Mushroom Science XVIII

Proceedings of the 18th Congress of the International Society for Mushroom Science

Editors: Jinxia ZHANG Hexiang WANG Mingjie CHEN

China Agriculture Press

图书在版编目（CIP）数据

第十八届国际食用菌大会论文集：英文／张金霞等编. —北京：中国农业出版社，2012.8
ISBN 978-7-109-16959-3

Ⅰ.①第… Ⅱ.①张… Ⅲ.①食用菌-国际学术会议-文集-英文 Ⅳ.①S646-53

中国版本图书馆 CIP 数据核字（2012）第 154158 号

中国农业出版社出版
（北京市朝阳区农展馆北路 2 号）
（邮政编码 100125）
责任编辑 黄 宇 阎莎莎 贺志清

中国农业出版社印刷厂印刷 新华书店北京发行所发行
2012 年 8 月第 1 版 2012 年 8 月北京第 1 次印刷

开本：889mm×1194mm 1/16 印张：61.25
字数：1 900 千字
定价：360.00 元
（凡本版图书出现印刷、装订错误，请向出版社发行部调换）

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Foreword

It is an honour and also a pleasure for me to write the Foreword for the Proceeding of the 18th Congress of International Society for Mushroom Science (ISMS).

There is an interesting short poem which speaks of something that occurs in a multitude of mushroom virtues:

The lotus is a flower of virtue which emerges from mud but is pure and unblemished (荷花花中君子,出污泥而不染)。

The mushroom is a fungus of wonder which rises up from decay but is nourishing and bountiful (蕈菌菌中神奇, 生朽物却溢香)。

S. T. Chang, 29 Sept. 1994.

The mushroom has always been a thing of mystery. In ancient times the seemingly miraculous manner of its growth without leave, without bud, without flower and without "seed", its sudden appearance after rain, its equally rapid disappearance and its curious umbrella-like shape gave rise to a wealth of allusions and mythologies. Today, it is continuing to perform feats of magic and versatility, not only as a source of food, tonic and medicine, but also by helping to convert agricultural and industrial organic wastes into useful matter.

It should be recognized at the outset that a broad definition of the term mushroom has been taken; namely, the mushroom is considered as a macrofungus with a distinctive fruiting body large enough to be seen with the naked eye and to be picked up by hand. Thus defined, a mushroom is not restricted to the class Basidiomycetes; it may be either epigeous or hypogeous; and it is not necessarily fleshy or edible. Mushrooms can be Ascomycetes as well. This definition is imperfect, however, it can be used as a working term for global mushrooms, including, in both, mushroom research and the mushroom industry.

Mushrooms have been found in fossilized wood 300 million years old. Almost certainly, prehistoric man used mushrooms collected in the wild as food. There are about 140,000 – 150,000 species of fungi, producing fruiting bodies of sufficient size and of suitability structure to be considered as macrofungi. They can be called mushrooms according to the above definition. Each mushroom species has its own special ecological niche and occurs only in certain habitats. According to recent reports, there is an aquatic mushroom, *Psathyrella aquatica*. The only known species of gilled mushroom that fruits under water, http://earthfix.opb.org./multimedia(slideshows)biologists-search-mountain-stream-for-oregons-und/.

Bringing together, farmers, scientists and business men and women with an interest in mushroom is essential for, open exchange of information of common interest. The location of the

18th Congress of International Society for Mushroom Science in Beijing is especially fitting, because mushroom production in China has seen steady and large increases. In 1978 the total production of mushrooms in China was only 60,000 tonnes, which accounted for less than 6% of total world mushroom production. In 2009, however, total production of mushrooms in China reached 20.2 million tonnes and accounted for over 80% of total world mushroom production. Furthermore, many new species of mushrooms have been recently cultivated and marketed in China.

This congress has its goal in the exchange of information about new findings in the various aspects of mushroom biology and mushroom products, including cultivation technology, nutritional and medicinal aspects of mushrooms, bioconversion and composting, genetics and breeding, and business management and marketing. In addition to lectures, oral presentations and poster demonstrations on these topics, round table discussions have been arranged on various specialized topics. It is hoped that this congress will close the gap in knowledge between herbaceous habitat mushrooms and the woody habitat mushrooms in general and tropical mushrooms and the cold climate *Agaricus* in particular. Since 1950 these congresses have been organized every three or four years. All but one, were held in Western countries; in 1974 the 9th congress was held in Tokyo and Taipei. The early organizers of the congresses were almost exclusively involved with *Agaricus bisporus*. However, it was gradually discovered that *Agaricus* was not the only edible mushroom being grown commercially. At the 6th congress held in Amsterdam in 1965, Dr. K. Mori of Japan showed his film on the cultivation of several species of edible mushrooms in eastern Asia, which "opened the eyes" of the western world (P. J. Bels, 1976). The cultivation of *Pleurotus ostreatus* and of *Stropharia rugoso-annulata* started independently in Eastern Europe shortly after this congress. *Volvariella volvacea* was introduced in the 9th congress of ISMS in 1974 and medicinal mushrooms *Ganoderma lucidum*, was showed in 14th congress in 1995 in Oxford. However, it should be noted that the history of *Agaricus* mushroom research and cultivation shows the road for other mushrooms. I hope this proceeding will be received well by readers and I wish the production and consumption of both edible and medicinal mushrooms in the world a bright future.

28 – May – 2012

Canberra, Australia

S. T. Chang, OBE, Ph. D, FCUHK, FWAAS

Emeritus Professor of Biology

The Chinese University of Hong Kong

Preface

Papers published in Mushroom Science XVIII were all selected from those submitted to the 18th Congress of the International Society for Mushroom Science (18th ISMS Congress). 187 full papers were received worldwide, 122 of which from 31 countries were selected by Publishing Sub-committee after careful review. Most of the papers were submitted for oral or poster presentations according to authors' preferences. Some papers that will not be presented in the Congress were selected also for publications for their valuable contents. We deeply regret to having excluded some papers due to space constraints.

Mushroom Science XVIII covers almost all the aspects of mushroom science and industry, including molecular technology, genetic breeding, cultivation, marketing, industrial analysis, etc. I believe the proceedings will definitely give us a comprehensive view of mushroom research and developmental trend in the world.

During the process of 'call for papers', collecting, reviewing and editing, we received great and extensive support and assistance from many colleagues. I would like to express my special gratitude to Prof. S. T. Chang for his valuable suggestions and help. All the reviewers were also highly appreciated for their helpful reviews. Our appreciation also goes to all the colleagues in Publishing Sub-committee and Secretariat for their hardworking contributions. At last, I'm sincerely grateful to Ministry of Agriculture of China for their financial sponsorship for publishing the proceedings.

Great efforts were put to adjust tables, figures, and even the layout of references. Please accept our apologies if there are any errors that have been neglected or caused by editorial process.

Dr. Jinxia Zhang
Secretary General for 18th ISMS Congress
Institute of Agricultural Resources and Regional Planning,
Chinese Academy of Agricultural Sciences, China

Keynote Lectures

Session Ⅰ Germplasm Diversity

Session Ⅱ Biochemistry and Molecular Biology

Session Ⅲ Genetics and Breeding

Session Ⅳ Disease, Pest and Quality Control

Session V Nutritional and Medicinal Aspects

Session Ⅵ Technology of Cultivation and Spawn-Making

Session Ⅶ Mycorrhizal Mushrooms

Session Ⅷ Marketing and Business

Breathing New Life to a Malaysia Lost National Treasure —the Tiger-Milk Mushroom (*Lignosus rhinocerotis*)

Tan CS[1], Ng ST[2], Yeannie Yap HY[3], Lee SS[3], Lee ML[1], Fung SY[3], Tan NH[3] and Sim SM[4]

[1]Strategy Research Centre, MARDI, P. O. Box 12301, Kuala Lumpur 50774, Malaysia; [2]Ligno Biotech Sdn Bhd, Perindustrian Balakong Jaya 2, Balakong Jaya 43300, Malaysia; [3]Dept of Molecular Medicine; [4]Department of Phamarcology, Medical Faculty, University Malaya, Kuala Lumpur 50740, Malaysia.

Email: tanchonseng@gmail.com; ngszuting@gmail.com; yean_ny_nie@yahoo.com; s_shien_lee@hotmail.com; rimeili_lee@yahoo.com; fungshinyee@gmail.com; tanngethong@yahoo.com.sg; debrasim@um.edu.my

Abstract

Tiger-Milk Mushroom (*Lignosus rhinocerotis*), locally known as "cendawan susu rimau", is a well-known traditional medicinal mushroom in Malaysia, with the first record of its usage dating back to 1664. The medicinally useful part of the mushroom is the sclerotia. It is used to treat asthma, fever, cough, cancer, food poisoning, for wound healing and as a general tonic. To date, however, scientific data on this mushroom are very limited. Also, the supply of the sclerotia is very limited and could not meet the demand, because this mushroom can only be collected from jungle and its occurrence is rare. Earlier attempts to cultivate the mushroom were not successful and usually resulted in low yield. Thus, the full potential of the health and economic benefits of this 'national treasure' have never been achieved. Recently, however, we have successfully cultivated the mushroom in a controlled environment with high yield. We have been able to induce the formation of sclerotia which eventually lead to development of stem and formation of the mushroom cap. The life cycle of *L. rhinocerotis* is 2 – 3 years. We have also developed a manufacturing process to produce the Tiger-Milk Mushroom sclerotia as a commercially viable product. We believe the availability of the mushroom sclerotia in large quantity with consistent quality will breathe new life into the biotechnological exploitation of this Malaysian national treasure. This paper reported the 10 years journey of the development of the *L. rhinocerotis* cultivar and the investigations of its biopharmacological properties as the first step to validate the medicinal benefits of the mushroom as recorded by ethno-botanical surveys.

Keywords. *Lignosus rhinocerotis*, Cultivation, Medicinal Mushroom, Ethno-botany

Introduction

Some four hundred years ago, an important traditional medicinal product was given to an European government agent who sailed to this region, The Diary of John Evelyn (publication dated 22 June 1664) recorded the name of the medicinal product as "*Lac tygridis*" (meaning tiger's milk). It was described as a fungus used by local communities to treat diseases that European doctors could find no cure for. This fungus was also later reported by Sir Henry Nicholas Ridley, "the father of Malaya's rubber industry" (Ridley, 1890), as an important medicinal mushroom used by the local communities. In the year 1890, Cooke pioneered the scientific documentation of this fungus and named it as *Fomes rhinocerotis* based on a specimen

obtained from Penang. The scientific synonym commonly used currently is *Lignosus rhinocerotis / rhinocerus.*

The Tiger-Milk Mushroom (*Lignosus rhinocerotis*) is an important medicinal mushroom in Southeast Asia and is one of the most popular medicinal mushrooms used by indigenous communities of Peninsular Malaysia (Lee et al., 2009). The Tiger-Milk Mushroom (locally know as "Cendawan susu rimau (harimau)"), has traditionally been used by the Malays, Chinese and indigenous communities in Malaysia for treatment of cough, fever, chronic hepatitis, gastric ulcer, liver and breast cancer food poisoning and as a general tonic (Chang and Lee, 2004; Wong et al., 2009; Lee et al., 2009). Malaysia's former prime minister, Tun Dr. Mahathir, during his opening speech at Biomalaysia, 2002 mentioned that his chronic cough had been cured by this medicinal mushroom. In China, *L. rhinocerotis* has also been used in the traditional Chinese medicine to treat liver cancer, chronic hepatitis and gastric ulcer (Huang, 1999). Research findings have shown that the sclerotial polysaccharides from the mushroom demonstrates very high anti-inflammatory, antioxidant, anti-proliferative (Lai et al., 2008) and immuno-modulating effects (Wong et al., 2009; Wong et al., 2010; Guo et al., 2011).

Lignosus rhinocerotis is structurally characterised to have a central stipitate pileus arising from a distinct sclerotium (Ryvarden and Johansen, 1980). The sclerotium of *L. rhinocerotis* is the part with medicinal value. Its geographical distribution is only in the tropical rainforest in the region of South China, Thailand, Malaysia, Indonesia, Philippines, and Papua New Guinea (Núñez and Ryvarden, 2001, Cui et al., 2011). The existence of this mushroom in the jungle is always solitary and this makes the collection of its sclerotia a tedious and difficult task. As a result, supply of the sclerotia is limited as well as costly. Recently, we reported a breakthrough in the cultivation of the *Lignosus rhinocerotis* strain, LiGNO™ TM02 on agar, solid and spawn medium with high production yield (Tan et al., 2009), thus overcoming the supply problem.

The nutrition industry is ever growing, despite poor economic outlook. The market for functional foods remains bullish. In this modern society, functional food demand is driven by the desire for products that help build and maintain good health. Functional foods contain special ingredients with unique beneficial effects from cardiovascular to mental health function, immunity. The list of contemporary health issue of public concern is growing rapidly. The continuous demand for nutraceuticals creates new opportunities and commercial pressures. Hence, the nutrition industry is always looking for potential functional ingredients. The cultivated *L. rhinocerotis* has a huge potential to be exploited in this arena.

Isolation and Cultivation of Tiger-Milk Mushroom

Although *L. rhinocerotis* is recognized as a rare species, it still can be found in the jungle in Malaysia. The study started with *L. rhinocerotis* specimens collected from the forest in areas of Cameron Highland, Sungai Perak, Gerik, Hulu Langat, and Raub. The spore and the tissue from its sclerotium, stem and pileus were grown in a special formulated culture media. The mycelium growth was then subjected to many cycles of sub-culturing in order to obtain a clean, pure culture. Genetic marker was developed in order to authenticate the specimens collected (Tan et al., 2010). The *L. rhinocerotis* culture growth conditions in liquid and solid media were optimised. Since the sclerotia of *L. rhinocerotis* is the part that contained medicinal properties, cultivation factors and conditions for the formation of sclerotia were also determined and optimised. The cultivation process takes approximately 6 months (for the sclerotia formation) to up to 2 – 3 years (for stem and pileus formation).

At present, we are able to produce the sclerotia of *L. rhinocerotis* at commercial scale in a 1 000 sq feet environmentally controlled culture room. The production is about 300 kg per month and can easily be

expanded by increasing the parameters of its culture room.

Safety Assessment

In the development of any functional ingredient, food safety has to be established based on its historical use, its intrinsic nature, usage or based on information generally known and accepted by qualified experts.

Although historical records indicate that *L. rhinocerotis* has been extensively used for hundreds of years without any toxicity concerns, a scientific assessment of its potential toxicity remains essential. Preliminary toxicity study showed that oral administration of the cultivated sclerotial powder to mice at daily dose of up to 10% of the experimental animals' body weight continuously for three months did not show any adverse effects. Various toxicity studies in compliance with the OECD guidelines were subsequently conducted.

Acute toxicity study showed that there were no treatment-related acute toxicities in rats following oral administration at a high dose of up to 2000 mg/kg. 28 days sub-acute toxicity study (Lee et al., 2011) showed that oral administration of the sclerotial powder at daily dose of up to 1000 mg/kg had no adverse effects on the growth rate, hematological and clinical biochemical parameters. Histological studies showed that the treatments did not induce any pathological changes in the liver, kidney, heart, spleen and lung of the animals. As the highest tested dose of 1000 mg/kg was not associated with any toxicity concerns, the NOAEL dose is concluded to be higher than 1000 mg/kg.

The cultivated *L. rhinocerotis* was also subjected to corticosteroid screening by Toxicology Laboratory, National Poison Centre. The results confirmed the absence of corticosteroid as part of its ingredient.

Further toxicity studies including chronic toxicity, genotoxicity and mutagenicity of the sclerotial powder as well as effect of the sclerotia on fertility in rats and possible teratogenic effect on the offspring are in progress.

Regulatory and Product Registration

Traditional medicine is a system of medicine based on cultural beliefs and practices handed down from generations to generations. The practice continues until today and the World Health Organization (WHO) estimates that 65% – 80% of the world's population rely on traditional medicine for their primary health care needs.

Regulation of traditional medicine in Malaysia began in 1992 and applications for product registration have to be submitted to the National Pharmaceutical Control Bureau (NPCB). Only ingredients listed in the NPCB Traditional Medicine Active Ingredients List are eligible to be used in any registered products. Unfortunately *L. rhinocerotis* was not in the list prior to September 2010. In order to qualify as functional ingredient, we have submitted to NPCB the supporting documents on the safety, history of use and bioactivities of *L. rhinocerotis*. NPCB accepted *L. rhinocerotis* to be listed in the traditional medicine active ingredient list in September 2010. This enables *L. rhinocerotis* to be exploited commercially as a functional ingredient. The first finishing product, capsule of 250 mg of 100% cultivated Tiger-Milk Mushroom sclerotic was approved by NPCB as traditional medicine in April 2011.

Potential Functional Food Ingredient

Functional foods have entered the global market at full force in the past decade and have rapidly gained

market share as a value added product. This category is generally thought to include products that influence specific functions in the body and thereby offers benefit for health, well-being or performance, beyond their nutritional value. As natural substances are clearly preferred over chemical ones as food supplements, functional botanical ingredients are more popular than ever in the functional food market. Thus, *L. rhinocerotis* may be proposed as a new potential functional ingredient due to its high anti-inflammatory, immuno-modulating, anti-proliferative and antioxidant activity. Its bioactivities render it a valuable item to be incorporated in functional foods, beverages and nutraceuticals.

Biopharmacological Efficacy Assessment Based on Scientific Studies

Research on the pharmaco-properties of *L. rhinocerotis* is essential to generate evidence-based proofs to substantiate the ethno-botanical claims of this fungus.

Anti-inflammatory

The present study investigates the anti-inflammatory activity of the sclerotia of the cultivated *L. rhinocerotis* using carrageenan induced anti-inflammatory model (rat paw edema). The cold water extract has been demonstrated to exhibit strong anti-inflammatory effects comparable to standard drug, indomethacin (unpublished data).

Immuno-modulating

Animal studies showed that the sclerotial polysaccharides of *L. rhinocerotis* exhibit multiple bioactive properties. Extracts of *L. rhinocerotis*, and particularly the sclerotial polysaccharide constituents exhibit stimulatory effects on human innate immune cells. The constituents have also been associated with immune modulation in preclinical study, and are hypothesised to exert anti-tumor effects as a result of these immune properties (Wong et al., 2009; Wong et al., 2010; Guo et al., 2011).

Anti-oxidant

The findings from a recent *in vitro* study (unpublished data) suggested that the antioxidant capacity of the mushroom sclerotium is comparable to many other medicinal mushrooms, which is generally moderately low. The water extracts, however, exhibited strong superoxide anion scavenging activity, indicating that the extract might be helpful in preventing certain type of oxidative stress. Taken as a whole, the *L. rhinocerotis* sclerotial powder offer a promising source of functional ingredient potentially attributed to its antioxidant capacity, specifically the superoxide anion scavenging activity.

Anti-proliferative

Lai et al. (2008) was the first to investigate the anti-proliferative effects of the sclerotial polysaccharides of the mushroom. They reported that the hot water extract of *P. rhinocerotis* (synonym of *L. rhinocerotis*) exhibited anti-proliferative activity against different kinds of leukemic cells. Recently, Lee et al. (2012, in press) reported that the cold water extract of the sclerotia of the cultivated *L. rhinocerotis* exhibited significant anti-proliferative activity against the breast cancer cell MCF-7 and lung cancer cell A549. Their results also showed that the cold water extract was essentially not cytotoxic against the normal breast and lung cells. Its cytotoxic action is due to a high molecular weight fraction isolated from the cold water extract, and that the cytotoxic action is mediated via apoptosis. The anti-proliferative action against MCF-7 cells provides a

plausible scientific basis for the traditional use of *L. rhinocerotis* sclerotia in breast cancer treatment by the Malaysian indigenous communities.

Efficacy Assessment Based on Functional Observation

We have conducted survey among the volunteers who had taken Tiger-Milk Mushroom for various health concerns. The volunteers were given 0.5g of Tiger-Milk Mushroom per day continuously for 1 – 2 weeks and feedbacks were collected after the treatment was completed. The collected volunteer testimonials can be categorized in the following groups.

Relief of Respiratory-related Illness

Tiger-Milk Mushroom improves breathing in patients with respiratory health concerns. It also enables the patients to expel the phlegm more easily.

Relief of Asthmatic Symptoms

One of the most prominent benefits of Tiger-Milk Mushroom is its ability to relieve asthmatic symptoms. It improves the patients' breathing and also reduces their inhaler usage frequency. For those patients suffering from asthmatic attack, Tiger-Milk Mushroom could actually shorten their recovery period and it was also observed that it decreases the recurrence of asthmatic attack.

Treatment of Chronic Cough

Tiger-Milk Mushroom had successfully cured many chronic cough cases, and the testimony of Malaysia former Prime Minster, Tun Dr. Mahathir (himself a medical doctor by training) was one of the most prominent cases.

Relief of Allergy

Tiger-Milk Mushroom helps to relief various allergy symptoms including respiratory allergy such as nasal & sinus symptoms. In the case of skin allergy such as eczema, it has been reported that the rashes subsided after a week of treatment.

Treatment of Joint Pain

Tiger-Milk Mushroom was found to be effective in the treatment of joint pain developed as a result of dengue fever, after one week consumption. It is also effective in relieving joint pain in the elderly. This is attributed to its anti-inflammatory activity.

Improvement of Stamina

Tiger-Milk Mushroom was also found to improve stamina and alertness in many healthy subjects. It generally improves the subjects "Qi"。

Conclusion

The potential of the high medicinal and nutritional values of *L. rhinocerotis* have never been fully realised due to its limited supply. With the successful cultivation technology at commercial scale, the supply problem is a thing of the past. Our on-going research has provided scientific evidences for its safety and

biopharmacological efficacy and substantiated its claims as a highly beneficial functional ingredient.

Acknowledgments

The authors thank MOA for providing the TechnoFund (TF0109M004) grant for the pre-commercialisation of *L. rhinocerotis* and to MARDI, University of Malaya, and LiGNO Biotech Sdn Bhd for collaborative work on *L. rhinocerotis*.

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