IUMS Outreach Program on Food Safety and International Conference on Mycotoxin

Program

November 14-15, 2014

Kamarijani-Soenjoto Auditorium

Faculty of Agricultural Technology
Universitas Gadjah Mada
Yogyakarta, Indonesia

Organized by:

Faculty of Agricultural Technology, Universitas Gadjah Mada

International Union of Microbiological Societies

In collaboration with:

Indonesian Association of Food Technologists
Patpi
Perhimpunan Ahli Teknologi Pangan Indonesia

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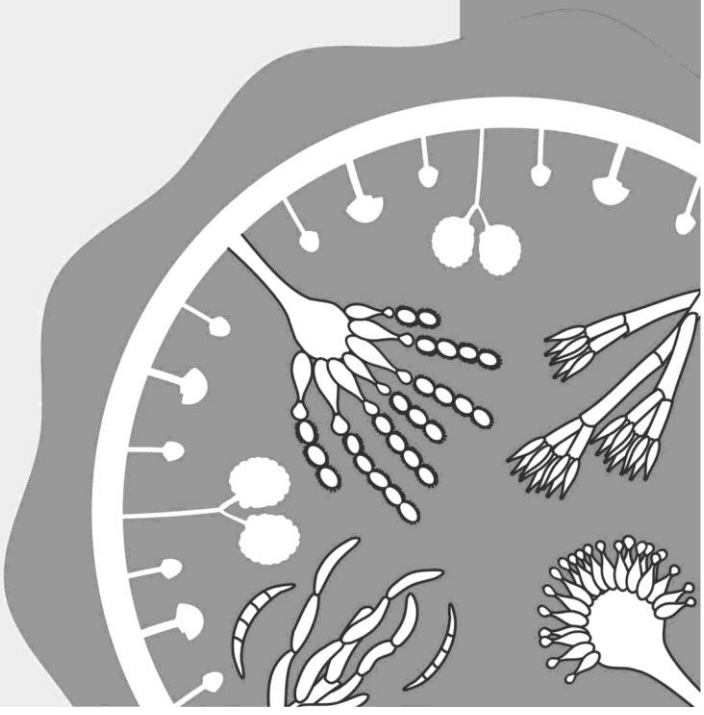

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IUMS OUTREACH PROGRAM ON FOOD SAFETY AND
INTERNATIONAL CONFERENCE ON MYCOTOXIN

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Funds Venture

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INTRODUCTION

In support of its mission to enhance the scientific background and professional effectiveness of basic and applied microbiologists, the International Union of Microbiological Societies (IUMS) is embarking on a program of educational outreach to developing countries and their microbiologists. The Union envisions an IUMS series of courses that will be offered to groups of microbiologists that may include graduate students, postdoctoral fellows, and practicing professionals from developing countries within a given geographic region. These will be offered periodically in various regions and on different topics of interest and importance.

The first IUMS Regional Course was offered in Singapore during June 14-16, 2010, and served microbiologists from the surrounding Asian countries. Singapore was chosen as the site, because of its proximity to the countries of Asia. IUMS made a contribution to the subsistence of the successful applicants as far as the finances allow. It is expected that this experience will boost the capability of the attendees in their microbiologic work after they return home, and we shall endeavor to forge a network of the attendees, so they can continue to communicate with each other and the instructors by e-mail.

The second IUMS Regional Course on Food Safety was offered in Bali (Indonesia) 22 - 24 June 2011 and organized in collaboration with the Indonesian Society for Microbiology (PERMI), the International Commission on Food Mycology (ICFM) and the International Committee on Food Microbiology and Hygiene (ICFHM). The third IUMS outreach conference on Antimicrobial Resistance took place in Havana, Cuba on November 14-16, 2013. The fourth course in Yogyakarta will focus on food safety and mycotoxins

The Faculty of Agricultural Technology Universitas Gadjah Mada (FTP-UGM) via its Center of Excellence on Mycotoxin Studies (CEMycoS) in collaboration with International Life Sciences Institutes (ILSI) Southeast Asia Region, and supported by SEAMEO BIOTROP Indonesia, ISPA Italy, CBS Netherlands, Universiti Sains Malaysia (USM) Penang Malaysia, and the International Society for Mycotoxicology (ISM) organized "the International Conference on Mycological Aspects of Food and Feed Safety" (IC-MAFFS) on 27-29 June 2013 at Universitas Gadjah Mada Yogyakarta, Indonesia. The objectives of the conferences was to share the latest science and updates, as well as to review, discuss and address important issues concerning to mycology and mycotoxins and their relation to food and feed safety aspects. This conference was attended by more than 200 scientists and practitioners from Indonesia, ASEAN countries, other Asia countries, including Japan, India and Saudi Arabia.

This year FTP UGM will take an opportunity to be the local organizer for the fourth IUMS outreach program, with the title of the activity "IUMS Outreach Program on Food Safety and International Conference on Mycotoxins". This program is designed for two days, 14-15 November 2014, held at the Faculty of Agricultural Technology UGM. This outreach program and conference, will be open for scientists from ASEAN countries, and other countries of ASIA, to participate by presenting their paper or poster. We will expect about 200 participants from all over ASEAN countries. To organize this program FTP-UGM is supported by two related societies in Indonesia, PERMI (Indonesian Society for Microbiology) and PATPI (Indonesian Association of Food Technologists), as well as National Agency of Drug and Food Control, Republic of Indonesia (BPOM).

PREFACE

Chairperson of Organizing Committee
By Endang S Rahayu

Dear distinguished guest, participants and colleagues, welcome to Yogyakarta, Indonesia and to the IUMS Outreach Program and International Conference on Mycotoxin. I am extremely happy to greet the honorable guest speakers and participants, and wish you all the best while staying in Yogyakarta.

First of all, I would like to thanks to the International Union of Microbiological Societies (IUMS) via Dr. Rob Samson for the opportunity given to the Faculty of Agricultural Technology Universitas Gadjah Mada, to organize the IUMS Outreach Program of Food Safety combined with International Conference on Mycotoxin.

This conference in fact could not be separated with similar conference which was organized last year (International Conference on Mycological Aspects of Food and Feed Safety, June 27-28, 2013) that had been participated by scientist from ASEAN countries. Nowadays, food safety and mycotoxins are still being the important issues, so conference related to these topics will attract the attention of researcher from the university, government agencies, as well as practitioners from industries, who want to get the latest information on it.

The IUMS Outreach Program on Food Safety and International Conference on Mycotoxin is attended by more than 200 participants. There are 17 lectures given by guest lectures from IUMS and 4 lectures from Indonesia. Two lectures will be delivered by Dr. Emilia Rico from USA; 13 lectures by our guest speakers from Europe, i.e., Dr. Su-Lin Leong from Sweden; Dr. Giancarlo Perrone from Italy; Prof. Ludwig Niessen from Germany; Prof. Naresh Magan from UK; Prof. Jens C. Frisvad and Prof. Ulf Thrane from Denmark; and other two lectures are delivered by Dr. Warapa Mahakarnchanakul and Dr. Latiffah Zakaria from Thailand and from Malaysia, respectively. Lecture will be started by Chairman of Indonesian Association of Food Technologist, Prof. Rindit Pambayun in 14 November 2014, and the last lecture (21st) will be delivered by the Head of National Agency for Drug and Food Control of The Republic of Indonesia (BPOM), Dr. Roy Sparringa. Wrap up of the program will be summarized by Prof. Robert A. Samson, from the Netherlands, as a representative delegates of IUMS.

The organizing committee has received 42 abstracts, 17 of them will be presented orally, and the remaining will be presented in poster. Full paper will be publish in online journal *Indonesian Food and Nutrition Progress,* journal published by Indonesian Association of Food Technologists in collaboration with Faculty of Agricultural Technology, Universitas Gadjah Mada.

According to the list of participants, we have 23 foreign participants from ASEAN countries, i.e., Thailand, Philippines, Malaysia, Singapore and Timor Leste; 93 Indonesian participants from Universitas Gadjah Mada and 58 participants from other universities; 26 participants from research institutes and government agencies (BPOM), and 23 participants from industries.

On behalf of the organizing committee, I would like to express my sincere thanks to IUMS, to all guest speakers, oral and poster presenters, participants, as well as, sponsors, for their contribution to the success of the outreach program and conference. The Conference Committee have tried their best in order to make this event meaningful and pleasant one. Please do not hesitate to let us know, if you have any suggestions or require any assistance during the course of your short stay.

At last, I would like to take this opportunity to thanks all the colleagues, the steering committee, and organizing committee (students) for their never ending precious cooperation that made this event possible.

Endang S. Rahayu (Organizing Committee)

PREFACE

Center of Excellence on Mycotoxin Studies (CEMycoS)
Faculty of Agricultural Technology, Universitas Gadjah Mada
By Suparmo

Your Excellency Head of The National Agency of Drug and Food Control, Dr. Roy Sparringa, Rector of UGM, Dean of Faculty of Agricultural Technology UGM Prof. Lilik Sutiarso, Distinguished guests from IUMS, Dr. Giancarlo Perrone (Institute of Sciences of Food Production, National Research Council, Bari, Italy), Prof. Dr. Jens C. Frisvad (Technical University of Denmark, Lyngby Denmark), Prof. Dr. Ludwig Niessen (Technische Univ. München, Freising Germany), Prof. Dr. Naresh Magan (Cranfield University, Bedford, United Kingdom), Dr. Rico Emilia (BCN Research Laboratories, Inc. USA), Prof. Dr. Robert A. Samson (CBS, The Netherlands), Dr. Su-Lin Leong (Swedish University of Agricultural Sciences, Sweden), Prof. Dr. Ulf Thrane (Technical University of Denmark), Dr. Warapa Mahakarnchanakul (Kasetsart University, Bangkok, Thailand), and Dr. Latiffah Zakaria (University of Sains, Penang, Malaysia).

Ladies and Gentlemen, Participants of the IUMS Outreach Program on Food Safety and Conference on Mycotoxin.

On behalf of the Center of Excellence on Mycotoxin Studies (CEMycoS), I wish first of all to extend our heart-felt thanks for your presence at this conference. Appreciation and sincere thanks are also addrressed to the IUMS for the timely response in terms of wisdom, finance, as well as efficient contributions and the industrious work in coordination with the Organizing Committee lead by Prof. Endang S. Rahayu. On behalf of the CEMycoS, and Universitas Gadjah Mada, I would like to convey our high appreciation and sincere thanks, through Dr. Samson and friends, to the Director of IUMS, for the attention to our institution and the generous financial support. The contribution make it possible for many highly regarded world-class scientists to come to Universitas Gadjah Mada, to share their expertice to many young Indonesian mycologists in this outreach program. Please convey our gratitude to the IUMS Office.

The objectives of the conferences are to share the latest science and updates, as well as to review, discuss and address important issues concerning to mycology and mycotoxin and their relation to food safety aspects.

This seminar is so significant, especially for Indonesians, as well as nations in the South East Asian Region, in a sense that this coming year, 2015, will mark the beginning of ASEAN Single Economic Community, when free trade in the area will be highly encouraged. We understand that the subject of this seminar, the mycotoxins, are parts of the Sanitary-PhytoSanitary contaminants that become one of the obstacle in international food trades. I do hope that, through this seminar and the outreach program, we are all will have better vision to the mycotoxin problems and become wiser in dealing with them.

Actually, not too long ago, research activities in the Department of Food and Agricultural Product Technology, Universitas Gadjah Mada, were focused to deal with problems of aflatoxins in foods. We encouraged masters and doctoral students to do researches on mycotoxins in food and feed. The researches were vary from survey related to the toxin occurence in primary and secondary food products, their fate during processing, effort in detoxification, estimate level of ingestion by people in the region that consume corn-based staple food, including collection of their urine samples for molecular trace of mycotoxin ingestion.

We deeply appreciate collaboration with colleagues from University Sains Malaysia through Dr. Latiffah Zakaria, Kasetsart University Thailand through Dr. Warapa Mahakarnchanakul and ISPA-CNR Bari Italy through Dr. Angelo Visconti and Dr. Antonio Logrieco that made it possible for several of our students and staffs to work in their laboratories. Thanks again for the chance of sharing both the expertice and laboratory facilities.

Our activities in the area gained a great deal attentions from the government, communities, as well as indutries that led us to open up discussion forums on aflatoxin, called Aflatoxin Forum Indonesia, the AFI. We conducted the AFI discussion annually from AFI_1 to AFI_8 wich were always fully attended by entusiastic participants. Following each of the AFI discussions, we also ran workshops on capacity building in mycological food safety procedures.

We enhanced our laboratory capability, which in 2013, we received ISO 17025 Certification for testing Aflatoxin in peanut. The CEMycoS initiated to organize testing laboratory network among several laboratories in the country and tried to do profeciency test on Aflatoxin, with the help of Romer Lab in Singapore, who provided standard samples. At the moment we are attempting to broaden our analitical capability to Ochratoxins in coffee samples.

In as much effort that we tried to be productive in the mycotoxin studies, we realize that local mycologists still have a lot to catch-up with our colleagues overseas. We welcome any collaboration that enable us to move forward faster. That is why Outreach Program on Food Safety and Conference on Mycotoxin of the IUMS is highly appreciated.
Thank you for your attention.

Suparmo, Dr.
Coordinator of CEMycoS
Faculty of Agricultural Technology, Universitas Gadjah Mada
www.cemycos.tp.ugm.ac.id

PREFACE

Indonesian Society for Microbiology (PERMI)
By Fedik A Rantam

Dear Colleagues,

We praise and gratitude to God Almighty who has given grace so we can attend a series of activities of the International Union of Microbiology Society (IUMS) Outreach Program on Food Safety and International Conference on Mycotoxin, at Universitas Gajah Mada, Indonesia.

Mycotoxin problems in Indonesia is largely unknown, a previous report indicated that aflatoxin-contaminated feed increases dramatically during the wet season. Mycotoxin contamination like aflatoxin has been well known as a worldwide health threatening problem in tropical countries including Indonesia. Contamination of commercial foods with high levels of aflatoxin is a very important issue for Indonesia since those foodstuffs are very popular among children.

Based on the global mycotoxin survey in Indonesia, showed that samples contained at least two mycotoxins while 10-17% contained 6 or more mycotoxins. These problems are a big challenges especially in tropical countries because it can induce deficiencies of most nutrients impair the immune response and increase susceptibility to diseases. Severe nutrient deficiencies are particularly deleterious to the immune system when they occur early in life. Then it can influence performance, impaired immunity, vaccine failure, and also decreased resistance to infectious diseases by human and animal health.

However, it is clear that many gaps remain to be filled for a comprehensive picture of the global situation with mycotoxin contamination and its health consequences become more important. Hopefully this event can be a way to exchange new approach or strategy finding and information among researchers, private sector (business) and government.

On behalf of the Indonesian Society for Microbiology I would like to extend my gratitude and thank to steering and organizing committee and who have supported this event.

Vice President of Indonesian Society for Microbiology,
Prof. Dr. Fedik A Rantam, DVM.

PREFACE

Dean of the Faculty of Agricultural Technology, Universitas Gadjah Mada
By Lilik Sutiarso

Dear all delegates,

I would like to express a very warm welcome to all participants of the IUMS Outreach Program on Food Safety and International Conference on Mycotoxin, which is held from 14 to 15 November 2014 at the Faculty of Agricultural Technology, Universitas Gadjah Mada in Yogyakarta, Indonesia and wish you a pleasant stay in our city.

Mold contamination and subsequent production of mycotoxin can occur in the field, at harvest, during postharvest, as well as in storage of agricultural commodities, such as grains and seeds. Certainly, it has a great significance for human's health and livestock, which still being a major problem throughout the world. As we all know, agricultural product commodities, in this hot and humid climate, may carry toxins if not handled properly. Failure in preventing as well as controlling the mycotoxin contamination may lead the food safety reduction and loss in economical profitability. Therefore, it is necessary to share the current research findings, knowledge, as well as experience regarding mycotoxin and to strengthen national and international networking among researchers, universities, government agencies, and industrial partners.

Faculty of Agricultural Technology UGM has declared as a Center of Excellent on Mycotoxin Studies (CEMycoS) since 4 years ago. Referring to its mission, the faculty takes an opportunity to be the local organizer for IUMS outreach program through this conference to strengthen our faculty's outreach program. This is the second time for the faculty to hold international conference as part of scientific activity of the CEMycoS. Through this program, it is expected that many benefit can be brought to public to develop the scientific interest into international contribution related to the mycological aspect focusing on mycotoxin and their relation to food safety.

Last but not least, once again I extend my sincere thanks to guest speakers, all the participants, companies, and other parties for the contributions and supports to this conference. Furthermore, I wish you an enriching and inspiring conference and hope that this conference will be a great success. Enjoy your time in Yogyakarta, Indonesia

Faculty of Agricultural Technology, Universitas Gadjah Mada,
Dean,
Prof. Dr. Lilik Sutiarso

PREFACE

Steering Committee
By Robert A Samson

IUMS is one of the 31 Scientific Unions of the International Council of Science (ICSU). It was founded in 1927 as the International Society of Microbiology, and became the International Association of Microbiological Societies affiliated to the International Union of Biological Sciences (IUBS) as a Division in 1967. It acquired independence in 1980 and became a Union Member of ICSU in 1982.

The objectives of the Union are to promote the study of microbiological sciences internationally: initiate, facilitate and coordinate research and other scientific activities which involve international cooperation; ensure the discussion and dissemination of the results of international conferences, symposia and meetings and assist in the publication of their reports; represent microbiological sciences in ICSU and maintain contact with other international organizations.

The major goal of IUMS is to promote research and the open exchange of scientific information for advancement of the health and welfare of humankind and the environment and strongly discourages any uses of knowledge and resources to the contrary.

The scientific activities of the Union are conducted by the three Divisions of Bacteriology & Applied Microbiology (BAM), Mycology and Eukaryotic Microbiology and Virology and by six specialist international committees, eight international commissions and two international federations. Their major activities include the classification and nomenclature of bacteria, fungi and viruses, food microbiology, medical microbiology and diagnostics, culture collections, education, and biological standardization.

IUMS also started to organize the IUMS outreach program. The first workshops were held in Singapore (2010), Bali (2011, in collaboration with the Indonesian Society of Microbiology), Havana, Cuba on November 14-16, 2013. A workshop on Food safety and mycotoxins is now taking place in 2014 in Yogyakarta.

PROGRAM

Friday (14 November 2014)	
07.00 – 07.45	Registration, Breakfast, and Coffee Morning
07.45 – 08.15	Opening Ceremony • Coordinator of CEMycoS (Suparmo) • Dean of the Faculty of Agricultural Technology UGM (Lilik Sutiarso) • Indonesian Society for Microbiology (PERMI) (Fedik Abdul Rantam) • International Union of Microbiological Societies (IUMS) (Robert A Samson)
08.15 – 08.25	Balinese Dance (Margapati Dance)
08.25 – 08.30	Introduction of speakers and participants (Endang S. Rahayu)
	Lecture 1 Moderator: Suparmo / Endang S. Rahayu
08.30 – 09.00	1. Rindit Pambayun – *Current research and technological application in food safety in Indonesia*
09.00 – 09.30	2. Warapa Mahakarnchanakul – *Mycotoxins regulations in Thailand*
09.30 – 10.00	3. Ulf Thrane – *Are all fungal metabolites toxic?*
10.00 – 10.30	4. Jens C Frisvad – *Mycotoxins and exometabolites in foods*
	Lecture 2 Moderator: AA Rahmianna / Nanik Suhartatik
10.30 – 11.00	5. Naresh Magan – *Mycotoxin regulations, sampling issues: The global context*
11.00 – 11.30	6. Su-Lin Leong – *Biocontrol of mycotoxins: Strategies and obstacles*
11.30 – 12.00	7. Emilia Rico – *Good Sanitation Practices (GSP) and Environmental Monitoring Program (EMP) to prevent pathogen contamination and mold spoilage of Ready-to-Eat (RTE) foods*
12.00 – 13.00	Break
13.00 – 13.50	Poster Session
13.50 – 14.00	Group photo session
	Lecture 3 Moderator: Latiffah Zakaria / Winiati P. Rahayu
14.00 – 14.30	8. Ludwig Niessen – *Application of molecular biological methods for detection of mycotoxin producing fungi in food*
14.30 – 15.00	9. Giancarlo Perrone – *Mycotoxigenic fungi and mycotoxins in corn*
15.00 – 15.30	10. Naresh Magan – *Ecology of mycotoxigenic fungi and possible prevention strategies*
15.30 – 16.00	11. FMC Sigit Setyabudi – *CEMycoS: Current, prospect of research & community outreach*
16.00 – 18.15	Technical Session for Mycotoxin Presentation I Moderator: Sardjono / Heni Adhianata
18.15 – 19.00	Welcome Dinner and Gathering
19.00 – 20.00	Traditional Performance (*Gathutkaca Gandrung*)

Saturday, 15 November 2014	
07.00 – 08.00	Registration, Breakfast, and Coffee Morning
08.00 – 10.00	Technical Session for Mycotoxin Presentation II Moderator: Harsi Dewantari Kusumaningrum/ Tyas Utami
10.00 – 10.30	Break
	Lecture 4 Moderator: Warapa Mahakarnchanakul / Gayuh Rahayu
10.30 – 11.00	12. Su-Lin Leong – *Identification of foodborne yeasts and moulds: A guide for users*
11.00 – 11.30	13. Jens C Frisvad – *How do we secure correct identification of mycotoxins and the fungi which produce them?*
11.30 – 12.00	14. Ludwig Niessen – *Detection of mycotoxins using affinity-based technologies*
12.00 – 12.30	15. Ulf Thrane – *Fusarium toxins*
12.30 – 13.30	Break
	Lecture 5 Moderator : Sigit Setyabudi / Nampiah Sukarno
13.30 – 14.00	16. Emilia Rico – *Molds isolated from the processing environment and their significance in spoilage of heat-processed beverages and juices*
14.00 – 14.30	17. Endang S. Rahayu – *Traditional fermented foods and their safety*
14.30 – 15.00	18. Giancarlo Perrone – *Black aspergilli and their mycotoxin production*
15.00 – 15.30	Break
	Lecture 6 Moderator: Endang S. Rahayu / Chusnul Hidayat
15.30 – 16.00	19. Latiffah Zakaria – *Mycotoxins in Malaysia*
16.00 – 16.30	20. Naresh Magan – *Climate change, food security and mycotoxins: do we know enough?*
16.30 – 17.00	21. Roy Sparringa – *Indonesian food safety: Regulation and challenge*
17.00	Wrap up and Closing: Robert A. Samson (IUMS)
17.30	Heading to Purawisata
19.00 – 20.00	Dinner at Purawisata
20.00 – 21.30	Ramayana Performance

PROGRAM FOR TECHNICAL SESSION

Friday (14 November 2014)	
16.00 – 18.00	Technical Session for Mycotoxin Presentation I Moderator: Sardjono / Heni Adhianata
16.00 – 16.15	1.1. Nampiah Sukarno – *Toxigenic Aspergillus flavus population detected by its aflatoxin genes in peanut kernel*
16.15 – 16.30	1.2. Okky Setyawati Dharmaputra – *Aspergillus flavus infection and aflatoxin contamination in stored nutmeg (Myristica fragrans) at various stages of the delivery chain in North Sulawesi Province*
16.30 – 16.45	1.3. Nova Wahyu Pratiwi – *Airbone fungi and aflatoxin-producing Aspergillus flavus group on Gaplek storage warehouse in Gunung Kidul, Yogyakarta, Indonesia*
16.45 – 17.00	1.4. Yeyen Wanita – *Aflatoxin content in some peanut (Arachis hypogaea L.) post-harvest handling in Gunung Kidul, DIY*

17.00 – 17.15	1.5. Ani Widiastuti – *Molecular identification of Fusarium species from maize kernels in several maize production area in Central and East Java, Indonesia*
17.15 – 17.30	1.6. Yunika Mayangsari – *Occurrence of ochratoxin A in cocoa powder and method validation*
17.30 – 17.45	1.7. Fitri Nadifah – *Identification of potatoes-contaminating fungi in traditional market of Condong Catur, District of Sleman, Yogyakarta*
17.45 – 18.00	1.8. Vita Meylani – *Mould, bacteria and heavy metals contamination in ground coffee*
18.00 – 18.15	1.9. Heru Susanto – *Evaluation of reduction fumonisin contamination in corn in the stage of making Sekelan that soaked with lime water and lactic acid bacteria*
Saturday, 15 November 2014	
08.00 – 10.00	Technical Session for Mycotoxin Presentation II Moderator: Harsi Dewantari Kusumaningrum / Tyas Utami
08.00 – 08.15	2.1. Betty Nurhayati – *Anticandida activities of ethyl acetate extract, fractions and compounds from Lactobacillus plantarum IBL-2 fermentation product*
08.15 – 08.30	2.2. Dadik Pantaya – *Low pH enhances rumen absorption of aflatoxin B1 and ochratoxin A in sheep*
08.30 – 08.45	2.3. Jessil Ann Pajar – *Within-host interactions between Metarhizium anisopliae and two Aspergillus spp. : evaluation of constructive implications on biocontrol strategies*
08.45 – 09.00	2.4. Hoa Bui Thi Quynh – *Efficacy on elimination of Listeria spp., Salmonella spp. and Pseudomonas spp. in single and mixed biofilms by hydrogen peroxide pre-treatment and cleaning process*
09.00 – 09.15	2.5. Betty Sri Laksmi Suryaatmadja Jenie – *Effect of co-culturing of Endomycopsis burtonii in angkak fermentation by Monascus purpureus on citrinin and red pigment production*
09.15 – 09.30	2.6. Endang Kusdiyantini – *Pigment production of Monascus sp. isolated from angkak in Semarang Region, Central Java, Indonesia*
09.30 – 09.45	2.7. Isworo Rukmi*, Kempong – a traditional fermented food in Karangpucung Kidul Village, Linggapura Bumiayu, Central Java: Fermentation agent and their roles*
09.45 – 10.00	2.8. Gayuh Rahayu – *Does microbial diversity of Indonesian tempeh determine its safety?*
10.00 – 10.15	2.9. Annytha Detha – *Natural Antimicrobial Compound in Sumba Mare's Milk*

LIST OF ABSTRACTS

No.	Speaker (Authors)	Address	Title	Note
INVITED SPEAKER				
1	Giancarlo Perrone	ISPA, Bari, Italy	Black Aspergili and Their Mycotoxin Production	IS-18
			Toxigenic Fungi and Mycotoxin in Corn	IS-9
2	Jens C. Frisvad	Technical University of Denmark	Mycotoxin and Exometaboities in Foods	IS-4
			How do We Secure Correct Identification of Mycotoxin and The Fungi which Product Them?	IS-13
3	Latiffah Zakaria	Universiti Sains Malaysia	Mycotoxin in Malaysia	IS-19
4	Ludwig Niessen	Technische Univ. Munchen Freising, Germany	Application of Molecular Biological Methods for Detection of Mycotoxin Producing Fungi in Food	IS-8
			Detection of Mycotoxins Using Affinity-Based Technologiest	IS-14
5	Naresh Magan	Cranfield University, Crandfield, Bedford, UK	Mycotoxin Regulations, Sampling Issues – The Global Context	IS-5
			Climate Change, Food Security ang Mycotoxins: Do We Know Enough?	IS-20
			Ecology of Mycotoxigenic Fungi and Possible Prevention Strategies	IS-10
6	Rico Emilia	International Commision on Food Mycology, USA	Good Sanitation Practices (GSP) and Environtment Monitoring Program (EMP) to Prevent Pathogen Contamination and Mold Spoilage of Ready to Eat (RTE) Foods	IS-7
			Mold Isolated from The Processing Environment and Their Significance in Spoilage of Heat Processed Beverages and Juices	IS-16
7	Rindit Pambayun	Universitas Sriwijaya, Indonesia	Current Research and Technological Application in Food Safety in Indonesia	IS-1
8	Roy Sparringa	National Agency of Drug and Food Control, Indonesia	Indonesian Food Safety: Regulation and Challenge	IS-21

9	Su-Lin Leong	Swedish University of Agricultural Sciences, Sweden	Biocontrol-Strategies and Obtacles	IS-6
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4	Yeyen Wanita	Balai Pengkajian Teknologi Pertanian, Yogyakarta, Indonesia	Aflatoxin Content in Some Peanut (*Arachis hypogaea* L.) Post-Harvest Handling in The Gunungkidul, DIY	O-1.4
5	Ani Widiastuti	Departement of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada, Indonesia	Molecular Identification of *Fusarium* Species from Maize Kernels in Several Maize Production Area in Central and East Java, Indonesia	O-1.5
6	Yunika Mayangsari	Faculty of Agricultural Technology, Universitas Gadjah Mada, Indonesia	Occurrence of Ochratoxin A in Cocoa Powder and Method Validation	O-1.6

7	Fitri Nadifah	Study Program Diploma of Health Analyst, Health Science College Guna Bangsa, Indonesia	Identification of Potatoes-contaminating Fungi in Traditional Market of Condong Catur, District of Sleman, Yogyakarta	O-1.7
8	Vita Meylani	Siliwangi University, Indonesia	Mould, Bacteria and Heavy Metals Contamination in Ground Coffee	O-1.8
9	Heru Susanto	Departement of Food Technology and Agricultural Product, Faculty of Agricultural Technology, Universitas Gadjah Mada, Indonesia	Evaluation of Reduction Fumonisin Contamination in Corn in The Stage of Making *Sekelan* That Soaked with Lime Water And Lactid Acid Bacteria	O-1.9
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11	Dadik Pantaya	France and Clermont Universite, France and Departement of Animal Science, State Polytechnic Jember, Indonesia	Low pH Enhances Rumen Absorption of Aflatoxin B1 and Ochratoxin A in Sheep	O-2.2
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13	Hoa Bui Thi Quynh	Departement of Food Science and Technology, Agro-Industry Faculty, Kasetsart University, Thailand	Efficacy on Elimination of *Listeria Spp., Salmonella Spp*. and *Pseudomonas Spp*. in Single and Mixed Biofilms by Hydrogen Peroxide Pre-Treatment and Cleaning Process	O-2.4
14	Betty Sri Laksmi Suryaatmadja Jenie	Departement of Food Science and Technology, Bogor Agricultural University, Indonesia	Effect of Co-culturing of *Endomycopsis burtonii in* Angkak Fermentation by *Monascus purpureus* on Citrinin and Red Pigment Production	O-2.5
15	Endang Kusdiyantini	Departement of Biology, Faculty of Science & Mathematics, Diponegoro University, Indonesia	Pigment Production of *Monascus* sp. Isolated From Angkak in Semarang Region, Central Java, Indonesia	O-2.6

16	Isworo Rukmi	Departement of Biology, Diponegoro University, Indonesia	*Kempong*, a Traditional Fermented Food in Karangpucung Kidul Village, Linggapura Bumiayu, Central Java: Fermentation Agent and Their Roles	O-2.7
17	Gayuh Rahayu	Departement of Biology, Faculty of Mathematics and Natural Science, Bogor Agricultural University, Indonesia	Does Microbial Diversity of Indonesian Tempeh Determine Its Safety?	O-2.8
18	Annytha Detha	Faculty of Veterinary Medicine, Nusa Cendana University, Indonesia	Natural Antimicrobial Compound in Sumba Mare's Milk	O-2.9
19	Sukumar Debnath	Rural Based Preventive Oncology Research Centre	Studies on Mycroflora Associated with Dried Areca Nut in Assam	cancelled
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1	Harsi Dewantari Kusumaningrum, Danik Dania Asadayanti, Betty Sri Laksmi Suryaatmadja Jenie, Novie Nurhidayat	Department of Food Science and Technology, Bogor Agricultural University, Indonesia	Citrinin and Pigment Production by Indigenous *Monascus purpureus* strains	P-1
2	Heni Adhianata, Warapa Mahakarnchanakul, FMC Sigit Setyabudi, Sardjono	Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Indonesia	Mycoflora of Fermented and Unfermented Cocoa Beans and Their Susceptibility Difference of Ochratoxin A and Aflatoxins Production in High Relative Humidity Storage	P-2
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5	Tyas Utami, Angga P. Nugroho Hutapea, Endang S. Rahayu	Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia	Reduction of Aflatoxin B1 by *Lactobacillus paracasei* SNP-2 during Peanut Milk Fermentation	P-5
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7	Hanim Z. Amanah, Tyas Utami, Endang S.Rahayu	Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia	Study on Factors Binding Aflatoxin B1 by Lactic Acid Bacteria *Lactobacillus paracasei* SNP-2	P-7
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9	Phakpoom Kooprasertying, Warapa Mahakarnchanakul, Thanapoom Maneeboon, Chananya Chuaysrinule	Department of Food Scinece and Technology, Faculty of Agro-Industry, Kasetsart University Thailand	Using in-House Immuno Affinity Column (KU-AF2) to Assess The Risk of Aflatoxin in Peanut in Thai Consumption	P-9
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11	Andika Sidar, Jaka Widada, Latifah Zakaria, Endang S. Rahayu	Graduate school of Biotechnology, Universitas Gadjah Mada, Indonesia	Detection and Cluser Analysis of Gene Encoding *vacuolar serine protease* Allergen in *Penicillium* Species isolated from Hospital Indoor Air in Yogyakarta Indonesia	P-11

12	Lilis Suryani	Departement of Microbiology, Faculty of Medicine and Health Science, Muhamadiyah Yogyakarta University, Indonesia	Identification of Fungus Caused Otomycosis	P-12
13	Marlia Singgih Wibowo, Isra Muzaqiyah, Betty Nurhayati, Tjokorde I. Armina, Padmasawitri, Yantyati Widyastuti, Tutus Gusdinar	School of Pharmacy, Institut Teknologi Bandung, Indonesia	Production and Utilization of *Lactobacillus plantarum* IBL-2 Bacteriocins as Meat Product Biopreservatives	P-13
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15	Indun Dewi Puspita, Ustadi, Mgs. Muhammad Prima Putra	Departement Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Indonesia	Isolation of Chitinolytic Bacteria from Fermented Shrimp Product and Screening for Antifungal Activity	P-16
17	Tutus Gusdinar Kartawinata, Betty Nurhayati, Tjokorde I. Armina Padmasawitri, Aniendha D. Ramadhani, Yantyati Widyastuti, Marlia Singgih Wibowo	School of Pharmacy, Institut Teknologi Bandung, Indonesia	Used in the Growth Inhibition of Foodborne Pathogenic Bacteria	P-17
18	Rohmatussolihat, Mika Miyashita, Yopi, Puspita Lisdiyanti, Hiroko Kawasaki and Ken-Ichiro Suzuki	Research Center for Biotechnology-LIPI, Indonesia	Isolation of Lactic Acid Bacteria from Indonesian Fermented Food	P-18
19	Unnop Tassanaudom, Warapa Mahakarnchanakul, Chidchom Hiraga	Institute of Food Research and Product Development, Kasetsart University, Thailand	Lactic Acid Bacteria Co-Culture Induction to Enhance the Activity of Antimicrobial Compounds Inactivation of *C. perfringens* on Dried Pepper by Washing with Oxidizing Agents	P-19

20	Susana Ristiarini, M. Nur Cahyanto, Jaka Widada, Latiffah Zakaria, Endang S Rahayu	Universitas Gadjah Mada, Indonesia	Color Value, Citrinin Content and Genetic Variation of from *Monascus purpureus* Angkak in Indonesia	P-20
21	Deni Pranowo, Romsyah Maryam, Nuryono, Ali Agus, FMC Sigit Setyabudi	Department of Chemistry, Faculty of Mathematic and Natural Sciences, Universitas Gadjah Mada	Application of Silica from Rice Hull Ash in Immobilization of Polyclonal AFB1-Antibody for Immunoaffinity Column Clean-Up	P-21
22	Hasim Munawar, Veronica Lattanzio, Biancamaria Ciasca, Giuseppe Panzarini, Sri Rachmawati, Michelangelo Pascale	IRCVS, IAARD, Ministry of Agriculture	Simultaneous Determination of Co-occurring Mycotoxins in Maize from West Java by Liquid Chromatography/Tandem Mass Spectrometry	P-22
23	Ma. Aussielita L. Lit	Analytical Solutions and Technical Services, General Santos City, Phillipines	Aflatoxin Levels of Foods and Feeds in the Philippines	P-23

INVITED SPEAKER'S ABSTRACTS

Current Research and Technology Application in Food Safety in Indonesia

Rindit Pambayun
Professor of Food Science and Technology at Sriwijaya University
The President of Indonesia Association of Food Technologists (IAFT, PATPI)
Address: Jl. Raya Palembang-Prabumulih Km. 32 Ogan Ilir, Inderalaya, 30662, Sumatera Selatan Tel. +62711-580664 Fax. +62711-320310
E-mail: rpambayun@yahoo.com

Abstract

Foods are considered to have high quality if they are safe, nutritious, palatable, and healthy. For these factors, safety is the most important factor of foods. Regarding the safety of food, the food has been correlated with mycotoxin. Some tropical foods of Indonesian origin have high risk with mycotoxin. Common foods that contain the mycotoxins include groundnuts (peanuts), maize (corn), rice, yams, cassava, soyabeans, fruits, vegetables, spices, cacao, and coffee. These food stocks have seriously health risks when they are heavily contaminated by mycotoxin. It will be happened when those foods are storaged under warm and humid conditions. In terms of food safety, the mycotoxins include aflatoxins (B1, B2, G1, G2, and M1), ochratoxin A, patulin, and toxins produced by Fusarium molds such as fumonisins (B1, B2, and B3), trichothecenes (nivalenol and deoxynivaleonol, T-2 and HT-2 toxin) and zearalenone can be exist in some daily foods. In Indonesia, the recent researches are focused on the safety of foods including in the raw materials, the technology of processes as well as the storage of food products.

Keywords: Food, technology, safety, mycotoxin

IS-2

Mycotoxin Regulation in Thailand

Warapa Mahakarnchanakul
Faculty of Agro-Industry, Kasetsart University, Thailand
E-mail: fagiwpm@ku.ac.th

Abstract

Prevention and control of mycotoxins in food and agricultural products in Thailand are managed by three major agencies. Local food products including imported goods are regulated by the Food and Drug Administration under the Ministry of Health. Department of Medical Sciences will be in charge of mycotoxins analysis in suspected foods. According to Thai Food Act or law (1979) if total amount of aflatoxins exceed the limit at 20 ppb, food will be classified as contaminated food. While agricultural products focusing on fruit and vegetables, either domestic and for export, fresh or processed, will be controlled by the Ministry of Agriculture and Cooperative. Laboratory Central Thai company which is the certified Laboratory under this ministry will be in charge of health certification for exporting. Bureau of Food and Agricultural Commodity and Food Standards (ACFS) also set voluntary standard for risky product as peanut in order to encourage industry to implement. While mandatory standard on pet foods and animal feeds are regulated by the Department of Livestock and certified by the Bureau of Quality Control of Livestock. The last agency involved with Thai community products standard, so called one tumbon one product (OTOP) voluntary standards, these are set by Ministry of Industry in order to support the development of quality and safety of food community products. In this present in term of national food law only amount of total aflatoxin is limited in food for human consume, however many mycotoxins in foods and raw materials are determined and monitored upon the requirement of each buyers or customers. Although many potentially contaminated foods have not been regulated such as coffee, wine, wheat and corn derived food, but setting of mycotoxins limit for regulate and control safety in food, based on Thai food safety risk assessment studies, to establish national standards is in progress.

Keywords: Thailand, food law and regulation, mycotoxins, one tumbon one product, food safety

IS-3

Are All Fungal Metabolites Toxic?

Ulf Thrane
DTU Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark
E-mail: ut@bio.dtu.dk

Abstract

Filamentous fungi have a significant impact on human life as spoilers of food and feed by degradation and toxin production. Mycotoxins and other exo-metabolites are part of the exo-metabolome in filamentous fungi, which comprises more than 30,000 known metabolites. Are they all toxic or otherwise undesirable? No! Filamentous fungi are well known for their production of many biotechnological products such as enzymes, and primary and secondary metabolites being organic acids, pigments, fragrances, pharmaceuticals, polyunsaturated fatty acids and many more. Many of these compounds are used as food ingredients; however, despite the huge chemical diversity among species of filamentous fungi only a few species are used by industry as cell factories. In some cases the fungus in itself is used as food or as part of fermented food. A careful chemical profiling of the exo-metabolome at species level is an important part of the phenotypic characterization of fungi. This has resulted in a palette of fungal strains from several species producing many different food ingredients. In addition to the useful compounds, many fungal species, including known production strains used in biotech industry, also produce undesired compounds such as mycotoxins; however, through the chemotaxonomy it is possible to select fungal strains with no known production of mycotoxins. All together, a multidisciplinary approach in fungal systematics with focus on the exo-metabolome and incorporation of information on the origin of the fungal cultures, their cultural and physiological characteristics as well as the genotypic information gives a complete picture of the organisms with the very best opportunities to explore and exploit the fungi as safe cell factories of the future.

IS-4

Mycotoxin and Exometaboities in Foods

Jens C. Frisvad
Department of Systems Biology, Technical University of Denmark, Søltofts Plads B 221, 2800 Kongens Lyngby, Denmark
E-mail: jcf@bio.dtu.dk

Abstract

Profiles of extrolites are highly species specific and fungal species are specifically associated to types of food, and therefore extrolite production can be qualitatively predicted, and pave the way for the optimal analytical techniques to use when analyzing foods chemically. All species in the major toxigenic genera produce a significant number of families of exometabolites (Ems) and it is possible that some of these EMs act synergistically or show the "Gulliver effect", i.e. that they may be less toxic alone, but would give a toxic response if ingested at the same time. Some of these extrolites are toxic to vertebrates (mycotoxins), other have bioactivities that may influence human and animal health in alternative ways. The most important mycotoxins are in general aflatoxins, fumonisins, ochratoxins, patulin, trichothecenes, zearalenone, sterigmatocystin, 3-nitropropionic acid, cyclopiazonic acid, penitrem A, verrucosidin and penicillic acid. However other extrolites may be important. Penicillin, produced by *Penicillium rubens, P. chrysogenum*, *P. nalgiovense* and *P. griseofulvum*, may be produced in foods and may make a contribution to penicillin resistance in bacteria. Mycophenolic acid, produced by the common food-borne fungi *P. brevicompactum*, *P. bialowiezense*, *P. roqueforti* and *P. carneum*, is a very efficient immune-system inhibitor, and thus pave the way for bacterial infections if accumulated in foods. Compactin, produced by *P. solitum* is a very effective cholesterol-lowering compound. These may be seen as positive contributions to healthy foods, but they are not under medical control, as they would be if prescribed by a general practitioner. All these extrolites can be produced on standard media such as Czapek yeast autolysate (CYA) agar and yeast extract sucrose (YES) agar, and these mycotoxins are often also produced on the foods the fungi are associated with. However mycotoxin/extrolite production on CYA and YES is only qualitatively indicative for what could be produced on foods, so examples will be given on how to optimally analyze for mycotoxins in pure culture and in foods using combinations of UHPLC-DAD-fluorescence, UHPLC-triple Quad MS and UHPLC-QTOF.

IS-5

Mycotoxin Regulations, Sampling Issues – The Global Context

Naresh Magan
Cranfield University, Applied Mycology Group, Cranfield, Bedford MK43 0AL, U.K
E-mail: n.magan@cranfield.ac.uk

Abstract

One of the key drivers of research on mycotoxins is the strict legislation which exists in different regions of the world, especially the EU. Indeed, in the EU, the RASFF system at its borders shows that 30% of commodities are rejected because of contamination with mycotoxins (e.g., cereals, nuts, spices, juices, dried fruits). Unfortunately, while the EU has the strictest limits world-wide, this is not the same in other regions of the world. I will show that relative types of legislation in different continents and discuss these issues. The other key problem area is with regard to taking representative samples. The EU has specific sampling plans for different types of commodities and this must be implemented for importing commodities into the EU. Because mycotoxins may be spatially present in grain in pockets or uniformly it is difficult to obtain a true representative sample. This paper will discuss representative sampling issues and where errors occur in the process and some of the problems associated with sampling.

IS-6

Biocontrol of mycotoxins - Strategies and Obtacles

Su-Lin Leong
Dept of Microbiology, Swedish University of Agricultural Sciences, Uppsala, Sweden
E-mail: -

Abstract

Micro-organisms have the potential to prevent or ameliorate the effects of mycotoxins, by acting at multiple stages of the food or feed chain, e.g. by preventing mycotoxin formation in the field or during storage, or by binding or degrading toxins during processing or even in the gut. Yet, despite hundreds of scientific publications on the topic, and much continuing research activity, few products are in commercial use. The drivers, regulatory aspects, and commercialization models for uptake of biocontrol agents to control mycotoxins will be discussed, using as examples the Afla-guard® (USA) / Aflasafe™ system (Nigeria, Kenya, and other African countries), and biopreservation of moist-stored cereals using the yeast *Wickerhamomyces anomalus* (Sweden, Cameroon). At the end of the session, participants are invited to give a snapshot of the Asian situation regarding implemention of biocontrol for mycotoxins.

IS-7

Good Sanitation Practices (GSP) and Environtment Monitoring Program (EMP) to Prevent Pathogen Contamination and Mold Spoilage of Ready to Eat (RTE) Foods

Emilia Rico-Munoz
BCN Research Laboratories, Inc., 2491 Stock Creek Blvd., Rockford, TN 37853, U.S.A.
E-mail: emilia.rico@bcnlabs.com

Abstract

Good Sanitation Practices (GSP) and an effective Environmental Monitoring Program (EMP) are essential to prevent pathogen contamination of ready-to-eat (RTE) foods. They are also essential to prevent mold spoilage of these foods. A strict sanitation program and the use of the right sanitizer is necessary to keep food safe and to prevent spoilage. Molds spoiling foods can produce mycotoxins, thus becoming a food safety issue. Environmental monitoring is an evaluation of the effectiveness of the microbial controls (pathogens and spoilage organisms) to prevent contamination of food products. It is not only a validation of the sanitation program, but an evaluation of multiple programs, including but not limited to sanitary design, personnel practices, and operational methods among others. The best practice is to use the four-zone system when determining what areas to take samples from. The four-zone system begins at the product-contact surfaces and extends to areas outside of rooms in which product is exposed. When monitoring for pathogens, the least amount or no testing should be done in Zone 1. If pathogens are found in Zone 1, it is likely a recall situation and it is too late. When monitoring for spoilage microorganisms, most of the sampling takes place in Zone 1. Establishing GSP and an effective EMP will be discussed in this presentation.

IS-8

Application of Molecular Biological Methods for Detection of Mycotoxin Producing Fungi in Food

Ludwig Niessen
Technische Universität München, Lehrstuhl für Technische Mikrobiologie, Gregor-Mendel-Straße 4, 85354 Freising, Germany
E-mail: niessen@wzw.tum.de

Abstract

Molecular biology provides interesting new tools for the specific, sensitive and rapid detection and identification of microorganisms in pure cultures and in food sources as an alternative to classical microbiological analysis. The polymerase chain reaction (PCR) can be used to specifically amplify the DNA sequence positioned between two primer binding sites in the target DNA. Amplification events can be analyzed using agarose gel electrophoresis. Using intercalating fluorescent dyes or fluorescently labelled probes, the signal can be quantified to determine specifically the concentration of target DNA in a sample. PCR has been applied to the detection and identification of a wide range of fungi, among which mycotoxin producers such as *Aspergillus carbonarius* (Ochratoxin A) or *Aspergillus flavus* (Aflatoxin B1) are the most important for food safety. Loop-mediated isothermal amplification (LAMP) has been developed as an alternative technology for DNA amplification. It is operated at constant temperature of 65 °C and can therefore be applied with low level equipment making it particularly useful for on site applications in medical settings, in agricultural and quarantine testing or in the food processing industry. The application of LAMP-based assays will be exemplified through applications for the diagnosis of fungal species producing aflatoxins as well as for trichothecene producers which can be detected in a group specific manner.

Mycotoxigenic Fungi and Mycotoxins in Corn

Giancarlo Perrone
Institute of Sciences of Food Production - CNR, Bari – Italy
E-mail: giancarlo.perrone@ispa.cnr.it

Abstract

Corn is the world's third most important crop after rice and wheat; about half is grown in developing countries where it is important food for human consumption. In industrialized countries, corn is largely used as livestock feed and as raw material for industrial products (feed and food). Respect to mycotoxins and their producing fungi occurring on corn a lot of studies have evidenced that all the principal mycotoxigenic genus, mainly *Aspergillus* and *Fusarium*, but subsequently *Penicillium* and *Alternaria* can occur and contaminate maize with a different profile of mycotoxins. The most important are mycotoxins contaminating corn are aflatoxins and fumonisins. Aflatoxins are highly toxic and the most potent carcinogenic mycotoxins, they are produced in corn mainly by different morphotype and chemotypes of *Aspergillus flavus* group; rarely other aflatoxigenic species like *A. parasiticus*, *A. nomius* etc. are reported as potential aflatoxigenic species on corn. Fumonisins are strong hepatotoxic and nephrotoxic mycotoxins, classified as possible carcinogenic to human; and widely contaminating corn. They are produced by *Fusarium* species causal agents of the "pink-ear rot" of maize (*F. verticillioides* and *F. proliferatum*); recently their presence has been also associated to occurrence in corn of species belonging to *Aspergillus niger* group. Other mycotoxins contaminating corn at less extent are moniliformin, zearalenones and type-B trichothecenes produced by the *Fusarium* species responsible of "red ear rot" of maize. A brief overview on the occurrence, biodiversity, ecology and their toxigenic potential is here presented.

IS-10

Ecology of Mycotoxigenic Fungi and Possible Prevention Strategies

Naresh Magan
Cranfield University, Applied Mycology Group, Cranfield, Bedford MK43 0AL, U.K
E-mail: n.magan@cranfield.ac.uk

Abstract

The ecology of specific mycotoxigenic fungi in relation to boundary conditions for growth and mycotoxin production shows that water activity x temperature ranges for growth are wider than those for mycotoxin production. This information is very important in understanding the relative risk of contamination of staple commodities with mycotoxin in each specific food chain. This also helps where to target the prevention of control strategies in the food chains. This lecture will discuss the production flow of key commodities (cereals, coffee, groundnuts, grapes/dried vine fruits) and discuss the key components which need to be controlled to minimise mycotoxin production and contamination.

IS-11

CEMycoS: Current, Prospect of Research & Community Outreach

FMC Sigit Setyabudi, Sri Raharjo, Endang S. Rahayu, Chusnul Hidayat, and Suparmo
Faculty of Agricultural Technology, Universitas Gadjah Mada, Indonesia
E-mail: francis.setyabudi@ugm.ac.id

Abstract

A research group in the Faculty of Agricultural Technology (FAT) namely Center Excellence on Mycotoxins Studies (CEMycoS) has been established in 2011. Nevertheless, the research and community service on mycotoxins subject were already initiated by Department of Food and Agricultural Technology - FAT within continuous collaboration of national and abroad institution partners since year of 2000's. During 14 years, this research group has mostly studied on the occurrence of mycotoxins in agricultural product supply chain from farmers to users and their fate during post harvest and processing of the corresponding derived products. The estimation of mycotoxin ingestion in corn-based staple food and tracing in human urine samples were done. Several efforts of toxin decontamination under physical, mild chemical and biological treatments were also studied in traditional food process and indigenous fermented products. Studies of a non-destructive toxin detecton method under vision instrument and support material for solid phase extraction using immunoassay were also developed for mycotoxins analysis. Moreover, the mycological studies have been performed to isolate the fungi producing toxins from agricultural and crop estate as well as their derived products. The identification of fungi producing toxins were also conducted by morphologycal and bio-molecular methods.

This research group is also focused in community outreach through development of academician, business, government and community network. Those stakeholders hold important role in the dissemination and application of science and technology in the context of sustainable community empowerment. CEMycoS observes that elaboration among researches and community service should be a backbone for the future activities. The outcome of research activities will emerge when CEMycoS provide research outputs to community with problem solving and benefit for community in term of knowledge-based development.

Since 2011, CEMycoS Laboratory have initiated inter-laboratory networks dealing with mycotoxins analysis in national level. CEMycoS already developed mycotoxins testing laboratory and achieved ISO 17025 certification for aflatoxin analysis in maize and maize-based products in 2013. Currently, we already extend scope of analysis to other mycotoxins. CEMycoS opens opportunity to collaborate with quarantine laboratory around country in strengthening their capacity in SPS contaminants analysis, especially for mycotoxin analysis.

IS-12

Identification of Foodborne Yeasts and Moulds: A guide for Users

Su-Lin Leong
Swedish University of Agricultural Sciences, Sweden
E-mail: -

Abstract

The stages for polyphasic identification of foodborne fungi will be described, from isolation, purification of cultures, selection of media and incubation conditions for morphological identification, DNA extraction, selection of primers for sequencing, sequence matching in databases, and finally, weighing up molecular and morphological results to come to a final conclusion. Useful tips and potential pitfalls at the various stages will be pointed out. The workflow will also be framed in the context of how to train students in these techniques.

IS-13

How Do We Secure Correct Identification of Mycotoxins and The Fungi Which Produce Them?

Jens C. Frisvad
Department of Systems Biology, Technical University of Denmark, Søltofts Plads B 221, 2800 Kongens Lyngby, Denmark
E-mail: jcf@bio.dtu.dk

Abstract

Mycotoxins are fungal exometabolites that are toxic to vertebrates when introduced in small amounts via a natural route (via the mouth, via the lungs or via the skin). Analytical methods for mycotoxins have improved considerably, but both effective separation from the matrix and other fungal exometabolites and identification via spectrometric methods are important to secure correct qualification and quantification. The analytical chemical methods used are often UHPLC coupled with diode array detection (DAD) or high resolution mass spectrometric (HR-MS-MS) detection. In general it is recommended to verify the identification of the mycotoxin with at least three different methods, for example correct retention time as compared to an authentic standard, a correct UV spectrum and a correct mass spectrum (preferably MS-MS). However some fluorescing mycotoxins can be determined by quite simple methods, such as thin layer chromatography, but this simple and inexpensive method can give false positive and negative results, so HPLC-DAD or HPLC-MS verification is strongly recommended. Certain additional data may help verifying the identification of an important mycotoxin: Foods have an associated mycobiota, and thus the presence of a mycotoxin producer in a certain food commodity increases the risk of one of its mycotoxins being produced. Mycological analysis of the food product is therefore a guide to the mycotoxins that may be present and should be analyzed. This does require correct identification of the fungi in the foods and appropriate methods for the isolation of the food-borne fungi. In some groups of fungi, identification is difficult, and polyphasic identification is recommended: The use of micro- and macromorphology, ecophysiology, extrolite profiles and sequences of appropriate house-hold genes will secure a correct identification (β-tubulin, calmodulin, trans elongation factor 1α, RPB2). DNA sequences should be used for a BLAST search in the part- database RefSeq, whereas a search in general in GenBank is problematic, and can often give misleading results. Furthermore ITS sequences are often not sufficient for identification at the species level, but may be used as a first bar-code attempt at superficial identification. Examples will be given on how to identify important mycotoxigenic fungi and their mycotoxins, with an emphasis on *Penicillium*, *Aspergillus* and *Talaromyces*.

IS-14

Detection of Mycotoxins Using Affinity-Based Technologies

Ludwig Niessen
Technische Universität München, Lehrstuhl für Technische Mikrobiologie, Gregor-Mendel-Straße 4, 85354 Freising, Germany
E-mail: niessen@wzw.tum.de

Abstract

Analysis of mycotoxins and other small molecules is routinely based on chemical detection and identification using high tech lab equipment, e.g. HPLC, GC, TLC. As an alternative, a variety of affinity based assay formats have been developed to speed up and simplify detection. Such assays make use of the specific binding between an analyte molecule and a receptor molecule. Recently the detection of new affinity molecules and mechanisms has opened attractive opportunities for the development of new and highly sensitive formats for assays and sample cleanup that have also been applied to the analysis of mycotoxins. During the presentation different affinity based approaches to the analysis of mycotoxins will be exemplified: Application of classical antibody based assays, nucleic acid based affinity assays, and the application of technologies based on chemical affinity will be elucidated. Focus will be set on immunoassay formats based on biosensors as well as on very recently developed aptameric assays for mycotoxin detection.

Fusarium Toxins

Ulf Thrane
DTU Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark
E-mail: ut@bio.dtu.dk

Abstract

Fusarium species are very well known for their ability to produce biologically active metabolites including mycotoxins, such as the trichothecenes, zearalenones, fumonisins, moniliformin, and beauvericins and other cyclic peptides. The metabolite production is highly influenced by the growth conditions and this information is of high value to feed and food safety, as mycotoxins are unwanted in agricultural crops. The available information on mycotoxins is overwhelming. A search for scientific peer-reviewed papers using the keywords "*Fusarium* and mycotoxin" retrieve >350 papers published in 2013! This equals one scientific *Fusarium*-mycotoxin paper every single day. In addition, these search engines do not cover all journals and books, patents, congress proceedings and technical reports! Unfortunately the quality of the information is variable and in this context both the identification of mycotoxin and the producing organisms are the weak points. It has been demonstrated that *Fusarium* mycotoxins are chemically altered by growing plants in the field as part of plants' defence against fungal invasion and harmful xenobiotics. Plants often detoxify mycotoxins by forming a conjugation between the toxins and one or more sugar molecules. The occurring metabolites are less or not toxic and are sometimes referred to as "masked mycotoxins", which partly may explain the mode of action of plant resistance. The masked mycotoxins are transferred into food and it is of concern that thehydrolysis of these metabolites back to their toxic parents seems to occur during mammalian digestion. The result can easily be that the total mycotoxin load of consumers is underestimated. Another concern is new mycotoxin-commodity combinations due to climate changes and the increasing worldwide trading of agricultural seeds and crops that together will impact the mycobiota. Long-term surveys have provided evidence that *Fusarium* has a great plasticity and capability to continuously select new genotypes demonstrating higher aggressiveness and mycotoxin production. Today more and more genes coding for biosynthetic pathways of non-household products are sequenced that add to the increasing information on the genetics behind metabolite productions. However, a whole-hearted integration of fungal phenetics, ecology, and genomics, as well as critical evaluation of the exceptional amount of metabolite data being generated is crucial to ensure safe fungal products without *Fusarium* toxins.

IS-16

Molds Isolated From the Processing Environment and Their Significance in Spoilage of Heat-processed Beverages and Juices

Emilia Rico-Munoz
BCN Research Laboratories, Inc., 2491 Stock Creek Blvd., Rockford, TN 37853, U.S.A.
E-mail: emilia.rico@bcnlabs.com

Abstract

Heat-processed beverages and juices can be spoiled by heat-resistant fungi (HRM) and spore forming bacteria. They can also be environmentally contaminated by a variety of non-heat resistant microorganisms such as *Fusarium oxyporum, Exophiala* sp., *Cladosporium* sp., *Aureobasidium pullulans*, yeast, lactic acid bacteria (LAB) and acetic acid bacteria (AAB) at the filler, capper, cooling tunnel and other areas of the processing environment. Heat-resistant molds produce ascospores that can not only survive the heat treatment given to these beverages and juices, but can also be activated and grow during storage. It is known that HRM ascospores are found in ingredients and in empty PET bottles. There is not much information on the role that the processing environment plays on the spoilage of these beverages and juices. This study was undertaken to determine the extent of the contamination by HRM ascospores of the beverage processing environment. More than 2,000 environmental samples were taken in 15 beverage/juice processing plants and tested for HRM ascospores. The areas with the highest counts of ascospores were the palletizer area, fork lifts/pallet jacks, pallets and the stretch wrap around the empty bottle pallets. Ascospores were also found at the depalletizers, slip sheets in between layers of empty bottles, the airveyors, the fillers, the batching areas, cooling tunnel areas, conveyors, rinsers and even at the cap tracks. The most common HRM isolated was *Byssochlamys spectabilis* followed by *Neosartorya fischeri*. Ascospores of *Neosartorya spp., Byssochlamys spp., Talaromyces spp., Hamigera avellanea, Thermoascus spp., Humicula fuscroata* and *Eurotium spp.* were also isolated. The significance of these molds in the spoilage of heat-processed beverages and juices and their safety will be discussed in this presentation.

IS-17

Traditional Fermented Foods and Their Safety

Endang S. Rahayu[1*)], Susana Ristiarini[2)], and Tyas Utami[1)]
1)Faculty of Agricultural Technology, Universitas Gadjah Mada
2)Faculty of Food Technology, Widya Mandala Surabaya Catholic University
*E-mail: endangsrahayu@yahoo.com

Abstract

Fermentation of foods have been done centuries ago and considered as simple method for preservation. However, since organoleptic properties and nutritional value of the products are enhanced, as well as, functionality, fermentation foods remain developed in line with the development of science and technology. In developing countries, fermentation of foods are carried out by small scale (home) industry using conventional method, spontaneous (without addition of starter cultures), simple, based on local materials. Traditional fermentation has several disadvantages, such as, quality is inconsistent and safety is not assured. There are at least three causes why traditional fermented food become unsafe. (1) Raw material contain hazard material that still exist in the product; (2) Contamination by pathogenic bacteria during processing; (3) Toxin produced by important microorganisms during fermentation. Many fermented foods are produced using molds (i.e., tempeh, oncom, soy-sauce, angkak), therefore, the risk of mycotoxin contamination should be considered. There are several questions to be discussed in this paper. Why is tempeh produced by fermentation using *Rhizopus oligosporus* and other *Rhizopus* claimed as safe food? Why are *Aspergillus oryzae* and *A. sojae* the closed related species with the well-known aflatoxin producers *A. flavus* and *A. parasiticus* considered as safe when used for koji making? *Aspergillus oryzae* and *A. sojae* were believed to be domesticated strains of *A. oryzae* and *A. sojae*, respectively and capability in synthesizing of aflatoxin loss in domesticated strains. On the other hand, why was citrinine still detected in angkak (rice fermented product based on *Monascus purpureus*)? In this paper, traditional fermented foods based on molds and their safety will be presented.

Keywords: Traditional fermented foods, safety

Black Aspergilli and Their Mycotoxin Production

Giancarlo Perrone
Institute of Sciences of Food Production - CNR, Bari – Italy
E-mail: giancarlo.perrone@ispa.cnr.it

Abstract

Black Aspergilli, which comprise 27 accepted species belonging to *Aspergillus* Sect. *Nigri*, are spread worldwide with significant impact on food and feed both for beneficial and harmful effects. Many species cause food spoilage, and several are used in the fermentation industry, or candidate in the biotechnology industries. However, this group of fungi represent one of the most important source of mycotoxins contamination of foods and feeds. Major mycotoxins produced by this group of filamentous fungi are ochratoxin A (OTA) and fumonisins of the B series, in particular FB_2. OTA is a potent nephrotoxic and carcinogenic toxin produced by different species belonging to the genus *Aspergillus* and *Penicillium*, in particular within black aspergilli the OTA producing species are *A. carbonarius*, *A. sclerotioniger*, and a low percentage of *A. niger*, *A. welwitschiae* and, *A. lacticoffeatus*. Fumonisins are carcinogenic mycotoxins originally produced by *Fusarium* species (*F. verticillioides* and *F. proliferatum*) and recently (2007) identified in cultures of *A. niger* group. Only the two closely related phylogenetic species of *A. niger* and, *A. welwitschiae* out of the 11 species of the *A. niger* "aggregate" complex are able to produce fumonisins. A brief overview on the occurrence, biodiversity, ecology and their toxigenic potential is here presented.

IS-19

Mycotoxin Research and Food Safety in Malaysia

Latiffah Zakaria
School of Biological Sciences, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia
E-mail: lfah@usm.my

Abstract

Research on mycotoxin in Malaysia started in 1960s which was mainly focussed on aflatoxins contamination due to disease outbreak of pig farms caused by contaminated feed. Another hazard of aflatoxins contamination was around 1988 of which contaminated flour used to make noodles caused death of 13 children. Since then, studies on aflatoxins and other mycotoxins have been conducted extensively especially on food and feed commodities. In Malaysia, research on mycotoxin are carried out by research institutions such as Institute of Medical Research and Malaysian Agricultural Research Development Institute, and several local universities. Important mycotoxins that have been reported in different types of food and feed are aflatoxins (AF), ochratoxin A (OTA), fumonisins (FUMs), deoxynivalenol (DON) and zearalenon (ZEN). Among the food and feed commodities analyzed were cereal grains such as rice, corn and barley, peanuts and peanuts products, spices and herbal medicines. Due to high temperatures (27 – 31^{o}C) dan high relative humidity (70 – 90%), food and feed commodities are easily contaminated by mycotoxin producing fungi if the commodities are not properly stored. Contamination can also occurred by improper processing techniques. Food safety issues in Malaysia are handled by Food Safety and Quality Division, Ministry of Health which deal all matters relating to food safety and nutrition as well as strengthen the efforts of all agencies involved in food safety

IS-20

Climate Change, Food Security and Mycotoxins – Do We Know Enough?

Naresh Magan
Cranfield University, Applied Mycology Group, Cranfield, Bedford MK43 0AL, U.K
E-mail: n.magan@cranfield.ac.uk

Abstract

There is interest in how the changes in climate change will affect mycotoxigenic fungi and mycotoxin contamination of staple foods. It is known that changes in water availability drought stress, temperature (+2-4^{o}C) and 2x or 3x existing CO_2 (700, 900 ppm) will occur in different regions of the world. There is some data which suggests that pest reproduction will increase damaging crops leading to more fungal infections and perhaps mycotoxin contamination. This lecture will cover the key aspects of these parameters, discuss the predictive modelling aspects of mycotoxin contamination of staple crops. The most recent data which we have done on the effect of interacting climate change factors on growth and aflatoxin production by *A. flavus*, and the effect on *A. carbonarius*, *A. niger* and *A. westerdijkiae* in relation to growth and ochratoxin will also be presented. The potential impact of climate change on food security could be significant and this will be discussed.

Indonesian Food Safety: Regulation and Challenge for Mycotoxins

Roy Sparringa
National Agency for Drug and Food Control Republic of Indonesia
E-mail: roy.sparringa@pom.go.id, sparringa@gmail.com

Abstract

Everyone has the right to access a safe, quality and nutritious food for the health and well-being of himself and of his family. Mycotoxin is one of major contributors for foodborne disease, which cause significance loss not only in agriculture area, but also public health sector. Approximately 25% of world food crops, mainly in developing countries, affected by the mycotoxins each year. Aflatoxins, one of mycotoxins, are estimated to cause 4.6-28.2% of total annual hepatocellular carcinoma (HCC) cases worldwide. Regulation and standard of mycotoxins are adopted to limit consumers' exposure to the mycotoxins. The permitted maximum levels as Food Safety Objective (FSO) of mycotoxins vary greatly among countries which are influenced by various factors, such as level of exposure, sociological, political and economic factors. Risk-based regulation on mycotoxins control along the food chain is of importance to assure the acceptable risk to the consumers' health. However to achieve the FSO is a great challenge. One of the major challenges to control mycotoxins is a fact that Indonesia lies in equator line where the climate is favorable for the growth of mycotoxin producing fungi. Several assessments showed high aflatoxin B1 (AFB1), mainly in peanut and corn along food chain. A case study of risk assessment of aflatoxin (AFB1) in peanut and corn and their products using the consumption data of the National Socio-Economic Survey (2011) and the AFB1 concentration of peanut and corn and its respective products reported by The National Agency of Drug and Food Control (2014) showed that the AFB1 dietary exposure mean was about 38.32 ng AFB1/kg bw/day, and estimated of HCC in Indonesia was more than 3500 cases annually. This figure is likely to be lower estimate, because the consumption data of staple foods (e.g. rice) which may be attributable to AFB1 contamination is not considered in the calculation. It is recommended that mycotoxin control should be in a single national integrated food policy. Food safety practices must be implemented along food chain based on risk management program through partnership alignment including Public Private Partnership Programs.

Keywords: *mycotoxin regulation, aflatoxin B-1, risk assessment, estimated hepatocellular carcinoma*

ORAL SPEAKER'S ABSTRACTS

O-1.1

Toxigenic *Aspergillus flavus* Population Detected by Its Aflatoxin Genes in Peanut Kernel

***)Nampiah Sukarno, Sri Listiyowati and Kemala S. Nagur**
Biology Department, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Indonesia.
*E-mail: nampiahsukarno@yahoo.com

Abstract

Aspergillus flavus is one of main aflatoxin producer in peanut kernel in Indonesia. The aim of this research was to study toxigenic *A. flavus* population grown in peanut kernel in Bogor, Depok and Jakarta, Indonesia using morphological and molecular approaches. Samples were collected from traditional markets. Fungal isolation was carried out using AFPA specific medium, and identification was done by FVAVIQ1/FLAQ2 and AFLA-F/AFLA-R specific primers for *A. flavus.* Four pairs of primer were selected for detection of aflatoxin genes in the fungal isolates, namely *apa2, nor1, ver1* and *omt1*. Aflatoxin production was determined by observation of fluorescence under UV light on coconut agar medium. Populations of *A. flavus* obtained were 5.55 x 10^4, 2.1 x 10^4, and 0.01 x 10^4 CFU/gram for sample from Bogor, Depok and Jakarta, respectively. Total eighteen isolates of *A. flavus*, which consist of 12, 4 and 2 isolates were successfully isolated based on variation of bright yellowish orange intensity on AFPA medium from Bogor, Depok and Jakarta, respectively. All isolates carried *apa2* and *nor1* genes, whereas the presence of *ver1* and *omt1* was vary. The presence of *omt1*, despite of *apa2* and *nor1*, determined the expression of aflatoxin genes detected by UV light. All of isolates derived from samples collected in Jakarta were toxigenic, whereas toxigenic isolates from Depok and Bogor, were 50 and 92%, respectively.

Keywords: aflatoxin genes, Aspergillus flavus, peanut, population, molecular identification

O-1.2

Aspergillus flavus Infection and Aflatoxin Contamination in Stored Nutmeg (*Myristica fragrans*) at Various Stages of the Delivery Chain in North Sulawesi Province

Okky Setyawati Dharmaputra[1*)], Santi Ambarwati[2)], Ina Retnowati[2)], and Nijma Nurfadila[2)]
[1)]SEAMEO BIOTROP, Jalan Raya Tajur Km. 6, Bogor 16134, Indonesia; and Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Dramaga Campus,Bogor, Indonesia
[2)]SEAMEO BIOTROP, Jalan Raya Tajur Km. 6, Bogor 16134, Indonesia
*E-mail: okky@biotrop.org

Abstract

A survey to obtain information on postharvest handling of nutmeg and to investigate *Aspergillus flavus* infection and aflatoxin contamination of stored nutmeg were conducted at farmer, collector and exporter levels in North Sulawesi province in April and May 2013. The moisture contents and percentage of damaged kernels were also determined. Twenty five, 22 and 27 dry nutmeg samples were collected randomly from 14 farmers, 8 collectors and 4 exporters, respectively. Thus, a total of 74 dry nutmeg samples were collected. The moisture content of nutmeg (based on wet basis) was determined using distillation method. The percentage of damaged kernels were determined by weighing them and dividing the weight of working sample used for damaged kernel analyses. *Aspergillus flavus* was isolated using dilution method on Dichloran 18% Glycerol Agar (DG18). Aflatoxin contents were determined using HPLC post-column derivatisation method. The results showed, that in general the method of postharvest handling conducted by farmers and collectors were not appropriately. The moisture contents of nutmeg kernels collected from farmers, collectors and exporters were 9.98, 10.49 and 9.33%, respectively. The percentage of damaged kernels collected from farmers, collectors and exporters were 70.55, 76.7, and 69.66%, respectively. The percentage of samples infected by *A. flavus* collected from farmers, collectors and exporters were 40.0, 40.9 and 33.3%, respectively. The percentage of samples contaminated by total aflatoxin collected from farmers, collectors and exporters were 64.0, 45.5 and 66.7%, respectively. Total aflatoxin contents in nutmeg samples collected from farmers, collectors and exporters were 220.47, 4.73 and 1.18 ppb, respectively. Aflatoxin B_1 contents in nutmeg samples collected from farmers, collectors and exporters were 208.33, 4.18, and 0.80 ppb, respectively.

Keywords: aflatoxin, Aspergillus flavus, distribution chain, Myristica fragrans, nutmeg, North Sulawesi province

O-1.3

Airborne Fungi and Aflatoxin-Producing *Aspergillus flavus* Group on *Gaplek* Storage Warehouse in Gunung Kidul, Yogyakarta, Indonesia

Nova Wahyu Pratiwi[1)], Latifah Zakaria[2)], Purnama Darmadji[1)], Endang Sutriswati Rahayu[1*)]
[1)] Food Science and Technology Study Program, Faculty of Agricultural Technology,
[1*)]Universitas Gadjah Mada, Yogyakarta, Indonesia
[2)] Pusat Kajian Ilmu Kajihayat, Universiti Sains Malaysia, Malaysia
*E-mail: endangsrahayu@ugm.ac.id

Abstract

This study aimed to determine the diversity of airborne fungi in *gaplek* storage in Gunung Kidul, Yogyakarta and find out the presence of *Aspergillus flavus* aflatoxin-producing genes. Identification of fungi was performed by macroscopic, microscopic as well as molecular characteristics. In this study 109 isolates were obtained and based on the macroscopic and microscopic characteristic were identified as *Aspergillus flavus*, *A. niger*, *A. tamarii*, *A. versicolor, A. wentii, A. fumigatus, A. sydowii, Cladosporium marcocarpum*, *C. sphaerospermum*, *Eurotium herbariorum*, *Penicillium rugulosum*, *P. polonicum*, *P. griseofulvum*, *Talaromyces erythromellis*, and *Trichoderma viride*. Thirthy one isolates showed reverse orange-colored colonies on media *Aspergillus flavus-parasiticus* Agar (AFPA), and 26 among of them had the ability to produce aflatoxin, showed the blue fluorescense in Coconut Cream Agar (CCA). Based on the β-tubulin gene sequences, 19 isolates had 99% similarity to *A. flavus* and 16 among of them had *nor-1, aflR, and omtB* gene that played a role in producing aflatoxin. On the other hand, there were 3 isolates had no these genes which was correlated to the absence of blue fluorosence of *A. flavus* as it's observed in CCA. One of the dominant fungi found in indoor airborne of *gaplek* warehouse in Gunung Kidul, Yogyakarta, Indonesia was *A. flavus* which had aflatoxigenic properties and capability of producing aflatoxin.

Keywords: diversity, airborne fungi, Aspergillus flavus, aflatoxin, gaplek

O-1.4

Aflatoxin Content in Some Peanut (*Arachis hypogaea* L.) Post-Harvest Handling in Gunungkidul, DIY

Yeyen Prestyaning Wanita and Sri Wahyuni Budiarti
Yogyakarta Assessment Institute for Agricultural Technology, Yogyakarta, Indonesia
E-mail: yeyen_world@yahoo.com

Abstract

Gunungkidul is the largest producer of peanuts (*Arachis hypogaea* L.), in Yogyakarta. The problem was poor farmer's post-harvest handling, resulting in a reduction in quality, especially the increase of aflatoxin content. The research was carried out in January-December 2014. Research conducted at Sediyo Mulyo Farmers Group, Sogo Hamlet, Candirejo Village, Semanu, Gunungkidul and Post Harvest Laboratory and Agricultural Machinery, Yogyakarta IAIT. The experimental design used in this study is completely randomized design with two treatments and seven replications. The treatment used were farmer post-harvest handling peanut and the introduction. The difference of the two treatments were in the process of sorting, drying, and storage. The results showed that the peanuts improvement of post-harvest handling: 1) could suppress the aflatoxin content of the third month of storage, ie from 360 ppb to 20 ppb, making it safe for consumption. 2) improved the quality of peanuts into a quality II which is in accordance with SNI 01 3021 1995.

Keywords: peanut, post-harvest handling, farmer, the introduction, and the aflatoxin content

O-1.5

Molecular Identification of *Fusarium* Species from Maize Kernels in Several Maize Production Area in Central and East Java, Indonesia

Ani Widiastuti[1*)], Tri Joko[1)], and Kurnia Ritma Dhanti[2)]

[1)] Laboratory of Plant Health Clinic, Dept. of Plant Protection, Fac. of. Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia

[2)] Graduate School of Biotechnology, Universitas Gadjah Mada, Yogyakarta, Indonesia

*E-mail: aniwidiastuti@ugm.ac.id

Abstract

Fusarium spp. which infect maize in the field will continue its infection in the post-harvest period. Some *Fusarium* spp. produce mycotoxin, while different species of *Fusarium* can produce different toxin. The mycotoxins cause harmful effect on human and animal health. Research focused on *Fusarium* mycotoxin of maize is still limited in Indonesia. Therefore, this research aims to reveal the presence of *Fusarium* spp. from maize and identify them based on molecular analysis. Samples of maize were collected from several production areas in Central and East Java. *Fusarium* spp. were isolated and grown in potato dextrose agar (PDA) medium and molecular identification was conducted by PCR assay using species-specific primer of estimated *Fusarium* spp. species. The result showed that from twenty sample isolates, there were ten isolates *Fusarium verticilloides,* two isolates *F. proliferatum,* three isolates *F. graminearum* and five isolates were not identified yet. Those unidentified isolates are now being prepared for sequencing-based analysis.

Keywords: molecular identification, maize kernel, Fusarium verticilloides, F. proliferatum, F. graminearum.

O-1.6

Occurrence of Ochratoxin A in Cocoa Powder and Method Validation

Yunika Mayangsari[1*], Deni Pranowo[2] and Muhammad Khak[3]
[1),3)] Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Indonesia
[2)] Department of Chemistry, Faculty of Science, Universitas Gadjah Mada, Indonesia
*E-mail: yunikamayangsari@gmail.com

Abstract

The purpose of this research was to determine the occurrence of Ochratoxin A (OTA) in cocoa powder which are marketed in Indonesia and also to perform a single laboratory validation for the analysis of OTA. Spiked samples with levels from 10.00 to 50.00 µg/kg for cocoa powder had an average recovery rate of 86.67%. The limits of detection and quantification in cocoa powder were 0.16 µg/kg and 0.54 µg/kg respectively. A good correlation (r = 0.9987) was found for this method. Three Indonesian markets of cocoa powder products were investigated to determine the presence of OTA, which was extracted by Ochraprep® immunoaffinity columns for cleaning up and analysed by high performance liquid chromatography (HPLC). The results showed that allof the cocoa powder products were contaminated with OTA at different levels and the average level of OTA contamination was 3.08 µg/kg.

Keywords: Ochratoxin A, cocoa powder, method-validation

O-1.7

Identification of Potatoes-Contaminating Fungi in Traditional Market of Condong Catur, District of Sleman, Yogyakarta

[*)]Fitri Nadifah, Yuliana Prasetyaningsih and Sri Rahayu
Study Program Diploma of Health Analyst, Health Science College Guna Bangsa, Yogyakarta
*E-mail: fitri.nadifah@gmail.com

Abstract

Potato (*Solanum tuberosum L*.) is one of the five basic sources of carbohydrates. It consumed by many people in the world. One of the constraints in potato production is the presence of fungal diseases. Fungi that cause diseases in potato crops include *Phytophthora infestans* which causes late blight, *Fusarium oxysporum* which cause fusarium wilt, *Alternaria solani* Sor. Which caused brown spot disease, and *Aspergillus niger* which infect bulbs and produce aflatoxin. Identification of potatoes-contaminating fungi can lead the farmers to get a better potatoes production. This research goal was to identify potatoes-contaminating fungi in Traditional Market of Condong Catur, District of Sleman, Yogyakarta. This research used descriptive method with laboratory examination. We took 30 defected potatoes which was suspected of being infected by fungi. Samples were taken from each potato aseptically and then cultured in Saboraud's Dextrose Agar (SDA) media. Fungal identification was held after 24 hours incubation. Based on the laboratory examination, there were fungal infections on all potatoes. These were identified as *Phytopthora infestans* (26.67%), *Fusarium oxysporum* (86.67%), *Alternaria solani* Sor. (6.67%), and *Aspergillus niger* (13.33%). *Phytophthora infestans*, *Fusarium oxysporum*, *Alternaria solani* Sor., and *Aspergillus niger* were identified as potatoes-contaminating fungi in Traditional Market of Condong Catur, District of Sleman, Yogyakarta.

Keywords: fungi, potatoes, contaminant

O-1.8

Mould, Bacteria and Heavy Metals Contamination in Ground Coffee

Vita Meylani[1*)] and Harsojo[2)]
[1)]Departement of Biology Education FKIP Siliwangi University, Tasikmalaya, Indonesia
[2)] Center of Isotop and Radiation Application, BATAN, Jakarta, Indonesia
*E-mail: meylani.vita@yahoo.co.id

Abstract

Coffee is one of many beverages favored by almost everyone around the world and coffee is also one of the main of export commodities beside non-oil and gas foreign exchange.The ground coffee has possibility of being contaminated by molds and bacteria as well as containing heavy metals that has met the threshold standards. The puspose of this research were to study the mould, bacteria contamination and also the content of heavy metals in ground coffee. Parameters used in this study were the total amount of mould, aerobic bacteria and coliform bacteria, while the heavy metals measured were Pb and Cu. The total number of mould and bacteria were analyzed using Total Plate Count while heavy metals using Atomic Absorption Spectrophotometri. The results showed that the total number of mould and aerobic bacteria were still less than recommended value from National Standardization Agency. No coliform bacteria were found in all samples observed. The Pb and Cu metals content were still less than recommended value from National Standardization Agency.

Keywords: mould, bacteria, heavy metal, ground coffee, AAS

O-1.9

Evaluation of Reduction *Fumonisin* Contamination in Corn in the Stages of Making *Sekelan* That Soaked With Lime Water and Lactic Acid Bacteria

Heru Susanto[1)], FMC Sigit Setyabudi[1)], Warapa Mahakarnchanakul[2)] and Sardjono[1*)]

[1)]Department Of Food And Agricultural Product Technology , Faculty Of Agricultural Technology, Universitas Gadjah Mada, Indonesia

[2)]Department Of Food Science And Technology, Faculty Of Agro-Industry, Kasetsart University-Thailand

*E-mail: sardjon@yahoo.com

Abstract

Corn as the main ingredient in the making *sekelan* (alternative food) in its storage are susceptible to mycotoxin contamination which reducing the corn quality. Fumonisin is one of mycotoxins that could potentially cause disease in humans and animals. Fumonisin produced mainly by *Fusarium verticilloides, Fusarium proliferatum* and other genera were often found in corn. The recommended maximum levels for fumonisins in human foods and in animal feeds regulated by Food and Drug Administration (FDA). For human foods, the maximum levels for fumonisins are 2-4 ppm. This study aims to determine the level of fumonisin contamination in corn as an ingredient *sekelan* and reduction at every stage of making *sekelan* marinated with lime water and lactic acid bacteria. Corn was inoculated with *Fusarium verticiloides* then made *sekelan* with three soaking treatments using water, with lime water and lactic acid bacteria (*Lactobacillus plantarum*). The content of fumonisin in each stage were analyzed using HPLC. The results showed that the milling process was able to reduce the contamination of fumonisin B1 from 14.47 μg/g to 0.69 μg/g and the largest part of fumonisin B1 content was in the skin and corn bran. In the milling process, followed by soaking with water, lime water, and lactic acid bacteria decreased fumonisin B1 97.72%, 100% and 98.67%, respectively and was not found fumonisin B2.

Keywords: *corn, sekelan, Fusarium verticilloides, mycotoxins, fumonisin B1*

O-2.1

Anticandida Activities of Ethyl Acetate Extract, Fractions and Compounds from *Lactobacillus plantarum* IBL-2 Fermentation Product

Marlia Singgih Wibowo[1*)], Betty Nurhayati[1)], Tjokorde I. Armina Padmasawitri[1)], Aniendha D. Ramadhani[1)], Yantyati Widyastuti[2)] and Tutus Gusdinar[1)]

[1)]School of Pharmacy, Institut Teknologi Bandung, Indonesia

[2)]LIPI, Jl. Raya Bogor Km. 46-Cibinong, Indonesia

*E-mail: marlia@fa.itb.ac.id

Abstract

Candida albicans is one of the causes of opportunistic infection in human. The bio-therapeutic development from antimicrobial compounds of Lactic Acid Bacteria (LAB), such as *Lactobacillus plantarum,* is targeted to be one of the alternatives for effective and non-toxic candidiasis therapy. This study was aimed to identify the anticandida compounds derived from *L. plantarum* IBL-2. The method used in the production process was fermentation of *L. plantarum* IBL-2 under optimal condition. For further study, extract of acid and cell-free supernatant was prepared using liquid-liquid extraction and column vacuum chromatography. Anticandida compounds were identified from the hexane: EtOAc (4 : 6) fraction, utilizing GC-MS. Anticandida activity of the compounds was measured using agar diffusion and microdilution method. The optimum incubation period for anticandida compounds production from *L. plantarum* IBL-2 was 72 hours, which produce acid and cell-free supernatant with inhibitor zone diameter against *C. albicans* of 9 mm. Ethyl acetate fraction from the liquid-liquid extraction produced the highest anticandida activity, with inhibitor zone diameter of 14 mm. Column vacuum chromatography was resulted in anticandida compounds which produced inhibitor zone diameter of 17 mm using diffusion agar method and 16 titer using microdilution method. Utilizing the GC-MS, several organic acids from anticandida compounds were identified. Identification of anticandida compound derived from *L. plantarum* IBL-2 showed that the compound consisted of several organic acids such as propanoic acid, 2-hydroxycaproic acid, pentanoic acid 4-metil, pentanoic acid 3- metil, lactic acid dimer, benzene propanoic acid, 3-ureidopropionic acid, hexadecanoic acid, oleic acid,octadecanoic acid and 1,2 benzenedicarboxylic acid.

Keywords: lactic acid bacteria, anticandida, biotherapy

O-2.2

Low pH Enhances Rumen Absorption of Aflatoxin B1 and Ochratoxin A in Sheep

Dadik Pantaya[1),2)], Diego P Morgavi[1)], Mathieu Silberberg[1)], Cécile Martin[1)], Suryahadi[3)], Komang G Wiryawan[3)] and Hamid Boudra[1)]

[1)]INRA, UMR1213 Herbivores, F-63122 Saint Genès-Champanelle, France and Clermont Université, VetAgro Sup, UMR Herbivores, BP 10448, F-63000, Clermont-Ferrand, France

[2)]Department of Animal Science, State Polytechnic Jember, Jember, Indonesia

[3)]Faculty of Animal Science, Bogor Agricultural University, Bogor, Indonesia

*E-mail: -

Abstract

The objective of this study was to determine whether the ruminal disappearance rate of aflatoxin B1 (AFB1), ochratoxin A (OTA) and fumonisin B1 (FB1) is affected by acidic rumen pH conditions. Disappearance was measured using a temporally isolated rumen model. A buffered solution containing AFB1, OTA and FB1 at pH 5 or 7 was incubated for up to 2 h in the rumen of three adult rumen-cannulated sheep. The mean pH of the solution during the 2-h incubation in the rumen was 6.8±0.15 and 5.7±0.25 for the neutral and acid conditions, respectively. AFB1 and OTA were readily absorbed in the rumen, particularly at acid pH. The fractional disappearance rates at acid and neutral pH for AFB1 were, respectively, 1.98±0.52 and 1.42±0.57/h ($p<0.019$) and for OTA were 0.16±0.10 and 0.06±0.03/h ($p<0.058$). OTA disappearance from the rumen was followed by a concomitant increase of OTA concentration in plasma throughout the 2-h incubation. In contrast, FB1 was not absorbed in the rumen. In conclusion, acid pH in the rumen increases the absorption of AFB1 and OTA, potentially contributing to an exacerbated toxic risk.

Keywords: *ruminal acidosis, mycotoxins, ruminal absorption, sheep*

Within-Host Interactions between *Metarhizium anisopliae* and Two *Aspergillus spp.*: Evaluation of Constructive Implications on Biocontrol Strategies

Jessil Ann L. Pajar*), Joey Genevieve T. Martinez and Franco G. Teves
Department of Biological Sciences, College of Science and Mathematics,
Mindanao State University, Philippines
*E-mail: jessilann.pajar@g.msuiit.edu.ph

Abstract

The negative aspects of traditional pest control, which involve the use of harmful chemicals, have led to the scrutiny of alternative methods such as the utilization of non-hazardous biocontrol agents. *Brontispa longissima* is a noxious coconut pest, whose infestation has been responsible for the reduced fruit production and damage of more than three million coconut trees across the country. The pathogenic capabilities and within-host interactions of *Metarhizium anisopliae* var. *F52* (M.a) and two *Aspergillus* spp. isolates on *B. longissima* were evaluated. These *Aspergillus* spp., identified as representative of sections *Fumigati* (Asp01) and *Flavi* (Asp02) were previously assessed for aflatoxin production via cultural method. Asp01 was found to have pathogenic effects comparable to M.a, causing 52% and 57% mortality on adult beetles respectively, 48 hours after inoculation. Antagonistic and mutualistic relationships were observed in mixed infections, whereby a reduced mortality of adult beetles was observed when M.a was mixed with Asp01 (19%), while 57% mortality was recorded on the treatment with the virulent M.a mixed with avirulent Asp02. Surprisingly, surface mycosis on cadavers was predominantly consisted of the avirulent Asp02. In this study, these relationships were investigated in the light of its effect on *B. longissima* and to the coconut crop *per sé*, its implications on pest management were then deliberated. This serves as a preliminary investigation, which may pave the way towards exploitation of new and more efficient control strategy to specifically curb *B. longissima* infestation–the first study to report *Aspergillus* spp. infection on *B. longissima*.

Efficacy on Elimination of *Listeria spp., Salmonella spp*. and *Pseudomonas spp*. in Single and Mixed Biofilms by Hydrogen Peroxide Pre-Treatment and Cleaning Process

Bui Thi Quynh Hoa[1)], Warapa Mahakarnchanakul[1*)], Tanaboon Sajjaanantakul[1)], Vichien Kitpreechavanich[2)]

[1)]Department of Food Science and Technology, Agro-Industry Faculty, Kasetsart University, Thailand

[2)]Department of Microbiology, Faculty of Science, Kasetsart University, Thailand

*E-mail: fagiwpm@ku.ac.th

Abstract

Prevention and control of biofilm formation in food processing environment has remained a challenge for food industry in term of food safety. This study was designed to investigate the efficacy of hydrogen peroxide pre-treatment combination with the regular procedure using daily cleaning in shrimp plant. Single and mixed species biofilm of *Listeria spp., Salmonella spp*. and *Pseudomonas spp*. were used as the model. In laboratory single biofilm on stainless steel coupons (SS) were formed under nutrient stress and harvested at 3 days and 7 days to assess the 4 cleaning procedures. The result showed that single biofilms of *Listeria* and *Salmonella* were completely eliminated by using 2% alkaline detergent 10 min following with 2 type of QUAT based sanitizers. However this procedure could eliminate *Pseudomonas*, high potential biofilm formation, by 3-4 log reduction but 5 log reduction was obtained when replaced with acid detergent. Then mixed species biofilm study was done on 3 materials, stainless steel, Teflon and rubber. The condition was simulated as continuously 7 days process conditions. H_2O_2 concentration of 1 and 2% at 5 and 10 min as pre-treatments were subjected to mixed biofilm prior to the regular cleaning procedure. Hydrogen peroxide 2% as pre-treatment reduced population of bacteria by 6 log (CFU/cm^2). No significant different in pre-treatment with cleaning process between 5 min of 2% H_2O_2 and 10 min of 1% H_2O_2, and mixed biofilm on stainless steel was removed the easiest compare to the others. Applying hydrogen peroxide as the pre-treatment following with the regular cleaning process in plants needed in removing and controlling biofilm, particular mixed species biofilm.

Keywords: mixed biofilms, Listeria spp., Salmonella spp., Pseudomonas spp., hydrogen peroxide, food safety

O-2.5

Effect of Co-culturing of *Endomycopsis burtonii* in Angkak Fermentation by *Monascus purpureus* on Citrinin and Red Pigment Production

Betty Sri Laksmi Suryaatmadja Jenie[1)], Danik Dania Asadayanti[2)], Harsi Dewantari Kusumaningrum[1*)], and Novik Nurhidayat[3)]

[1)] Department of Food Science and Technology, Bogor Agricultural University, Bogor, Indonesia

[2)] Vocational Education Development Center for Agriculture, Cianjur, Indonesia

[3)] Research Center for Biology, Indonesian Institute of Sciences, Cibinong, West Java, Indonesia

*E-mail: harsikusumaningrum@yahoo.com

Abstract

Monascus purpureus are commonly used in angkak fermentation to produce the red pigment (angkak). During angkak fermentation, this mold can also form a mycotoxin called citrinin. The objectives of this study were to increase the red pigment production and to reduce the citrinin formation by co-culturing *M. purpureus* strains with an indigenous isolate of *Endomycopsis burtonii* during angkak fermentation at different time of inoculation. Sterilized rice was used as substrate and three strains of *M. purpureus* (JmbA, TOS and AID) at concentration of 10^7 CFU/ml were used for angkak fermentation performed at room temperature. Suspension of *E. burtonii* was added at three different concentrations (10^3, 10^4 and 10^5 CFU/ml) at day 2, 4, and 6 of fermentation period of 14 days. Citrinin were detected after 14 days of angkak fermentation analyzed by HPLC with Nucleosil 100-5 C-18 column and fluorescent detector. The intensity of red pigment was measured by spectrophotometry at 500nm. The results showed that the highest production of red pigment was achieved by strain *M. purpureus* TOS that co-cultured with 10^4CFU/ml of *E. burtonii* added at day 6. The intensity of red pigment by this co-culturing procedure was 1.75 times higher than that without co-culturing. Furthermore, strain TOS formed relatively less citrinin after co-culturing with 10^4 CFU/ml of *E. burtonii* added at day 6, i.e. from 0.54 ppm to 0.47 ppm. Although, the other strains (JmbA and AID) also indicated an increasing trend of red pigment intensity by co-culturing with *E. burtonii*, however, the formation of citrinin was also increase. These results suggested that co-culturing with *E. burtonii* can improve the red pigment production and reduce the citrinin formation. However, the effect of co-culturing with *E. burtonii* was varied, likely depending on the *M. purpureus* strain involved.

Keywords: angkak, citrinin, red pigment, Endomycopsis burtonii, Monascus purpureus

Pigment Production of *Monascus* sp. Isolated From Angkak in Semarang Region, Central Java, Indonesia

Soni Nugraha, Arina Tri Lunggani, and *)Endang Kusdiyantini
Department of Biology, Faculty of Sciences and Mathematics, Diponegoro University, Indonesia
*E-mail: kusdiyantini@undip.ac.id

Abstract

The development of the food processing industry led to the highly used of dyes, especially the type of synthetic dyes that can be harmful to consumers because of its toxicity. Natural dyes to be one of the alternatives used in the field of food. One of the natural dyes is widely used as a food coloring that is red yeast rice. Red yeast rice is rice that is overgrown by the mold *Monascus* sp. that produces pigment. This study aims to obtain pure isolates of red fungus rice from angkak in Semarang. The growth and the red pigment production of the fungal isolates in the different source of N and pH were evaluated. The treatment was done by inoculating fungal isolates in PDB (potato dextrose broth) medium which was treated at pH 3, 5, 7, 9 and added with nitrogen source Ammonium Chloride 1%, Ammonium Nitrate 1%, as well as Peptone 1% for optimization. Analysis of pigments was conducted using a spectrophotometer with a wavelength (λ) of 500 nm and analysis of dry cell was done based on mycelia weight (g/l). The results showed that the highest pigment concentration occured at treatment of pH 7 with 0.812 absorbance value and the highest value of the cell dry weight at pH 7 was 1.232 g/l. Results of optimization with different nitrogen sources showed the highest pigment levels occurred in the addition of a nitrogen source Ammonium Chloride 1% with 0.821 absorbance valve and the dry weight of most cells in Ammonium Nitrate was 2.556 g/l.

Keywords: pigment, angkak, isolate fungus, pH, nitrogen.

O-2.7

Kempong, a Traditional Fermented Food in Karangpucung Kidul Village, Linggapura Bumiayu, Central Java: Fermentation Agent and Their Roles

Devia Kusmawati Arfina, Endang Kusdiyantini and *)Isworo Rukmi
Department of Biology, Diponegoro University.
*E-mail: rukmi_is@yahoo.com

Abstract

Kempong is a traditional fermented food found in South Karangpucung Linggapura Bumiayu village, Central Java prepared from palm kernel cake. This is traditional fermented food which exclusively found in that region, and consumed mostly everyday by the people in the village. To examine the important mold and their roles in *kempong* fermentation. Mold isolation was done by direct isolation on PDA medium from *kempong* product. The proteolytic, amylolitic, lipolytic activity of the isolates were also observed by hydrolysis assay on agar media. Proximat analysis of kempong were also conducted. *R. oryzae*, *A. chevalieri,* and *A. tamarii* have been isolated from kempong product. All isolates showed good enzyme activities, particularly proteolytic and amylolytic. The proximate analysis of kempong showed that the carbohydrate, protein, fat, ash, and water content were 16.67%, 5.77%, 2.80%, 0.75%, 74.03% d.w respectively. *R. oryzae* isolate showed a high proteolytic activity, which indicates that this species might be the main agent in *kempong* fermentation. The nutritional value of kempong was lower than the raw substrate.

Keywords: kempong, mold, fermentation, palm kernel

O-2.8

Does Microbial Diversity of Indonesian Tempeh Determine Its Safety?

Gayuh Rahayu[1*], Iman Hidayat[2], and Tatik Hartanti[3]
[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Darmaga Campus, Bogor, Indonesia
[2]Research Center for Biology, Indonesian Institute of Sciences (LIPI), Indonesia
[3]Department of Biology, Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia
*E-mail: gayuhrahayu@gmail.com

Abstract

Tempeh is a popular Indonesian traditional fermented food that can be used as source of macro and micronutrient, isoflavon and dietary fibers. Tempeh is mainly made from soybean and fermented by *Rhizopus* spp. to produce soybean cake. Recent findings on *Rhizopus* on tempeh from Indonesia indicated that the diversity of *Rhizopus* associated with tempeh was reduced to only *R. microsporus* and *R. delemar*. Further, phylogenetic study based on the ITS sequence indicated that the *R. microsporus* from Indonesian tempeh nested in the same clade with clinical strains *R. microsporus.* While *R. microsporus* may harbour endosymbiotic *Burkholderia rhizoxinica* and *B. endofungorum,* which the toxin is harmful for human. Nevertheless, no report on toxicated people in Indonesia due to consuming tempeh. This raise the question whether *R. microsporus* of Indonesian tempeh hosted toxin producing bacteria and thus correlate with tempeh safety. This study find out that tempeh samples from Bogor were free from *Salmonella* and aflatoxin, but contain *E. coli.* Hyphal washing method indicated no bacteria exists both within and on the surface of the hyphae of *R. microsporus* isolated from fresh tempeh. This result was supported by PCR amplification of 16S RNA of the crude extract of some *R. microsporus* isolates. A hyphothetical answer to tempeh safety based on metagenomic approach is discussed.

Keywords: DNA, food safety, metagenomic, Rhizopus, tempeh

A Review Article: Natural Antimicrobial Compound in Sumba Mare's Milk

Annytha Detha
Faculty of Veterinary Medicine, Universitas Nusa Cendana, Kupang-East Nusa Tenggara, Indonesia
E-mail: annytha.detha@gmail.com

Abstract

Mare's milk has long been used as a health drink and has a therapeutic effect. Sumba horses is the original horse in Indonesia, has a high number of population. According to statistics, the population of Sumba horses reach one-eighth of the total population of horses in Indonesia. Sumba horses are typically used in cultural ceremonies, transportation equipment, agricultural equipment, and horserace, but Sumba mare's milk have not been utilized. The aims of this study were to assess the potential utilization of Sumba mare's milk associated with horse care system maintenance, the condition of the area and population of Sumba horse; to determine the composition of Sumba mare's milk; and to identify and fractionate antimicrobial activity. The study was conducted through collection of journal and data about the Sumba mare's milk. The results of the review showed that maintenance system and horse population in large numbers on the island of Sumba, became an indication of the utilization of mare's milk as a nutritious food source. Sumba mare's milk can also be a new revenue source as a food that improves the economy of the community. Based on the data, it can be conclude that Sumba horse had a great potential in producing mares milk. The average of sumba mare's milk contained protein, fat, lactose and total solids in respectively 1.82%, 1.67%, 6.48% and 11.37%. Identification of antimicrobial compounds using HPLC method, there are six main peaks with different polarities and retention times. Fractionation results of six fractions with different polarity levels were tested of antimicrobial activity against causative agent of subclinical mastitis. The conclusions of present study showed that utilization of Sumba mare's milk have the potential to be developed on the Sumba area. Sumba mare's milk nutritional value, namely protein, fat, lactose, and total solid were balanced and compounds in whey protein had antimicrobial activity against causative agent of subclinical mastitis.

Keywords: Sumba, mare's milk, fractionation, antimicrobial

O-

Studies on Mycoflora Flora Associated with Dried Areca Nut in Assam

S.Debnath[2*)] and S.K Debnath[1)]

[1)]CEO, Rural Based Preventive Oncology Centre, Bokakhat-785612 Assam. India

[2)]Research Director, Peak Chemical Industries Limited, Sevok Road, Siliguri-734001. West Bengal, India

*E-mail: sukumardebnath@rediffmail.com

Abstract

Mycoflora associated with dried betel nut commonly known as supari is poorly understood particularly with the occurrence of mycotoxigenic *A. flavus* in Assam, India. The present work was carried out to examine the quantitative as well as qualitative composition of mycoflora in ten betel nut samples collected from different shops at Bokakhat, Assam. There were 2200-6310 cfu of mycoflora/g of dry betel nut. It was observed that *Aspergillus niger* dominated the fungal population followed by Blue *Penicillium* and yeast. Occurrence of *Mucor* and other hyaline forms are also observed in direct plating of dried areca nut. There were a total of 4578 colonies of fungi recorded during the study and *A. niger* represented 73% of total fungal flora. *A. flavus* was found in three samples. Other fungal species found in this study were pink yeast (25.25%), *Aspergillus flavus* (1.5%), *Trichoderma viride* (0.61%), an ash coloured *Aspergillus* (0.34%), blue *Penicillium* (0.24%), *Mucor* (0.06%) and white *Penicillium* (0.02%). The occurrence of *A. flavus* in betel nut is a significant risk factor which contribute to incidence of various types of oral cancer in human in state of Assam and has been discussed in the light of existing epidemiological evidences.

POSTER SPEAKER'S ABSTRACTS

Citrinin and Pigment Production by Indigenous *Monascus purpureus* strains during Angkak Fermentation

Harsi Dewantari Kusumaningrum[1*)], Danik Dania Asadayanti[2)], Betty Sri Laksmi Suryaatmadja Jenie[1)], and Novik Nurhidayat[3)]

[1)] Department of Food Science and Technology, Bogor Agricultural University, Bogor, Indonesia
[2)] Vocational Education Development Center for Agriculture, Cianjur, Indonesia
[3)] Research Center for Biology, Indonesian Institute of Sciences, Cibinong, West Java, Indonesia
*E-mail: harsikusumaningrum@yahoo.com

Abstract

This study detected citrinin and pigment production by indigenous *M. purpureus* strains during angkak fermentation. Citrinin is a mycotoxin originally isolated from *Penicillium citrinum*, which is also found to be produced by other variety of other fungi. Three strains of *M. purpureus* isolated from angkak commercially produced in Jember (JmbA), Pontianak (TOS) and Medan (AID) were used. The cultures were grown on Malt Extract Agar media at 30 ^{o}C for 7 days. Fermentation of angkak was conducted by adding of 10% (v/w) starter suspension to sterilized rice, followed by incubation at room temperature (25-32 ^{o}C) for 14 days. The red, orange and yellow pigments were measured by spectrophotometry at wave length of 500 nm, 470 nm and 410 nm, respectively. Citrinin were detected after 14 days of angkak fermentation, using HPLC with Nucleosil 100-5 C-18 column and fluorescent detector. The results indicated that the growth curves of all strains were similar, with level of 6.0 to 7.0 Log_{10} CFU/g reached after 6 days and was relatively constant up to 14 days. All strains produced red, orange and yellow pigments. The highest red pigment formation as indicated by the highest measured intensity were found in descending order from high to low by *M. purpureus* strain JmbA, TOS, and AID. All strains also formed citrinin, i.e. 1.76 ppm by JmbA, 0.54 ppm by TOS, and 0.34 by AID. These levels are lower than LD_{50} that has been reported for oral administration as about 50 mg/kg, 35-58 mg/kg and 19 mg/kg to the rat, mouse, and rabbit, respectively. Although there is no established limit in humans, however, caution should be suggested when consuming products containing citrinin.

Keywords: citrinin, pigments, Monascus purpureus, angkak

P-2

Mycoflora of Fermented and Unfermented Cocoa Beans and Their Susceptibility Difference of Ochratoxin A and Aflatoxins Production in High Relative Humidity Storage

Heni Adhianata[1)], Warapa Mahakarnchanakul[2)], FMC Sigit Setyabudi[1)] and Sardjono[1*)]

[1)]Department of Food and Agricultural Product Technology, Faculty of Agricultural Technolgy, Universitas Gadjah Mada, Yogyakarta, Indonesia

[2)]Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand

*Email: sardjon@yahoo.com

Abstract

Most of Indonesia's cocoa beans have been produced through fermentation. Various metabolites such as alcohol, lactic acid, and acetic acid are produced during cocoa beans fermentation. These metabolites would induce different chemical characteristic of fermented cocoa beans. Lactic and acetic acid are fermentation products with antimicrobial activity and capable to suppress fungal growth. In the other hand, ochratoxin A (OTA) and aflatoxins (AFs), mycotoxins produced by mycoflora *Aspergillus* and *Penicillium* species, usually found as cocoa beans contaminants. This study was the first research on fermented and unfermented cocoa beans mycoflora diversity and their susceptibility difference of OTA and AFs production. In this research, parameters evaluated were mycoflora, OTA, and AFs contamination to determine the correlation between susceptibility of fermented and unfermented cocoa beans on OTA and AFs production and the diversity of mycoflora during storage. To investigate the susceptibility, this research was conducted on storage simulation with 91% of relative humidity. This study was divided into 2 parts. In part I, enumeration using direct plating method was used to determine the level of cocoa beans mycoflora contamination from field and during storage. To investigate the potential of OTA production, and possibility of AFs formed during storage simulation, spore suspension of *Aspergillus ochraceus* (4.32×10^8 cfu/ml) was inoculated in fermented and unfermented cocoa beans with a ratio of 2 ml spore suspension per 100 grams of cocoa beans. Cocoa beans were incubated at 91% RH environment to support mycoflora growth and OTA and AFs production. In part II, enumeration using dilution and plating method was used to monitor the growth of inoculated *A. ochraceus* and other species population which grow during storage simulation. Enumeration result indicated that mycoflora contamination in unfermented cocoa beans was higher than those in fermented cocoa beans. Mycoflora identification result showed the presence of *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor*, and *Eurotium*. Fermented cocoa beans were more susceptible to AFs production with dominant contaminants from *flavus* group. The highest AFs production was found in fermented inoculated cocoa beans after 10 days storage. Unfermented cocoa beans were more susceptible to OTA production with dominant contaminants from black *Aspergilli*. The highest OTA production was found in unfermented inoculated cocoa beans after 15 days storage.

Keywords: ochratoxin A, aflatoxins, mycoflora contamination, fermented and unfermented cocoa beans

P-3

Survey of Aflatoxin B_1 Contamination in Rice from Thailand

Jiratchaya Kuanpan[1)] Parichat Narongplain[1)] Kanin Suksomsak[1)] Pichitpon Mungkasem[2)] Saowalak Adunphatcharaphon[2)] and Awanwee Petchkongkaew[2*)]

[1)] Princess Chulabhorn's College, Thailand

[2)] Department of Food Science and Technology, Faculty of Science and Technology, Thammasat University (Rangsit campus), Thailand

*E-mail: awanwee@tu.ac.th

Abstract

Aflatoxin B_1 (AFB_1) is a highly toxic, mutagenic, teratogenic and carcinogenic compound that exhibits immunosuppressive activity and acute and chronic toxicity in both humans and animals. Currently, AFB_1 contamination in food and feed is a danger to human health. Rice is one of Thailand's staple foods, consumed almost on a daily basis. The per capita rice consumption per annum was estimated at 119 kg of milled rice. Unfortunately, research focusing on aflatoxin B_1 contamination in rice is quite limited. Therefore, the main objective of this work was to investigate the aflatoxin B_1 contamination levels in rice by Enzyme-linked Immunosorbent Assay (ELISA). Forty two rice samples including milled rice (6 samples), glutinous rice (5 samples), brown rice (19 samples), Hom Ma Li rice (8 samples), red rice (3 samples), and purple rice (1 sample) were collected from local stores or supermarket in Thailand. Results indicated that aflatoxin B_1 contamination level was in the range of 0-19.91 ppb. The highest aflatoxin B_1 contamination level has been detected in one brown rice sample. Only three rice samples showed negative results. Fortunately, all the detected levels were under the regulation limit of Thailand.

Keywords: Aflatoxin B_1, rice, Thailand

P-4

Isolation of *Aspergillus spp.* from Thai Husked Rice and Their Ability to Produce Aflatoxin B1

Panrapee Iamtaweejaroen[1),4*)] , Warapa Mahakarnchanakul[2)], Thanapoom Maneeboon[3)] and Chananya Chuaysrinule[3)]

[1)]The Graduate School, Kasetsart University, Bangkok, Thailand

[2)]Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand

[3)]Scientific Equipment and Research Division, Kasetsart University Research and Development Institute, Kasetsart University, Bangkok, Thailand

[4)]Center for Advanced Studies for Agriculture and Food (CASAF), Kasetsart University, Bangkok, Thailand

*E-mail: ammy184@hotmail.com

Abstract

The consumption of husked rice is increased but the quality and safety of husked rice is still an issue. Aflatoxin B1 (AFB1) contamination has been investigated between July 2011- Sept 2012 and found AFB1 contamination on 240 rice samples ranging from 0.09 to 26.61ppb. Thus, the ability of mold isolates from husked rice was investigated. Xerophilic mold on husked rice collected from Central and Northern East regions of Thailand (120 samples) was counted on DG18 and the population ranged from 2.1-4.0 and 1.2-3.7 logCFU/g respectively. The results showed no correlation between amount of AFB1 and the fungal population in rice samples. Identification was done by sequencing method with calmodulin gene fragments. Fifty six of *Aspergillus* spp isolates was identified as 5 major species namely *flavus* (44)*, fijiensis* (4), *tamarii* (3)*, brunneoviolaceus* (3) and *fumigatus* (2), all isolates showed potential to produce AFB1. The highest potential of AFB1 production cultured on media was *A. flavus* (1,004 ppb) while the lowest was *A. tamarii* (0.5 ppb). Although the results from the survey revealed that Thai husked rice was safe from AFB1 contamination, however poor storage without relative humidity control may promote the growth of toxin producing fungi. Since potential aflatoxin-producing fungi may exist in rice, therefore good practices in post-harvest particularly appropriate dry process, good sanitary condition of storage have to be emphasized in farm.

Keywords: Aflatoxin B1, Aflatoxin, husked rice, Aspergillus spp., food safety

P-5

Reduction of Aflatoxin B_1 by *Lactobacillus paracasei* SNP-2 during Peanut Milk Ferm entation

Tyas Utami, Angga P. Nugroho Hutapea, *)Endang S. Rahayu
Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia
*Email: endangsrahayu@yahoo.com

Abstract

The objective of this study was to study the ability of *Lactobacillus paracasei* SNP-2 to reduce aflatoxin B_1 (AFB_1) content during fermentation of peanut milk. *Aspergillus flavus* contaminated peanuts were extracted to obtain peanut milk. The amounts of AFB_1 were analyzed during peanut milk production. AFB_1 contaminated peanut milk was inoculated with 24 h culture of *L. paracasei* SNP-2, and incubated at 37°C for 12 h. Viable cell, pH and reduction of AFB1 content were monitored during fermentation. Processing of peanut milk from raw peanuts reduced AFB_1 concentration for about 62%. During peanut milk fermentation cells grew from 9.8×10^7 CFU/ml to 1.6×10^9 CFU/ml, and the pH decreased to 3.50. Fermentation of peanut milk by *L. paracasei* SNP-2 resulted in the reduction of 44.44% AFB_1.

Keywords: Aflatoxin B_1, reduction, lactic acid bacteria, peanut milk fermentation

Binding of Aflatoksin B_1 to *Lactobacillus paracasei* SNP-2 and The Stability of Bacteria/AFB_1 Complex

Rohula Utami[1)], Tyas Utami[2)] Suparmo[2)], and Endang S Rahayu[2*)]

[1)]Department of Food Science and Technology, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Indonesia; [2]Department of Food and Agricultural Product Technology, [2)]Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia

*E-mail: endangsrahayu@yahoo.com

Abstract

The aim of this research was to study the binding ability of Aflatoxin B_1 (AFB_1) to viable and non viable of *Lactobacillus paracasei* SNP-2 in phosphate buffered saline pH 7.3. Cells were grown in MRS broth at 37°C for 24 h, and then centrifugated at 1800 g for 20 minutes at < 10°C to get the pellet. Pellet was suspended in phosphate buffered saline pH 7.3 until the cell concentration was about 10^{10} CFU/ml. Viable cells, and heat-, and acid killed cells were evaluated their ability to bind AFB_1 in phosphate saline buffer pH 7.3. Stability of the *L. paracasei* SNP-2-AFB_1 complexes were evaluated by determining the amount of AFB_1 released to the phosphate buffered saline following five times washing. The results showed that AFB_1 binding by heated-and acid-killed bacteria were higher than that of by viable cells. More than 70% of bound AFB_1 was released from viable bacteria after five times washing. However, heat- and acid-killed cell treatments were significantly increased the complex stability of bacteria-AFB_1.

Keywords: Aflatoxin B_1, Lactobacillus paracasei SNP-2, binding of AFB_1

P-7

Study on Factors affected Binding Aflatoxin B1 by Lactic Acid Bacteria *Lactobacillus paracasei* SNP-2

Hanim Z. Amanah, Tyas Utami, *)Endang S.Rahayu
Faculty of Agricultural Technology, Universitas Gadjah Mada
*E-mail: endangsrahayu@yahoo.com

Abstract

The objectives of this research are (1) to evaluate effect of external factors such as AFB_1 concentration, acidity (pH), temperature, and incubation time on the binding of AFB_1 by *Lactobacillus paracasei* SNP-2, (2) to evaluate the effect of bacterial population in binding process. In this research, 10^{10} CFU/ml bacteria were incubated on phosphate buffered saline with various pH (3,0; 4,0; 5,0; 6,0; and 7,3) that contain various AFB_1 concentrations (20, 40, 60, 80 and 100 ppb). The variation of incubation times were 10 min, 1 h, 6 h, 12 h, and 24 h. To evaluate the effect of number of bacterial cells, three different concentration of bacterial cells i.e., 10^8 CFU/ml, 10^9 CFU/ml and 10^{10} CFU/ml, were incubated in the phosphate buffered saline (pH 5) that contain of 20 ppb AFB_1. The result indicated that increasing AFB_1 concentration in medium did not affect the percentage of AFB_1 binding but affect the binding speed. On the other hand, acidity affected the binding capacity and after 24 h of incubation time at temperature of 37°C showed the highest value. Approximately a minimum of $1x10^9$ CFU/ml was required for significant binding of AFB_1 by *Lactobacillus paracasei* SNP-2.

Keywords: AFB_1, Lactobacillus paracasei SNP-2,

P-8

A Rapid ELISA Test for the Detection of T-2 Toxin in Grain Samples

Karen Ong[1*)] Norasidah A. Rashit[1)] Georg Haeubl[2)] and Michael Z. Zheng[3)]
[1)]Romer Labs Malaysia Sdn. Bhd., Suite 218, Eureka Complex, Universiti Sains Malaysia, 11800 Penang, Malaysia
[2)]Romer Labs Diagnostic GmbH, Technopark 1, 3430 Tulln, Austria
[3)]Romer Labs Singapore Pte.Ltd.,
Jalan Bukit Merah #08-08, e-Centre@redhill building, Singapore 159471
*E-mail: karen.ong@romerlabs.com

Abstract

T-2 toxin is a type Atrichothecene and it is produced by fungi of the *Fusarium* genus. T-2 toxin can be found in grains such as wheat, maize, oats, barley, rice, beans and soybeans, as well as in some cereal-based products. T-2 toxin inhibits protein synthesis and affects the actively dividing cells such as those lining the gastrointestinal tract, skin, lymphoid and erythroid cells. The effects of T-2 toxin to animals include weight loss or poor weight gain, bloody diarrhea, dermal necrosis or beak lesions, hemorrhage and decreased production (weight gain, eggs, milk, etc.). An ELISA test, has been developed to detect T-2 toxin in grain samples. The test was performed as a solid phase direct competitive ELISA using a horseradish peroxidase conjugate as the competing, measurable entity. The toxin was extracted with 70% methanol and then mixed with enzyme conjugate before transferred to the antibody coated microwells. After incubation at room temperature for 10 minutes, the microwells were washed and enzyme substrate was added and allowed to incubate for an additional of 5 minutes. Stop solution was then added and the intensity of the resulting yellow colour was measured optically with a microplate reader at 450 nm with a differential filter of 630 nm. The test had a quantitation range of 20 – 500 $\mu g \cdot kg^{-1}$ with limit of detection10 $\mu g \cdot kg^{-1}$ for maize. Results obtained from internal studies assessing accelerated stability, accuracy and precision determined that test to be accurate, precise, sensitive and effective for measuring T-2 toxin in grain samples.

Keywords: T-2 toxin, ELISA, mycotoxin, grain

Using in-House Immuno Affinity Column (KU-AF2) to Asses the Risk of Aflatoxin in Peanut in Thai Consumption

Phakpoom Kooprasertying[1),4*)], Warapa Mahakarnchanakul[2)], Thanapoom Maneeboon[3)] and Chananya Chuaysrinule[3)]

[1)]The Graduate School, Kasetsart University, Bangkok, Thailand

[2)]Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand

[3)]Scientific Equipment and Research Division, Kasetsart University Research and Development Institute, Kasetsart University, Bangkok, Thailand

[4)]Center for Advanced Studies for Agriculture and Food (CASAF), Kasetsart University, Bangkok, Thailand

*E-mail: np_kool@hotmail.com

Abstract

Aflatoxins (AFs) and aflatoxin B1 (AFB_1) intake from peanuts in Thai was assessed by using commercial Immunoaffinity Column (IAC) and in-house IAC ("KU-AF2"). Sixty samples containing each 20 samples of raw peanuts, roasted peanuts and ground peanuts were determined. Samples were collected from 4 retail markets in Bangkok during November 2013-January 2014. Exposure assessment was done based on food consumption data from the National Bureau of Agricultural Commodity and Food Standards database (2006) and estimated the contribution of AFs and AFB_1 using @risk software. Roasted and ground peanuts were highly contaminated by AFs as 100% while raw peanut was 80%. The highest concentration of AFs found in ground peanuts was 362.5 ppb resulting in highest mean concentration as 68.2 ppb, the contamination was higher than Thai Food and Drug Administration, limit at 20 ppb. Similar pattern was found when using KU-AF B_1 detected ground peanuts (AFB1 323.4 ppb). The intake of AFB_1 on raw, roasted and ground peanuts in Thai consumption was 0.32, 0.29 and 1.12 ng/kg.bw/day, respectively. Although roasted peanuts contaminated with the lowest AFB_1 concentration but consumption of roasted peanuts was higher than raw peanut resulting in similar intake of AFB_1. The potential risk for cancer using a formula derived by the Joint Food and Agricultural Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA) was estimated at 0.0071-0.027 person/year/100,000 persons. According to the results implied the current intake of AFB_1 by peanuts consumption in Thai has potential effect on health and need be managed risk.

Keywords: exposure assessment, Aflatoxin B_1, peanuts, Aflatoxins intake, Thailand

P-10

Rapid Lateral Flow Test for Quantification of Aflatoxin M1 in Milk

Poh Hong Goh[1)] Michael Zheng[1*)] and Alois Schiessl[2)]
[1)]Romer Labs Singapore Pte Ltd, Jalan Bukit Merah #08-08, e-Centre@Redhill Building, Singapore 159471
[2)]Romer Labs Division Holding GmbH, Technopark 1, 3430 Tulln, Austria
*E-mail: Michael.zheng@romerlabs.com

Abstract

Aflatoxin M1 (AFM1) is produced as a metabolite of aflatoxin B1 and secreted in the milk of animals that have consumed feed contaminated with aflatoxin B1 (AFB1). AFM1 is a carcinogenic, teratogenic and mutagenic compound and may cause deleterious effects to the liver, kidney as well as brain. Regulations of aflatoxin M1 are in place in many countries worldwide. The European Commission has set a maximum level of 0.05µg/kg AFM1 in milk while the US Food and Drug Administration has placed an AFM1 limit of 0.5 ng/ml (ppt) for milk. AFM1 in milk samples can be analyzed using analytical methods, including ELISA (enzyme-linkedimmunosorbent assay) and HPLC (high pressure liquid chromatography). These methods are used inlaboratories and expensive instrumentsare required in cases wherethe HPLC method is used. The AgraStrip® Aflatoxin M1 quantitative lateral flow test was developed in Romer Labs Singapore for the screening of AFM1 in milk samples and can be potentially used for on-site testing. The test has been validated for its efficacy in detecting AFM1 on several types of milk samples such as raw milk, pasteurized milk and ultra-heat treated milk (UHT). In-house validation results showed that the test kit is accurate and precise in analyzing AFM1 in milk samples in its quantification range and results are comparable to results obtained from the reference HPLC method. The limits of detection for tests on raw milk, pasteurized milk and UHT milk samples have been determined at slightly lower than 50ppt, which meets the European Commission maximum level. The study collectively indicates that the AgraStrip® Aflatoxin M1 lateral flow test is a rapid and effective method for the screening of AFM1 in several milk samples in the quantification range of 50−600ppt.

Keywords: Aflatoxin M1, lateral flow strip test, raw milk, pasteurized milk, UHT milk

Detection and Cluster Analysis of Gene Encoding *Vacuolar serine protease* Allergen in *Penicillium* Species Isolated from Hospital Indoor Air in Yogyakarta Indonesia

Andika Sidar[1*)], Jaka Widada[1,2),3)], Latifah Zakaria[3)], and Endang S. Rahayu[4)]
[1]Graduate School of Biotechnology, Universitas Gadjah Mada, Yogyakarta Indonesia
[2]Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta Indonesia
[3]School of Biological Science, Universiti Sains Malaysia, Penang Malaysia
[4]Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta Indonesia
*E-mail: andikasidar@yahoo.com

Abstract

Penicillium is commonly found in indoor air and some of them are known as allergenic fungi. Vacuolar serine protease (VSP) was reported as an important allergen from *Penicillium.* Existence of allergenic fungi is considered as a risk factor for inhabitants inside the room, especially in hospital. So far, none studies about hospital airborne fungi in Indonesia. Therefore, this study was conducted to investigate the diversity of fungal isolates obtained in hospital indoor air and then focused on identification of *Penicillium* species bearing gene encoding VSP. Sampling airborne fungi in several hospital rooms was carried out using non-volumetric air sampling method, then gene encoding VSP allergen in *Penicillium* was detected based on PCR amplification using new degenerate primer and continued with species identification of allergenic *Penicillium* based on PCR amplification of β-tubulin gene. Cluster analysis was carried out reffered to RFLP analysis based on nucleotide sequence of gene encoding VSP allergen of *Penicillium* isolates. The result showed that fungal isolates obtained from indoor hospital room belong to 10 genera, there were *Penicillium, Aspergillus, Cladosporium, Eurotium, Curvularia, Alternaria, Paaecilomyces, Fusarium, Chaetomium,* and *Aureobasidium*. VSP gene was detected in *P. citrinum, P. steckii*, and *P. copticola.* The interesting of these finding was the existence of gene encoding VSP in *P. steckii* and *P. copticola* has not been reported previously. Phylogenetic tree constructed from RFLP analysis showed that VSP gene of the same *Penicillium* species is grouped in one cluster. It indicates that gene encoding VSP allergen might be classified as species-specific.

Keywords: Penicillium, vacuolar serine protease allergen, phylogenetic, indoor fungi, hospital

P-12

Identification of Fungus Caused Otomycosis

Lilis Suryani
Department of Microbiology, Faculty of Medicine and Health Science, Muhammadiyah Yogyakarta University, Yogyakarta, Indonesia
E-mail: lilis_fkumy@yahoo.co.id

Abstract

Otomycosis is an acute, sub acute or chronic fungal infection of in the ear, ear canal or outer auditory canal. The otomycosis found in tropical and sub-tropical countries has high degree of humidity around 70-80% and the air temperature around 15-30 °C in the etiology otomycosis, some species are classified as saprophytic fungi including: *Aspergillus niger, A. flavus, A. fumigatus, Allesceria boydii, Scopulariopsis, Penicillium, Rhizopus, Absidia and Candida sp*. There are about 200 species that cause opportunistic infection in humans. Although rarely life-threatening, but otomycocis is a challenge for the medical area because it requires long-term care, primarily to the high recurrence rate constraint. The aim of this study was to determine the frequency of otomycosis, clinical presentation, predisposing factors and the species fungus caused otomycosis. The study was an observational analytic using sectional study design. The study was conducted in the Microbiology, Faculty of Medicine and Health Science, Muhammadiyah Yogyakarta University, from January to August, 2012. Convenient sample comprising 86 patients of both sexes and all groups were selected from ENT clinic, Sari Asih, Yogyakarta. Materials and tools used were sabouroud dextrose agar, sterile physiological saline, lactophenol cotton blue, cotton stick sterile, test tubes sterile, petri dishes sterile, gloves, glass objects, and microscope. The data collected from patients with symptoms of inflammation of the outer ear. Patient ear discharge swabs samples were taken with sterile cotton stick and put into a test tube containing sterile physiological saline, and then cultural method was conducted in microbiological laboratory data were analysed using Chi-square. There were 86 patients with documented diagnosis of otomycosis. There were 38 (44%) males and 48 (56%) females. The age of patients ranged from 21 to 40 years old were 45%. Most common symptom was hearing loss (7%) followed by pruritis (17.4%), pain (47.7%), and tenderness (5.8%). The fungi caused otomycosis was *Aspergillus sp* (81.4%) and *Candida sp* (18.6%). The used of topical antibiotics increased the risk of otomycosis. The results showed that *Apergillus sp* and *Candida sp* are include as fungi caused otomycosis.

Keywords: *Otomycosis, fungi*

P-13

Production and Utilization of *Lactobacillus plantarum* IBL-2 Bacteriocins as Meat Product Biopreservatives

Marlia Singgih Wibowo[1*)], Isra Muzaqiyah[1)], Betty Nurhayati[1)], Tjokorde I.Armina Padmasawitri[1)], Yantyati Widyastuti[2)], and Tutus Gusdinar[1)]

[1)]School of Pharmacy, Institut Teknologi Bandung, Indonesia

[2)]LIPI ,jl.Raya Bogor Km. 46-Cibinong, Indonesia

*E-mail: marlia@fa.itb.ac.id

Abstract

Biopreservation is one of the alternatives to obtain safe food products. Bacteriocin produced by Lactic Acid Bacteria (LAB) is potential as biopreservatives, which is safe for consumption, since it is a protein degradable by proteolytic enzymes. This study aimed to optimize bacteriocin production from *L. plantarum* IBL-2 and to evaluate the effectiveness of bacteriocins in reducing the number of total plate count and *Salmonella typhimurium* in ground beef. Bacteriocin was produced through fermentation of *L. plantarum IBL-2*, under various conditions to yield the compound with the best antimicrobial activity. The total number of bacteria in ground beef after the addition of *L. plantarum* IBL-2 fermentation supernatant was determined. The result was compared with the sample without preservatives (control), and sample added with commercial Nissin. All three samples of ground beef were spiked with *S. typhimurium* and incubated for 0, 2, 6, 8, 12, 14 days at a temperature of 4-10 ° C. Total Plate Count (TPC) method was utilized to determine the number of bacteria in the samples. The fermentation process resulted in bacteriosin with the strongest antimicrobial activity when using low molecular weight liquid medium (LMWLM), followed by a series of refining process. From day 0-14, the number of *S. typhimurium,* in sample added with *L. plantarum* IBL-2 fermentation supernatant, was lower than control and sample added with Nissin. The most optimal antimicrobial activity of bacteriosin was obtained using LMWLM as fermentation media, and using a series of refining process consist of bacteriocin supernatant evaporation, membrane ultrafiltration, and gradual fractionation using 80% ammonium sulfate. Bacteriosin from *L. plantarum* showed antimicrobial activity against *S. typhimurium.*

Keywords: Bacteriocin Production, L.plantarum IBL-2, Biopreservative (meat product biopreservative)

P-14

Shelf-Life Analysis of Soft Cheese Stored at Ambient and Refrigerated Temperatures

Gener Gregorio
Central Luzon State University, Science City of Muñoz, Philippines
E-mail: gdgregorio@yahoo.com

Abstract

This study was conducted to determine the shelf-life of soft cheese stored at ambient and refrigerated temperatures. It aimed to correlate the effects of pH and microbial load to the general acceptability of soft cheese in both storage conditions. Batches of milk from PCC herd were collected and processed into soft cheese using abomasal extract of Murrah Buffalo as milk coagulant. These cheese samples were divided into two lots (A and B). Cheese samples in Lot A were stored at ambient temperature (26-32 °C) while cheese samples in Lot B were stored at refrigerated temperature (4 °C). The rate of bacterial growth, fungal growth (mold) and pH as well as sensory evaluation of the soft cheese stored in both conditions were observed. As the storage progressed, a decreasing trend in pH and fungal (mold) count and an increasing trend in bacterial count were observed. Soft cheese stored at ambient temperature had 22 hours shelf-life while soft cheese stored at refrigerated temperature had a prolonged shelf-life of up to 10 days. Based on the study, strict sanitation must be observed during cheese making and soft cheese must be stored at refrigerated temperature.

Keywords: microbial succession, decreasing trend in pH, anti fungal properties of LAB

The Comparison of Sensitivity of Aminoglycoside and Beta Lactam Antibiotics to *Avibacterium paragallinarum*

Elisabet Tangkonda
Faculty of Veterinary, Universitas Nusa Cendana, East Nusa Tenggara, Indonesia
E-mail: elisabet_tangkonda@yahoo.co.id

Abstract

Avibacterium paragallinarum is the aetiology of Infectious coryza (IC). Infectious coryza is an acute upper respiratory infection that affect birds with clinical symptoms of snoring, facial swollen and a foul-smelling nasal discharge. Infectious coryza is detrimental due to its high morbidity rate and decreasing egg production of layers. Antibiotics are commonly used as a treatment for infrectious coryza. The selection of appropriate antibiotics is useful for eradicating the disease. The aim of this study was to compare the effectiveness of aminoglycoside antibiotics (amikacin, gentamycin, kanamycin and neomycin) to beta- lactam antibiotics (ampicillin, penicillin and amoxicillin+clavulanic acid) against *Avibacterium paragallinarum*. This study used a descriptive analysis method to measure and compare the sensitivity level of *Avibacterium paragallinarum* toward two classes of beta-lactam and aminoglycoside antibiotics using Kirby's Baurer method. The results showed that beta-lactam antibiotics, namely ampicillin, penicilin and amoxicilin+clavulanic acid, were found to be sensitive. Within aminoglycoside class, only gentamycin and neomycin which were sensitive, whereas amikacin and kanamycin were resistant and intermediate respectively. In conclusion, most beta-lactam antibiotics are remain capable to counter *Avibacterium paragallinarum*. On the other hand, aminoglycoside antibiotics are less capable.

Keywords: Avibacterium paragallinarum, aminoglycoside, beta-lactam, antibiotics, sensitivity

Isolation of Chitinolytic Bacteria from Fermented Shrimp Product and Screening for Antifungal Activity

Indun Dewi Puspita[*)], Ustadi, and Mgs. Muhammad Prima Putra
[1)]Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada
*E-mail: dune_28@yahoo.com

Abstract

Exploration of bacterial chitinase from food products is beneficial in the term of food safety and the stability of enzyme when it is applied in the food system. Bacterial chitinase from several genus of Gram positive and Gram negative bacteria showed antifungal activity against fitopathogen *Fusarium* sp. Application of antifungal compound produced by food-isolated bacteria could be an alternative to control the growth of mycotoxin producers in food system. The objective of this research was to isolate chitinolytic bacteria from Indonesian fermented shrimp product. Isolation of chitinolytic bacteria was performed on colloidal chitin agar. Isolates which was surrounded by clear zone showed chitin hydrolyzing activity. Chitinolytic activity of cell free-culture supernatant was quantified by detection of the amount of N-acetyl glucosamine produced from the reaction of crude enzyme and colloidal chitin. Antifungal activity of isolates was examined against *Fusarium* sp. and *Aspergillus* sp. There were 44 bacteria isolated from Rusip, 40 fromerasi, and 34 from Petis. The highest chitinolytic activity from Rusip was shown by isolate KKT01, JKT33 from Terasi, and SDI23 from Petis. This result showed that traditional shrimp products inhabited with chitinolytic bacteria which potential for the production of antifungal compound. Further study required for the confirmation of antifungal mechanism against food-borne fungi especially in fisheries product.

Keywords: chitinolytic bacteria, antifungal activity, traditional shrimp products

Used in the Growth Inhibition of Foodborne Pathogenic Bacteria

Tutus Gusdinar[1*)] Betty Nurhayati[1)] Tjokorde I. Armina Padmasawitri[1)] Aniendha D. Ramadhani[1)] Yantyati Widyastuti[2)] Marlia Singgih Wibowo[1)]

[1)]School of Pharmacy, Institut Teknologi Bandung, Jl. Ganesha 10 Bandung 40132.

[2)]LIPI , jl. Raya Bogor Km. 46-Cibinong.

*E-mail: gusdinar@fa.itb.ac.id

Abstract

The activity of bacteriocin and non-bacteriocin antimicrobial compound derived from *L. plantarum* can be enhanced using multiple induction methods, including co-culture method with Lactic Acid Bacteria (LAB). The antimicrobial compound is targeted to be an alternative for effective and non-toxic food borne diseases bio-control. The aim of this study were to study antimicrobial activity enhancement of *L. plantarum* bacteriocin and non-bacteriocin compound through co-culture with LAB and to identify the antimicrobials activity of the compound in inhibiting the growth of foodborne disease indicator bacteria. Compatibility study was performed using agar disc diffusion method. Fermentation of *L. plantarum* IBL-2 under optimal condition was performed to produce antimicrobial compound. Neutralized cell-free supernatant of the fermentation product was prepared using ultrafiltration membrane. For non-bacteriocins antimicrobial study, extract of acid and cell-free supernatant was prepared using liquid-liquid extraction and column vacuum chromatography. Antimicrobials activity of the compound was measured using agar diffusion and microdilution method. The optimum co-cultures were *L. plantarum* IBL-2 and *Streptococcus thermophilus* since it had the lowest value of compatibility index (CI), which was 0.81 and 0.89, for bacteriocins and nonbacteriocins respectively. The result for bacteriocin activity against the foodborne disease indicator bacteria using microdilution and disc diffusion method were 4.8 and 10.1 mm respectively. The average of non-bacteriocin ethyl acetate fraction antimicrobial activity against the food borne disease indicator bacteria using microdilution method and disk diffusion were 153.6 and 30.3 mm respectively. The activity of antimicrobial compound derived from *L. plantarum* could be enhanced using co-culture method with *Streptococcus thermophilus.* Antimicrobials activity of the compound, both bacteriocins as well as non-bacteriocin, had the potential as bio-control alternative to inhibit the growth of foodborne disease bacteria.

Keywords: lactic acid bacteria co-culture, compatibilities, biocontrol, indicator bacterial of food borne disease

P-18

Isolation of Lactic Acid Bacteria from Indonesian Fermented Food

Rohmatussolihat[1*], Mika Miyashita[2], Yopi[1], Puspita Lisdiyanti[1], Hiroko Kawasaki[2] and Ken-Ichiro Suzuki[2]

[1]Research Center for Biotechnology-LIPI, Jl. Raya Bogor KM 46 Cibinong, Bogor 16911

[2]Biological Research Center (NBRC)-NITE, Kazusa-Kamatari, Chiba, Japan

*E-mail: rohmatussolihat@gmail.com

Abstract

This study was conducted to isolate the lactic acid bacteria (LAB) from Indonesian fermented food (FF). In 2012, thirty five of FF samples was collected from Yogyakarta (Sleman, Muntilan, Beringharjo, and Kranggan) and West Java (Bogor, Cirebon, Bandung, and Majalengka). In 2013, thirty three of FF samples was collected from Bali (Badung, Blahbat, Gianyar, and Gerobokan, Sukawati and Tabanan) and West Sumatra (Solok, Bukit Tinggi and Padang). The medium that were used to isolate the LAB from FF are MRS and TSYE, pH 6, with and without 10% NaCl. In 2013 medium MRS pH10 with 10% NaCl and skim milk pH 7 were also used to isolate the LAB. The 16S rRNA gene sequencing was used to identify the LAB isolates. From total of 164 isolates that were collected in 2012, we selected 94 isolates, 80 isolates belongs to LAB and 14 isolates belongs to *Staphylococcus*. In 2013, total of 228 isolates were collected, we selected 125 isolates, 101 isolates belongs to LAB, 20 isolates belongs to *Staphylococcus* and 4 isolates belongs to *Bacillus.*

P-19

Lactic Acid Bacteria Co-Culture Induction to Enhance the Activity of Antimicrobial Compounds Inactivation of *C. perfringens* on Dried Pepper by Washing with Oxidizing Agents

Unnop Tassanaudom[1*)], Warapa Mahakarnchanakul[1)] and Chidchom Hiraga[2)]
[1)]Faculty of Agro Industry, Kasetsart University, Thailand
[2)]Institute of Food Research and Product Development, Kasetsart University, Thailand
*E-mail: u_tassana@hotmail.com

Abstract

Increasing demand of dried pepper as an indispensable ingredient in Asian cuisine is apparent, but it raises an awareness of quality and safety of this product. Now microbiological standards of dried pepper in European Community legislation have not been set, however products should be free from pathogenic microorganisms at levels that may represent a hazard to health and requirements. Thus, the objective of this study was to investigate the *C. perfringens*, the mainly bacterial contamination, on dried pepper and the appropriate intervention to inactivate this bacteria. Results showed that 86% of dried pepper sold in Thai markets (N=100) were contaminated with *C. perfringens*, ranging from 5–2,150 CFU/g. High positive correlation between *C. perfringens* and anaerobic proteolytic spore-formers ($r=0.545$, $p<0.01$) was found and may possible use amount of anaerobic spore-formers to refer *C. perfringens* load in dried peppers. The effective washing in dried pepper process was necessary in order to inactivate *C. perfringens*. Washing with three types of oxidizing agents were done on artificially contaminated dried spur peppers (*Capsicum annuum* Linn. Var *acuminatum* Fingerh.). Washing dried peppers with 200 ppm sodium hypochlorite (NaOCl) solution or 50 ppm acidic electrolyzed water (AcEW) for 10 min were found to be the most efficient treatment to reduce *C. perfringens* vegetative cells (2.0 or 3.2 log CFU/g, respectively) from the initial load of 4.5 log CFU/g with no significant difference ($p>0.05$). Whereas, 0.5 ppm ozonated water and tap water were similar in their ability (reduction by 1.8 and 1.5 log CFU/g, $p>0.05$). According to these findings, dried peppers and derived products could be the major source of *C. perfringens*, therefore food producers must concern the quality and safety of dried peppers by applying the appropriate washing to reduce *C. perfringens* contamination to ensure the use as the safety ingredient in food process.

Keywords: C. perfringens, *anaerobic spore-formers, dried pepper, oxidizing agents, food safety*

P-20

Color Value, Citrinin Content and Genetic Variation of from *Monascus purpureus* Angkak in Indonesia

Susana Ristiarini[1*)], M. Nur Cahyanto[2)], Jaka Widada[3)], Latiffah Zakaria[4)], Endang S Rahayu[2)]
[1)]Faculty of Food Technology, Widya Mandala Surabaya Catholic University, Surabaya, Indonesia
[2)]Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia
[3)]Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia.
[4)]School of Biological Science, Universiti Sains Malaysia, Penang, Malaysia
*E-mail: s_ristiarini@yahoo.com

Abstract

Angkak is fermentation product from *Monascus* that used rice as its substrate. This product is used as natural food coloring and seasoning. The aims of this research were to examine the correlation between red color with citrinin content and to characterize *Monascus* isolates from angkak in Indonesia. Thirty samples were collected from different sites in Indonesia. Color values assesment using chromameter were composed of lightness (L*), redness (a*) and yellowness (b*). The red color (a*) of angkak was around 13.06 ± 0.677 – 19.03 ± 0.547. Meanwhile using ELISA method, we found that the angkak samples contain citrinin around 25.85 $\pm$ 5.33 ppm – 93.13 $\pm$ 3.97 ppm. This result indicated that there were no correlation between red color value and citrinin content of angkak. Forty five isolates were obtained during this study, and based on phenotypic characters such as colony diameter, pigmentation, aerial mycelium, cleistothecium diameter, size spore and ascomata produced, these isolates are diverse, however according to molecular character no species diversity. Based on molecular characteristics, either using primer ITS or partial beta tubulin, all isolates were identified as *Monascus purpureus*. Meanwhile, determination of genetic variation analysis were carried out using RAPD method. This result indicated that there were diversity of these isolates.

Keywords: *angkak, Monascus purpureus, red color, citrinin*

P-21

Application of Silica from Rice Hull Ash in Immobilization of Polyclonal AFB1-Antibody for Immunoaffinity Column Clean-Up

Deni Pranowo[1)], Romsyah Maryam[2)], Nuryono[1)], Ali Agus[3)], and FMC Sigit Setyabudi[4*)]

[1)] Department of Chemistry, Faculty of Mathematic and Natural Sciences, Universitas Gadjah Mada

[2)] Toxicology and Mycology Research Group, Indonesian Research Centre for Veterinary Science

[3)] Department of Animal Science and Husbandry, Faculty of Animal Husbandy, Universitas Gadjah Mada

[4)] Department of Food and Agricultural Product Technology, Faculty of Agicultural Technology, Universitas Gadjah Mada

*E-mail: francis.setyabudi@ugm.ac.id

Abstract

Aflatoxin B1 (AFB1) is the most harmful mycotoxin that often contaminate agricultural product used as feedstuffs such as palm kernel cake (PKC) and copra meal (CM). High performance liquid chromatography – fluorescence (HPLC-FLD) usually applied in AFB1 analysis. However, the HPLC-FLD require purification process to eliminate matrix interferences and concentrate the analyte using immunoaffinity column (IAC). IAC consists of organic polymer sorbent such as sepharose gel to immobilize AFB1 antibody. However, the affinity of organic polymer sorbent and antibody is unstable and easily degraded due to organic solvent during elution process. This study was aimed to produce solid phase extraction column from silica material modified with AFB1 polyclonal antibody (SiA-AFB1) under sol-gel method. The source of silica was originated from rice hull ash and successful to apply for the support matrix of immobilization AFB1 antibody in SiA-AFB1 column. Immobilization of AFB1 antibody under sol-gel method provide appropriate time (5-10 minutes) in order to add and maintain the stability of AFB1 (pH~7) prior to addition on sodium silicate achieving aqua gel phase. Reducing water content in aqua gel phase to reach xerogel phase was taken under cold temperature (4°C) and acceptable incubation time (48 hours). This study was able to provide SiA-AFB1 column that potential to apply in the purification method based on immunoassay prior AFB1 analysis using HPLC-FLD. The average recovery were 56.5, 93.7 and 99.3% for batch 2, 3, and 4 respectively but the first batch columns were very low recovery. The best elution solution for application of the column was MeOH/H2O/HoAc=50/49/1.

Keywords: Immunoafinity Column, Silicate, Aflatoxin B1, Rice Hull Ash

P-22

Simultaneous Determination of Co-occurring Mycotoxins in Maize from West Java by Liquid Chromatography/Tandem Mass Spectrometry

Hasim Munawar[1)], Veronica Lattanzio[2)], Biancamaria Ciasca[2)], Giuseppe Panzarini[2)], Sri Rachmawati[1)], Michelangelo Pascale[2)]

[1)]Toxicology Laboratory, Indonesian Research Centre for Veterinary Science (IRCVS)
Jalan R.E. Martadinata No 30 Bogor 16114, Indonesia

[2)]Institute of Sciences of Food Production (ISPA), National Research Council (CNR),
Via Amendola, 122/O - 70126 Bari (BA), Italy

*E-mail: -

Abstract

The presence of mycotoxins in animal feed can affect not only animal health but also quality and safety of animal products such as milk, eggs and meat. The aim of the research was to investigate the co-occurrence of mycotoxins, namely aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2), nivalenol (NIV), deoxynivalenol (DON), T-2 and HT-2 toxins, zearalenone (ZEA), fumonisins (FB1 and FB2), and ochratoxin A (OTA) in maize samples by LC-MS/MS after immunoaffinity column clean-up. A total of twenty-four samples collected in West Java, Indonesia, were selected from a large number of samples on the basis of aflatoxin contamination, between 1 and 430 µg/kg total aflatoxins, previously determined by HPLC-FD (AOAC Official Method No. 2005.08). Ground samples were extracted by two sequential extractions with water then methanol and cleaned up by multi-antibody immunoaffinity columns (Myco6in1+TM, Vicam, a Waters Business). Furthermore, multi-mycotoxin analysis were carried out with a QTrap MS/MS system (Applied Biosystems) equipped with an electrospray (ESI) interface and a micro-LC system (Agilent Technologies). Results showed that all samples were contaminated by AFB1, AFB2, FB1 and FB2 with mean values of 116 µg/kg (median 78 µg/kg), 9.6 µg/kg (median 5.6 µg/kg), 1330 µg/kg (median 733 µg/kg), 763 µg/kg (median 382 µg/kg), respectively. OTA was found in twelve samples with mean value of 6.5 µg/kg (median 1.2 µg/kg). Only three samples resulted contaminated with DON and ZEA (mean values of 62 µg/kg and 22 µg/kg, respectively) and one sample with T-2 and H-2 toxins at levels of 7 µg/kg and 10 µg/kg, respectively. AFG1, AFG2 and NIV were not found in all samples. In conclusion, maize samples were found to be contaminated not only by aflatoxins but also by other mycotoxins naturally occurring in maize. Therefore, more attention should be paid in national monitoring programmes, not only to aflatoxins but also to other major mycotoxins

Keywords: maize, mycotoxins, co-occurrence, LC-MS/MS

P-23

Aflatoxin Levels of Foods and Feeds in the Philippines

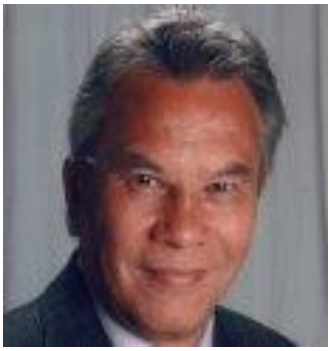
Ma. Aussielita L. Lit
ASTS Analytical Solutions & Technical Services
General Santos City, Philippines
E-mail: ma.aussielita.lit@gmail.com

Abstract

Globally, climate change and weather disturbances have brought increased risk in food consumption. In the Philippines, where environmental conditions of high temperature and relatively high humidity exist, the production of mycotoxin-free food and feed products can never be attained. However, to ensure limited risk in the consumption of food and feed products placed in the market, the production process adapted by manufacturing facilities by the exercise of Good Manufacturing Practice (GMP), Hazard Analysis Critical Control Point (HACCP) and Food Safety Management Systems (FSMS) enabled them to produce food products with acceptable levels of mycotoxin. Mycotoxin contaminated grains, seeds and groundnuts posed a serious problem in the Philippine export. Rice, corn, coconut and peanuts comprised bulk of Philippine export products to US and Europe with set limit of 20 ppb and <5.0 ppb aflatoxin level, respectively. Brown or unpolished rice was found to have higher aflatoxin level of 3.0 ppb compared to polished rice of < 1.0ppb. The highest total aflatoxin level of 11 ppb was contained in the hull, bran and settled dust after the milling process. The milling process reduced aflatoxin level by 68% in regular milled rice and 82% in well milled rice. In oilseed products like coconuts, severity of aflatoxin contamination is high as well as the likelihood of its occurrence. In the process of manufacture, sorting reduced contamination significantly. Results showed that 80 to 95% of the aflatoxin was found in the meal and when pressing is applied, aflatoxin in crude oil was higher. Further reduction of aflatoxin level of 25 to 65% was achieved by the bleaching and deodorizing steps. The final product of coconut oil was found within the acceptable level of <5.0 ppb. Of equal importance to rice and coconut is corn, being utilized for food and feed to humans and animals. Corn is grown all throughout the Philippines with aflatoxin and fumonisin to be the most dominant to occur during storage with maximum levels as high as 270 ppb and 1820 ppb. Detoxification of corn can be achieved during its processing however the incorporation of Bt genes in corn also showed reduction in levels of aflatoxin and fumonisin due to the inhibition of growth the aflatoxin producing fungi. As the global market demand for high quality, safe and nutritious foods, the food and feed industry deem it necessary that strict adherence to agricultural and manufacturing processes be carried out during production. Most steps in production ensure reduction on mycotoxin levels of each commodity for animal and human consumption.

CURRICULUM VITAE

	**Emilia Rico-Munoz, Dr.** BCN Research Laboratories, Inc., 2491 Stock Creek Blvd., Rockford, TN 37853, U.S.A. (800) 236-0505/(865) 573-7511 emilia.rico@bcnlabs.com
	**Giancarlo Perrone, Dr.** Institute of Sciences of Food Production-CNR, Bari - Italy +390805929363 ; +39.3288781051 giancarlo.perrone@ispa.cnr.it
	**Jens C. Frisvad, Prof. Dr.** DTU Systems Biology, Søltofts Plads, Building 221, Technical University of Denmark (DTU), DK-2800 Kgs. Lyngby, Denmark, Phone +45 45 25 26 26; Fax +45 45 88 49 22 jcf@bio.dtu.dk
	**Latiffah Zakaria, PhD** School of Biological Sciences, Universiti Sains Malaysia, 11800 USM,Penang, Malaysia Phone +604-6533506 (office) / +6 013-4238090 Fax +6 04-6565125 Lfah@usm.my / latiffahz@yahoo.com

	**Ludwig Niessen, Prof. Dr.** Technische Universität München, Lehrstuhl für Technische Mikrobiologie, Gregor-Mendel-Straße 4, 85354 Freising, Germany niessen@wzw.tum.de
	**Naresh Magan, Prof. DSc** Environmental Science and Technology Department School of Applied Sciences, Cranfield University, Cranfield, Bedford MK43 0AL, U.K. +44 (0)1234758308 n.magan@cranfield.ac.uk
	**Rindit Pambayun, Prof. Dr.** Professor of Food Science and Technology at Sriwijaya University The President of Indonesia Association of Food Technologists (IAFT, PATPI) Address: Jl. Raya Palembang-Prabumulih Km. 32 Ogan Ilir, Inderalaya, 30662, Sumatera Selatan Tel. +62711-580664 Fax. +62711-320310 rpambayun@yahoo.com
	**Robert Archibald Samson, Prof. Dr.** CBS Fungal Biodiversity Centre Uppsalalaan 8, 3584 CT Utrecht, The Netherlands +31302122656 r.samson@cbs.knaw.nl ; rasamson@zonnet.nl

	**Roy Alexander Sparringa, Dr.** National Agency of Drug and Food Control Republic of Indonesia roy.sparringa@pom.go.id , sparringa@gmail.com
	**Su-Lin Leong, Dr.** Department of Microbiology, Swedish University of Agricultural Sciences, Upssala, Sweden
	**Ulf Thrane, Prof. Dr.** DTU Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark ut@bio.dtu.dk
	**Warapa Mahakarnchanakul, PhD** Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, 50 Ngamwongwan road, Jatujak, Bangkok, Thailand 10900 +66832596363 fagiwpm@ku.ac.th

	**Endang Sutriswati Rahayu, Prof.** Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada Jl. Flora, Bulaksumur, Yogyakarta 55281, Indonesia +628122690013 endangsrahayu@ugm.ac.id ; endangsrahayu@yahoo.com
	**FMC Sigit Setyabudi, Dr.** Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada Jl. Flora, Bulaksumur, Yogyakarta 55281, Indonesia francis.setyabudi@ugm.ac.id ; francis.setyabudi@gmail.com
	**Suparmo, Dr.** Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada Jl. Flora, Bulaksumur, Yogyakarta 55281, Indonesia suparmo@ugm.ac.id
	**Lilik Sutiarso, Prof. Dr.** Dean of Faculty of Agricultural Technology, Universitas Gadjah Mada Jl. Flora, Bulaksumur, Yogyakarta 55281, Indonesia lilik-soetiarso@ugm.ac.id

	**Sri Raharjo, Prof. Dr.** Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada Jl. Flora, Bulaksumur, Yogyakarta 55281, Indonesia +62818460890 sraharjo@ugm.ac.id.
	**Fedik Abdul Rantam, Prof. Dr.** Department of Veterinary Science and Microbiology, Faculty of Veterinary, Universitas Airlangga, Surabaya, Indonesia fed-ar@unair.ac.id
	**Chusnul Hidayat, Dr.** Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada Jl. Flora, Bulaksumur, Yogyakarta 55281, Indonesia chusnulhi@ugm.ac.id ; chusnulhi@yahoo.com

LIST OF INVITED SPEAKER, ORAL PRESENTER, POSTER PRESENTER AND PARTICIPANT

No.	Name	Instansi	Email	Phone
1	Aan Sofyan	Faculty of Agricultural Technology, Universitas Gadjah Mada	sofyanspt@gmail.com	087836464054
2	Abdi Christia	Universitas Surya, Jl. Boulevard Gading Serpong Blok O/1, Summarecon Serpong, Tangerang	abdi.christia@surya.ac.id	081259137285
3	Abdul Syukur Lumbessy	Universitas Gadjah Mada	abdulsyukur.ugm@gmail.com	081281489492
4	Adalfredo Do Rosario Ferreira	Ministry Of Agriculture And Fisheries (Timor-Leste), Comoro Road, Comoro, Dili, Timor-Leste	ferreira310@gmail.com	67077312325
5	Afnita Nur Amalina	Faculty of Agricultural Technology, Universitas Gadjah Mada	afnita.amalina@gmail.com	081328025184
6	Agnes Murdiati	Faculty of Agricultural Technology, Universitas Gadjah Mada	amurdiati@yahoo.com	08122703108
7	Agung Nur Mustofa	PT Tiga Pilar Sejahtera, Sangiran, Solo		
8	Agustina Asri Rahmianna	Res. Inst. For Legume and Tuber Crops, Balitkabi, Indonesia	anna_rahmianna@yahoo.com	
9	Agus Setiyoko	Faculty of Agricultural Technology, Universitas Gadjah Mada	elraka77@gmail.com	085743834043
10	Agus Subagyo	Balai Besar Pengawas Obat dan Makanan di Semarang, Jl. Madukoro Blok AA-BB No. 8 Semarang	likpomsm@yahoo.com	08122819165
11	Agus Sudono	Universitas Pendidikan Indonesia, Jl. Dr. Setiabudhi No. 229 Bandung	sudono@upi.edu	0853 20083513
12	Agus Wijanarka	Jurusan Gizi - Politeknik Kesehatan Kemenkes Yogyakarta, Jl. Tata Bumi No. 3 Yogyakarta	agusw_jogja@yahoo.co.id	08164225088

13	Agussalim M	Politeknik Pertanian Negeri Pangkep, Jl. Poros Makassar-Parepare Km 83 Mandalle Pangkep Sulawesi Selatan	agussalim_matti@yahoo.com	085299914017
14	Ahmad Safarudin	Faculty of Agricultural Technology, Universitas Gadjah Mada	semartobat.adv@gmail.com	0857 43207817
15	Ahmad Yudis Mahardhika	Microbiology of Agriculture Universitas Gadjah Mada	dhika.dhikong@gmail.com	0838 67699307
16	Aldila Din Pangawikan	Faculty of Agricultural Technology, Universitas Gadjah Mada	pangawikan@gmail.com	085878135607
17	Alfi Nur Rochmah Rochmah	Faculty of Agricultural Technology, Universitas Gadjah Mada	finur88@yahoo.com	085725120133
18	Amalia Wahyuningtyas	Faculty of Agricultural Technology, Universitas Gadjah Mada	amaliawahyuningtyas31@gmail.co.id	085768257781
19	Andika Sidar	Faculty of Agricultural Technology, Universitas Gadjah Mada	andikasidar@yahoo.com	085743436223
20	Andre Putra	Universitas Gadjah Mada	andre.tp89@gmail.com	085743001687
21	Angga Pratama	Universitas Gadjah Mada	Anggapratama422@ymail.com	082373543342
22	Ani Widiastuti	Faculty of Agriculture, Universitas Gadjah Mada	aniwidiastuti@ugm.ac.id	
23	Aninda Sidar	Dept of Agricultural Microbiology, Faculty of Agriculture, Universitas Gadjah Mada	nindasidar@yahoo.com	085643402731
24	Anny Yanuariati	Faculty of Agricultural Technology, Universitas Gadjah Mada	annyyanuariati@yahoo.com	081367740860
25	Annytha Detha	Nusa Cendana University, Kupang	annytha.detha@gmail.com	089624348969
26	Aprilita Kusumawardhani	Faculty of Agricultural Technology, Universitas Gadjah Mada	aprilitakusumawardhani@gmail.com	087738306929
27	Apriyanto D Nugroho	Badan Ketahanan Pangan, Kementrian Pertanian, Jakarta	apriyanto.dnugroho@gmail.com	

28	Ardiba Rakhmi Sefrienda	Faculty of Agricultural Technology, Universitas Gadjah Mada	ardiba2@gmail.com	085327858828
29	Awanwee Petchkongkaew	Department of Food Science and Technology,Faculty of Science and Technology, Thammasat University, 99 Mhu 18 Paholyothin road, KhongLuang, Pathumthani, 12120, Thailand	awanwee@tu.ac.th	66872545445
30	Ayu Arum	Universitas Gadjah Mada	ayu.23.cute@gmail.com	082245399099
31	Bagus Fajar Pamungkas	Fakultas Perikanan dan Ilmu Kelautan Universitas Mulawarman, Jl Gn. Tabur Kampus Gn. Kelua Samarinda	fajar_gus@yahoo.com	08125550855
32	Baiq Rani Dewi Wulandani	Mataram University, Majapahit 62 Mataram-West Nusa Tenggara	rani.wulandani@gmail.com	082138438432
33	Bambang Dwi Wijatniko	Faculty of Agricultural Technology, Universitas Gadjah Mada	nicodw92@gmail.com	085266978630
34	Bangun Nusantoro	Faculty of Agricultural Technology, Universitas Gadjah Mada	bangun.nusantoro@ugent.be	087738347775
35	Beta Alfisyahri Putri	Faculty of Agricultural Technology, Universitas Gadjah Mada	beta.alfisyahriputri93@gmail.com	085641422277
36	Betty Nurhayati	Institut Teknologi Bandung, Jalan Ganesa no. 10 Bandung	betty.nurhayati@gmail.com	082129049111
37	Betty Sri Laksmi Suryaatmadja Jenie	Department of Food Science and Technology, Bogor Agricultural University, Bogor	harsikusumaningrum@yahoo.com	
38	Bondan	PT. DIPA Puspa Labsains	bondan.sugiarto@dipa.co.id	08881683989
39	Calvin Jatmika Pang	PT Megah Sejahtera Scientific	marketing@megahsejahtera.com	08385890092
40	Carolina Sisca	Faculty of Agricultural Technology, Universitas Gadjah Mada	ceesde.carolina.sisca@gmail.com	085725104495

41	Caroline H. Santoso	PT. Thermalindo Sarana Laboratoria Jakarta Head Office Jl. Daan Mogot II No. 100 D-E-J, Jakarta 11510, Indonesia	caroline@thermalindo.com	081808810598
42	Catur Budi Handayani	University of Veteran Bangun Nusantara Sukoharjo, Jalan Letjen S. Humardani No. 1 Jombor Sukoharjo		085728420771
43	Chusnul Hidayat	Faculty of Agricultural Technology, Universitas Gadjah Mada	chusnulhi@ugm.ac.id	081578715684
44	Citra	PT. DIPA Puspa Labsains	Citra.apriliasanti@dipa.co.id	085742228929
45	Citra Merryna Kinanthi	Brawijaya University, Jl. Veteran, Malang, Jawa Timur 65145	melodykinanthi@gmail.com	085646443464
46	Dadik Pantaya	Department of Animal Science, State Polytechnic Jember, Jember, Indonesia, 68121		
47	Damiana Candrasari	Fakultas Farmasi, Universitas Sanata Dharma, Campus III Paingan, Maguwoharjo, Depok, Sleman, Yogyakarta 55282, Indonesia	damiana.candrasari@gmail.com	081578061531
48	Deni Pranowo	Department of Chemistry, Faculty of Mathematic and Natural Sciences, Universitas Gadjah Mada	maspranowo@yahoo.com	
49	Denny Nyotohadi	PT Megah Sejahtera Scientific	technical.support@megahsejahtera.com	087881749149
50	Devi Yuni Susanti	Faculty of Agricultural Technology, Universitas Gadjah Mada	deyusan11@gmail.com	031327196595
51	Dewi Anita Bakara	Orang Tua Group, Jl. Daanmogot km 14 no 26, Jakarta Barat	dewianitabakara@yahoo.com	08111730327
52	Diana Nur Fitri	Faculty of Agricultural Technology, Universitas Gadjah Mada	dyananf@gmail.com	085779337757

53	Dinda Maulida	PT AraSains, Kompleks Ruko Gading Bukit Indah Blok F No.5 Jl.Bukit Gading Raya. Kelapa Gading, Jakarta 14240		
54	Dita Kristanti	Faculty of Agricultural Technology, Universitas Gadjah Mada	dita.kristanti@gmail.com	087738448967
55	Donowati Tjokrokusumo	Badan Penerapan dan Pengkajian Teknologi, Jakarta	dtjokrokusumo@yahoo.com	08161437096
56	Eka Mulyanti	Balai Besar Pengawas Obat dan Makanan, Pontianak	sweet_coster@yahoo.com	0817 465945
57	Eko Basuki	University of Mataram, Jl.Majapahit no 62 Mataram 83125	ekobasuki10@gmail.com	081907727455
58	Elisa Nurnawati	FMIPA UNSRI, Kampus Indralaya Ogan Ilir Sumatera Selatan	nurnawati@yahoo.com	08127382564
59	Elisabet Tangkonda	Fakultas Kedokteran Hewan Universita Nusa Cendana, Jl. Adi Sucipto Penfui Kupang, Nusa Tenggara Timur	elisabet_tangkonda@yahoo.co.id	082147574489
60	Ella Saparianti	Brawijaya University, Jl. Veteran, Malang, Jawa Timur 65146	ella_thpub@yahoo.com	0818531560
61	Emily Tay	DuPont Company (S) Pte Ltd, 1 Harbourfront Place, #11-01, Harbourfront Tower One Singapore 098633	Emily-Boon-Hui.Tay@dupont.com	6598522897
62	Emma Riftyan	Faculty of Agricultural Technology, Universitas Gadjah Mada	emmariftyan@gmail.com	081513744337
63	Endang Kusdiyantini	Fac. of Science & Mathematics, Diponegoro University, Jl. Jl. Prof. Sudarto SH, Tembalang Semarang 50275	kusdiyantini@undip.ac.id	0811288428
64	Endang S. Rahayu	Faculty of Agricultural Technology, Universitas Gadjah Mada	endangsrahayu@yahoo.com	08122690013
65	Enny Purwati Nurlaili	Untag Semarang, Jl. Pawiyatan Luhur, Bendan, Semarang	enny.purwati@gmail.com	08122869216

66	Eny Orinda	Faculty of Agricultural Technology, Universitas Gadjah Mada	e.orinda@yahoo.com	087839412389
67	Erlina Nafulani	Faculty of Agricultural Technology, Universitas Gadjah Mada	erlinanafulani@gmail.com	085729334941
68	Erna Ayu D	PT Tiga Pilar Sejahtera, Sangiran,Solo		
69	Fenty Nurtyastuti Eka Pertiwi	Brawijaya University, Jl. Veteran, Malang, Jawa Timur 65147	fentyastuti@gmail.com	085647409373
70	Fika Almira	Faculty of Agricultural Technology, Universitas Gadjah Mada	almirafika@gmail.com	089692112141
71	Fitri Agustina	Balai Pengawas Obat dan Makanan Serang, Jl. Syech Nawawi Al Bantani Cipocok Jaya Serang Banten	fitri.mudhofir@gmail.com	085729913248
72	Fitri Komala Sari	Faculty of Agricultural Technology, Universitas Gadjah Mada	fikom.mala@gmail.com	08565332867
73	Fitri Nadifah	Prodi D3 Analis Kesehatan STIKES Guna Bangsa Yogyakarta, Jl. Ring road Utara, Condong Catur, Depok, Sleman	fitri.nadifah@gmail.com	081328280691
74	Florentinus Dika	Fakultas Farmasi Universitas Sanata Darma, Paingan Maguwoharjo Depok Sleman	dikaocta@usd.ac.id	085729071377
75	FMC Sigit Setyabudi	Faculty of Agricultural Technology, Universitas Gadjah Mada	francis.setyabudi@gmail.com	08985127938
76	Fungki Sri Rejeki	Universitas Wijaya Kusuma Surabaya, Dukuh Kupang XXV/54 Surabaya	fungki_sby@yahoo.com	08165449820
77	Gabriela Elysia Hetharia	Faculty of Agricultural Technology, Universitas Gadjah Mada	gelysia1705@gmail.com	081344657605
78	Gayuh Rahayu	Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural9University, Darmaga Campus, Bogor 16680, Indonesia	gayuhrahayu@gmail.com	

79	Gener Gregorio	Department of Biological Sciences, Central Luzon State University, Phillipines	gdgregorio@yahoo.com	639173433883
80	Giancarlo Perrone	Institute of Sciences of Food Production, Bari, Italy	giancarlo.perrone@ispa.cnr.it	393288781051
81	Hanim Zuhrotul Amanah	Faculty of Agricultural Technology, Universitas Gadjah Mada		
82	Harsi Dewantari Kusumaningrum	Department of Food Science and Technology, Bogor Agricultural University, Darmaga Campus, PO Box 220 Bogor 16002	harsikusumaningrum@yahoo.com	081311210180
83	Harsojo	PATIR-BATAN, Jl. Lebak Bulus Raya 49 Jakarta Selatan	apu.harsojo@yahoo.com	08121895046
84	Hasim Munawar	IRCVS, IAARD, Ministry of Agriculture, Jl.R.E.Martadinata No 30 Bogor 16114	seemunawar@gmail.com	087873790639
85	Hatifah Prapti L	Balai Besar Pengawas Obat dan Makanan, Yogyakarta	hatifah_pl@yahoo.com	0817 465945
86	Heni Adhianata	Universitas Gadjah Mada	heni_adhianata@yahoo.co.id	085646747821
87	Hermin	PT Megah Sejahtera Scientific	sales.megahsejahtera@gmail.com	081553125220
88	Herni Purwantari	Faculty of Agricultural Technology, Universitas Gadjah Mada	herniptari@gmail.com	081341752990
89	Heru Susanto	Faculty of Agricultural Technology, Universitas Gadjah Mada		
90	Hibi Koma	Faculty of Agricultural Technology, Universitas Gadjah Mada	maukobihiuma@yahoo.co.jp	081227148445
91	Hikmah Yulisari	Universitas Gadjah Mada	hikmahyuliasari@gmail.com	083863332809
92	Hilary Evi	PT Megah Sejahtera Scientific	hilary.tan@megahsejahtera.com	

93	Hoa Bui Thi Quynh	PhD student, Food Science and Technology, Kasetsart University, 50 Ngamwongwan Road, Lad Yao, Chatuchak, Bangkok 10900, Thailand	btqhoa71@gmail.com	6682070668
94	I Gusti Ayu KD Diah Puspawati	Kampus Universitas Udayana Bukit Jimbaran, Badung 80361, Bali	dipa_ftp@yahoo.com	081237455665
95	Ichda Chayati	Yogyakarta State University, Karangmalang Yogyakarta	ichdach@yahoo.co.id	085291817100
96	Idya Rachmawati	Faculty of Agricultural Technology, Universitas Gadjah Mada	dyarachma@gmail.com	085655025077
97	Indun Dewi Puspita	Dept. Fisheries, Faculty of Agriculture, Universitas Gadjah Mada	dune_28@yahoo.com	08164225599
98	Indyah Sulistya Utami	Faculty of Agricultural Technology, Universitas Gadjah Mada	indyahsulistya@yahoo.com	08121559788
99	Iskandar Azmy	Faculty of Agricultural Technology, Universitas Gadjah Mada	iskandar.azmy@gmail.com	085664226001
100	Isnaillaila Paramasari	Faculty of Agricultural Technology, Universitas Gadjah Mada	paramasarii@gmail.com	085727375770
101	Isti Handayani	Universitas Jenderal Soedirman, Jl. HR Boenyamin 708 Purwokerto 53122.	isti_handayaniunsoed@yahoo.co.id	08122733084
102	Isworo Rukmi	Diponegoro University, Jl. Prof. Sudarto SH. Tembalang, Semarang 50275	rukmi_is@yahoo.com	0811288428
103	Ita Zuraida	Fakultas Perikanan dan Ilmu Kelautan Universitas Mulawarman, Jl Gn. Tabur Kampus Gn. Kelua Samarinda	itazuraida@gmail.com	081346563692
104	Jacquelyn Peran	Department of Science and Technology IV-A, Jamboree Rd. Timugan Los Banos, Laguna, Phillipines	jacque_peran@yahoo.com	639167113435
105	Jaka Widada	Dept of Agricultural Microbiology, Faculty of Agriculture, Universitas Gadjah Mada	jwidada@gmail.com	081578800227

106	Jeannie Setiyanti Siagian	Faculty of Agricultural Technology, Universitas Gadjah Mada	siagian.jeannie@yahoo.com	081344035635
107	Jens C. Frisvad	Technical University of Denmark	jcf@bio.dtu.dk	
108	Jessil Ann Pajar	MSU-Iligan Institute of Technology, Andres Bonifacio Avenue, Tibanga, 9200 Iligan City, Philippines	jessilann.pajar@g.msuiit.edu.ph	639262432326
109	Jiratchaya Kuanpan	Chulabhorn Science High School Lopburi, 216 Mhu 1 Huaypong Khoksamrong Lopburi 15120 Thailand	parn_jiratchaya@hotmail.com	66868040176
110	Juli Santoso	UPN "Veteran" Jawa Timur, Jl. Raya Rungkut Madya, Gunung Anyar, Surabaya	julipikir.upn@gmail.com	08123224601
111	Karen Ong	Romer Labs Malaysia Sdn. Bhd., Suite 218, Eureka Complex, Universiti Sains Malaysia, 11800 Penang, Malaysia	karen.ong@romerlabs.com	60165342282
112	Khairul Aripin	Faculty of Agricultural Technology, Universitas Gadjah Mada	khairularipin@yahoo.com	0852 28190148
113	Khanin Suksomsak	Chulabhorn Science High School Lopburi, 216 Mhu 1 Huaypong Khoksamrong Lopburi 15120 Thailand	kon-321-@hotmail.com	66861256453
114	Kurnia Ritma Dhanti	Master Students, Biotechnology, Universitas Gadjah Mada	geopelia_dhanti@yahoo.com	085728272762
115	Laras Rianingsih	FPIK Universitas Diponegoro, Jl Prof Soedharto SH. Tembalang Semarang	larasrianingsih2008@gmail.com	081328685010
116	Latiffah Zakaria	Universiti Sains Malaysia	lfah@usm.my	
117	Lia Astriana	Sekretariat Jejaring Intelijen Pangan (JIP) Direktorat Surveilan dan Penyuluhan Keamanan Pangan, Badan Pengawas Obat dan Makanan, Jl Percetakan Negara 23 Jakarta, Telp. 021-42803516 Fax. 021-42878701	liastriana@yahoo.com	021-42803516

118	Lia Ekawati	PT. Thermalindo Sarana Laboratoria Jakarta Head Office Jl. Daan Mogot II No. 100 D-E-J, Jakarta 11510, Indonesia	lia@thermalindo.com	081325030985
119	Lilis Suryani	Universitas Muhamadiyah Yogyakarta, Jalan Lingkar Barat, Taman Tirto, Kasihan, Bantul, Yogyakarta	lilis_fkumy@yahoo.co.id	0274387656
120	Ludwig Niessen	Technische Universität München, Lehrstuhl für Technische Mikrobiologie, Gregor-Mendel-Straße 4, 85354 Freising, Germany	niessen@wzw.tum.de	
121	Luis De Almeida	Seeds Of Life Program (Timor-Leste), Comoro Road, Comoro, Dili, Timor-Leste	almeida_laclubar@yahoo.com	0670 77262971
122	M. Arya Pradana	Dept of Agricultural Microbiology, Faculty of Agriculture, Universitas Gadjah Mada	muhammadarya.arya@gmail.com	085268401409
123	Ma. Aussielita L. Lit	Analytical Solutions and Technical Services, Unit 206-207 Valley High, General Santos City, Phillipines	ma.aussielita.lit@gmail.com	639155784740
124	Maridian Widiyasworo	PT AraSains, Kompleks Ruko Gading Bukit Indah Blok F No.5 Jl.Bukit Gading Raya. Kelapa Gading, Jakarta 14241	maridian.woro@arasains.co.id	082123440722
125	Marischa Lissanta	Faculty of Agricultural Technology, Universitas Gadjah Mada	mlissanta@gmail.com	087738669731
126	Marlia Singgih Wibowo	Institut Teknologi Bandung, Jalan Ganesa no. 10 Bandung	marlia@fa.itb.ac.id	0816611886
127	Martono Wiyono	PT. Garuda Food, Jl. Bintaro Raya No. 10 A Keb. Lama, Jakarta Selatan	martono.wiyono@garudafood.co.id	087876305999
128	Marwati Munir	Universitas Mulawarman Fakultas Pertanian, Kampus Gunung Kelua Samarinda - Kalimantan Timur	marwatiwawa.unmul@gmail.com	081328675998
129	Mary Astuti	Faculty of Agricultural Technology, Universitas Gadjah Mada	ana_food@yahoo.com	085729707222

130	Mastiur Hutagaul	National Agency of Drugs And Foods Controls, Percetakan Negara No. 23. Johar Baru Jakarta Pusat 10560	mastiur.hutagaol25@gmail.com	081383612167
131	Mercy Taroreh	Fakultas Pertanian USRAT, Jl. Kampus USRAT Bahu, Monado	mercytaroreh@yahoo.com	081225433762
132	Michelle Yit	PT AraSains, Kompleks Ruko Gading Bukit Indah Blok F No.5 Jl.Bukit Gading Raya. Kelapa Gading, Jakarta 14240	michelleyit@arasains.co.id	
133	Muhammad Irkham Huseini	Microbiology of Agriculture Universitas Gadjah Mada	irkham.mikro@gmail.com	08562851740
134	Muhammad Nur Cahyanto	Faculty of Agricultural Technology, Universitas Gadjah Mada	mcahyanto@yahoo.com	085228064602
135	Nampiah Sukarno	Bogor Agricultural University, Biology Department, FMIPA-IPB, Darmaga Campus, Bogor16880, Indonesia	nampiahsukarno@yahoo.com	0811116376
136	Nani Ratnaningsih	Yogyakarta State University, Kampus Karangmalang Yogyakarta	nratnaningsih@yahoo.com	085643011397
137	Nanik Suhartatik	Fakultas Teknologi Pertanian, Universitas Slamet Riyadi Surakarta, Jl. Sumpah Pemuda 18 Joglo Kadipiro Surakarta 57136	n_suhartatik@yahoo.com	081578800227
138	Naresh Magan	Cranfield University, Applied Mycology Group, Cranfield, Bedford MK43 0AL, U.K.	n.magan@cranfield.ac.uk	
139	Nazarni Rahmi Syamsuri	Faculty of Agricultural Technology, Universitas Gadjah Mada	nazarni.rahmi@yahoo.com	08152137489
140	Nicole Angelique Sanchez	UPLB, Jose R Velasco Ave, Los BaÃ±os 4031,Laguna, Philippines	nikisanchez@outlook.com	639179769033
141	Niken W. P.	Faculty of Agricultural Technology, Universitas Gadjah Mada		

142	Ninoek Indriati	Reserach Center Development Marine & Fisheries Product Processing & Biotechnology, Jl. KS. Tubun Petamburan VI Jakarta Pusat	indriati_ninoek@yahoo.co.id	08161812817
143	Nova Wahyu Pratiwi	Universitas Riau	nova.wahyu.pratiwi@gmail.com	
144	Novento Wijayaputra	PT Tigaraksa Satria, Tbk, Jln. Cangkringan km 1.5 Dhuri Tirtomartani Kalasan Sleman Yogyakarta 55571	n-wijayaputra@tigaraksa.co.id, veven.wijaya@gmail.com	081326122172
145	Nugroho Indrotristanto	Sekretariat Jejaring Intelijen Pangan (JIP) Direktorat Surveilan dan Penyuluhan Keamanan Pangan Badan Pengawas Obat dan Makanan Jl Percetakan Negara 23 Jakarta	Nindrotristanto@gmail.com	021-42803516
146	Nuki Bambang Nugroho	Balai Pengkajian Bioteknologi - BPPT, Gedung 630, Kawasan PUSPIPTEK, Setu, Tangerang Selatan 15314	nbnugroho@gmail.com	08159607599
147	Nur Arifah Qurota A'yunin	Faculty of Agricultural Technology, Universitas Gadjah Mada	nurarifahqurota@ymail.com	085227849600
148	Nur Fajriani	Faculty of Agricultural Technology, Universitas Gadjah Mada	fary_potter07@yahoo.com	085242190499
149	Nurin Imana	Universitas Gadjah Mada	nurin.imana@gmail.com	085743868301
150	Okky Setyawati Dharmaputra	SEAMEO BIOTROP, Jalan Raya Tajur Km.6, Bogor 16134, Indonesia	okky@biotrop.org	08161373690
151	Panrapee Iamtaweejaroen	Kasetsart University, 50 Ngam Wong Wan Rd, Lat Yao Chatuchak Bangkok 10900 Thailand	ammy184@hotmail.com	66988291559

152	Parichat Narongplain	Princess Chulabhon's Collage Lopburi, 216 Sub-district : Huaypong, District : Khok-sam-rong Province : Lopburi 15120, Thailand	parichat_tan12@hotmail.com	823598062
153	Peni Setyowati	Faculty of Agricultural Technology, Universitas Gadjah Mada	penisetyawati@yahoo.com	081328778366
154	Pepita Haryanti	Universitas Jenderal Soedirman, Jl. HR Boenyamin 708 Purwokerto 53122.	pita_thpunsoed@yahoo.com	085692521534
155	Phakpoom Kooprasertying	Kasetsart University, 50 Ngam Wong Wan Rd, Lat Yao Chatuchak Bangkok 10900 Thailand	np_kool@hotmail.com	66812598095
156	Poh Hong Goh	Romer Labs Singapore Pte Ltd, Jalan Bukit Merah #08-08, e-Centre@Redhill Building, Singapore 159471	goh.pohhong@romerlabs.com	6591306015
157	Purnama Darmadji	Faculty of Agricultural Technology, Universitas Gadjah Mada		08122787347
158	Qurrotul A'yun	Faculty of Agricultural Technology, Universitas Gadjah Mada	ayunahmada@yahoo.com	085702465252
159	Ratna Wulandari	National Agency of Drug and Food Control, Republik Indonesia, Jl. Percetakan Negara No.23 - Jakarta 10560 Indonesia	nuhamufidah02@gmail.com	0817 465945
160	Retno Widyastuti	Faculty of Agricultural Technology, Universitas Gadjah Mada	widyaastuti_r@yahoo.com	085647358727
161	Retty Ikawati	Faculty of Agricultural Technology, Universitas Gadjah Mada	rettyikawati@gmail.com	081328012655
162	Riana Rachmawati	Faculty of Agricultural Technology, Universitas Gadjah Mada	rianarachmawati@live.com	085747737716
163	Rico Emilia	BCN Research Laboratories, Inc., 2491 Stock Creek Blvd Rockford, TN, USA	emilia.rico@bcnlabs.com	8002360505
164	Rindit Pambayun	Universitas Sriwijaya	rpambayun@yahoo.com	

165	Rini Yanti	Faculty of Agricultural Technology, Universitas Gadjah Mada		
166	Rio Aprianto	Faculty of Agricultural Technology, Universitas Gadjah Mada	rhiyo_ap@yahoo.co.id	081377776030
167	Rita Khairina	Faculty of Fishery Lambung Mangkurat University Banjarbaru, Jl. A.Yani km 36 Banjarbaru	ritasyaiful@yahoo.co.id	081351901904
168	Rizkina Fitriyani	Universitas Gadjah Mada	rizkinafitri@yahoo.co.im	085742022899
169	Robert A. Samson	CBS,The Netherlands	r.samson@cbs.knaw.nl ; rasamson@zonnet.nl	
170	Rohadi	FTP Universitas Semarang, Jl. Soekarno Hatta Semarang	rohadij@yahoo.com	08156520917
171	Rohmat Triyadi	Balai Pengawas Obat dan Makanan Denpasar, Jl. Tjut Nya Dien No. 5 Renon Denpasar	rohmat.triyadi@gmail.com	081804092194
172	Rohmatussolihat	Research Center for Biotechnology-LIPI, Jl. Raya Bogor KM 46 Cibinong	rohmatussolihat@gmail.com	08128310395
173	Rohula Utami	Sebelas Maret University, Jl. Ir. Sutami 36A Kentingan, Surakarta, Jawa Tengah	rohula_utami@yahoo.com	081329669292
174	Rosyidin Tri Brata	PT ISM Tbk - Divisi Bogasari, Gedung Kunci Biru, Bagian Training, Jl. Raya Cilincing No. 1 Tg. Priok Jakarta	isneeny.yatman@bogasariflour.com	
175	Roy Sparringa	National Agency of Drug and Food Control, Republic of Indonesia	roy.sparringa@pom.go.id	
176	Sakina Yepti Kiptiyah	Faculty of Agricultural Technology, Universitas Gadjah Mada	sakinayetikiptiyah@gmail.com	08561799683
177	Santi Ambarwati	SEAMEO BIOTROP, Jalan Raya Tajur Km. 6, Bogor 1634, Indonesia	ambarwati@biotrop.org	08129505270
178	Saptini Mukti Rahajeng	Balai Besar Pelatihan Pertanian Ketindan, Jl. Ketindan No.1 Lawang, Malang	saptinimukti@gmail.com	082141700181

179	Sardjono	Faculty of Agricultural Technology, Universitas Gadjah Mada	sardjon@yahoo.com	081903729556
180	Satriya Abrian	Faculty of Agricultural Technology, Universitas Gadjah Mada	satriyaabrian@gmail.com	087812830745
181	Sellen Gurusmatika	Faculty of Agricultural Technology, Universitas Gadjah Mada	sellen.gurusmatika@mail.ugm.ac.id	081336030072
182	Setyaningrum Ariviani	Departement of Agricultural Technoloy, Faculty of Agriculture, Sebelas Maret University, JL. Ir. Sutami No 36A Kentingan Surakarta	setya_ariviani@yahoo.com	0817271176
183	Silma Awalia	National Agency of Drug and Food Control, Republik Indonesia, Jl. Percetakan Negara No.23 - Jakarta 10560 Indonesia	silma_awalia@yahoo.com	0812 90129110
184	Silvia Agustriana	Faculty of Agricultural Technology, Universitas Gadjah Mada	silviaagustriana80@gmail.com	082374673981
185	Simao Margono Belo	Seeds Of Life Program (Timor-Leste), Comoro Road, Comoro, Dili, Timor-Leste	simaobelo@yahoo.co.id	67075610410
186	Siti Nur Purwandhani	Widya Mataram Yogyakarta University, Ndalem Mangkubumen KT III / 237	maharanividya@gmail.com	08122766074
187	Siti Nurjannah	Faculty of Agricultural Technology, Universitas Gadjah Mada	akar_dandelion@yahoo.com	085743504248
188	Sri Anggrahini	Faculty of Agricultural Technology, Universitas Gadjah Mada	sri_anggrahini2006@yahoo.com	08122798139
189	Sri Hartati	Balai Besar Pengawas Obat dan Makanan di Semarang, Jl. Madukoro Blok AA-BB No. 8 Semarang	srihartati.bpom@gmail.com	08156565215
190	Sri Hartati	Faculty of Agriculture Veteran Bangun Nusantara University Sukoharjo, Jl. Letjen S.Humardani no1 Jombor Sukoharjo	tatik_univet@yahoo.com	085327570375

191	Sri Kanoni	Faculty of Agricultural Technology, Universitas Gadjah Mada	sri_kanoni@yahoo.com	0817272016
192	Sri Rahayu	Faculty of Agricultural Technology, Universitas Gadjah Mada		
193	Sri Naruki	Faculty of Agricultural Technology, Universitas Gadjah Mada	sri_naruki@yahoo.com	08562867478
194	Srie Wulan Wignjo Djoenarto	PT. Thermalindo Sarana Laboratoria Jakarta Head Office Jl. Daan Mogot II No. 100 D-E-J, Jakarta 11510, Indonesia	wulan@thermalindo.com	0811820868
195	Srirana Kongtago	Princess Chulabhorn's College, 216 Mhoo 1, Huaypong, Khok samrong, Lopburi 15120, Thailand	Sriranapccl@gmail.com	0804 358011
196	Sukumar Debnath	Rural Based Preventive Oncology Research Centre, RBPORC, Bokakhat Assam 785612 (Cancelled)	sukumardebnath@rediffmail.com	919954213745
197	Su-Lin Leong	Swedish University of Agricultural Sciences, Dept of Microbiology Uppsala, Sweden		
198	Sumartini Tini	Faculty of Agricultural Technology, Universitas Gadjah Mada	Tinny.sumardi@gmail.com	085604666011
199	Suparmo	Faculty of Agricultural Technology, Universitas Gadjah Mada	S_parmo@ugm.ac.id	0811283497
200	Supasalit Chapunya	Princess Chulabhorn's College, 216 Huaypong Khoksamrong, Lopburi 15121, Thailand	ball8762@gmail.com	0875394840
201	Supatmi	PT Tiga Pilar Sejahtera, Sangiran,Solo		
202	Susana Ristiarini	Widya Mandala, Catholic University, Surabaya	s_ristiarini@yahoo.com	
203	Sutardi	Faculty of Agricultural Technology, Universitas Gadjah Mada	sutardi@yahoo.com	

204	Suwatio Santun	Orang Tua Group, Jl. Daanmogot km 14 no 26, Jakarta Barat	suwatio@yahoo.com	08111730331
205	Thammawit Thampitee	Princess Chulabhorn's College, 216 Huaypong Khoksamrong, Lopburi 15120, Thailand	tham_mat@hotmail.com	907988776
206	Thrane Ulf	DTU Systems Biology, Technical University of Denmark, Lyngby, Denmark	ut@bio.dtu.dk	
207	Titiek Farianti Djafaar	The Assessment Institute for Agricultural Technology, Yogyakarta 55584, Indonesia	titiekfd@yahoo.co.id	081578882867
208	Titik Budiati	State Polytechnic of Jember, Jl. Mastrip PO BOX 164 Jember	titik.budiati@gmail.com	081946628655
209	Tri Astuti Rahmawati	Balai POM Pangkal Pinang, Jl. Pulau Bangka, Air itam, Pangkal Pinang	rahmadehade@gmail.com	08117118910
211	Tri Marwati	Balai Besar Penelitian dan Pengembangan Pasca Panen Pertanian Departemen Pertanian, Jl. Tentara Pelajar No. 12 Kampus Penelitian Pertanian Cimanggu Bogor 16111 - Jawa Barat	watipasca@yahoo.com	
212	Tomy Indra Kurniawan	PT Megah Sejahtera Scientific	tomy@biochem-tech.com	085649410707
213	Tutus Gusdinar Kartawinata	Institut Teknologi Bandung, Jalan Ganesa no. 10 Bandung	gusdinar@fa.itb.ac.id	0811237125
214	Tyas Utami	Faculty of Agricultural Technology, Universitas Gadjah Mada	tys_utami@yahoo.com.sg	081328850909
215	Ummu Aimanah	STTP Gowa, Jl. Romanglompoa, Gowa, Sulawesi Selatan	ummuitpn_unhas@yahoo.co.id	081342940280
216	Unnop Tassanaudom	Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, 50 Ngam Wong Wan Rd, Lat Yao Chatuchak Bangkok 10900, Thailand	u_tassana@hotmail.com	0869262662

217	Veriani Aprilia	STIKES Alma Ata Yogyakarta, Jl. Ring Road Barat Daya No 1, Tamantirto, Bantul, Yogyakarta	aprilia.triwanto@gmail.com	081328004575
218	Victor Basuki	DuPont, Jalan Ampera Raya No.9-10, Jakarta Selatan 12550	victor.basuki@dupont.com	08111630280
219	Viphavee Liangraksa	Princess Chulabhorn's College, 216 Huaypong Khoksamrong, Lopburi 15122, Thailand	atom_696@hotmail.com	0841553180
220	Vita Fitriani	Faculty of Agricultural Technology, Universitas Gadjah Mada	vita.fitriani22@gmail.com	085292126983
221	Vita Meylani	Siliwangi University	meylani.vita@yahoo.co.id	085327122811
222	Wagiman	Faculty of Agricultural Technology, Universitas Gadjah Mada	ayuafif@ugm.ac.id	08164223655
223	Wahidah Mahanani	Faculty of Agricultural Technology, Universitas Gadjah Mada	wahidah.mahanani@gmail.com	0812 15575277
224	Warapa Mahakarnchanakul	Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, 50 Ngam Wong Wan Rd, Lat Yao Chatuchak Bangkok 10900, Thailand	fagiwpm@ku.ac.th	0832596363
225	Winarni	PT. DIPA Puspa Labsains	winarni@dipa.co.id	08886130209
226	Winiati P. Rahayu	Bogor Agricultural Institute	wini_a@hotmal.com	
227	Y Wuri Wulandari	Universitas Slamet Riyadi (UNISRI), Jl. Sumpah Pemuda 18, Kadipiro, Surakarta	yustinawulandari@yahoo.co.id	08122755169
228	Yeyen Wanita	The Assessment Institute for Agricultural Technology, Yogyakarta 55584, Indonesia	yeyen_world@yahoo.com	

229	Yuliani	Universitas Mulawarman, Fak.Pertanian UNMUl, Jl.Pasir Balengkong Kampus Gunung Kelua Samarinda	yulicnd@yahoo.com	085250514456
230	Yunan Kholifatuddin Sya'di	Faculty of Agricultural Technology, Universitas Gadjah Mada	yunan_k@ymail.com	085264011481
231	Yunika Mayangsari	Faculty of Agricultural Technology, Universitas Gadjah Mada	yunikamayangsari@gmail.com	085881087093
232	Yus Maria Novelina	Balai Besar Industri Agro, Kementrian Perindustrian, Jl. Ir. H. Juanda no 11 Bogor	yusmaria_ns@yahoo.com	08128130849
233	Yustina W. W	Faculty of Agricultural Technology, Universitas Gadjah Mada		
234	Zhulfani Nur Latifatunissa	Faculty of Agricultural Technology, Universitas Gadjah Mada	fanilatunissa@yahoo.co.id	08997437387
235	Agus Suyanto	Semarang Muhammadiyah University, Indonesia	agussuyanto.kh@gmail.com	08562760919
236	Asih Susanti	Faculty of Agricultural Technology, Universitas Gadjah Mada		
237	Igantius Srianta	Widya Mandala Chatolic University Surabaya, Indonesia	srianta_wm@yahoo.com	083831215763
238	Muhammad Syafrudin	Faculty of Agricultural Technology, Universitas Gadjah Mada	syafrudin_kumai@yahoo.co.id	08125160644
239	Pita Kumudasari	PT. Thermalindo Sarana Laboratoria	pita@thermalinda.com	0315674739
240	Siti Aminah	Faculty of Agricultural Technology, Universitas Gadjah Mada		
241	Siti Tamaroh Cahyono Murti	Mercu Buana University, Indonesia	sititamaroh65@gmail.com	085228226660
242	Tri Untari	Faculty of Animal Medicine, Universitas Gadjah Mada	t_untari@yahoo.com	081328629822
243	Vembriarto Jati Pramono	Faculty of Animal Medicine, Universitas Gadjah Mada	vjatipramono@gmail.com	08562897033
244	Zaki Utama	Faculty of Agricultural Technology, Universitas Gadjah Mada		

INTERNATIONAL CONFERENCE ON MYCOLOGICAL ASPECTS OF FOOD AND FEED SAFETY (IC-MAFFS) 2013

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IC-MAFFS 2013 PARTICIPANTS

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