fungi with polysaccharides, proteins and small molecules active substances from medicinal fungi by collecting, studying and analyzing literature and data. There are great diversity and different structures in antitumor active ingredient of medicinal fungi. The research and development of antitumor medicinal fungi with high efficiency and low toxicity have a good application prospect and practical significance.

ROLE OF CORDYCEPS IN MILD TO MODERATE COVID INFECTION

SESSION 06

LECTURE 04

Siddharth P. Dubhashi¹, Sagar Galvankar², Vibha Dutta³, Sarman Singh⁴, Sagar Sinha⁵,
Jaishree Ghanekar⁶, Sameer Kadam⁷, Parineeta Samant⁸, Irshad H. Chaudry⁹,
Harshawardhan Kelkar¹⁰, Sankalp Dwivedi¹¹, Rakesh Mishra¹², Amit Agrawal¹³

1 Department of Surgery, All India Institute of Medical Sciences (AIIMS), Nagpur, India

2 Department of Emergency Medicine, Sarasota Memorial Hospital, Sarasota, FL, USA

3 Director, All India Institute of Medical Sciences, Nagpur, India

4 Former Director, All India Institute of Medical Sciences, Bhopal, India

5 Department of Emergency Medicine, MGM Medical College, Navi Mumbai, India

6 Department of Medicine, MGM Medical College, Navi Mumbai, India

7 Department of Surgery, MGM Medical College, Navi Mumbai, India

8 Department of Biochemistry, MGM Medical College, Navi Mumbai, India

9 Department of Surgery, University of Alabama at Birmingham, AL 35294, USA

10 Ayurveda expert

11 Department of Neurosurgery, All India Institute of Medical Sciences, Bhopal, India

12 Department of Neurosurgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

13 Department of Neurosurgery, All India Institute of Medical Sciences, Bhopal, India

Objectives:

To evaluate efficacy and safety of Cordyceps as an adjunct to standard treatment in mild (symptomatic) to moderate COVID-19 infection

Methods:

Randomized, double-blind, placebo-controlled study, approved by IRB. Patients with either mild symptomatic or moderate Category COVID-19 infection were included. Randomized to one of two groups to receive either Cordyceps capsules as an add-on therapy to standard treatment protocol (Arm 1 = 33) or placebo plus standard treatment protocol (Arm 2 = 32). All received standard of care treatment in both groups. Individual patients' participation was for 30 days. 500 mg cordyceps capsules or placebo was administered three times a day after food for 15 days. Evaluation was done for efficacy and safety. Statistical analysis performed

ROLE OF CORDYCEPS IN MILD TO MODERATE COVID INFECTION

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Results:

Proportionately higher number of patients recovered in Cordyceps group 18 (60%) as compared to Placebo group 15 (53.6%) on Day 5. Patients receiving Cordyceps had mean improvement of clinical symptoms earlier than patients receiving Placebo (6.6 ± 2.8 days Vs 7.0 ± 3.3 days). Proportionately higher number of patients showed RT-PCR negative result in Cordyceps group 17 (56.7%) as compared to Placebo group 13 (46.4%) on Day 10. There was significant change in the mean values of MCPiP, CXCL10 and IL-1 β levels at Day 5 and Day 10 as compared to baseline (Day 1) values in patients receiving Cordyceps capsules. The mean IgG levels at Day 16 was 26.86 ± 27.45 in Cordyceps group and 16.52 ± 22.86 in Placebo group. None of the subjects developed any serious adverse event in this study.

Conclusion:

Cordyceps appears to be an efficacious and safe immunological adjuvant in the treatment of patients with mild to moderate COVID-19 infection.

SHORT ORAL PRESENTATION / session NO 6

LECTURE 01 WOOD-DECAYING FUNGI AS PLANT PROTECTION AGENTS AGAINST FUSARIUM OXYSPORUM

Urszula Waszczuk, Ewa Zapora

Institute of Forest Sciences, Bialystok University of Technology, Poland

Biologically active fungal substances attract more and more attention not only because of its medicinal action, but also because of the unconventional use of their metabolites in biological plant protection. The intensive use of synthetic fungicides in the fight against phytopathogens raises considerable concerns, mainly due to the toxicity and carcinogenicity of their chemical compounds. This is important, especially in research into new organic fungicides based on fungal substances that can be a form of biological control.

Some species of fungi that decompose dead wood have a wide range of secondary metabolites that limit the growth of pathogenic soil fungi. White and brown rot fungi are considered to be particularly active.

One of the most dangerous soil pathogens is the fungus *Fusarium oxysporum*. This fungus causes damping-off disease, which is particularly dangerous for forestry. It also attacks adult plants. Research in recent years has proven that *F. oxysporum* can be one of the causes of the mass dieback of oak, ash and also alder trees. Limiting the growth of this pathogen is extremely difficult due to its high adaptability, various infective spore forms and the wide spectrum of host plants.

The aim of the study was to evaluate whether fungal extracts (concentrations: 5-100%) of *Fomitopsis pinicola*, *Ganoderma applanatum*, *Trametes versicolor* are able to prevent and/or inhibit the growth of *F. oxysporum* on Scots pine seedlings. For this purpose, tests were carried out in various variants differing both in the method and time of fungal extracts application (prevention and treatment). All tested fungal extracts showed a curative or preventive effect against *F. oxysporum*. Significant reduction of necrosis on the roots of Scots pine seedlings infected with *F. oxysporum* was observed. The extracts of *G. applanatum* and *T. versicolor* were the most effective (already at a concentration of 5%). The most significant reduction of necrosis was observed in the procedure in which the extract was spotted directly on the root apex 24 hours after *F. oxysporum* infection (curative treatment).

Wood decay fungi are potentially effective remediation agents that can form the basis of new, multi-functional preparations used in biocontrol. Isolation and testing of active compounds may be one of the future topics of research on induced plant resistance.

ANTICANCER POTENTIAL OF LAETIPORUS SULPHUREUS

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LECTURE **02**

**Halina Car¹, Anna Sadowska¹, Diana Sawicka¹, Ewa Zapora², Emilia Sokotowska¹,
Marek Wołkowycki²**

1 Department of Experimental Pharmacology, Medical University of Białystok, Poland

2 Department of Silviculture and Forest Use, Institute of Forest Sciences,
Białystok University of Technology, Poland

e-mail: zfarmdosw@umb.edu.pl

Mushrooms are edible in many countries as culinary delicacy, especially because of their taste. However, in recent years, scientists have expanded the area of research to also include other uses of mushrooms, mostly for medical purposes and food preservation. In terms of medicinal value, wild mushrooms have become an important source of antioxidant compounds, which is valuable due to free radicals elimination.

These, in turn, can cause structural damage to cells and are associated with the development of many chronic diseases, such as various types of cancer, cardiovascular diseases and diabetes. Medicinal mushrooms can be a very important element in the prevention and treatment of many diseases and ailments. Nowadays, thanks to the development of new research techniques, it is possible to introduce the arboreal fungi, which were so far used only in alternative therapies. Numerous studies indicate the high therapeutic activity of extracts and compounds obtained from polyporoid fungi, i.e. large-fruited fungi growing mainly on living or dead wood of trees and shrubs.

Laetiporus sulphureus (Bull.) Murrill is an edible mushroom belonging to the class of Basidiomycetes, also known as Chicken Mushroom. It is widespread in Asia, Europe and North America from tropical to subtropical zones. The fungus is commonly found on both deciduous and coniferous trees. It can be harvested as an edible mushroom and has long been used only in Asian folk medicine. Analysis of multiple studies has shown that nutritionally *L. sulphureus* provides many key ingredients such as carbohydrates, proteins, amino acids, vitamins, minerals, fatty acids, and fiber, which can provide an excellent opportunity to promote public health.

Consequently, the traditional use of fruiting bodies to treat fever, cough, stomach cancer and rheumatism is increasingly being followed in European countries. It is anticipated that natural products, as well as their synthetic derivatives, may play an important role in the development of innovative resources, including those which can inhibitor prevent cancers.

ANTICANCER POTENTIAL OF LAETIPORUS SULPHUREUS

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Our study focused on the anticancer potential of *L. sulphureus* collected from various host tree species (*Salix caprea*, *Populus tremula*, *Fraxinus* sp.). The study material was collected in Bialowieza Forest (Poland). Species were identified on the basis of dichotomous keys used as standard in the taxonomy of fungi, analyzing the macroscopic features of fruiting bodies and elements of the microscopic structure of the hymenium.

The cytotoxic effect of extracts was examined on the Ht-29 colorectal cancer cell line and human enterocytes CCD112Co-N. Studies were conducted on six extract concentrations (10, 50, 100, 200, 400 and 600 µg/ml). Cytotoxicity was assessed by MTT test with the use of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. Measurements were performed after 24-hour incubation of the cells with fungus extracts. The results showed that tested extracts exerted significant inhibitory effect on colon cancer cells but not on enterocytes. These preliminary results indicate that the extract is not toxic to physiological cells of the colon, whereas decrease the viability of cancer cells, which identify the need to provide further investigation.

IMPACT OF THE THERAPEUTIC PROPERTIES OF EUROPEAN MUSHROOMS ON HUMAN CORNEAL EPITHELIAL CELLS AND T LYMPHOCYTES TO TREAT EYE DISEASESSHORT
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LECTURE 03

Alexander Areesanan¹, Sven Nicolay¹, Ulrike Grienke², Judith M. Rollinger² and Carsten Gründemann¹**1** Translational Complementary Medicine, Department of Pharmaceutical Sciences, University of Basel**2** Division of Pharmacognosy, Department of Pharmaceutical Sciences, University of Vienna

In times of modernisation and increased environmental and sensory influences, such as LED lighting, media-induced blue light radiation or more intense ultraviolet (UV) radiation, there is an increased prevalence of inflammatory eye diseases, such as dry eye disease (DED). The application of mushrooms for different inflammatory health purposes has a long tradition in Asian countries and it is becoming increasingly popular in the Western hemisphere. Due to this medical potential, 11 European mushroom extracts from the genera of Fomes, Fomitopsis, Ganoderma, Gloeophyllum, Inonotus, Laetiporus, Laricifomes, Lignosus and Piptoporus were investigated in cellular systems capable of simulating dry eye pathology.

Their cytotoxicity, cytoprotective and antioxidant properties were investigated using an immunological technology platform to investigate the human corneal epithelial cell line (HCE-T), as well as the activity and function of human immunocompetent cells, two cells that are prevalent in DED pathology. At high, non-physiological concentrations, the tested extracts demonstrated no cytotoxic effects on the investigated cells. Furthermore, several extracts have shown an impact on UV-B-induced cytoprotection and superoxide radical-scavenging properties in HCE-T cells at defined concentrations. Moreover, distinct mushroom extracts have the capacity to lower NF- κ B expressions in lipopolysaccharide (LPS)-exposed monocytic NF- κ B reporter cells, but they have no significant impact on specifically diminishing inflammatory mediator release from primary human T lymphocytes.

A crucial factor in the development of DED is a reduction of the protective lipid layer of the tear film. Therefore, we will present data to show the ability to produce lipid after exposure to the mushroom candidate extracts in an immortalized human meibomian gland epithelial cells (IHMGEC) model using fluorescence microscopy. Overall, the results indicate that extracts from distinct mushrooms interfere with the function of the cells involved in DED pathology, thus providing a rationale for their potential as a medicinal mushroom.

HETEROBASIDIUM ANNOSUM ACTIVITY ON COLORECTAL CELL LINES

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LECTURE 04

Anna Sadowska¹, Diana Sawicka¹, Ewa Zapora², Emilia Sokotowska¹,
Marek Wołkowycki², Halina Car¹

1 Department of Experimental Pharmacology, Medical University of Białystok, Poland

2 Department of Silviculture and Forest Use, Institute of Forest Sciences,
Białystok University of Technology, Poland

e-mail: zfarmdosw@umb.edu.pl

Colorectal cancer is one of the most common malignant neoplasm in Poland. So far, numerous factors contributing to the development of colorectal cancer have been identified. There are genetic and environmental factors, such as bad eating habits, excessive alcohol consumption and smoking. Despite the abundance of data from scientific studies, prevention is still insufficient, as the only practical recommendation is to assess a potential genetic mutation contributing to the disease and to change the diet. Currently, there are no preparations or foods aimed at reducing the incidence on the market. For the above-mentioned reasons, the incidence of colorectal cancer is constantly increasing. The basic and priority treatment is surgery involving the excision of the neoplastic lesion within healthy tissues with a safety margin and the surrounding lymph nodes. Currently, the anticancer drugs on the market have numerous side effects, which emphasizes the need to introduce new, effective and, above all, less toxic preparations, e.g. from natural products.

Medicinal mushrooms can be a very important element in the prevention and treatment of many diseases. therapy of diseases with substances obtained from fungi is called mycotherapy or fungotherapy. Therefore, it is expected that medicinal mushrooms and their synthetic derivatives will play an important role in the development of innovative agents in the prevention or treatment of cancer.

Their use originates from traditional folk medicine. Currently, through the development of modern research techniques, it is possible to subject Polypores fungi so far used only in alternative therapies. Numerous research works carried out mainly in Japan, China and the USA indicate the high therapeutic activity of extracts and compounds obtained from polyporoid fungi. They possess the following activity confirmed in experimental studies: antitumor, antibacterial, antifungal, antiviral, anti-inflammatory, anti-allergic, cytostatic and immunomodulating.

HETEROBASIDIUM ANNOSUM ACTIVITY ON COLORECTAL CELL LINES

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Heterobasidion annosum (Fr.) Bref. (HA) is a widespread fungus, therefore it is easily accessible, and the process of producing the extract is not expensive. In our previous studies, inhibition of DLD-1 cell line growth and inhibition of DNA synthesis have been observed. The significant result was also a weak activity reducing the viability of healthy fibroblasts, which indicated little toxicity to healthy cells. Therefore, the aim of our present study was to evaluate its anticancer potential on different colorectal cancer cell line - Caco-2 and human enterocytes CCD112Co-N as physiological cells of colon. The results of our study showed significant decrease in viability of cancer cells (approximately 72-82% for HA concentrations 0.1, 1, 5 and 10 µg/ml) but only a slight reduction of normal enterocytes viability (90-99%). This results confirm our previous findings and provide a basis for further research concerning antitumor activity of Heterobasidion annosum extract.

Key words: Heterobasidion annosum, colorectal cancer, enterocytes, cytotoxicity

EVALUATION OF MUSHROOMS AND FERMENTED WHEAT GRAIN FOR ACTIVITY AGAINST COLORECTAL CANCER CELLS

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LECTURE 05

Maximiliano Bidegain¹, Pablo Postemsky¹, Józef Kaczor², Wojciech Rzeski²

1 Centro de Recursos Naturales Renovables de la Zona Semiárida (CERZOS),
 Universidad Nacional del Sur (UNS)-CONICET, Bahía Blanca, Argentina

2 Department of Functional Anatomy and Cytobiology, Maria Curie-Skłodowska University, Lublin, Poland

Colorectal cancer (CRC) is the third most common cause of cancer in the world. It is known that there are some factors in the diet that can both increase and decrease the risk of CRC. That is why the use of natural chemopreventive agents from the diet to reverse, suppress or prevent carcinogenic progression is an interesting strategy worth studying.

Within the edible mushrooms there are genera such as *Grifola* and *Ganoderma* with recognized medicinal properties. Various compounds obtained from these fungi have shown antiproliferative and antimetastatic properties against different lines of human cancer. Since mushrooms contain hundreds of bioactive compounds, they can act synergistically in multiple processes related to cancer.

On the other hand, cereals, including wheat, are one of the main sources of food in the world. The fungal mycelium can grow and colonize cereal grains providing numerous potentially medicinal compounds, thus creating a new functional food in the process.

The objectives of this study were to obtain and characterize wheat flours biotransformed by different species of *Grifola* and *Ganoderma* and evaluate their antitumor potential against different cancer cells. Two kinds of biomass sources were employed: fruiting bodies (FB) and mycelium biotransformed wheat grains (BWG). Twelve milled samples (1 commercially available *Ganoderma lucidum* (GL) FB, 2 *Grifola frondosa* (GF) BWG, 3 GL BWG, 4 wheat grains (control), 5 *Grifola sordulenta* (GS) BWF, 6 *Grifola gargar* (GG) BWF, 7 GG FB, 8-12 GL FB cultivated on sunflower or with rice side streams with different supplements) were produced by Cerzos-UNS/Conicet (Argentina).

Twelve ethanolic extracts were prepared and evaluated for anticancer activity on human colon cancer cell model. Human colon adenocarcinoma HT-29 (ATCC, No HTB-38) and as a control normal human epithelial CCD841 CoTr (ATCC No CRL1807) were used for experiment.

In order to examine the influence of ethanolic extracts on cell proliferation, the MTT method was used. A neutral red (NR, (3-amino-7-dimethylamino-2-methylphenazine hydrochloride) assay was performed to verify the toxicity of ethanolic extracts.

**EVALUATION OF MUSHROOMS AND FERMENTED
WHEAT GRAIN FOR ACTIVITY AGAINST COLORECTAL
CANCER CELLS**

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Samples 8-12 obtained from *Ganoderma lucidum* E47, indicated the highest antiproliferative potential against colon cancer (HT-29 cells). However, similar activity occurs for normal CCD 841 CoTr colon cells. We observed a decrease in the viability of CCD 841 CoTr cells (at concentration above 100 micrograms/mL) for samples 8-12. Tested extracts exhibited low toxicity for tumor cells.

An interesting fact is that all samples from supplemented *G. lucidum* (8-12) have higher anticancer activities than the commercially available mushroom (1) and control (4), and the best ratio between the antiproliferative activity against cancer and loss the viability of the normal cells.

Funding by

Argentina government (Conicet PUE Cerzos and MECCyT Cofecyt PFIPI ESPRO)

In collaboration between Maria Curie-Skłodowska University Lublin Poland and CERZOS, Universidad National del Sur - CONICET, Argentina

THE ACTIVITY OF COMPONENTS OF TRUE TINDER MUSHROOM, CHAGA INONOTUS OBLIQUUS (FR.) PIL. AGAINST CORONAVIRUS SARS COV-2SHORT
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LECTURE 06

Tamara V. Teplyakova, Oleg V. Pyankov, Maksim Skarnovich, Alena Ovchinnikova, Alevtina Poteshkina, Alexandr Safatov

SRC Virology and Biotechnology Vector, Koltsovo, Novosibirsk region, 630559

E-mail: teplyakova@vector.nsc.ru

The spread of the new coronavirus that caused the COVID-19 pandemic embraced all countries of the world. In addition to vaccine development, the search for antiviral compounds against the new coronavirus, SARS-CoV-2, is also relevant.

Our work is devoted to screening some components from pharmaceutical raw materials of chaga mushroom for their ability to inhibit the replication of coronavirus, strain nCoV/Victoria/1/2020, in Vero and Vero E6 cell cultures. The virus with an infectious titer of 5.0 ± 0.29 (± 0.57) lg TCD₅₀/ml was obtained from the State Collection of Pathogens of Viral Infections and Rickettsioses of the Federal State Budgetary Institution SRC VB Vector of Rospotrebnadzor.

Aqueous extracts of the fungus *I. obliquus* were shown to have a high antiviral activity against SARS-CoV-2. Extracts without prolonged temperature exposure were more effective. Under optimal preparation conditions, aqueous extracts of chaga showed an inhibitory concentration of IC₅₀ = 0.75 µg/ml in replication of the SARS-CoV-2 coronavirus with low toxicity and a high selectivity index of SI = 155.5. Aqueous extracts of chaga obtained at 50-95°C for 1.0 to 72 hours showed an inhibitory concentration, which in the range of 0.75-11.6 µg/ml exhibited 50% antiviral activity against SARS-CoV-2. Befungin, a pharmaceutical preparation produced from chaga by extraction with heated water (68-72°C) in three reactors and mixing with cobalt salts and alcohol showed weak or no activity [patent RU 2741714 C1, 2021; Teplyakova T.V. et al., 2022].

Melanin was isolated from natural chaga by the alkaline hydrolysis method. The best melanin sample to inhibit coronavirus replication in Vero cell culture was the one obtained in an autoclave during 30 minute exposure and an excess pressure of 0.7 atm followed by removal of an aqueous ammonia solution from the product at +30-40°C. The inhibitory concentrations of IC₅₀ µg/ml in two samples dried at +50°C were 19.405 and 22.992 µg/ml with the same toxicity of 666.7, while in samples dried at a temperature not higher than +30-40°C this concentration was in the range of 6.2-8.5 µg/ml [patent RU 2747018 C1, 2021].

**THE ACTIVITY OF COMPONENTS OF TRUE TINDER
MUSHROOM, CHAGA INONOTUS OBLIQUUS (FR.)
PIL. AGAINST CORONAVIRUS SARS COV-2**

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Since chaga contains humic substances and melanin involved in their creation, the mechanism of action of chaga components may be related to its sorption capacity and the direct effect of its active components on pathogenic organisms. It is known that chaga contains a complex of various compounds that are formed in a close interaction of the birch and the mushroom.

Taking into account the results obtained on antiviral activity, low toxicity, the ability to improve blood rheology with respect to platelet aggregation, aqueous extracts and melanin from the fungus chaga *I. obliquus* can be considered as the basis for obtaining therapeutic and preventive drugs against COVID-19.

Key words: *Inonotus obliquus*, chaga, extracts, melanin, antiviral activity, coronavirus, SARS-CoV-2.
This work was supported by the State Assignment of Rospotrebnadzor.

**ANTITUMOR ACTIVITY OF CYTOKININ-CONTAINING
XXTRACTS FROM MYCELIUM OF MEDICINAL MUSHROOMS
GANODERMA LUCIDUM AND LENTINULA EDODES IN VITRO**SHORT
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LECTURE 07

**Vedenicheva N.P., Al-Maali G.A., Bisko N.A.1, Kosakivska I.V.,
Garmanchuk L.V., Ostapchenko L.I.****M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine 2ESC
"Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv**

Most basidiomycetes contain biologically active substances in fruiting bodies and cultured mycelium that enhance innate and acquired immune responses and show antitumor activity in animals and humans. They also produce phytohormones, in particular cytokinins, in significant quantities. Cytokinins in vitro inhibit the growth and cause apoptosis of many types of cancer cells. It could be assumed that the therapeutic properties of medicinal mushrooms depend on cytokinins synthesized in their cells in combination with fungal-specific metabolites. *Ganoderma lucidum* Karst and *Lentinula edodes* (Berk.)

Pegler are well known medicinal mushrooms with an extremely wide range of medicinal properties including an antitumor effect. The aim of the current study was to identify the biological activity of extracts from the mycelial biomass of these mushrooms, containing cytokinins, on model lines of cancer cells. Mycelial biomass of *G. lucidum* strain 1900 and *L. edodes* strain 712 was obtained by cultivation in liquid nutrient medium. The content of cytokinins in mycelial biomass was determined using HPLC-MS. The effect of crude fungal extracts and purified cytokinin fractions on the growth of tumor cells of different degrees of malignancy in vitro was analyzed.

Trans-zeatin and zeatin riboside prevailed in mycelial biomass of both mushroom species. The total content of cytokinins in *L. edodes* was almost twice as high as in *G. lucidum*. All tested samples showed a cytotoxic effect at a concentration of 5 mg/ml on human colon adenocarcinoma cells (Colo 205). The effect of crude extracts was about 10% higher compared with purified cytokinin fractions. The greatest cytotoxic influence on the Colo 205 cells was caused by the crude extract from the mycelial biomass of *G. lucidum*, while the maximum antiproliferative effect was observed with the addition of the *L. edodes* crude extract. The crude extracts of both mushroom species did not affect the degree of attachment of cervical cancer cells (HeLa) cells to the substrate, whereas the purified cytokinin fraction from the mycelial biomass of *G. lucidum*, at a concentration of 5 mg/mL, increased the level of adhesion of HeLa cells to the substrate at a concentration of 5 mg/ml.

**ANTITUMOR ACTIVITY OF CYTOKININ-CONTAINING
EXTRACTS FROM MYCELIUM OF MEDICINAL MUSHROOMS
GANODERMA LUCIDUM AND LENTINULA EDODES IN VITRO**

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Both crude extracts and cytokinin fractions of *G. lucidum* and *L. edodes* changed the morphology of human hepatocellular carcinoma cells (HepG2). Cells became more solitary, especially under the influence of purified cytokinin fractions, filopodia were almost absent, there were single spherical cells detached from the substrate.

Our findings can be considered as indirect evidence that cytokinins could be one of the active components of medicinal macromycetes. The fact that crude mushroom extracts manifested a more pronounced negative effect on tumor cells than purified cytokinin fractions in some experiments indicates a complex synergistic nature of the action of biologically active substances produced by medicinal mushrooms.

Key words – medicinal mushrooms – mycelial biomass – cytokinins – Colo205 cells – HepG2 cells – HeLa cells

**ANTICANDIDAL AN ANTI-ASPERGILLUS EFFECTS
OF PLEUROTUS OSTREATUS AND LAETIPORUS
SULPHUREUS EXTRACTS**SHORT
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LECTURE 08

Milica M. Galić*, Jasmina Lj. Čilerdžić, Mirjana M. Stajić

University of Belgrade, Faculty of Biology, Takovska 43, 11000, Belgrade, Serbia

In the era of increasing antimicrobial resistance, fungal pathogens present an important threat to human and animal health, crops and other organisms. To overcome the problem of resistance there is a growing need for alternative sources such as various bioagents. Previous studies showed that edible and medicinal mushrooms are not only sources of vitamins, minerals, proteins but also bioactive compounds. Recently, *Pleurotus ostreatus* (Jacq ex Fr.) P. Kumm. and *Laetiporus sulphureus* (Bull: Fr.)

Murr. have gained significant attention due to their high nutritional value and many proven medicinal effects. Likewise, the use of mushroom extracts in prevention of numerous diseases has raised interest because of their broad spectrum of effects such as immunomodulatory, cytotoxic, antioxidative, antimicrobial, etc. The present study was performed to determine the antifungal activity of water extracts of *P. ostreatus* and *L. sulphureus* basidiocarps and mycelia against selected *Aspergillus* and *Candida* species using microdilution method. The results showed significant variability in antifungal activity of basidiocarp and mycelial extracts of both species against all tested micromycetes. Fungistatic activity of the extract of *P. ostreatus* basidiocarps at a concentrations of 0.5 mg/mL was detected against *C. krusei*, *C. parapsilosis*, while concentrations of 2.0 mg/ml, 8.0 mg/ml and 16.0 mg/ml, were effective toward *C. albicans* BEOFB604m, *C. tropicalis* and *C. albicans* BEOFB605m, respectively. Extracts of *L. sulphureus* basidiocarp was even more active.

The tested extracts of *P. ostreatus* and *L. sulphureus* basidiocarp against *Aspergillus* spp. showed similar results where the minimal inhibitory concentrations (MICs) for the most species were 16.0 mg/mL. Water extract of *L. sulphureus* basidiocarp gave the highest activity against *A. terreus* (MIC = 2.0 mg/mL). Contrary to basidiocarp extracts, mycelial extracts inhibited the growth of only *C. tropicalis* at the highest tested concentration while even stimulatory affected the growth the *C. albicans* BEOFB604m, *C. krusei* and *C. parapsilopsis*. The growth of another *C. albicans* strain was stimulated only with mycelial extract of *P. ostreatus*, while the *L. sulphureus* extract showed this effect on *A. sydowii* and *A. terreus*.

The obtained results showed the potential use of examined extracts as a source of natural compounds signifying as promising as an antifungal agent and therefore could prevent the development of some important pathogenic fungi. The use of mushroom extracts could be alternative substitution to the current antifungal products being ineffective due to fungal resistance and causing many side-effects.

Corresponding author: galic.m@bio.bg.ac.rs

EFFECT OF ERINACINE A ENRICHED HERICIUM ERINACEUS SUPPLEMENTATION ON COGNITION – A PILOT DOUBLE-BLIND PLACEBO-CONTROLLED STUDYSHORT
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LECTURE 09

Katja Bezek¹, Dominika Češek¹, Maša Černelič Bizjak¹, Saša Kenig¹, Andrej Gregori², Erik Bird², Zala Jenko Pražnikar^{1*}

1 University of Primorska, Faculty of Health Sciences, Polje 42, 6310 Izola, Slovenia

2 MycoMedica d.o.o., Podkoren 72, 4280 Kranjska Gora, Slovenia

Heridium erinaceus is an edible and medicinal mushroom with a vast spectrum of pharmacological activities. Traditionally, it has been used against diseases of the digestive tract and for the regeneration of nerves in neurodegenerative diseases. While the prevalence of neurodegenerative diseases and their associated cognitive deficits is highly associated with the aging population, many efforts have been made to test the neuroprotective effects of various naturally occurring compounds. Erinacines and hericenones are known as the main groups of compounds from *H. erinaceus* exhibiting neuroprotective properties.

The aim of the present study was to test the effect of a food supplement containing a known concentration of erinacine A in healthy individuals over 55 years of age. The study was organized as an 8-week double-blind one with two parallel groups in which eligible healthy participants were randomly assigned to *H. erinaceus* supplement group (N=18; 67% F, 33% M), and the placebo group (N=14; 67% F, 33% M). A food supplement produced from *H. erinaceus* fruiting bodies by MycoMedica d.o.o., Slovenia, was used in this trial with a 3.44 mg daily dose of erinacine A against placebo capsules containing corn starch. Anthropometric measurements and analyses of selected biochemical parameters were performed at two-time points, before and after the intervention. Simultaneously, the feces samples were collected in order to determine gut microbiota and chitinase activity. The cognitive function was assessed by two non-verbal speed tests, Test of Perception Speed "Patterns" (THP) and Test of Series (TN), measuring the perception and mental speed, working memory and the speed of information processing.

After the intervention, there were no statistically significant differences in anthropometric or biochemical parameters between the experimental and placebo groups. An increased serum level of Brain-Derived Neurotrophic Factor (BDNF) was detected in the experimental group, when compared to the placebo group. Moreover, using a model with cognitive abilities as the dependent variable with the consideration of gender, age and initial cognitive ability of each participant, a statistically significant improvement in cognitive abilities measured by the THP parameter was shown.

**EFFECT OF ERINACINE A ENRICHED HERICIUM ERINACEUS
SUPPLEMENTATION ON COGNITION – A PILOT DOUBLE-
BLIND PLACEBO-CONTROLLED STUDY**

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When constructing a model that included average fecal chitinase 3-like-1 (CHI3L1) values, the effect of *H. erinaceus* intervention on the levels of neuropeptide Y (NPY; $F=5.3$, $p=0.039$), values of THP ($F = 5.3$, $p = 0.040$) and TN ($F = 9.3$, $p = 0.010$) cognition tests was observed, while there was no effect in the placebo group. On the basis of this study supplementing the diet with *H. erinaceus* was recognised as a safe and well-tolerated intervention with a positive neurocognitive benefit.

Keywords: erinacine A, *Hericium erinaceus*, cognition

**EXTRACTION OF CORDYCEPIN FROM CORDYCEPS MILITARIS
AND ITS CHARACTERIZATION BY HPLC
AND POTENTIOMETRIC TITRATIONS**SHORT
ORAL
PRES

08

LECTURE 10

Mateja Zotler^a, Andrej Gregori^b, Gregor Marolt^a**a** University of Ljubljana, Faculty of Chemistry and Chemical Technology,

Večna pot 113, 1000 Ljubljana, Slovenia

b MycoMedica d.o.o., Podkoren 72, 4280 Kranjska Gora, Slovenia

In the last decade, an interdisciplinary approach in modern chemical sciences is progressively encouraged by traditional Chinese medicine, part of which is based on the use of natural or cultured *Cordyceps militaris* fungus fruiting bodies. One of its main bioactive compounds is cordycepin, also known as 3' deoxyadenosine. On the basis of profound studies, its beneficial effects on health as well as curing abilities have been observed.

For this reason, isolation, and the use of cordycepin as potential bioactive agent has so far attracted many interests and remains a research challenge [1]. Therefore, the main aim of our work was to evaluate the optimal extraction procedure for isolation of cordycepin from *C. militaris* and its determination and characterization by the use of HPLC and potentiometric titrations, including its acid-base properties in order to find the most efficient way for the purification of extracts.

In the scientific literature, there is little to none reliable data regarding pKa values of cordycepin available. Therefore, we used potentiometric approach to obtain the protonation stability constants. In this regard, the range between pH 2 and 13.5 was investigated by alkalimetric titration technique. Similarly, as chemically related adenosine molecule, which has been more widely investigated, it is believed that cordycepin acts as multiproton acceptor/donor due to the presence of amino and hydroxyl groups, as well as purine ring's nitrogen atoms [2]. There is also a great potential for the application of developed electrochemical method for preliminary detection of cordycepin in purified eluates and crude extracts.

The second part of the research was focused on the optimization of extraction procedure by investigation of various experimental conditions, including: i) volumetric ratio between ethanol and water solvents, ii) ration between dried *C. militaris* mass and total extraction solvent volume, iii) extraction time, iv) temperature, v) type of mixing (mechanical vs. ultrasonic), and vi) purification procedures (centrifugation, filtration etc.). The purified extracts were analysed for the cordycepin content by HPLC chromatography, using Hypersil C18 separation column and UV-detection at 260 nm and 282 nm.

EXTRACTION OF CORDYCEPIN FROM CORDYCEPS MILITARIS AND ITS CHARACTERIZATION BY HPLC AND POTENTIOMETRIC TITRATIONS

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In addition to cordycepin concentration, the amount of the organic and inorganic impurities was evaluated as well, and it has been shown that the use of higher ethanol contents (over the water) in the extraction solvent significantly reduced the amount of impurities in extracts.

Key words:

Cordycepin, Cordyceps militaris, Potentiometric titrations, Extraction, HPLC chromatography.

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**CORDYCEPIN AS A POTENTIAL PHARMACOLOGICAL
INHIBITOR OF PRO-FIBROTIC PATHWAYS
IN SYSTEMIC SCLEROSIS**SHORT
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LECTURE 11

Matija Bajželj^{1,5}, **Andrej Gregori**^{2,3}, **Snežna Sodin-Šemrl**^{1,4}, **Katja Lakota**^{1,4}**1** University Medical Centre Ljubljana, Department of Rheumatology, Ljubljana, Slovenia,**2** Mycomedica Ltd., Podkoren, Slovenia**3** Biotechnical faculty, University of Ljubljana, Ljubljana, Slovenia**4** Faculty of Mathematics, Natural Sciences and Information Technologies,
University of Primorska, Koper, Slovenia**5** Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Cordycepin (3'-deoxyadenosine) is an adenosine analogue of fungal origin contained in different entomopathogenic species i.e. *Cordyceps militaris*, *Ophiocordyceps sinensis*, as well as in some *Aspergillus* species. This secondary metabolite possesses various biological activities including immunomodulatory, antidiabetic, antitumor, anti-viral, and antibacterial effects. Cordycepin has been lately employed in the development of drugs and can be produced by submerged or solid-state fermentation and isolated from culture broth, mycelia or the fruiting bodies.

Cordycepin has already been studied *in vitro* and *in vivo* as a potential treatment for different organ fibrosis, such as kidney and renal fibrosis. In this study, we investigated the use of cordycepin as a potential treatment for lung and skin fibrosis in systemic sclerosis (SSc), a rare systemic autoimmune disease, characterized by autoimmunity, vasculopathy, inflammation, and fibrosis. While skin fibrosis is the main symptom of the disease, pulmonary artery hypertension and pulmonary fibrosis represent the major risk for mortality. Fibroblast activation by transforming growth factor- β (TGF- β) lies at the center of (SSc) pathophysiology, transforming them into myofibroblasts which express excessive amounts of extracellular matrix proteins (ECM), such as collagen type I (COL1A1) and fibronectin, that can result in organ dysfunction.

Human dermal and lung fibroblasts (DF and LF, respectively) from four different healthy adult controls were cultured and treated with TGF- β (5 ng/ml) with the different doses of cordycepin (1 μ M, 5 μ M, 10 μ M, 20 μ M, 40 μ M). The expression of pro-fibrotic mRNAs, such as COL1A1, α -smooth muscle actin (α -SMA) and fibronectin was analysed using qPCR. Protein levels of COL1A1 and α -SMA were detected by Western blot. RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay were used to measure their rates in real-time. In an *in vitro* setting after stimulation with TGF- β , cordycepin dose-dependently abrogated differentiation of skin and lung fibroblasts into myofibroblasts, as seen by a decrease of α -SMA mRNA expression levels to 0,04 FC of unstimulated cells.

**CORDYCEPIN AS A POTENTIAL PHARMACOLOGICAL
INHIBITOR OF PRO-FIBROTIC PATHWAYS
IN SYSTEMIC SCLEROSIS**

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Cordycepin also reduced the production of ECM protein components triggered by TGF- β , such as CO-L1A1 mRNA to 0,08 FC and protein levels (37 % decrease compared to unstimulated cells) as well as fibronectin mRNA to 0,15 FC by lung and skin fibroblasts. The utilized doses of cordycepin were not cytotoxic for the DF and LF. Cordycepin inhibits TGF- β -induced expression of pro-fibrotic markers on human DF and LF. This provides the basis for further investigations on the precise pharmacological mechanism of cordycepin in ex-vivo conditions.

SESSION 07

LECTURE 01 **SENOLYTIC EFFECT OF TRITERPENOIDS COMPLEX NT EXTRACTED FROM GANODERMA LUCIDUM ON SENESCENT LIVER CANCER CELLS**

Ahmed Attia Ahmed Abdelmoaty^{1,2}, Changhui Wu¹, Ye Li^{1*} and Jianhua Xu^{2*}

1 Fujian Xianzhilou Biological Science & Technology Co., Ltd, Fuzhou 350108, P. R. China;

2 Fujian Provincial Key Laboratory of Pharmacology of Natural Medicine, School of Pharmacy, Fujian Medical University, Fuzhou 350122, P. R. China

Hepatocellular carcinoma (HCC) is the most common type of liver cancer and a leading cause of death worldwide. Therefore, it is critical to identify novel therapeutic concepts for HCC. Cellular senescence is a process which refers to a permanent inhibition of cellular proliferation and differentiation without undergoing cell death. It was reported that cellular senescence plays a critical role in the progression of liver diseases, including liver cancer. Elimination of senescent cells or modulation of the senescence-associated secretory phenotype (SASP) in these cells with a senolytic agent might be useful to treat liver cancer. Ganoderma lucidum known as "Lingzhi" in China is a well-known medicinal mushroom and traditional Chinese medicine, which has been used for the prevention and treatment of hepatitis, immunological disorders and cancer.

The biologically active compounds as primarily triterpenoids and polysaccharides of *G. lucidum* have been reported to possess hepatoprotective, antioxidant, antitumor, immunomodulatory, and antiangiogenic activities. However, the senolytic activity of *G. lucidum* against senescent cancer cells has not been investigated. In this study, we determined the senolytic effect of the triterpenoids complex NT on senescent HCC cells. Here, senescence was induced in HCC cells by the chemotherapeutic agent Adriamycin (ADR) and subsequently, cells were treated with NT to analyze its senolytic activity. We showed that NT induced concentration-dependent inhibition in senescent HCC cells, which was investigated via the significant reduction in the levels of senescence markers. NT concentration-dependently induced caspase-dependent apoptosis in senescent HCC cells through the mitochondrial-mediated pathway. In addition, NT inhibited autophagy at the late stage in senescent HCC cells. We further demonstrated that NT induced inhibition of the SASP, especially IL-6, IL-1 β and IL-1 α in senescent HCC cells via the inhibition of NF- κ B, TFEB, P38, ERK and mTOR signaling pathways. These findings are of potential importance for elucidating a novel senolytic agent against therapy-induced senescent HCC cells and the elimination of senescent HCC cells and inhibition of HCC progression.

*Correspondence to: Jianhua Xu, email: xjh@fjmu.edu.cn; Ye Li, email: lee@xianzhilou.com

POTENTIAL APPLICATION OF SELENIUM-ENRICHED MUSHROOMS IN THE FOOD AND PHARMACEUTICAL INDUSTRY

SESSION 07

LECTURE 02

Milena Pantić^{1*}, Dunja Miletić¹, Danka Matijašević^{1,2}, Aleksandra Sknepnek¹, Miomir Nikšić¹

1 University of Belgrade, Faculty of Agriculture, Department of Food technology and Biotechnology, Nemanjina 6, 11080 Belgrade, Serbia

2 Laboratory for Molecular Microbiology, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444a, 11000 Belgrade, Serbia

** Presenting and corresponding author: Milena Pantić*

E-mail address (of the presenting/corresponding author): milenas@agrif.bg.ac.rs

Selenium (Se) is an essential micronutrient which bioavailability and toxicity depends on its form and concentration that is taken into the body. Se uptake from organic sources goes up to 90-95%, while from inorganic it's up to 10%. In addition, toxicity of organic Se compounds is low. In the last decades, the interest in functional foods and natural dietary supplements consumption has been growing. Since the mushroom market also constantly grows, bioactive compounds, isolated from higher fungi grown on fortified substrates, are their promising source. During last 15 years of research, the biological activity of Se-enriched macromycetes has been proven through in vitro tests by our group. The research was conducted on selenium enriched mushroom species (*Pleurotus* spp, *Lentinus edodes*, *Coriolus versicolor* and *Agaricus bisporus*) obtained using solid state and submerged cultivation techniques, and different sources of selenium for the growing substrate supplementation.

Chemical composition analysis revealed that, mostly, Se-enriched extracts and polysaccharides obtained from mycelia and fruiting bodies had higher content of glucans, total proteins and phenolic compounds compared to non-enriched samples. Also, a significant percentage of total accumulated Se was incorporated in the form of L-selenomethionine. Mushrooms enriched with Se from an organic source (selenourea, Se-yeast) showed significantly different Se-accumulation efficiency, biomass yield, content of the L-selenomethionine, Se content and different biological activity, compared to the mushroom samples enriched with inorganic salts (sodium selenate and sodium selenite).

The ability of some mushroom species to grow and accumulate selenium also varied on used cultivation technique and growth stage. In vitro test of the fresh fruiting bodies indicated the positive influence of Se from Se-modified zeolites on the activity of enzymes of primary antioxidant protection - superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT).

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Antioxidant activity of selenium enriched extracts depended on the Se concentration, form of the Se used for the supplementation, mushroom strain and type of the mushroom extract. An increase in the activity was noted with increasing concentration of Se in all tested samples.

Furthermore, antibacterial activity against food-borne pathogens and non-toxicity to moderate toxicity of extracts, with high content of Se, against normal MRC-5 cell line was proven. According to results, Se-enriched mushrooms in the form of fruiting bodies, mycelia, extracts or polysaccharide extracts could be a promising non-toxic dietary supplements or functional food/additives with prolonged shelf life and improved bioactivity.

Keywords: Biological Activity, Dietary Supplement, Functional Food, Mushrooms, Selenium.

GIVE ME MY DOSE-A RESEARCHER'S AND CONSUMER'S DIVE INTO THE MUSHROOM-BASED NUTRACEUTICAL INDUSTRY

SESSION 07

LECTURE 03

Jovana Vunduk*

* Institute of General and Physical Chemistry, University of Belgrade, Studentski trg 12/V,
11158 Belgrade, Serbia; vunduk.jovana@hotmail.com

As researchers step into the age of fungiceuticals followed by piling up the in vitro and in vivo data on mushroom benefits, the industry took its own tempo and style. The market time is now even if there are not enough data or definitive directions. However, mushrooms fulfill several requirements of industrial importance; the evidence of traditional use, numerous species, a broad scope of biological activities, they can be cultivated, processing can be scaled-up, and the evidence of adverse effects is scarce. On the other hand, there is a paradigm shift from curing to prevention, and consumers, especially Western ones, seek over-the-counter solutions.

This trend increased significantly during the COVID-19 pandemic due to the most marketed mushroom activity-immunostimulation. The problem is that we, the consumers, still do not know much about this type of product. We expect the industry to be fair, thus we believe that the capsule, powder or tincture we are ingesting is perfectly formulated. The fact is that medicinal mushroom science is booming but with many questions still to be answered. Fungal material identification presents one such issue with possibly significant outcomes like poisoning or adverse therapeutic effects. In science and industry, about one-third of samples are identified by morphological characteristics while another third of samples presented in scientific papers were never subjected to any kind of identification. Moreover, molecular identification provides higher security but it has its problems too, like choosing the wrong marker gene, contamination, wrong choice of primers, problems with online DNA sequence databases, etc. The quality and type of raw material is another important issue, especially if the final product is based on mycelium and the health claim on its packaging is derived from research conducted with solid state-cultivated fruit bodies.

The method of cultivation, conventional or organic, needs to be highlighted since mushrooms are well-known as metalloids accumulators as well as other chemicals like those used for pest management. Consumers are not aware of these details and pay almost no attention to them which enables the industry to continue with the non-transparent policy. Other parameters of importance for researchers, industry and consumers, which heavily affect the final product, are bioavailability, extraction parameters, dosing, drug delivery systems, quality, safety, and novel fields of application. In addition, the responsibility for the product's quality and safety differ from country to country without the universal wholesome principle.

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For example, methods for measuring the amount or type of polysaccharides or terpenes, as the main active ingredients, are not standardized leaving space for manipulation. This step is of high importance in the case of mushroom-based nutraceuticals since most of the products are mycelium-based while the material is cultivated using grains and cannot be separated from it later. Both materials contain polysaccharides, but of different structures and activity, however, they both contribute to the total polysaccharide amount.

Aside from the numerous production and standardization problems the evidence of mushroom's biological activity is growing with more and more high-quality clinical studies and insights into the novel avenues of application like treating neurodegenerative conditions, mood and sleeping disorders, novel viruses, or gut microbiota modulation.

Key words: fungiceuticals, fungal material identification, immunostimulation, mushroom-based nutraceuticals, standardization

**ISOLATION AND STRUCTURAL ANALYSIS
OF POLYSACCHARIDES FROM VARIOUS
MEDICINAL MUSHROOMS**

SESSION 07

LECTURE 04

Roman Bleha¹, Leonid Sushytskyi¹, Andriy Synytsya¹, Jana Čopíková¹, Pavel Klouček²

- 1** Department of Carbohydrates and Cereals, Faculty of Food and Biochemical Technology,
University of Chemistry and Technology in Prague, Technická 5, 166 28 Praha 6 Dejvice, Czech Republic
- 2** Department of Crop Production, Faculty of Agrobiological Sciences, Food and Natural Resources,
Czech University of Life Sciences Prague, Kamýcká 129, 165 00 Praha 6 Suchbátka, Czech Republic

blehar@vscht.cz

Mushroom fruiting bodies have been commonly used for centuries in natural medicine and today in the food and pharmaceutical industries, as a source for the preparation of food supplements (Tel-Çayan, 2020). It is due to their chemical composition, which includes terpenoids, alkaloids, polysaccharides, and proteins (Bleha, 2022). Most of these compounds have positive biological activity on the human body, such as anti-inflammatory, immunomodulatory, antitumor, and antioxidant (Baeva, 2020).

Polysaccharides like α - or β -glucans or galactomannans can be obtained by different types of extraction such as cold and hot water, alkali, in DMSO (Bleha, 2022; Baeva, 2019). In this way, it is possible to obtain polysaccharides with different sugar composition, degree of branching, and molecular weight (Hong, 2019). To obtain pure polysaccharide fractions from raw extracts, enzymes like α -amylase and pullulanase are needed to remove starch such as polysaccharides, pepsin, and pronase to decrease the amount of proteins (Bleha, 2022; followed by anion exchange chromatography (DEAE Sepharose Fast Flow) in combination with size exclusion chromatography (Sephadex G 75) (Hong, 2019; Sushytskyi, 2020).

It is very important to use several combination analytical techniques, such as FTIR, Raman, and NMR spectroscopy with GC/FID and GC/MS, for neutral sugar determination and methylation analysis. GPC/SEC are commonly used for molecular weight determinations (Bleha, 2022; Baeva, 2019; Hong, 2019). Our research is focused on the isolation and structural characterization of polysaccharides isolated from *Pleurotus ostreatus*, *Ganoderma resinaceum* and *Lucidum* and *Hericium erinaceum* mushrooms in different solvents like cold (20 °C) and hot (under reflux 100 °C) water extraction followed by alkali (1 mol L⁻¹ aqueous NaOH) and DMSO extractions. We have obtained several types of glucans and mannogalactans like branched O-2- β -D-mannosyl-(1 \rightarrow 6)- α -D-galactan and a highly branched (1 \rightarrow 3)(1 \rightarrow 4)(1 \rightarrow 6)- α -D-glucan from *Ganoderma resinaceum* and branched partially methoxylated mannogalactan and slightly branched (1 \rightarrow 6)- β -D-glucan from *Pleurotus ostreatus* and mannogalactan and glucan in *Hericium erinaceum*.

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Acknowledgments

This work was supported by the grants of Specific University Research of UCT Prague, project number 21-SVV/2019, 21-SVV/2020, 21-SVV/2021 and 21-SVV/2022 and by the Ministry of Agriculture of the Czech Republic (project QK1910209) are greatly acknowledged

MEDICINAL POTENTIAL OF THE INSOLUBLE EXTRACTED FIBERS ISOLATED FROM THE FOMES FOMENTARIUS FRUITING BODIES: A REVIEW.

SESSION 07

LECTURE 05

Kalitukha Liudmila^{a*}, Galiano Alvaro^b, Harrison Francisco^b

^a Good Feeling Products GmbH, Neuss, Germany

^b Innovative Drugs Regulatory Europe GmbH, Munich, Germany

** Address all correspondence to: Liudmila Kalitukha, Good Feeling Products GmbH, Hansemannstr. 7, 41468 Neuss, Germany; Tel.: +4921315129900; Fax: +492131512990299, E-mail: liudmila.kalitukha@gfpsl.de*

The tough, hoof-shaped fruiting bodies of the tinder conk mushroom, *Fomes fomentarius* (L.) Fr. (Polyporaceae, Agaricomycetes), were traditionally used all over the world as tinder to start fire, for ritual purposes, to make artworks like clothing, frames, ornaments, and also to cure various human diseases (wounds, gastro-intestinal disorders, liver-related problems, inflammations, various cancers, etc.).

The first wave of scientific interest in *F. fomentarius* in Europe dates back to the early 1970s with the discovery of the red-brown pigments of the *F. fomentarius* external layer. Since then, a number of research papers and reviews have mentioned the history of use, taxonomy, composition and medicinal properties of some *F. fomentarius* preparations, e.g., soluble extracts and their fractions, isolated cell walls, mycelia and compounds purified from the culture broth.

The present review is focused on the composition and benefits of the water-insoluble cell walls obtained from the *F. fomentarius* fruiting bodies.

Isolated cell walls of the tinder mushroom reveal a fibrous hollow structure with an average diameter of 3-5 μm and a wall thickness of 0.2-1.5 μm . Naturally, the fibers are composed of 25-38 % glucans, with a majority of β -glucans, around 30 % polyphenols, 6 % chitin and less than 2 % hemicellulose. The percentage of the main structural compounds can vary either slightly or considerably, depending on the extraction conditions.

According to in vitro, in vivo, ex vivo as well as clinical studies, *F. fomentarius* fibers can modulate the immune system, contribute to intestinal health, accelerate wound healing, absorb heavy metals, organic dyes and radionuclides, normalize kidney and liver function, and provide antibacterial, antiviral, antifungal, anxiolytic, anti-inflammatory and analgesic effects.

**MEDICINAL POTENTIAL OF THE INSOLUBLE EXTRACTED
FIBERS ISOLATED FROM THE FOMES FOMENTARIUS
FRUITING BODIES: A REVIEW.**

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Multiple action of the insoluble cell walls purified from the *F. fomentarius* fruiting bodies is particularly effective in the treatment of chronic, recurring, complicated multifactorial diseases. It is certainly worth exploring the medicinal potential and the practical application of these preparations further.

KEY WORDS:

Fomes fomentarius, medicinal mushrooms, cell wall, glucans, chitin, polyphenols, therapeutic properties

ABBREVIATIONS:

BW, body weight; DW, dry weight; IFN, interferon; IL, interleukin; LD50, median lethal dose; NK, natural killer; NOD2, nucleotide-binding oligomerization domain-containing protein 2; PAMPs, pathogen-associated molecular patterns; Th, T helper cells; TLR, Toll-like receptor; TNF, tumor necrosis factor

**SELENOPOLYSACCHARIDES OF MUSHROOM ORIGIN:
CONTROVERSY OVER EFFECTS ON THE HUMAN
IMMUNE SYSTEMM**

SESSION 07

LECTURE 06

Jadwiga Turło

Department of Drug Technology and Pharmaceutical Biotechnology,
Medical University of Warsaw, 1 Banacha St., 02-097 Warsaw, Poland

* E-mail: jadwiga.turlo@wum.edu.pl

Polysaccharides are extremely interesting macromolecules due to their remarkable structural diversity and, consequently, diverse biological activity. There are great differences in their physical and chemical properties, including solubility; viscosity; swelling capacity; resistance to acids, bases, and enzymes; and chemical character (acidic, basic, neutral), which contribute to their versatility. They also possess a wide range of pharmacological activities: antioxidative, antitumor, antimicrobial, antiviral, anti-obesity, hypolipidemic, antidiabetic, hepato-protective, and others.

Polysaccharides of mushroom origin are among the best known and most potent macromolecules with antitumor and immunomodulatory properties. The relationship between their activity and the chemical composition has been extensively investigated over the past three decades, however, there are some conflicting reports on this topic.

Interestingly, the modification of the polysaccharides' structure carried out by biotechnological or chemical methods may cause a significant change in their biological activity. Incorporation of selenium (Se) in the polysaccharide molecules is one of the currently explored methods of modifying the structure and activity of these compounds. The mechanism by which selenium exerts antioxidant, anticancer, and immunomodulating activity differs from that of polysaccharides, but a similar pharmacological effect suggests a possible synergy of these two agents. This has led to a growing research interest in Se-polysaccharides in recent years, particularly the methods of incorporating selenium into the carbohydrate structure and the structure- activity relationship of these compounds.

Although structure-activity relationship (SAR) among polysaccharides is not clear, it is assumed that determinants of their immunomodulatory activity are (e.g.) the monosaccharide composition, water solubility, molecular weight, branching degrees (DBs) and triple helical conformation. Uncertainty of the structural data on the atomic level considerably hinders this task. The next issue to be solved is the mechanism of immunomodulating activity of mushroom-derived polysaccharides. For example, one possible pathway of host-mediated actions from polysaccharide-drug lentinan is binding of β -glucans to iC3b-receptors (CR3, CD11b/CD18) of phagocytic cells and natural killer (NK) cells and stimulation of phagocytosis and/or cytotoxic degranulation.

**SELENOPOLYSACCHARIDES OF MUSHROOM ORIGIN:
CONTROVERSY OVER EFFECTS ON THE HUMAN
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We described in our papers the biosynthesis, isolation, and immunomodulatory activity of the selenium-containing polysaccharide fractions isolated from the mycelial cultures of *Lentinula edodes*. In our assumption, they were to be selenium-containing analogues of the immunostimulatory drug lentinan.

The results of the structural studies were unexpected and have shown that the fraction was a mixture of high molar mass polysaccharides α - and β -glucans, completely different structurally from lentinan. We defined four components of the Se- polysaccharide fraction, determined their primary structure, and examined the effect on T- and B-cell proliferation. The results of preliminary tests of immunomodulatory activity were also surprising: the effect of Se-polysaccharides on the proliferation of human blood lymphocytes demonstrated their selective immunosuppressive activity.

This activity seems to be opposite to immunostimulatory activity of *L. edodes* – derived polysaccharides, described in the literature. However, in-depth receptor binding studies currently being conducted indicate a more complex mechanism of action and do not rule out some mechanisms of immune system activation, despite a selective suppressive effect on T cell proliferation. So, to the question: the obtained Se-polysaccharides are immunostimulants or immunosuppressants, there is no simple and obvious answer yet.

HUNGARIAN MACRO FUNGI AS PROMISING SOURCE OF BIOACTIVE NATURAL PRODUCTS: SUMMARY OF A DECADE RESEARCH

SESSION 07

LECTURE 07

Attila Ványolós

Department of Pharmacognosy, Semmelweis University, Üllői u. 26, 1085 Budapest, Hungary

In the last decade we have launched an ample screening program for the evaluation of macro fungi indigenous to Hungary in terms of their potential antibacterial, antioxidant, xanthine oxidase (XOD) inhibitory and antiproliferative activities, among others. Our screening study carried out on the XOD inhibitory activity of 47 mushroom species demonstrated that several wild edible mushrooms (*Suillus grevillei*, *Tricholoma populinum* and *Clitopilus prunulus*) have remarkable enzyme inhibitory properties.

In the preliminary cytotoxicity experiments extracts of several species were investigated in vitro for their cytotoxic activity against several cancer cell lines, using the MTT assay. A total of 68 organic and water extracts of selected mushrooms were screened for their potential cell growth inhibitory activity. Our experiments revealed that 7 out of 17 species demonstrated notable antiproliferative activity against one or more cell lines. The antimicrobial activity of selected fungal species was examined against standard bacterial strains and clinical isolates, including multiresistant *Acinetobacter baumannii* and methicillin-resistant *Staphylococcus aureus* (MRSA). From 40 mushrooms included in this experiment, 16 species exhibited antibacterial effects with moderate to high potential, moreover some of them potentiated the action of cefuroxime against MRSA.

In our search for novel fungal metabolites with significant biological activity we have already identified compounds with a spectacular structural diversity. Among the isolated constituents one can find small molecular compounds e.g. steroids with ergostane and lanostane skeletons, ceramides and terphenyl quinones. Bioactivity-guided isolation led to the identification of two novel cyclopeptides from the mushroom *Gymnopus fusipes*, namely gymnopeptides A and B, which represent the largest cyclic peptides of mushroom origin being constituted of 18 amino acids. These unique peptides are highly methylated, the number of N-methylated amino acids is 10 out of 18. Gymnopeptides A and B exhibited remarkable cell growth inhibitory property with IC₅₀ values in nanomolar range.

Evaluation of the ion channel activity of mushroom metabolites isolated from brick cap mushroom (*Hypopholoma lateritium*) revealed that one of the isolated lanostane triterpenes demonstrated remarkable blocking activity on GIRK ion channel. Considering its intense blocking effect and high selectivity, this compound could be a potential promising agent in treatment of atrial fibrillation.

HUNGARIAN MACRO FUNGI AS PROMISING SOURCE OF BIOACTIVE NATURAL PRODUCTS: SUMMARY OF A DECADE RESEARCH

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In another study the examination of the edible *Meripilus giganteus* led to the isolation of two new cerebrosides, mericeramides A and B. Mericeramide A is the first representative of halogenated natural cerebrosides. Bioassay-guided fractionation of the chloroform extract of *Tapinella atrotomentosa* led to the isolation of secondary metabolites with significant antibacterial activity against multiresistant *Acinetobacter baumannii* and extended-spectrum β -lactamase-producing *Escherichia coli*.

The investigation of their activity in DPPH and ORAC assays revealed that they also have remarkable antioxidant properties. Recently a series of novel lanostane diesters named pholiol A-D along with other known fungal metabolites, with significant chemo-sensitizing activity on cancer cells have been identified in *Pholiota populnea*.

The results of our research project unambiguously demonstrate that mushrooms native to Hungary represent an inexhaustible reservoir of biologically active natural product with a significant therapeutic potential.

INVESTIGATION OF THE EFFECT OF BEER WITH GANODERMA LUCIDUM ON BLOOD PRESSURE AND HEART RATE IN YOUNG MEN

SESSION 07

LECTURE 08

Saša Despotović^{1*}, Mirjana M. Platiša², Mile Veljović¹, Viktor Nedović¹, Natalija Velić³,
Janez Gorenšek⁴, Ljiljana Gojković-Bukarica²

1 Faculty of Agriculture, University of Belgrade, Belgrade, Serbia / sdespot@agrif.bg.ac.rs

2 Faculty of Medicine, University of Belgrade, Belgrade, Serbia

3 Food and Technology Faculty Osijek, University of J.J. Strossmayera in Osijek, Osijek, Croatia

4 Institute of Applied Mycology and Biotechnology, Celje, Slovenia

A large number of studies have dealt with the effect of alcohol on the cardiovascular system. It is assumed that there is no significant difference between the alcoholic beverages consumed but that the only relevant parameter is the amount of alcohol consumed, i.e., its positive effect on the cardiovascular system in cases of moderate consumption. No consensus has been reached at the world level on safe amounts of alcohol, but each country has its guidelines. In most countries, recommended daily doses for men range from 10 to 40 g, limited to a maximum of 160 g per week. These values are reduced to 8 and 20 g per day for women. In this regard, the Institute for Mental Health of Serbia recommends up to two alcoholic drinks per day (330 ml of 5% beer) for men and one for women. Pharmacological in vivo studies were conducted on 18 men aged 26 ± 1 , whose body fat index was $24,5 \pm 0,5$ kg/m².

They were all in good general health, with no symptoms or history of heart disease, hypertension, or diabetes, a normal clinical picture, and no addiction problems. All examinations were conducted under the Ethics Committee of the Faculty of Medicine in Belgrade, while each individual voluntarily signed the consent for participation. Electrocardiogram, heart rate, systolic and diastolic blood pressure were recorded. Furthermore, the time between the depolarization of ventricles until their repolarization (the QT interval) and time between two successive ventricular depolarizations (the RR interval) were determined. The obtained results show that, immediately after consumption, control, and beer, there is a sudden jump in heart rate, diastolic and systolic blood pressure. However, after ten minutes, their values return to the initial level.

By analyzing the obtained electrocardiograms, it can be seen that the QT variability was significantly increased after beer consumption with *Ganoderma lucidum* extract, after which it returned to normal. This drink has an acute and delayed effect on the entire cardiovascular system, which could be monitored through changes in blood pressure and heart rate. The results also indicate a decrease in the parasympathetic control of the heart and altered dynamics of the QT interval.

Keywords: alcohol, beer, heart rate, *Ganoderma lucidum*, electrocardiography, adult.

LION'S MANE MUSHROOM (HERICIUM ERINACEUS) AS GOOD SOURCE OF BIOACTIVE CONSTITUENTS

SESSION 07

LECTURE 09

Se Hwan RYU,¹ Sang Won YEON,¹ Ayman TURK,¹ Sung Min KO,² Beom Seok KIM²,
Bang Yeon HWANG,¹ and Mi Kyeong LEE¹

1 College of Pharmacy, Chungbuk National University, Cheongju, 28160, Korea,

2 C&G Agricultural Association, Sejong 30067, Korea

E-mail: mklee@chungbuk.ac.kr

Hericium erinaceus, commonly called lion's mane mushroom, is an edible and medicinal mushroom that has been traditionally used for the treatment of metabolic disorders, gastrointestinal diseases and memory impairment. In this study, isolation and structural characterization were conducted with chromatographic techniques and spectroscopic analysis. In addition, anti-hyperglycemic activity of the isolated compounds was evaluated. Ten compounds including four new compounds, erinacenols A-D (1–4), were isolated from the fruiting bodies of *H. erinaceus*. Investigation of the anti-hyperglycemic effect of isolated compounds demonstrated that erinacenol D (4), 4-[3',7'-dimethyl-2',6'-octadienyl]-2-formyl-3-hydroxy-5-methoxybenzylalcohol (6), hericene A (7), hericene D (8) and hericenone D (9) strongly inhibited α -glucosidase activity with IC₅₀ values of < 20 μ M. Further analysis by molecular docking demonstrated the interaction of α -glucosidase and isolated compounds and suggested the importance of long side chain for α -glucosidase inhibitory activity.

For the development of mushroom with high content of active compounds, culture conditions were optimized by measuring the content of hericene A (HA), one of the active constituents of *H. erinaceus*, depending on different culture conditions. The highest HA production was achieved by agricultural by-products combination of sawdust 42% + cottonseed meal 12% + rice bran 7% + cottonseed hulls 19% + beet 13% with the value of 3.46 mg HA/g dry mushroom. The incubation days for fruiting bodies affected the HA content whereas little effect of mycelia growth periods. In addition, *H. erinaceus* grown in darkness contained a high amount of HA than those under light conditions. Taken together, *H. erinaceus* is a good source of bioactive constituents and the development of *H. erinaceus* with increased HA content can be achieved by optimization of cultivation conditions.

Acknowledgments: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT, 2022R1A2C1008081)

**EFFECT OF ISOLATED AND PURIFIED CHEMICAL
CONSTITUENTS FROM THE CULINARY-
MEDICINAL MUSHROOM LEUCOCALOCYBE
MONGOLICA” FRUITING BODY ON MAMMARY
EPITHELIAL CELLS PROLIFERATION (IN VITRO STUDY)**

SESSION 07

LECTURE 10

Asmaa Hussein Zaki,^{a,b} & Bao Haiying***a** Key Laboratory of Edible Fungi Resources and Utilization, Ministry of Agriculture and Rural Affairs, Jilin Agricultural University, Changchun, Jilin, 130118, China**b** Department of Agricultural Chemistry, Faculty of Agriculture, Minia University, Minia 61111, Egypt

** Address all correspondence to: Bao Haiying, Key Laboratory of Medicinal Fungal Resources and Development and Utilization, Jilin Agricultural University, Changchun 130118, People's Republic of China; E-mail: baohaiying2008@126.com;*

The fruiting body of medicinal mushroom *Leucocalocybe mongolica* contains many bioactive compounds that have many pharmacological activities. The bioactive compounds in the fruiting body were extracted by petroleum ether and water extracts.

Then, gas chromatography-mass spectrum (GC-MS), preparative liquid chromatography, nuclear magnetic resonance (NMR) and ESI-MS analysis were performed to separate and identify bioactive molecules from the fractions. Two fractions of the petroleum ether extract were identified by GC-MS, which contained sixteen compounds including Pentadecanoic; Hexadecanoic acid, methyl ester; 9 acid Hexadecanoic acid, ethyl ester; Oleic eicosyl ester; Hexadecanedioic acid, 3-methyl- dimethyl ester; Ethyl Oleate; Octadecanoic acid, ethyl ester; N-(2-Phenylethyl)undeca-(2Z,4E)-diene-8,10-diynamide (3.beta.,22E)-; Rhodopin; Heptanoic acid, 2-methyl-6-oxo- methyl ester; Nonanoic acid, 9-oxo- methyl ester; 3,4-Dihydroisoquinolin-7-ol 6-methoxy-3,3-dimethyl-1-methylsulfanyl-; Tetradecanoic acid, ethyl ester. In addition to three compounds were identified from fraction (6) including Hexanedioic acid, bis (2-Ethylhexyl) ester, Dibutyl phthalate and Phthalic acid, 6-ethyloct-3-yl-2-Ethylhexyl ester. In addition, NMR and ESI-MS analysis were used to identify three other compounds from mushroom *L. mongolica*.

Two compounds were obtained from the petroleum ether extract, which was identified as β -sitosterol and ergosterol by NMR and ESI-MS analysis. Similarly, another compound was isolated from the water extract which was identified as mannitol. An in vitro experiment was established to evaluate the impact of β -sitosterol and mannitol on and mammary epithelial MCF10A cells.

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The cytotoxicity effect of β -sitosterol and mannitol on mammary epithelial MCF10A cells was assessed by CCK8 assay. However, immunofluorescence, real-time PCRs, and western blot investigated the expression levels of various proliferation-related proteins. The results indicated that β -sitosterol and mannitol remarkably increased mammary epithelial MCF10A cell proliferation compared to control by 22.5% and 21.9%, respectively.

According to the results, β -sitosterol and mannitol in mammary epithelial development upregulate the expression level of PRLR, PR, ER, and STAT-5a in MCF10A mammary epithelial cells. These results may offer insight into the effect of β -sitosterol and mannitol in the development of mammary epithelial cells in vitro.

IN VITRO CULTURE TECHNOLOGY OF OPHIOCORDYCEPS SINENSIS: A BOON FOR NUTRACEUTICAL & PHARMACEUTICAL INDUSTRY

SESSION 07

LECTURE 11

Ranjit Singh* and H.K.Pandey

Defence Institute of Bioenergy Research (DIBER) DRDO, Haldwani (Uttarakhand)

* sangwanranjit@rediffmail.com

Ophiocordyceps sinensis or Caterpillar Mushroom is a high value medicinal, non-toxic and entomogenous fungus, having many similar species world over, parasitic on insects of one sort or another like flies, wasps, ants, scale insects, spiders, moths etc. *Cordyceps* an Ascomycetous genus belonging to family Clavicipitaceae of class Pyrenomycetes.

The species of *Cordyceps* found in abundance include *C. sinensis*, *C. gansuensis*, *C. militaris*, *C. capitata*, *C. memorabilis*, *C. ophioglossoides*, *C. nepalensis*, *C. multiaxialis* and *C. cicadae*. A very few species of this fungus are also found growing on the underground fruiting bodies of another fungus. Now *Cordyceps sinensis* is renamed as *Ophiocordyceps sinensis*. It is locally known as Yarsha Gambo and Kira

Since it parasitises on the Lepidopteran insect larvae of the caterpillar *Thetarhodes* spp. Yarsha Gambo is derived from Tibetan name Yartsa Gunbu (dbyarrtswadgun 'bu) meaning "summer grass winter worm". Name 'keeraghas' is on the basis of its appearance above the ground like a blade of grass. It is one of the most important species growing in higher hills of the Himalayas including Nepal, Bhutan, China and India.

Defence Institute of Bio-Energy Research, Haldwani, Uttarakhand (India) has succeeded in establishment of the pure mycelium culture of *O. sinensis* in the laboratory. The broth culture of this fungus yields a thick mat of mycelium which could be converted into powder form after freeze drying. In vitro fruiting body formation has also been achieved after trials on 76 different media combinations. Bio-chemical contents of the natural and lab produced *O. sinensis* has been compared and found at par. Cordycepin content which is most important bio-active compound was also found at par in both the samples. Because of the limited availability of the natural *O. sinensis* and very high costs reach to the general public was limited. Now because of the cost effective indigenous in vitro culture technology development and its transfer to three industries for its multiplication and product development. Benefit of this invaluable medicinal fungus will be available to the common masses and that too at an affordable cost without disturbing the natural habitat of this medicinal mushroom.

Keywords: *Ophiocordyceps sinensis*, Himalayas, Keera Ghas, DIBER, In Vitro, mycelium culture

MINERAL ELEMENT ENRICHMENT OF MUSHROOMS FOR THE PRODUCTION OF MORE EFFECTIVE FUNCTIONAL FOODS

SESSION 07

LECTURE 12

Oyetayo, V.O.

Department of Microbiology
Federal University of Technology / Akure, Nigeria

In the last three decades, human awareness of promotion of good health through dietary intervention had increased. Hence, there has been advocacy for the promotion and consumption of health enhancing foods referred to as functional foods. Myconutrients and chemicals obtained mainly from higher fungi have also received attention as sources of bioactive compounds that can serve as functional foods. Moreover, the importance of mineral elements in our diet for the maintenance of human health cannot be over emphasized.

These elements are responsible for a lot of chemical and electrical processes that enhance stable homeostatic physiological state in the body and their absence normally result to deficiency diseases. These processes are highly specified and specialized and the human body can only function properly if the accurate balance of minerals and other elements are continually being supplied to look after our system. Most staple foods consumed by human are lacking in one essential mineral element or the other. It is therefore expedient to find a way to remedy this.

Edible and medicinal mushrooms that can easily absorb minerals and can bio-accumulate them as functional organic compounds during growth can therefore be used as vehicle to supply these mineral elements that are not in adequate amount in our diet. This will certainly be a definite strategy to solve the problem of mineral malnutrition. Some of these essential elements are selenium, iron, zinc, calcium and so on. This paper therefore highlights the potentials of mushrooms as a hydra headed vehicle for the supply of mycoactive compounds and essential minerals in our diets as a means of promoting human health.

Keywords: Health, Enhancing, Functional foods, Strategy, Mineral elements, Enrichment

SHORT ORAL PRESENTATION / session NO 7

LECTURE 01 EFFECT OF VITAMIN D2, SELENIUM AND GLUCANS ON ANTI-CANCER ACTIVITY IN PORTOBELLO MUSHROOM

Grifoll, V.^{1*}, Lavega, R.¹, Carrasco, J.¹, Garrido, P.², Vilariño, M.², Mirpuri, E.² Martínez, A.², Pérez-Clavijo M.¹

1 Centro Tecnológico de Investigación del Champiñón de La Rioja (CTICH), Autol, Spain

2 Centro de Investigación Biomédica de La Rioja (CIBIR), 26006 Logroño, Spain

* Presenting author: microbiologia@ctich.com

Mushrooms are rich sources of bioactive compounds. The potential health benefits associated with mushroom intake are well-known. The study of the role of mushroom bioactive compounds to fight diseases, including cancer, is of major interest for our society. In the present study we have focused on the Portobello variety of *Agaricus bisporus* mushrooms. The aim is to find out whether agronomic intervention during mushroom cultivation may increase the content of certain bioactive compounds in the mushrooms and ultimately increase its anticancer activity.

The present study evaluated the effects of vitamin D2, selenium and glucans on anticancer activity. For this purpose, several Portobello crops were grown on commercial phase II compost following standard cultivation practice to correlate the increase in vitamin D2 and the anticancer activity. For this purpose, ultraviolet radiation (UVA, UVB and UVC) was used to increase vitamin D2. UV-lamps were placed at 25 cm from the casing layer in the mushroom beds and treated with 6-18 J/cm² for periods ranging from 15 to 45 min. UVA and UVB were applied during cultivation while UVC radiation was applied to freshly harvested mushrooms. Mushroom samples were extracted by saponification process and the levels of vitamin D2 were determined by HPLC. A significant increase in vitamin D2 was detected when mushrooms were irradiated with UVB but was not found in UVA-irradiated specimens. This increase in vitamin D2 was not followed by a significant improvement on the anti-cancer activity, indicating that the antitumor activity was not due to vitamin D2 contents.

In addition, different crop trials were performed to evaluate the influence of selenium treatment during cultivation on the anti-cancer activity of cultivated Portobello. Sodium selenate was incorporated with irrigation water to raise the selenium contents of the mushrooms.

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Five separate treatments were performed through drench applications onto the casing layer prior to primordial formation, with water containing 10, 20, 25, 30 and 60 ppm of selenium. Treatments were added as follows: 10 ppm six/nine times, 20 ppm nine times, and other treatments six times. Results of in vitro assay with cell lines showed that selenium has a protective effect over tumor cells.

Finally, the addition of carbohydrates to irrigation water was evaluated to examine their impact on the total glucan content. Glucose (0.05%), galactose (0.05%), xylose (0.1% and 0.05%), and mannose (0.05%) were studied. Total glucans were determined using yeast and mushroom β -Glucan assay kit (Megazyme). There was no significant increase in glucan content.

For all the conditions, mushrooms were harvested, processed and freeze-dried. Samples were kept sealed in the dark at room temperature until further analysis. Anticancer activity was always assessed through in vitro assays with human lung cancer cell line A549, as well as a non-cancer cell line NL20. IC50 values were calculated using MTT assays.

The project leading to this report has received funding from Consejería de Agricultura, Ganadería y Medio Ambiente de La Rioja, Spain, through project grant no. 24P/18.

Keyword: anticancer activity, bioactive compounds, medicinal mushrooms



ASSESSMENT OF THE IN VITRO ANTIOXIDANT AND ANTI-INFLAMMATORY POTENTIAL OF ETHANOL EXTRACTS OF CULTIVABLE MUSHROOMS

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LECTURE 02

Grifoll, V.^{1*}, Lavega, R.¹, Siles-Sanchez, M.N.², Santoyo, S.² Pérez-Clavijo M.¹

1 Centro Tecnológico de Investigación del Champiñón de La Rioja (CTICH), Autol, Spain

2 Department of Production and Characterization of Novel Foods, Institute of Food Science Research-CIAL (UAM + CSIC), Campus de Cantoblanco, Universidad Autónoma de Madrid, 28049 Madrid, Spain.

* Presenting author: microbiologia@ctich.com

Mushrooms are a very relevant source of proteins, carbohydrates, amino acids, and minerals. They also contain many bioactive metabolites. For these features, mushrooms have been historically appreciated as tasty food and for medicinal purposes. Polysaccharides, terpenoids, phenolics, and many other low molecular weight compounds have been reported to be responsible for the antioxidant and anti-inflammatory effects detected in mushrooms. The health benefits observed in mushroom-based diets could be partially related to additive or synergistic antioxidant and anti-inflammatory properties of a wide group of bioactive compounds.

The aim of this study was to evaluate the antioxidant and/or anti-inflammatory potential of cultivated basidiomes of *Agaricus bisporus* (AB), *Agaricus brunneocens* (ABp), *Agaricus subrufescens* (ABz), *Lentinula edodes* (LE), *Agrocybe aegerita* (AA), *Pleurotus citrinopileatus* (PC) and stems of *Agaricus bisporus* that eventually could be employed as ingredients for nutraceuticals, pharmaceuticals, functional food, cosmetics, and animal feed.

The different mushroom species were grown using different substrates and following standardized cropping practices in our facilities. Mushrooms were harvested, frozen and freeze-dried for further analysis. Sequential extraction with ethanol was performed. All extracts had a quantifiable but moderate antioxidant activity measured by TPC (total phenolic compounds), DPPH, FRAP, and ABTS assays. The antioxidant activity was also assessed by a cellular method using Caco-2 as a cellular model. The Cellular antioxidant activity (CAA) assay showed that ABz and ABp have high antioxidant activity, followed by AB, LE, and AA. PC showed the lowest activity.

The anti-inflammatory activity of the ethanol extracts was evaluated on a model of inflammatory monocytes, human THP-1. After differentiation to macrophages (THP-1/M), cells were incubated with 0.01 µg/mL of LPS and 40 µg/mL mushroom extracts for 12 h. The release of TNF-α, IL-1β and IL-6 was measured in the supernatants of THP-1/M cells using ELISA kits.

**ASSESSMENT OF THE IN VITRO ANTIOXIDANT
AND ANTI-INFLAMMATORY POTENTIAL OF ETHANOL
EXTRACTS OF CULTIVABLE MUSHROOMS**

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LECTURE **02**

Our observations suggest that including industrially cultivated mushrooms in the diet may help to prevent diseases associated with oxidative damage and inflammation.

The project leading to this report has received funding from Consejería de Agricultura, Ganadería y Medio Ambiente de La Rioja, Spain, through project grant no. CT22_04.



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**EFFECT OF LED ILLUMINATION ON YIELD,
 QUALITY AND VITAMIN D2 CONTENT
 IN PLEUROTUS OSTREATUS**

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LECTURE **03**

Marina De Bonis*, Silvia Locatelli, Paolo Zanin, Paolo Sambo, Carlo Nicoletto

Department of Agronomy, Animal, Food, Natural resources and Environment (DAFNAE) –
 University of Padua, Viale dell'Università 16, Legnaro (PD) Italy

*marina.debonis@phd.unipd.it

LED lighting is an innovative technology characterized by different application areas that allow to improve the production and quality performance of fruit and vegetables. The world of cultivated mushrooms is also a potential target of this technology as a tool for biofortification. The application of led light in cultivated mushrooms has been mainly evaluated in *Agaricus bisporus*, whereas information on *Pleurotus ostreatus* is still limited requiring further investigation and confirmation. The aim of the research was to evaluate the effect of different integrative lighting treatments in order to improve the nutritional traits of *P. ostreatus*.

This study (funded by Project YESP - Veneto Rural Development Program 2014-2020; PON Green Themes - Action IV.5) aimed to observe the effect on yield, quality and nutrient content of vitamin D2 of oyster mushrooms under different LED lightening treatment (LLT). The first flush of two consecutive production cycles of *P. ostreatus* (P80 and P73 mycelium produced by Italspawn) under three different types of LLT (red, blue, red+blue) were considered. *P. ostreatus* was cultivated on straw substrate. During the cultivation cycles the emergence of primordia, yield, number of families, number of fruiting bodies and their dimension (diameter and thickness) were measured. Qualitative traits (pH, EC, °Brix, total phenol and antioxidant content) were also analyzed together with the amount of vitamin D2.

In the first cycle red light increased primordia earliness compared to other treatments. Red light was also characterized by an higher production of families per kg of substrate (0.66). Different artificial lighting affected morphological traits: treatment with red/blue and blue light increased the diameter of the mushroom body with an average of 10.74 and 9.86 cm respectively. In the second production cycle, there were no significant differences in the formation of primordia, but red light affected the production of fruiting bodies per family. Concerning the morphological characteristics, blue and red/blue light had an effect on fruiting body diameter with about (8.90 cm) on average.

**EFFECT OF LED ILLUMINATION ON YIELD,
QUALITY AND VITAMIN D2 CONTENT
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Light treatments did not affect the pH, electrical conductivity, total soluble solids and titratable acidity, but a higher concentration of vitamin D2 was found in mushrooms grown under blue and red/blue light treatments. In both cycles LLT did not influence *P. ostreatus* yield. In conclusion, different LLT do not affect the yield and quality of *P. ostreatus* production but have a significant effect on the formation of primordia and fruiting bodies, as well as the size of the mushroom. In addition, vitamin D2 is positively affected by light treatment with blue and red/blue light. It is also considered appropriate to study the combined effect during the production cycle of red-light and blue light, the first during primordia formation and the latter during the growth of mushroom bodies.

**ENHANCING "LONGEVITY VITAMIN" L-ERGOTHIONEINE
EXTRACTION FROM MUSHROOMS COLLECTED IN ITALY:
A PRELIMINARY STUDY**

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LECTURE 04

**Lorenzo Goppa¹, Paola Rossi², Federica Corana³, Anthea Desiderio¹,
Daniela Ratto², Elena Savino¹**

1 Department of Earth and Environmental Sciences (DSTA), University of Pavia,
Via S. Epifanio 14, 27100 Pavia, Italy

2 Department of Biology and Biotechnology "L. Spallanzani" (DBB), University of Pavia,
Via Ferrata 1, 27100 Pavia, Italy.

3 Centro Grandi Strumenti, University of Pavia, Via A. Bassi 21, 27100 Pavia, Italy.

L-(+)-Ergothioneine (ET) is an amino acid derived from histidine that shows a tautomeric structure, with the thione tautomer predominant at physiological pH; this makes the molecule unusually resistant to autoxidation. Thanks to its beneficial effects on humans, there is an increasing interest by scientific community to better understand the activity of this molecule on human health. ET has been shown to be able to extend lifespan and to protect neurons and preserve cognitive function, so it has recently been defined as the "longevity vitamin".

Humans cannot synthesize ET and need to uptake it from diet; mushrooms are the main food source of ET.

In the present work fungal species that, according to literature, could have high ET concentrations together with never investigated species were chosen for ET extraction and quantification; samples of sporophores (collected in the field or purchased depending on the species) and/or mycelia (isolated from sporophores and grown on malt extract broth) have been analyzed.

These preliminary steps were necessary to get research's goal: optimize the ET extraction procedures. Among 13 analyzed basidiomes, the highest ET concentration was found in the genus *Pleurotus* (Fr.) P. Kumm., and in particular *P. citrinopileatus* Singer, followed by *Hericium erinaceus* (Bull.) Pers.

In order to improve ET final yield we focus firstly on sample preparation, comparing drying with lyophilization and finding that the former allows a higher ET concentration than the latter.

Then, comparing ET content in different basidiomes' portions, pileus resulted to be richer than stipes.

For *Fomitopsis officinalis* (Vill.) Bondartsev & Singer, *H. erinaceus* and *P. citrinopileatus* also the difference between different growth stages such as basidiome and mycelium have been investigated, but no clear trend was found, it depends on the considered species.

**ENHANCING “LONGEVITY VITAMIN” L-ERGOTHIONEINE
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Finally, for these species three different extraction procedures have been tested by changing three fundamental extraction parameters like raw material/solvent ratio and incubation temperature and time. The results show that a raw material/solvent ratio of 1:20 and an incubation time of 24h or 48h along with an incubation temperature of 25°C are recommended to increase the final ET yield.

Keywords: L-Ergothioneine; mushrooms; extraction; *Fomitopsis officinalis*; *Heridium erinaceus*; *Pleurotus*; *Pleurotus citrinopileatus*.

**NUTRITIONAL PROPERTIES, CHEMICAL COMPOSITION
AND BIOLOGICAL PROPERTIES OF CRATERELLUS
CORNUCOPIOIDES**SHORT
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07

LECTURE 05

Dejan Stojković¹, Marina Kostić¹, Angela Fernandes², Lillian Barros², Jasmina Glamočlija¹**1** Institute for Biological Research "Siniša Stanković" - National Institute of Republic of Serbia, University of Belgrade, Bulevar despota Stefana 142, 11000 Belgrade, Serbia.**2** CIMO-ESA, Polytechnic Institute of Bragança, Campus de Santa Apolónia, Ap. 1172, 5301-855 Bragança, Portugal.

Craterellus cornucopioides (L.) Pers. (Cantharellaceae), or horn of plenty, is an edible fungi. It is also known as the black chanterelle, black trumpet, crna truba (Serbian), trompette de la mort (French), trombetta dei morti (Italian) or trumpet of the dead, djondjon (Haitian). *C. cornucopioides* growing wild in Serbia was chemically characterized in nutritional value and individual compounds (sugars, fatty acids, tocopherols, organic acids, and phenolic compounds).

Antioxidant (scavenging activity, reducing power and inhibition of lipid peroxidation), antibacterial and antifungal activities of its methanolic extract were evaluated in vitro. *C. cornucopioides* was shown to be rich in carbohydrates (79.32 g/100g dw), followed by proteins (8.36 g/100g dw), ash (9.43 g/100g dw), and fat (2.90 g/100g dw). The most abundant sugar compound was identified as mannitol (14.16 g/100g dw); four tocopherol isoforms (α -, β -, γ - and δ -tocopherols) were detected. Unsaturated fatty acids predominated over saturated ones, with oleic and linoleic acids as the most dominant. Five organic acids were quantified oxalic, citric, quinic, fumaric, and malic acid. The methanolic extract of the mushroom showed in vitro antioxidant and antimicrobial activities. Methanolic extract successively inhibited the growth of pathogenic bacteria.

Keywords: *C. cornucopioides*; chemical composition; antioxidant activity; antimicrobial activity.

Acknowledgments

This research is funded by the Serbian Ministry of Education, Science and Technological Development [Contract No. 451-03-68/2022-14/ 200007]. The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Programme PT2020 for financial support to CIMO (UIDB/00690/2020); national funding by FCT, P.I., through the institutional scientific employment program-contract for L. Barros's contract through the celebration of program-contract foreseen in No. 4, 5 and 6 of article 23° of Decree-Law No. 57/2016, of 29th August, amended by Law No. 57/2017, of 19th July.

ANTIOXIDATIVE AND ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACTS FROM REISHI AND REISHI/PROPOLIS

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LECTURE 06

**Sandra Konstantinović¹, Milica Stefanović¹, Dragan Troter¹, Dragan Stojiljković²,
Ljiljana Stanojević¹, Bojana Danilović¹**

1 Faculty of Technology, Bulevar Oslobođenja 124, 16000 Leskovac, Serbia

2 Super Natura, Južnomoravskih brigada 156E, 16000 Leskovac, Serbia

Ganoderma lucidum, also known as Reishi, is a polypore mushroom that belongs to the genus *Ganoderma*. Compounds, such as, triterpenoids, polysaccharides and peptidoglycans, are responsible for Reishi health effects. *G. lucidum* is used in treatment of different viral infections, bronchitis, asthma; heart, kidney and liver disease, high blood pressure and cholesterol and different types of cancer. Propolis and its extracts are known to have antiseptic, anti-inflammatory, antioxidant, antimicrobial activities.

In the present study antioxidative and antimicrobial activity of commercial ethanolic Reishi (*G. lucidum*) and Reishi/Propolis extracts were determined. The commercial extracts were purchased from Super Natura, Leskovac. Commercial ethanolic extracts of pure Reishi (70 v/v) and Reishi/Propolis (70:30 v/v) were given by Super Natura, Leskovac. All extracts were obtained from pure Reishi mycelium (Fungi Land, Leskovac, Serbia) and used for analysis. 100 mL of each extract was evaporated by rotary evaporator at 40 °C to dryness and dried at 50 °C until reaching constant weight ($\gamma = 4$ mg/mL).

The antioxidant activity of extracts was investigated spectrophotometrically by DPPH assay. Disc-diffusion method was used for antimicrobial activity investigation on following pathogenic microorganisms: *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC15313, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 8427, *Candida albicans* ATCC 2091, *Bacillus cereus* ATCC 11778, *Klebsiella pneumoniae* ATCC 700603 and *Bacillus luteus* in haus soj.

Reishi/Propolis extract showed better antioxidant and antimicrobial activity activity than the reishi extract. The degree of DPPH radical neutralization depends on the extracts concentration. The concentrations of Reishi and Reishi/Propolis extracts required to neutralize 50% of the initial concentration of DPPH radicals (EC50) after 20 minutes incubation and immediately after adding DPPH solution were 56 and 2 ug/cm³, respectively.

ANTIOXIDATIVE AND ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACTS FROM REISHI AND REISHI/PROPOLIS

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The pure Reishi extract showed no activity against *L. monocytogenes*, *B. cereus* and *B. luteus*, while it showed activity against *S. aureus*, *E. coli*, *P. aeruginosa* and *P. vulgaris* (inhibition zone 9, 10, 12 and 15 mm, respectively). Reishi/Propolis extract showed the antibacterial activity against *L. monocytogenes* and *B. luteus* (inhibition zone 20 and 15 mm, respectively). Reishi/Propolis extract showed the best activity against *S. aureus*.

The obtained results indicated that commercial ethanolic Reishi and Reishi/Propolis extracts could be used as sources of natural antioxidants and antimicrobial agents.

Acknowledgment: This work was supported by the Republic of Serbia - Ministry of Education, Science and Technological Development, Program for financing scientific research work, number 451-03-68/2022-14/200133.

FUNGI EXTRACT BANK – FROM INNOVATION TO APPLICATION

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LECTURE **07**

Ewa Zapora, Urszula Waszczuk

Institute of Forest Sciences, Białystok University of Technology, Poland

The Fungi Extract Bank is a project implemented by the Institute of Forest Sciences of Białystok University of Technology (Poland) in a modern Research-Scientific Centre in Hajnówka, located on the edge of the Białowieża Primeval Forest.

The Fungi Extract Bank (FEB) is an innovative research project. The basis for the establishment of the FEB was many years of experience as well as extensive national and international cooperation. The uniqueness of the Białowieża Forest region and the diversity of mycobiota present here provide opportunities to discover new species for science and the opportunity to examine activity of unusual fungal species.

The Fungi Extract Bank is a collection of extracts from several hundred species of Macromycetes – mostly from the morphological group of polyprotid fungi (suprapubic, suprarenal, mainly saprotrophs and parasites). It is a constantly growing collection. To create a collection of extracts, classic methods of solvent extraction are used, as well as belonging to green technologies - Supercritical Fluid Extraction (ScFE).

Fungi come mostly from the Białowieża Forest. The species are collected from different hosts, which can be important in the identification of chemical composition and further studies of their biological activity. In addition, there are also extracts from fungal species from other countries of Europe, as well as North and South America.

The main goals of the projects carried out at FEB is the screening of fungal extracts. The aim of this research is to search unknown so far activities of fungi, which may therefore prove to be candidates for new medicinal mushrooms in the future.

The FEB is the basis for collaborating and carry out research in the field of mycology and potential activity of fungi. The aim of cooperation is implementation of R&D projects (medical, veterinary, agricultural, forestry etc.), and the exchange of current knowledge and experience in interdisciplinary teams of experts.

The Fungi Extract Bank enables both research on unique, rare species of fungi and increases the applicability of the research results, which in turn enables their commercialization.

**A NEWLY DEVELOPED HERICIUM ERINACEUS FOOD
SUPPLEMENT WITH STANDARDIZED ERIANCINE
A CONTENT**

SHORT
ORAL
PRES

07

LECTURE 08

**Marija Gregori¹, Erik Bird¹, Jure Pohleven¹, Marko Dragan², Janez Gerdenc²,
Boštjan Jančar², Tinka Palkovič³, Katerina Naumoska³, Alen Albreht³, Andrej Gregori^{1,4}**

1 Mycomedica Ltd, Podkoren 72, 4280 Kranjska Gora, Slovenia

2 PharmaHemp Ltd, Koprška ulica 106c, 1000 Ljubljana, Slovenia

3 Laboratory for Food Chemistry, Department of Analytical Chemistry, National Institute of Chemistry,
Hajdrihova ulica 19, 1001 Ljubljana, Slovenia

4 Biotechnical Faculty, University of Ljubljana, Jamnikarjeva ulica 101, 1000 Ljubljana, Slovenia

Erinacine A is a medicinal compound with various neuroprotective properties, such as enhancing nerve growth factor release; reducing amyloid- β deposition, increasing insulin-degrading enzyme expression etc. As eriancine A content is of major importance and is mostly unknown in food supplements, we aimed to determine its content, as well as to develop a new, safe and effective product with standardized content of eriancine A.

Erinacine A standard was obtained from *Herichium erinaceus* by extract fractionation using Flash/Prep in first dimension, followed by second dimension fractionation using HPLC-UV. The identity of the isolated compound was proven by LC-MS/MS and the purity (against procedural blank) was determined by LC-CAD.

Pure erinacine A (98.56%) was used as an external reference standard for HPLC analysis with the aim of developing a standardized food supplement. Samples for analyses were prepared by extraction with 70% ethanol. Agilent 1260 Infinity II HPLC system with a Poroshell 120 SB-C18 4.6 x 150 mm 2.7 μ m column and a DAD detector at wavelength of 340 nm was used for quantification. HPLC method was developed and validated in an ISO 17025 accredited laboratory.

Over 50 commercial and wild *Herichium erinaceus* strains were screened for erinacine A content and the best of them were cultivated and fruited under optimized growing condition. Optimized were composition of growing substrate, its moisture content and particle size distribution, type and volume of cultivation vessel/bag, weight of substrate, type and size of breathing filters, temperature during substrate overgrowth and fructification, relative humidity in growing chambers, light regime and intensity as well as temperature and duration used for drying of an end product.

A NEWLY DEVELOPED HERICIUM ERINACEUS FOOD SUPPLEMENT WITH STANDARDIZED ERIANCINE A CONTENT

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After harvesting we aimed to increase extraction yields of erinacine A through optimizing extraction parameters, such as temperature, pressure, solvent type and concentration, solvent/material ratio, extraction time and extraction type (batch or continuous). After extraction liquid extracts were dried using different, mostly starch containing carriers.

After more than 2500 cultivation and extraction experiments a new standardized food supplement was developed containing concentrations of erinacine A higher than 1 mg/g. This product's prototype safety as well as significant improvement in cognitive abilities measured by THP parameter were already proven in a pilot double-blind placebo-controlled trial.

The project was co-financed by the Republic of Slovenia and the European Union from the European Regional Development Fund (ERDF).

Keywords: erinacine A, *Heridium erinaceus*, cultivation, fruiting bodies, food supplement

COPRO PROJECT: DEVELOPMENT OF A FIRST STANDARDIZED FOOD SUPPLEMENT FROM CORDYCEPS

SHORT
ORAL
PRES

07

LECTURE 09

Erik Bird¹, Marija Gregori¹, Jure Pohleven¹, Nina Prezelj², Marko Prezelj², Rok Pikon²,
Tilen Oražem², Andrej Gregori^{1,3}

1 Mycomedica Ltd, Podkoren 72, 4280 Kranjska Gora, Slovenia

2 Kobis Ltd, Ljubljanska 48, 1236 Trzin, Slovenia

3 Biotechnical Faculty, University of Ljubljana, Jamnikarjeva ulica 101, 1000 Ljubljana, Slovenia

The aim of this project was to develop a new Cordyceps food supplement with standardized content of cordycepin 10mg/g on an industrial level. As content of cordycepin and other adenosine analogues in most food supplements is mostly unknown or varies greatly (from 0,03mg/g to slightly above 1,5mg/g in tested food supplements) we developed a new food supplement with known as well as standardized content of cordycepin and with higher amount compared to any known existing food supplement on the market.

A HPLC analytical procedure was developed and validated. Samples were extracted in 20% methanol and analysed using BDS Hypersil C18; 2,4µm; 100 mm x 4,6 mm column (Thermo Fisher Scientific) and 0,007 M phosphate/methanol gradient.

Approximately 65 wild and commercial Cordyceps strains were screened for cordycepin and adenosine content and their cultivation parameters optimized through series of experiments. As strain degeneration rate plays a very important role, many steps were taken to use undegenerated strains and preserve them against degeneration. Optimized cultivation parameters include substrate type, its moisture content and particle size distribution, filling rate of cultivation vessels and substrate weight, cultivation vessel volume, its dimensions and type (bag, PP box, PP bag), type and size of breathing filters, temperature and RH in growing rooms, light regime and intensity during overgrowth and fructification.

After 86 cultivation/extraction experiments and more than 2100 samples tested, a new standardized food supplement named Cordyvit®Pro K was developed containing concentrations of cordycepin higher than 10 mg/g.

The project was co-financed by the Republic of Slovenia and the European Union from the European

Regional Development Fund (ERDF).

Keywords: cordycepin, Cordyceps sinensis, cultivation, fruiting bodies, food supplement, Cordyvit

SESSION 08

LECTURE 01 SELECTION OF WOOD DECAY FUNGAL STRAINS WITH MEDICINAL PROPERTIES USEFUL FOR DEVELOPMENT OF MYCO-MATERIALS

Cartabia M.^{1,5}, Savino E.¹, Baiguera R.M.¹, Buratti S.¹, Girometta C.E.¹, Auricchio F.³, De Donno C.³, Scalet G.³, Di Landro L.⁴, Benedetti A.⁴, Milanese C.², Vadivel D.², Babbini S.⁵, Dondi D.²

- 1 Dep. of Earth and Environmental Sciences (DSTA), University of Pavia, Via S. Epifanio 14, 27100 Pavia, Italy
- 2 Dep. of Chemistry, University of Pavia, Viale Taramelli 10, 27100 Pavia, Italy
- 3 Dep. of Civil Engineering and Architecture (DICAr), University of Pavia, Via Ferrata 3, 27100 Pavia, Italy
- 4 Dep. of Aerospace Engineering, Politecnico di Milano, Via La Masa 34, 20156 Milano, Italy
- 5 MOGU S.r.l., Via S. Francesco d'Assisi 62, 21020 Inarzo (VA) Italy

Recently researchers are focusing their attention on the potential of wood decay fungi (WDF) as a source for biotechnological and industrial applications. Chemical composition, mycelial texture, ease of cultivation, and lack of sporification make these organisms particularly suitable for developing myco-materials. In the Myco-Advanced leather materials (MATER) project different strains of wood decay fungi (many of them considered medicinal mushrooms too) were isolated using 2% malt extract agar (MEA) medium enriched with hydrogen peroxide and maintained at 4 °C and -80 °C. The identification of the strains was confirmed by molecular analysis of the ITS region. Based on growth rate, colour, homogeneity, consistency of the mycelium, at first 21 strains were chosen to be chemically characterised through scanning electron microscopy (SEM) and thermogravimetric analysis (TGA).

Three strains were selected in consideration of their different cell wall chemical composition (high content of α -glucans, β -glucans or chitin) to evaluate how these differences could influence the mechanical and chemical characteristics of myco-materials. The fungal strains were cultivated in liquid submerged dynamic fermentation (both flasks and bioreactor). Later on, chitin and glucans were crosslinked with acetic acid and plasticized with glycerol in order to obtain flexible sheets. *Abortiporus biennis*, *Fomitopsis iberica* and *Stereum hirsutum* strains resulted to be adapted to produce material with adequate flexibility. Thermogravimetric analysis (TGA) allowed us to evaluate the principal chemical components, providing a semi-quantitative indication on mat composition. The material obtained from each species was mechanically tested in terms of tear strength, elongation at break, and Young's modulus. Taking all the results into account, there was evidence of a correlation between chitin content and material mechanical response.

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In conclusion, a new typology of sustainable and 100% pure fungal-based raw materials have been produced from different wood decay fungi with officinal properties too. Further chemical and physical steps are needed in order to improve these materials for practical applications.

The in-depth analysis of fungal strains knowledge is an essential groundwork for any further study on this topic. Future interdisciplinary researches can give a real opportunity to significantly improve myco-materials.

Keywords: myco-materials; fungal strains; medicinal mushrooms; thermogravimetric analysis (TGA); electron microscopy (SEM)

This study was supported by Fondazione Cariplo and Regione Lombardia, Grant No. 2018-1765, through the project entitled "MYCO-ADVANCED LEATHER MATERIALS (MATER)", P.I. Professor D. Dondi

MYCELIUM-BASED COMPOSITES IN THE ARCHITECTURAL SCALE

SESSION 08

LECTURE 02

Hana Vašatko¹, Milena Stavic¹

¹ Graz University of Technology, Institute of Architecture and Media, Graz, Austria

The increased utilisation of mycelium-based composites in the last decade has made their implementation wider in architectural and design practices, but also some industrial applications. Such naturally grown materials present a sustainable alternative to several building elements, i.e. components. The current state of the art is investigated by looking into patents, scientific literature and own preliminary research. Scarcity and depletion of natural resources, the anthropocene and circular economy are one of the many factors that led to an increased interest in developing environmentally feasible materials. The construction industry is responsible for a large portion of the global CO₂ emissions and finding sustainable alternatives has become more important than ever. Consequently, bio-based materials, such as mycelium-based composites, have gained their significance in architecture and design.

The ability of hyphae to bind the substrate it grows on into a solid material opened up the possibility of developing objects that have the potential of substituting several building elements. Numerous artists and designers have developed and designed minor scale prototypes, which were made as temporary artefacts on a small scale for exhibitions, but also developed as alternative products, such as packaging material, furniture, insulation, pottery or sound absorbers. Most of these products were made by casting the substrate in a mould with the desired shape. As this way of shaping mycelium-based composites has its advantages in achieving precise geometries and repetitive production processes by usage of the same mould, it often remains limited when it comes to freeform shapes. Additional shaping methods developed through digital fabrication tools are also presented as an alternative to the standard tools of casting.

Although fabricating mycelium-based products on a scale of design objects presents an achievable goal, scaling up to an architectural and building context remains under discussion. Maintaining a sterile environment during the fabrication process of large elements and the exposure of the material in atmospheric conditions are still a limiting factor. Several projects that have tackled these issues and were successfully developed are presented in this lecture, with the focus on using different fabrication methods, depending on their design strategy.

VALIDATION OF LARGE-SCALE FOREST-FUNGI INOCULATION AND CHAGA CULTIVATION NETWORK IN THE NORDICS: A CIRCULAR BIOECONOMIC STRATEGY FOR SUSTAINABLE FOREST MANAGEMENT

SESSION 08

LECTURE 03

Peter Petros, Maria Suonpää, Casper Fransman, Heikki Eino Kiheri

KÄÄPÄ Biotech Oy, Karjalohja, Finland University of Helsinki, Department of Microbiology,
Helsinki, Finland Natural Resources Institute Finland, Helsinki, Finland

Chaga is a high-value, fungi-derived biomass that forms from unique metabolic biochemical processes between the white rot fungus, *Inonotus obliquus*, and its host tree. Chaga is increasingly sought after and harvested for use in global industries, primarily the rapidly growing food supplement industry. Found only in northern hemispheric regions, a single wild specimen of chaga can take several years to develop into a mature form, resulting in low natural availability. To date, the feasibility of successful cultivation of chaga has been questioned. Herein, we present an industry-led initiative for the validation of large-scale, decentralized inoculation of fungi and cultivation of chaga as a valuable economic forest-based strategy for sustainable forest management.

The method utilizes myceliated dowels colonized with a native strain of *Inonotus obliquus* and applied via inoculation to living tree stands otherwise felled for secondary forest-value chains. Monitored tree stand inoculations commenced from 2018 via a forest inoculation network. In 2021, 278 inoculated living trees (227 *Betula pubescens*; 50 *Betula pendula*; 1 *Alnus incana*) across 12 managed sites across Finland were randomly-selected, examined and characterized. The observed inoculation success rate after 3 years averaged 92% and the probability of chaga bulging averaged 68.6% for the 12 sites. Annual wild chaga observations revealed highly variable annual growth rates in measured biomass volume (min: -30%, max: +165%, average: ~50%), reflecting the need for ongoing monitoring and data collection of chaga cultivations. Preliminary results provide strong support for this approach to offer commercially-viable, large-scale, sustainable cultivation of high value fungal-crop derivatives as an alternative bio-circular economic practice for sustainably managed forests. Countries with extensive forest cover of *Betula* spp. such as Finland, provide an ideal setting for a chaga cultivation network. The cultivation network presented herein is an essential practice to enable further optimization of large-scale forest-fungi cultivation methods at scale to provide future sustainable solutions for consumer markets and beyond. KÄÄPÄ Biotech has developed the largest commercial chaga network with over 225 hectares of managed forest containing over 100,000 inoculated trees and over 400,000 chaga dowels utilized via inoculations to date.

Keywords: chaga, sustainable forest management, inoculation, fungi, circular economy, *Inonotus obliquus*, cultivation.

EFFECT OF SEA BUCKTHORN PRESS CAKE ON THE CULTIVATION OF INONOTUS OBLIQUUS MYCELIUM AND ITS POLYSACCHARIDES

SESSION 08

LECTURE 04

Gabriele Beltrame¹, Jarl Hemming², Jani Trygg¹, Hongbo Yang³, Zenghua Han³,
 Baoru Yang¹

1 Food Chemistry and Food Development, Department of Life Technologies, University of Turku,
 Turku, Finland

2 Wood and Paper Chemistry, Åbo Akademi University, Turku, Finland

3 Institute of Microbiology, Heilongjiang Academy of Sciences, Harbin, China

Inonotus obliquus is a basidiomycete, obligate parasite of birch, found above the 40th parallel north, mainly in North America, Northern Europe, Siberia, Northern China, and Japan. During its infection, it causes the formation of a black sterile conk from the tree bark. This sterile conk, called Chaga, is a renowned traditional remedy and a resource of interest for the production of nutraceutical products. Multiple biological activities have been assigned to Chaga extracts and isolated molecules. Among these molecules, polysaccharides have demonstrated multiple bioactivities and constitute relevant ingredients of commercial products. In contrast with the increasing industrial interest for Chaga, its harvesting is hampered by its slow growth and the logistical problems related to collection and transportation.

The mycelium of this basidiomycete, obtained after submerged cultivation, has been considered a feasible alternative to the sterile conk for the production of polysaccharides. Sea buckthorn press cake, fibrous sidestream of juice industry rich in oil, has been selected in our work as cultivation supplement to increase the mycelium yield of *I. obliquus*. Different supplement dosages and two cultivation times were used. Exopolysaccharides from culture media and intracellular polysaccharides extracted from mycelia were investigated. Supplementation increased the cultivation yield of *I. obliquus* and positively affected the yield and molecular weight of exopolysaccharides.

However, at higher dosages, the supplementation reduced content and molecular weight of mycelial polymers. The pectins in the sea buckthorn press cake affected to different extent the monomer composition of polysaccharides. Polymers extracted from mycelium had high glycogen content, lacked in phenolic compounds, and were polydispersed. On the contrary, polymers obtained from wild sterile conk were monodispersed and absent in glycogen but rich in phenolic compounds. The differences in structure and molecular properties among the polysaccharides of mycelium and sterile conk are likely associated with differences in bioactivities and, therefore, in nutraceutical potential.

**AMAZING GRACE HEATH PRODUCTS –
NATURAL PHELLINUS MUSHROOM -
NEW VISIONS OF A MODERN ELIXIR OF LIFE**

SESSION **08**

LECTURE **XX**

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EFFECT OF VITAMIN D2, SELENIUM AND GLUCANS ON ANTI-CANCER ACTIVITY IN PORTOBELLO MUSHROOM

SESSION 08

LECTURE 01

Grifoll, V.1*, Lavega, R.1, Carrasco, J.1, Garrido, P.2, Vilariño, M.2, Mirpuri, E.2 Martínez, A.2, Pérez-Clavijo M.1

11 Centro Tecnológico de Investigación del Champiñón de La Rioja (CTICH), Autol, Spain

2 Centro de Investigación Biomédica de La Rioja (CIBIR), 26006 Logroño, Spain

* Presenting author: microbiologia@ctich.com

Mushrooms are rich sources of bioactive compounds. The potential health benefits associated with mushroom intake are well-known. The study of the role of mushroom bioactive compounds to fight diseases, including cancer, is of major interest for our society. In the present study we have focused on the Portobello variety of *Agaricus bisporus* mushrooms. The aim is to find out whether agronomic intervention during mushroom cultivation may increase the content of certain bioactive compounds in the mushrooms and ultimately increase its anticancer activity.

The present study evaluated the effects of vitamin D2, selenium and glucans on anticancer activity. For this purpose, several Portobello crops were grown on commercial phase II compost following standard cultivation practice to correlate the increase in vitamin D2 and the anticancer activity. For this purpose, ultraviolet radiation (UVA, UVB and UVC) was used to increase vitamin D2. UV-lamps were placed at 25 cm from the casing layer in the mushroom beds and treated with 6-18 J/cm² for periods ranging from 15 to 45 min. UVA and UVB were applied during cultivation while UVC radiation was applied to freshly harvested mushrooms. Mushroom samples were extracted by saponification process and the levels of vitamin D2 were determined by HPLC. A significant increase in vitamin D2 was detected when mushrooms were irradiated with UVB but was not found in UVA-irradiated specimens.

This increase in vitamin D2 was not followed by a significant improvement on the anti-cancer activity, indicating that the antitumor activity was not due to vitamin D2 contents.

In addition, different crop trials were performed to evaluate the influence of selenium treatment during cultivation on the anti-cancer activity of cultivated Portobello. Sodium selenate was incorporated with irrigation water to raise the selenium contents of the mushrooms. Five separate treatments were performed through drench applications onto the casing layer prior to primordial formation, with water containing.

SESSION 09

LECTURE **PSILOCYBIN & MOVEMENT:** **01 GAME CHANGER FOR THE HEALTH SOCIETY?**

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**Dr. Lucas Pawlik**  
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Mycoverse Foundation, Vaduz, Liechtenstein  
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Due to the ongoing global crisis since the COVID-19 pandemic and related lockdowns, we are experiencing a dangerous rise in diseases and disorders. Obesity, physical inactivity, and a rise in suicide, existential anxieties, and depression are mutually reinforcing, and their increase destabilizes our global health.

This article discusses the current research and the related process of legalizing the "Break-Through-Drug" psilocybin to mitigate this escalating global health crisis. It shows psilocybin could revolutionize treatment options for neurohormonal brain disorders such as depression, addiction, and anxiety by improving and modulating our neuroplasticity. Further research may lead to a new paradigmatic understanding of the interplay between mind and body and the associated mental disorders that impose the largest costs on the health care system.

Due to decades of illegalization hindering the introduction of the medication of psilocybin, medical and public education efforts are needed for psilocybin to be accepted by the public and patients as a curative medication.

The variety of psychedelic experiences associated with regular doses of psilocybin presents a challenging opportunity for individuals and society. It requires protected space and a clear framework for creating favorable internal and external conditions during the active phase of use and the subsequent integration phase.

Although psilocybin is among the safest medications known to science, studies show that psychedelic experiences can occasionally be experienced as one of the greatest challenges in one's life. In addition, mystical experiences, significant in predicting healing effects, indistinguishable from classical mystical-religious experiences, occur when psilocybin is taken in regular doses.

After explaining neurogenesis and functional hyperconnectivity, the two processes crucial for psilocybin's effects, further applications of psilocybin, and the essences of psilocybin-containing mushrooms are outlined. Further, the microdosing of psilocybin in combination with physical activity is being explored as a potentially cost-effective way to treat obesity and its associated leading cause of death in the healthcare system: chronic disease.

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01 **PSILOCYBIN & MOVEMENT:
GAME CHANGER FOR THE HEALTH SOCIETY?**

It discusses the role of psychedelic and mystical experiences not only as a barrier to legalization but also as a transdisciplinary opportunity for a transcultural research design to understand and explore what humanness means for psychophysical and ecological health and transcultural understanding. As a transdisciplinary autistic researcher, the author shows the demands of the autistic community not to be pathologized but to be recognized in their otherness as partners in dialogue as a pioneering chance for designing participatory research ethics.

For psilocybin to be used globally as a medication, it must be able to be associated with lived values and therapeutic practices like freedom, social equality, integration, health, movement, productivity, empathy, and meditation.

Just as the individual has to work on mindset, setting, and framework conditions to integrate the psilocybin experience, our society has to develop suitable framework conditions for psilocybin research, therapy, and legalization. Then psilocybin can be expected to contribute to the global health system and the advancement of science and a humane society.

Keywords: legalization, obesity, depression, physical inactivity, functional hyperconnectivity, neurogenesis, microdosing, mystical experiences, autism, ethics

MICRODOSING WITH MAGIC MUSHROOMS – CURRENT AND FUTURE PERSPECTIVES AS OF 2022 THE REVOLUTION AS BEGUN!

SESSION 09

LECTURE 02

A talk by Alexis Kaiser, lawyer and magic mushrooms activist

The revolution is happening. A quick review of today's press articles on Magic Mushrooms or "PCM" suffices to explain the exploding general interest for these mushrooms. Microdosing is a more elaborate use of Magic Mushrooms with a growing number of applications. After discussing the benefits currently observed with microdosing magic mushrooms, we will go into more details about the different type of mushrooms, individual posology and the information needed in relation with microdosing.

A review of multiple user's feedback will bring a more empirical knowledge about the incredible benefits of microdosing as well as common noted side-effects. In a second part, we will explain how the influence of business interests are highly intertwined with current research and how it will shape the future legal landscape in Europe and in the world (Saudi Arabia, UAE, Russia). Because the domain of application goes way beyond the treatment of depression, a lot of ventures are jumping in this field.

Canada and the USA are pioneering in the way, but many other countries will follow-suit. We will then discuss the new domains of application for microdosing : mood lifter for depressed seniors, ideal treatment of seasonal depression, coping with work and also for the creative professions like DJ's and artists, as well as pain and anxiety reliever for certain type of medical treatments.

The EU legal environment will be decisive in the widespread use of MD. The impact on the perception of fungi has radically changed over the last 20 years and we are in the beginning of this massive change. After studying the legal challenges to decriminalize magic mushrooms in Europe (they are controlled substances under criminal laws defined at a national level). We will evoke the role of media and TV networks in rehabilitating magic mushrooms and how activism will significantly help to change public perception and shape more adapted laws for the use of these amazing substances.

Introduction

The revolution is happening. A quick review of press articles on Mushrooms is exploding. Business interests are highly intertwined with research on Magic Mushrooms because the domain of application goes way beyond the treatment of depression.

Review of ascertained health benefits of PCM and European empirical studies. New domains of application: seniors, seasonal depression, intensive work period, cope with stress, DJ's and the artistic professions.

MICRODOSING WITH MAGIC MUSHROOMS – CURRENT AND FUTURE PERSPECTIVES AS OF 2022 THE REVOLUTION AS BEGUN!

SESSION 09

LECTURE 02

Legal environment will be decisive in the widespread use of MD. The impact on the perception of fungi.

Questions:

- Who hasn't heard of MD?
 - Everyone has heard about MD but what it is all about
 - What are the typical uses: review of current user profiles
 - Is MD safe? Is there any addiction potential or withdrawal symptoms?
 - What type of mushrooms you use. Key differences of applications between PCM dosage and uses (micro vs. macro). Migraines, depression, Pain,
- Part 1 –Overview of Microdosing (MD) effects and applications

Key benefits:

- Elevated mood throughout the day, promotes concentration & flow states, better focus and procrastination killer. Better problem-solving skills.- Help to better cope with stress and reduce social anxiety.
- Considerable reduction of cravings associated with quitting smoking or drinking alcohol
- Increases willpower and enhanced performance both physically and mentally
- help adopt healthier habits and lifestyles over the long-term (6 months MDP and more).
- Enhances spiritual well-being for those eager to reconnect with nature.

Plays a major role in the treatment of depression. Fantastic natural mood-enhancer – again, we found the adaptogen properties of the mushrooms.

But the main benefit is that you can go on with your work and daily obligations. Fantastic potential for the treatment of senior people depression. Significant potential for the treatment of Parkinson's disease, Alzheimer and Dementia.

Current noted side-effects: increase of emotional response – increased of perceived stress if taken during a period of high stress. A few physical symptoms (dry mouth). All are reversible effects. Can reveal accumulated fatigue for over-stressed people (stress/ adrenaline response reduce fatigue). No known lasting side-effects.

MICRODOSING WITH MAGIC MUSHROOMS – CURRENT AND FUTURE PERSPECTIVES AS OF 2022 THE REVOLUTION AS BEGUN!

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Type of mushrooms:

Golden teachers, truffles but also amanita muscaria and amanita Panthera. Current research in Russia. The importance of dosage and to accommodate user's expectations. Combination with Lion's mane. How to handle existing medication? A key issue in relation with MD. The problem of heavy depression treatment and opioids medication. Additional benefits of MD lays in its essence: it's micro-dosing!

Posology: The importance of guidelines and following a protocol adapted to the person's specific health condition, age, gender and individual sensitivities. LSD protocol vs. PCM protocols. Positive effects on sleep patterns not possible with LSD. How long should people microdosing? Recent trends in use. Combination with other mushrooms (Heridium).

Research: review of the research and published studies in Europe. Key organisations. The role of media hype in demystifying and dedramatizing magic mushrooms vs. the "Amsterdam Syndrome" (reckless use by teenagers in Amsterdam).

Research on Psilocybin vs. Research on Magic Mushrooms. The key difference and the difficulty of standardisation for clinical trials.

Which bring us to the next part. How are we going to use MD in the future and how the legal setting is going to be.

MICRODOSING WITH MAGIC MUSHROOMS – CURRENT AND FUTURE PERSPECTIVES AS OF 2022 THE REVOLUTION AS BEGUN!

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PART 2 – OVERVIEW OF WHAT IS HAPPENING IN THE US AND CANADA

In a research paper published in 1998, it stated that “the literature tentatively suggests that there are few, if any, long-term neuropsychological deficits attributable to hallucinogen use. To better resolve this issue, however, it will be important to study larger samples of chronic, frequent hallucinogen users...”

Business interests will force a change of regulations

Business appetites in the sector of Magic mushrooms (Canada / US). Greed vs. Health. The IPO syndrome vs. the need to bring affordable solutions to the public. Push to use MD for everything (diet, etc,...).

The paradox of European regulations. Directives for supplements but criminal laws are still under national determination. Importance of local cultural factors.

Review of the legal framework in Europe. Current overview of regulations and the coming decriminalisation of psychedelics. Production remains illegal everywhere!

What type of uses? Impact on the perception of mushrooms.

A word on recreational use. Let's stop the hypocrisy here! Macro dosing is as important as MD! How microdosing has the potential to become mainstream within 3-4 years.

The role of activism. Key players. Change of public and media perception.

How to change your mind is having a very large reach! Change of mentalities (via heavy lobbying!) will trigger a change in regulations.

Tales from the field – review of hundreds testimonies on MD

Use of MD is safer than having a large dosage of Magic mushrooms.

Conclusion & Q&A (10')

**MICRODOSING WITH MAGIC MUSHROOMS –
CURRENT AND FUTURE PERSPECTIVES
AS OF 2022 THE REVOLUTION AS BEGUN!**

SESSION 09

LECTURE 02

ALEXIS KAISER

SHORT PROFILE



Alexis Kaiser grew up in France and graduated in international business law at the Sorbonne University in Paris in 1998 before studying economics at the university of St. Gall in Switzerland. After a few years in the financial industry, working in London, Geneva and Moscow, he moved to Zurich in 2009 and worked since then as an international business lawyer specialized on the legal structuring of assets for wealthy clients. In 2012, he turned his focus on the legal status of psychedelics -obtaining a short victory with the temporary legalisation of Ayahuasca in 2012 - before the Swiss police lobbied heavily before regulatory authorities to prohibit its use. In the following years, he continued to defend criminal cases in relation with the possession and import of Ayahuasca, LSD or mushrooms in Switzerland. He worked extensively with different plant medicines in Europe and South America and developed a keen interest for mushrooms in 2014, after discovering their extraordinary benefits for human health, animals and our planetary ecosystem.

He specializes today on problems associated with the regulations of psychedelics in Europe and the necessary need to reform our drugs policies and laws. Alexis has become passionate about medicinal mushrooms and is committed to promote their therapeutical use and health benefits among the public in a scientific and entertaining way. He recently co-founded the foundation Mycoverse in Liechtenstein, a charity focused on gathering existing research and publications on fungi to create the largest digital library on the topic, inform the public and train medical professions about the incredible benefits of medicinal and magic mushrooms. The foundation intends to promote scientific research and the recognition of mycotherapy as a new medical discipline, among other activities.

PSILOCYBIN SOLUTIONS FOR THE GLOBAL HEALTH CRISI

Dr. Dirk Proeckl

Mycoverse Foundation, Vaduz, Liechtenstein

Due to the ongoing global crisis since the COVID-19 pandemic and related lockdowns, we are experiencing a dangerous rise in diseases and disorders. Chronic diseases, obesity, physical inactivity, and rise in suicide, existential anxieties, and depression are mutually reinforcing and their increase destabilizes our global health.

This article discusses the current research and the related process of legalizing the "Break-Through-Drug" psilocybin to mitigate this escalating global health crisis. It shows psilocybin could revolutionize treatment options for neurohormonal brain disorders such as depression, addiction, and anxiety disorders by increasing and modulating our neuroplasticity. This current research findings could yield a new paradigmatic understanding of the mind-body interplay and its disorders.

However, due to decades of illegalization hindering the introduction of the medication of psilocybin, medical and public education efforts are needed for psilocybin to be accepted by the public and patients as a curative medication.

The variety of psychedelic experiences associated with regular use of psilocybin presents both a challenge and an opportunity for individuals and for society as a whole, as it requires a protected space and a clear framework for creating favorable internal and external conditions during the active phase of use and during the subsequent integration phase.

Although psilocybin is among the safest medications known to science, studies show that patients have experienced psychedelic experiences, occasionally, as one of the most challenging of their lives. In addition, mystical experiences indistinguishable from classical mystical-religious experiences frequently occur when taking psilocybin, which are significant in predicting its healing effects.

After explaining neurogenesis and functional hyperconnectivity, the two processes crucial for psilocybin's effects, further applications of psilocybin and drug essences of psilocybin-containing mushrooms are outlined.

The article explains how microdosing could help introducing Psilocybin's potential health benefits and its combination with physical activity could be a cost-effective method to help treating obesity and its related chronic diseases, which are the main causes of death in the global health care system.

PSILOCYBIN SOLUTIONS FOR THE GLOBAL HEALTH CRISI

It also discusses the role of psychedelic and mystical experiences not only as a barrier to legalization but also as a transdisciplinary opportunity for a transcultural research design to understand and explore what being human means for psychophysical and ecological health and transcultural understanding.

For psilocybin to be used globally as a medication, it must be able to be associated with lived values and therapeutic practices like vitality, health, movement, weight loss, productivity, empathy, and meditation. The individual has to frame mind-set, setting and develop means to integrate the psilocybin experience into their lives for psilocybin to deliver its game changing effects. In the same manner the successful integration of psilocybin can only happen if those who integrate the "Break-Through-Drug" psilocybin into society, also deliver the mind-sets and settings for its social integration.

If suitable framework conditions for psilocybin research, therapy and legalization are created, psilocybin can be expected to contribute to the stabilization of the global health system and the advancement of science and human society.

Keywords: psilocybin legalization, obesity, depression, physical inactivity, functional hyperconnectivity, neurogenesis, microdosing, mystical experiences

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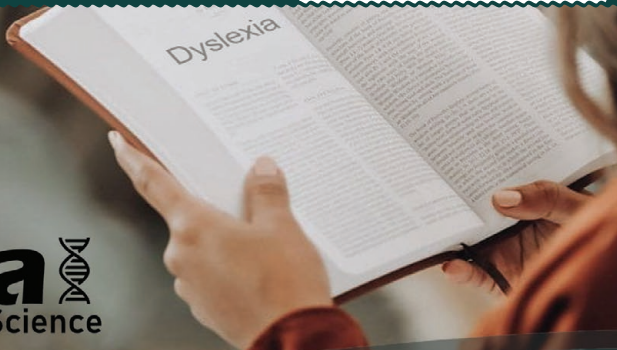
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