Biological resources and breeding for improvements in the production of the button mushroom in small-scale farming

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Contents

1. Biological resources and breeding for improvements in the production of the button mushroom in small-scale farming.

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Gourmet mushrooms may contribute to the development of a new agriculture by addressing the consumer demand and some of the non-nutritional uses of agricultural productions. The value of recycling with gourmet and medicinal mushrooms is clearly understood and wastes can be viewed as positive products, at least in term of providing new economic opportunities and positive environmental consequences. Important cultivated edible and medicinal mushrooms are members of the *Agaricus* genus. They are saprophytic fungi acting as secondary decomposers in forest litter. The button mushroom is *Agaricus bisporus* cultivated as a gourmet mushroom since the 18th century and high yields are now obtained in sophisticated farms in western countries. Its potential as a medicinal mushroom has recently been shown. Throughout the world the

cultivation of this mushroom is developing in small and mid-sized farms, but the technical knowledge and the strains have been developed for cultivation in high-tech large farms. Needs for small-scale farming are simple cultivation processes using rustic strains able to fruit under various conditions, and strains with stable contents in active ingredients, whatever the cultivation conditions. By using available biodiversity and breeding, one can expect to develop homogeneous, vigorous and rustic strains with good productivity and commercial quality. Potentials are explored in this article.

AGARICUS BISPORUS AS A SOURCE OF ACTIVE BIOMOLECULES

Recent evidence suggests that *A. bisporus* contains high levels of substances of possible medicinal importance such as tyrosinase, aromatase inhibitors and immunomodulating and antitumor polysaccharides [1]. Ergosterol, vitamin D2 content, and various antioxidants are also proposed as interesting components for the development of *A. bisporus* as a nutraceutical for tomorrow [2, 3]. *Agaricus bisporus* was shown to interfere with processes critical to atherogenesis and cardiovascular disease and to have a potential to minimize risks [4].

Very few studies have reported the tissue distribution of bioactive metabolites in mushrooms during development, despite growing evidence that maturation affects the concentration of natural compounds in mushrooms [5-7]. For *A. bisporus*, recent data provide guidance for optimized harvesting time of mushrooms and maximized health benefits [8, 9].

FIGURE 1: White and brown strains of *Agaricus bisporus* cultivated on an experimental scale

BIODIVERSITY AND GENETIC VARIABILITY

Agaricus bisporus has been cultivated in Europe since the beginning of 18th Century and expended to other continents later. The cultivars used by growers over the world are suspected to come from the same restricted pool of strains, and the genetic base of all the present day hybrids is very narrow. The first commercial crossbred *A. bisporus* strains (Horst-U1) were developed in The Netherlands in the 1980's. In a recent study with 75 cultivated genotypes provided by European spawn makers, isolated from 1990 to 2005, and maintained under liquid nitrogen,

six groups were identified which corresponded to the five ancestral lineages and the hybrids belonging to either Horst-U1 or Horst-U3 sub-group of strains. For three hybrids that seemed to be genetically identical to Horst-U1 at the heterokaryotic level, the analyses of each constituting nuclei have demonstrated allelic rearrangement [10]. Such a study shows the narrow genetic variability among the cultivars of this mushroom. That is why a collection of wild strains appeared necessary to researchers. Crop wild relatives and local varieties are the elements of agricultural biodiversity most likely to contain the necessary novel, unique and high level of genetic diversity needed to sustain innovations in breeding programs. Since the end of the 80's, two major collections, the ARP (*Agaricus* Resource Program, [11]) in the USA, and the CGAB (Collection du Germoplasme des Agarics à Bordeaux, [12]) at INRA-Bordeaux contain hundreds of wild isolates originating from various habitats and numerous geographic origins. Among the various identified populations, one was identified in Greece with some white strains that are very rare in the wild and another one in the Sonoran Desert in California. This isolated population is composed by a specific variety, *A. bisporus* var *burnettii* having as specific life cycle and other phenotypic characteristics that can be used in breeding programs when crossed with *A. bisporus* var *bisporus* to which belong all the cultivated and most of the wild strains.

Wild germplasm thus may represent a useful resource available for developing *Agaricus* strains with improved quality for the production of substances with possible medicinal importance. Studies on both genotypic and phenotypic variability stress the interest of these collections for introducing new traits in the cultivated strains. However, until recently, there are only a few developments of new cultivars using the potentiality of wild strains.

BREEDING OF *AGARICUS* MUSHROOMS

Knowledge of the life cycle and reproductive strategies is necessary to develop breeding of mushrooms. Only a few genetic studies have been carried out concerning *A. bisporus*, due to its particular life cycle. It is an amphitallic species with a homothallic or heterothallic cycle depending on the ploïdy level of the spores that can be heterokaryotic (n + n) or homokaryotic (n), respectively. Cultivated strains and most of the wild strains belong to *A. bisporus* var. *bisporus* with a predominantly pseudohomothallic life cycle, producing mainly bisporic basidia. This behavior, equivalent to a pseudoclonal inbreeding system, hampers outcrossing and limits breeding successes. The discovery of the heterothallic *A. bisporus* var. *burnettii* among the wild genetic resources in a specific population [13] makes it possible to overcome these limitations. In fact, this variety is predominantly heterothallic and produces mainly tetrasporic basidia necessary for homokaryotic spore production. Introducing the dominant tetrasporic allele [14] into mushroom pedigrees greatly facilitates the breeding process. A third variety, *A. bisporus* var. *eurotetrasporus*, produces mainly tetrasporic basidia but is homothallic [15].

Outcrossing of homokaryons is the method generally used for the development of crossbred mushroom strains. The hybrid obtained is cultivated to produce fruiting

bodies that generate numerous spores as offspring. Depending on the life cycle, the spores either are auto-fertile and able to fruit or are homokaryons needing to be crossed with a tester for producing fruiting bodies on which phenotypic traits are assessed. These methods allowed performing genetic analyses of quantitative traits that are under polygenic inheritance. The quantitative genetic approaches are based on a global analysis of phenotypic trait variation through variance components, heritabilities and least number of effective factors, but the individual genetic effects of these factors remained inaccessible. This method has been proven to be effective for important production traits in mushroom industrial cultures such as yield, quality or resistance to diseases [16, 17]. To our knowledge, there is no published work on genetic characteristics of traits affecting the production of bioactive molecules in *A. bisporus.*

The development of molecular markers and the construction of genetic linkage maps are essential tools for genetic studies of quantitative traits and improve the efficiency of breeding programs. These tools are mainly used in plants and can be applied to mushrooms. An expanded genetic linkage map of an intervarietal *A. bisporus* var. *bisporus* x *A. bisporus* var. *burnettii* hybrid, based on AFLP, SSR and CAPS markers, has recently been published and it sheds light on the recombination behaviour of the species [18]. This linkage map contributes to the understanding of genome organisation and is a reference for the development of powerful tools for establishing efficient breeding programs with *A. bisporus* in addition to information from its recent genome sequencing. Marker assisted selection is currently used by breeders of *A. bisporus*, but for only a couple of traits such as color or for identifying homokaryons.

DEVELOPMENT OF RUSTIC *AGARICUS* STRAINS FOR CULTIVATION IN SUSTAINABLE SMALL-SCALE FARMING

Up until now, the management of the temperature during fruiting of *Agaricus* mushrooms is critical. It could necessitate expensive facilities and consume large amounts of energy. That is why it is a challenge to have strains that can be cultivated under various temperatures.

We screened wild strains representative of the major geographical populations of *A. bisporus* for their ability to produce mature fruiting bodies at the normal temperature used for cultivation (around 17°C) and at a higher temperature, set around 24°C [19]. One quarter of the strains of *A. bisporus* var *bisporus* were able to fruit at high temperature, but for most of those, yields were significantly lower than they were at the lower temperature. They represented all studied populations. All *A. bisporus* var. *burnettii* strains produced fruiting bodies with high yields both at 17°C and 25°C. However the mushrooms produced were small, opened rapidly and were far from the standard of commercialization in Europe. Apparently this variety is not dependent on or is less susceptible to temperature for fruiting and that would be an advantage for small-scale farming, especially if the objective is to produce medicinal extracts. Hybrids can be obtained by crossing homokaryons of *A. bisporus* var. *burnettii* and *A. bisporus* var. *bisporus*. The first genetic map of the species was built from such a cross [17]. Improved hybrids

including the lower susceptibility to temperature could be produced rapidly by using marker assisted selection tools.

IMPROVEMENT OF THE MUSHROOM QUALITY AS SOURCE OF MEDICINAL COMPOUNDS USING THE GENETIC RESOURCES AND BREEDING

Improving the quality of mushroom products as source of raw extracts or purified compounds having medicinal effects is a challenge for the development of new activities and products. The objective may be reached by mushroom breeding. In addition to a large genetic and phenotypic variability and possibility to cross strains, it is necessary to be able to screen the properties of a large number of strains and hybrids. For instance, offspring of at least 100 individuals is necessary for genetic analyses of a specific trait and one can expect to obtain significant improvements without affecting other important traits by studying several offspring. That renders impossible *in vivo* tests on animals, and *in vitro* tests on cellular models would be very expensive and time consuming. The more promising way is the chemical analysis of a specific metabolite, even if it should be difficult for polysaccharides with biological activity depending on their chemical structure and spatial arrangement.

There is no significant study on the variations in the potentials of health benefits with the genotypes of a large number of strains. Higher concentrations of ergosterols and antioxidant activities were found in a brown cultivated strain than in a white hybrid [9] and higher radical scavenging activities correlating with higher phenolic contents were found in extracts of two wild strains than in a white hybrid cultivated under the same conditions [8]. Sabourin et al. [20] published an interesting work on agaritine content of 50 *A. bisporus* strains from wild germplasm. Substantial natural variation exists, that potentially could be exploited to manipulate agaritine levels in novel hybrid strains. Each of the New World population samples had lower mean agaritine levels than any of the Old World population samples, and cultivars had intermediate agaritine levels corresponding to the lower-middle range recorded from European strains. This example shows that there is a potentially useful resource available for developing *Agaricus* strains with high or low contents of a specific component.

CONCLUSION

Whereas *A. bisporus* is mainly used as an edible mushroom it has an interesting potential as a medicinal mushroom and sources of biologically active molecules in its extracts. The research work performed on the biology of this mushroom clearly states that there is an available biodiversity inside the species that could be a source of interesting traits for the development of a small-scale mushroom farming dedicated to the production of medicinal mushroom extracts. The biological translation of the practical needs for developing this activity is: homogeneous, vigorous and rustic strains able to produce high quantities of fruiting bodies rich in active ingredients under various conditions. The examples of current works on the

effect of temperature on the mushroom development and the content in some compounds given is this article open opportunities to use the biodiversity and breeding programs to reach the objective of proposing new strains adapted to the conditions of small scale farming. The selection criteria are different from those used today for the development of edible strains used by large farms and a specific breeding program should be built for supporting the development of this new farming activity. However, breeding work can be very long-term. The development and use of marker-assisted selection is a promising way nowadays because a genetic linkage map is available [17] and has proved its efficiency [21, 22]. Furthermore, the whole sequence of *A. bisporus* has been released (http://genome.jgi-psf.org) for the scientific community and is going to be analysed [23].

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2. Current status of Indian edible mushroom industry

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3. Mushroom production in Brazil: Current situation and future perspectives

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Brazil is not included among the statistics relating to worldwide mushroom production. The country does not even appear in the data provided by the ERS (Economic Research Service/U.S. Department of Agriculture) in the Mushroom Industry Report (1) although it produces more mushrooms than some of the countries that are listed. According to official IBGE (Instituto Brasileiro de Geografia e Estatística, 2) statistics, Brazil produced 5,894 tons of mushrooms in 2006 which, in keeping with the Mushroom Industry Report cited above would place Brazil in 34th position among the group of 70 listed countries.

Mushroom cultivation in Brazil has a high growth potential although there is no established tradition of mushroom production and consumption. This potential is evidenced by official statistics that, while occasionally lacking accuracy, clearly demonstrate growth of the activity within the country. According to the IBGE, mushroom production in 2006 was 33.2% higher compared to 1996 and, while this might be perceived as a relatively small increase over a ten year term, it represents a significant improvement considering the challenges faced by farmers during that period.

In addition to the lack of a fully developed internal market, Brazilian farmers faced strong competition from processed mushrooms imported from China which, contrary to European and North American preferences, are favored by Brazilian consumers. In 1998, the Brazilian government introduced an antidumping measure to protect the country's farmers against imported Chinese mushrooms. Although this resulted in a decline in mushroom imports, official statistics revealed that these continued throughout the period of the antidumping measure, which has now expired and has not been renewed. In the light of such competition, the growth in Brazilian mushroom production attained during the last decade has been significant and auspicious.

In order to understand the reality surrounding mushroom production in Brazil, and how it has survived despite great challenges, it is necessary to recognize its various peculiarities. Firstly, mushroom cultivation in Brazil was initiated by Asian

immigrants, mainly Chinese and Japanese, who introduced button mushroom (*Agaricus bisporus*) cultivation using very simple facilities. For small farmers, this reality persists today, restricting cultivation of this mushroom to regions with a mild climate and high relative humidity. Despite the poor conditions, low production costs guaranteed the farmers' survival. At the same time, specialized companies appeared in the country, which incorporated modern technology in terms of both compost production and mushroom cultivation techniques. These advances allowed the companies to maintain the continuous mushroom production required to ensure supermarket contracts and establish distribution chains (3).

Processed mushrooms provided a major stimulus to the domestic market, turning the mushroom into a relatively common food for the Brazilian consumer, mainly for the preparation of special dishes. However, as the market became established, the mushroom distributors began to seek new suppliers in China, who offered lower priced but often inferior quality products. In order to survive this new challenge, Brazilian farmers had to readapt and improve their competitiveness. A key point for the more well-structured farmers was the processing and sales of preserved mushrooms, which guaranteed a higher value-added product. Consequently, many farmers began to supply pre-processed mushrooms to the industry, which then finalized the processing and packaged the product. Other farmers created their own product brands and distributed these directly to the supermarket chains. With the elimination of the distributor intermediary, these farmers became more competitive and, at the same time, received more income for their work.

Despite this advance, Brazilian farmers continued to face serious problems. A major concern was the extensive use of sodium bisulfite in the mushroom preservation process. This practice had already been banned in other countries, and constituted a barrier for the more discerning consumers. Although sodium bisulfite was not essential for the traditional canning processes in which autoclaving is used, farmers considered use of the chemical to be essential when the mushrooms were distributed in non-autoclavable plastic containers. Therefore, in order for the Brazilian farmer to produce a healthier product for the more demanding consumers, alternatives to the use of sodium bisulfite in the preservation process are essential.

Another major problem faced by Brazilian farmers has been the lack of any official registration of pesticides for the control of pests and diseases. Officially, only low pesticide volumes are used, and the manufacturing companies are not interested in registering their products for mushroom cultivation due to high costs involved. Therefore, it is necessary for public organs, along with the research institutions, to seek a solution to this problem. Until this happens, Brazilian farmers will use pesticides in a clandestine way, without adequate guidelines and without official controls. This situation, besides damaging the farmers themselves, hampers the development and supervision of appropriate good practices, ultimately resulting in harm to the Brazilian consumer.

Official statistics do not discriminate between the different types of mushrooms cultivated in Brazil but, in terms of volume, the button mushroom is responsible for the bulk of production. Nevertheless, Brazilians have also been consuming other mushroom species. For example, during the 1990s, there was considerable publicity about the medicinal mushroom, *Agaricus subrufescens*, popularly known as the Sun Mushroom, which is sold today in Brazilian markets in dehydrated form for the preparation of tea, or in the form of capsules as a dietary supplement and as a stimulant of the immunological system (4). Since that time, many consumers began to see mushrooms, not only as a source of food, but also as a benefit to health, thereby awakening an interest in other mushrooms. Consequently, the shiitake and oyster mushrooms, previously restricted to the Asian communities, began to occupy other niches in the market. Market growth was greatly favored by the fact that these mushrooms are preferably sold fresh, thereby benefiting domestic producers as they were less exposed to international competition.

Nowadays, there is evidence of a continuous expansion of shiitake and oyster mushroom production, which demand higher prices in markets compared to the button mushroom. Contrary to the situation relating to the button mushroom, Brazilians have learned to consume specialty mushrooms in their natural (fresh), rather than processed, form. Since these mushrooms run a smaller risk of competition from imports, many farmers are shifting from button mushroom production to shiitake and oyster mushroom cultivation. Therefore, the increase in total mushroom production recorded in the official data published in 2006 may have originated in large part from specialty mushroom cultivation. Although there are no official data, present demand for mushrooms among Brazilian consumers appears to exceed current production levels, which indicates that future investment interest in this sector will increase.

The Brazilian government has demonstrated immense interest in projects that benefit family-based agriculture, which is characterized by relatively small land requirements and household-sourced labor. In this context, shiitake and oyster mushroom cultivation are ideal since only small cultivation areas are required, and the added value they represent is superior to any other horticultural product. Therefore, it is essential that Brazilian research institutions develop projects that allow higher public investment aimed at developing this sector as a model for family agriculture, thereby bringing social benefits and contributing to the growth of the mushroom industry in Brazil.

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4. Message from Dr. Manjit Singh, President, Local Organizing Committee, 8th ICMBMP, New Delhi

Dr. Manjit Singh
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The First International Conference on Mushroom Biology and Mushroom Products was held at the Chinese University of Hong Kong in August 1993, since when subsequent conferences have been organized at regular intervals in several countries. After the successful organization of the 7th ICMBMP at Archachon, France, the 8th ICMBMP is scheduled to be held in India during 19-22 November, 2014. This will be a unique opportunity to interact with mushroom researchers from different countries and to gain exposure to mushroom research and development in India.

India is a country of diverse climate and culture. Founded on an ancient civilization, the country is endowed with heritage sites of great historical value and aesthetic beauty, and a wide range of landscapes - deserts and rivers, forests and mountains, plateaus and plains. Delhi, the venue of the conference, has long been the capital of India and is sprinkled with captivating ancient monuments, fascinating museums and art galleries, architectural wonders, a vivacious performing-arts scene, fabulous eating places and bustling markets.

This conference aims to increase interaction and the exchange of information among researchers working on various facets of mushrooms including (i) Biodiversity and Conservation, (ii) Mushroom Biology & Biotechnology (iii) Genomics, Genetics & Breeding, and (iv) Nutritional & Medicinal Aspects, and Innovative Mushroom Products. Not only will the conference provide the opportunity to meet colleagues and friends but will also serve to strengthen collaboration between mushrooms scientists, farmers and mushroom entrepreneurs, including people from industry. Accordingly, the programme of the 8th ICMBMP will comprise plenary lectures together with presentations/displays of the research achievements of young scientists. A trade exhibition, to run concurrently with the scientific programme, will provide an opportunity for the mushroom industry to showcase their services, products and technologies.

The Local Organising Committee consists of members of the Directorate of Mushroom Research and the Mushroom Society of India. For further details, please contact: icmbmp8.manjit@gmail.com or icmbmp8.secretariat@gmail.com. We are seeking people from different countries to join the scientific committee with responsibility for developing the scientific programme and reviewing manuscripts

submitted for inclusion in the conference proceedings. Interested researchers are requested to email their bio-data to icmbmp8.manjit@gmail.com. A dedicated 8th ICMBMP website is under construction and will be accessible in due course. Until then, further information will be made available on the website of Mushroom Society of India: http://www.mushroomsociety.in/. The Organising Committee extends a warm welcome to you to attend this conference on Mushroom Biology and Mushroom Products in New Delhi.

Wait for the first call for papers soon

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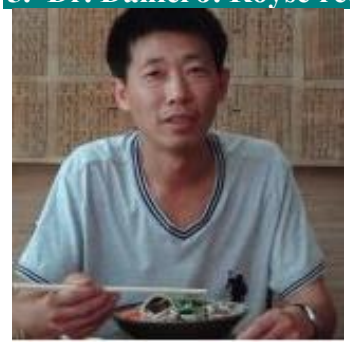
5. Dr. Daniel J. Royse retired



Dr. Qing Shen
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Dr. Daniel J. Royse retired in June 2012 after 34 years service as a Professor and Mushroom Extension Specialist in the Department of Plant Pathology, the Pennsylvania State University (USA). Throughout his tenure, Dan's research has been focused on improving the production efficiency and quality of edible mushrooms in order to minimize production costs and maximize farm profits.

Dr. Royse is notable for his many scientific achievements, including over 300 scientific publications and innumerable conference lectures at both national and international level. He has given outstanding service to his university and to the mushroom community at large and, in 2011, was awarded the Outstanding Research Award at the International Conference on Mushroom Biology and Mushroom Products held in Arcachon, France.

Dr. Daniel J. Royse during a trip to Tropical Mexico in 2010.

Dr. Royse is an individual who has had a profound influence on the life of many people, and has used his expertise and experience to help countless other researchers achieve their goals. He has travelled to many countries to offer advice and counsel that has benefitted mushroom growers worldwide. As a university professor, not only was he an outstanding mentor but also a good friend, and his students will never forget the many hours spent with them both in and out of the laboratory.

Although retired, Dan is not yet ready to leave the mushroom field, and we believe that his passion for mushroom research will continue to carry him to even greater heights. We truly wish him good luck and good health in his retirement, and hope that he will finally find more time to do the things he likes, such as sitting back and having a glass of wine during the day. Cheers!

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6. The International School of Advanced Studies on Mushroom Biotechnology and Bioengineering (ISASMBB) held at Pitesti, Romania

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Biotechnology and bioengineering are two complementary disciplines that are focused primarily on the application of concepts and methods involving biological systems (microorganisms, plant and animal cells/tissues), and secondarily on the use of biophysics, biochemistry, mathematics and computer science to solve real-world problems related to life sciences. These disciplines can be applied to *in vitro* studies directed at discovering new knowledge relating to the complexity of the mushroom life cycle through the use of pure cultures grown in the laboratory or by employing industrial-scale robotic systems able to drive and control all the physical and chemical parameters that influence mushroom development bioprocesses.

Viable solutions to some of the unsolved problems were presented, and prospective applications were discussed, by established experts working at prestigious universities and research institutes worldwide within the framework of the International School of Advanced Studies on Mushroom Biotechnology and Bioengineering (ISASMBB), organized by and held at the University of Pitesti from 23rd to 27th September, 2012. This event was supported by the Consiliul Naţional al Cercetării Ştiinţifice din România (National Council of Scientific Research from Romania) - Executive Agency for Higher Education, Research, Development and Innovation Funding (CNCS–UEFISCDI) through project No. PN-II-ID-SSA-2012-2-012.

The ISASMBB was addressed principally to the needs of young researchers (i.e. doctoral and master's students) working in the biological sciences, biotechnology, bioengineering and related specialisations. The programme of scientific lectures covered the following topics: a modern view on the current status, future trends and unsolved problems relating to medicinal mushroom research, the cultivation, biochemistry and molecular biology of the edible straw mushroom *Volvariella volvacea*, characteristics of new biotechnological procedures for high efficiency mushroom cultivation, cultivation of *Ganoderma lucidum* and *Grifola frondosa* and production of their pharmaceutically-active components, natural biodiversity and molecular genetics for breeding *Agaricus* sp., the cultivation of *Agaricus subrufescens* in Europe, the major diseases affecting *Agaricus bisporus* and their biological control, the exploitation of the biotechnological potential of agricultural by-products through mushroom cultivation, the use of spent mushroom substrate for degrading organochlorine pesticides and low input technology for pasteurizing substrate for oyster mushroom production.

To disseminate the scientific data presented during the event, all the contributions to the ISASMBB have been complied into a single volume entitled "Mushroom Biotechnology and Bioengineering" and published by CD Press Publishing House of Bucharest. We trust this volume will contribute to a better understanding of new trends in applied biotechnology and bioengineering involving the use of edible and medicinal mushroom species as the main biological tools in the agro-food industry,

medicine and health care, biopharmaceutical production, molecular genetics and environmental protection.
ISASMBB Joint Chairmen: Assoc. Prof. Dr. Marian Petre and Prof. Dr. Marin Berovic.

Figures, from left to right: a) Welcome dinner for Keynote Speakers b) Lecturers visiting Dr. Ionel Didea Rector of the University of Pitesti c) Delegates attending the School of Advanced Studies on Mushroom Biotechnology and Bioengineering D) Walking around Pitesti.

7. About this bulletin

The WSMBMP Bulletin is the official electronic publication of the World Society for Mushroom Biology and Mushroom Products. The bulletin is intended to keep members informed about Council activities and to share general information about mushrooms. It is designed to allow communication between society members and alert them about new topics and opportunities related to mushrooms. Society members and general public are kindly invited to submit letters, comments and information of interest for the mushroom community to be published in the bulletin. Please submit your contributions electronically in free format to the editors José E. Sanchez esanchez@ecosur.mx, John Buswell jabuswell2003@yahoo.co.uk, Daniel J. Royse djr4@psu.edu or Helen Grogan helen.grogan@teagasc.ie.