# Program and Abstracts

## International Symposium of Mycotoxicology 2016

# <ISMYCO 2016>

**For mutual understanding of**
**mycotoxin problems**
**and strengthening Asian network**

November 30th - December 3rd, 2016
The University of Tokyo, Yayoi Auditorium, Tokyo, Japan

Under auspices of
Japanese Society of Mycotoxicology

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# International Symposium of Mycotoxicology 2016

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**November 30th – December 3rd, 2016**
**The University of Tokyo, Yayoi Auditorium, Tokyo, Japan**

Under auspices of
**Japanese Society of Mycotoxicology**

Endorsed by
**Food Safety Commission of Japan**
**Ministry of Agriculture, Forestry and Fisheries**
**Ministry of Health, Labour and Welfare**

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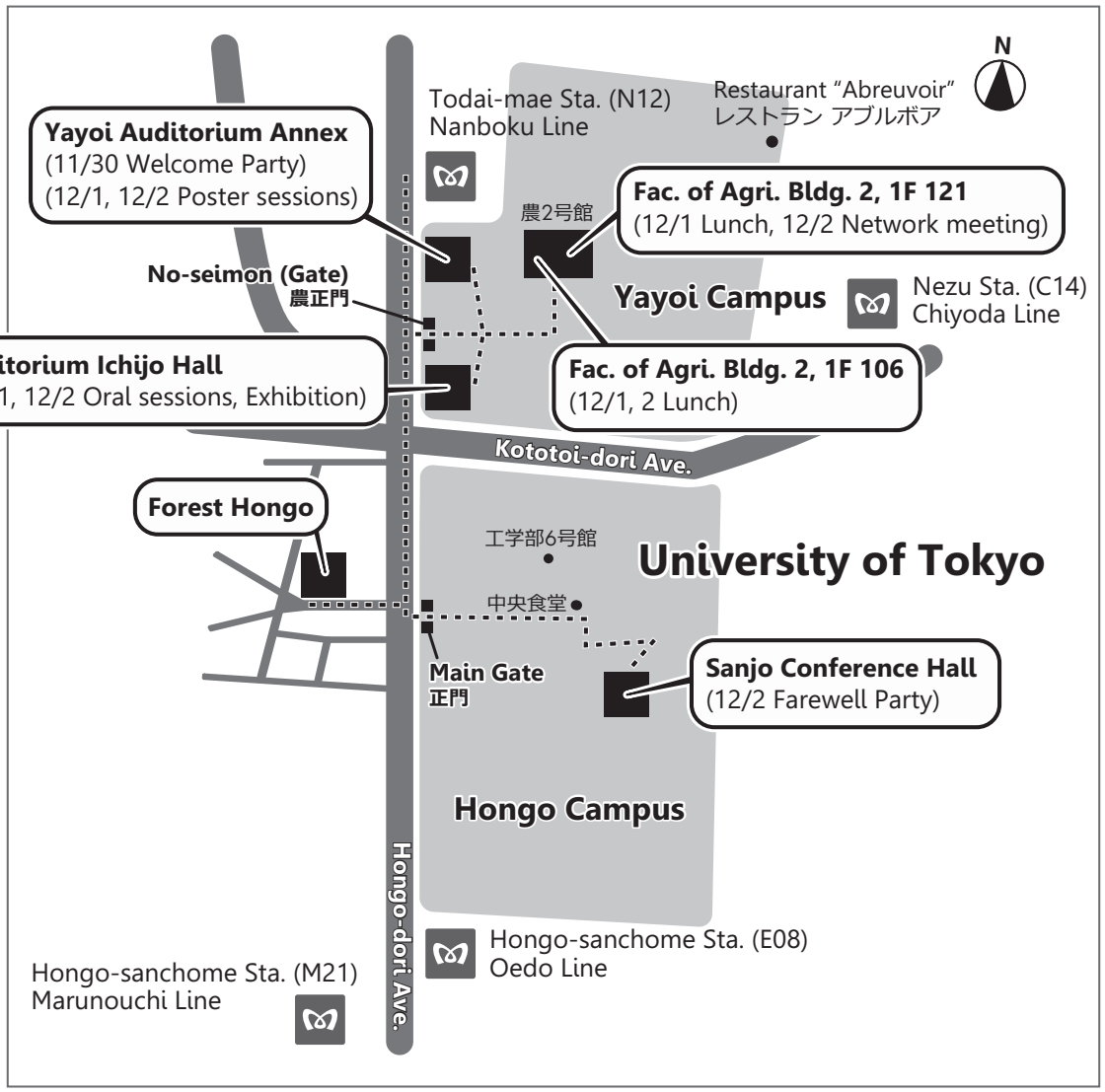
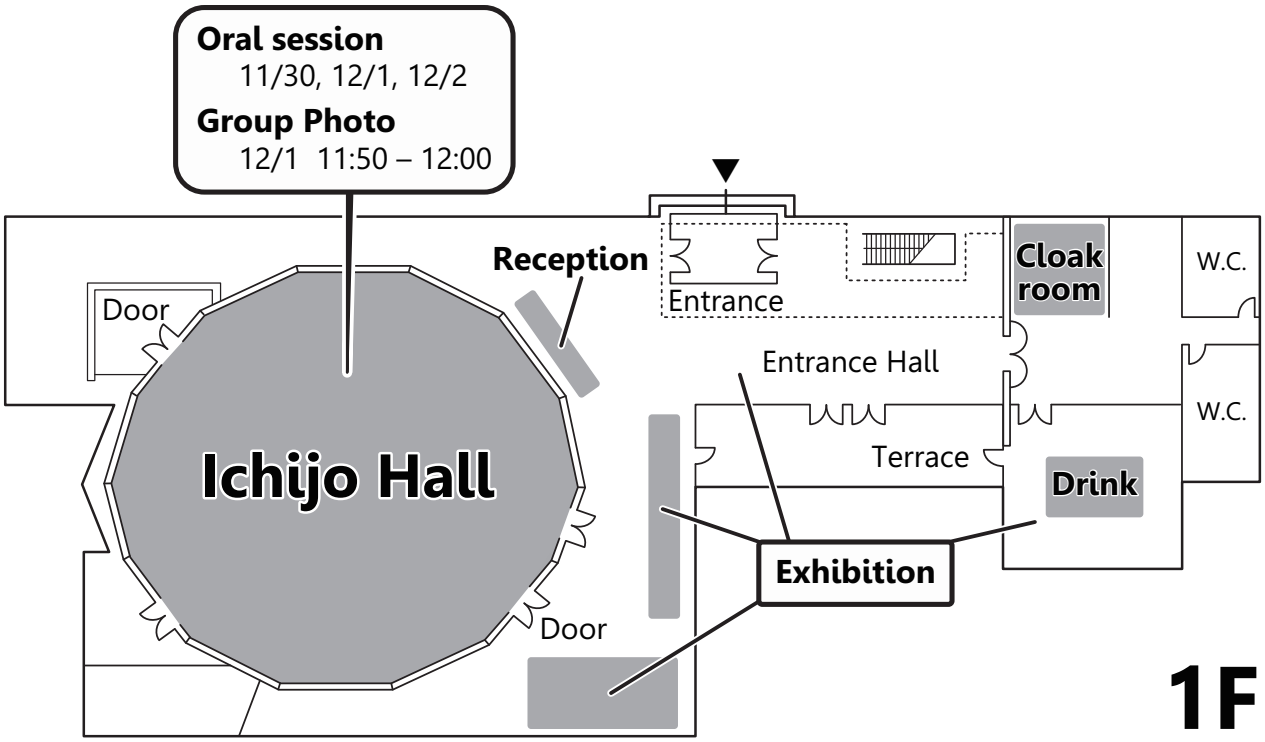
# Map surrounding the conference place (Yayoi Auditorium)

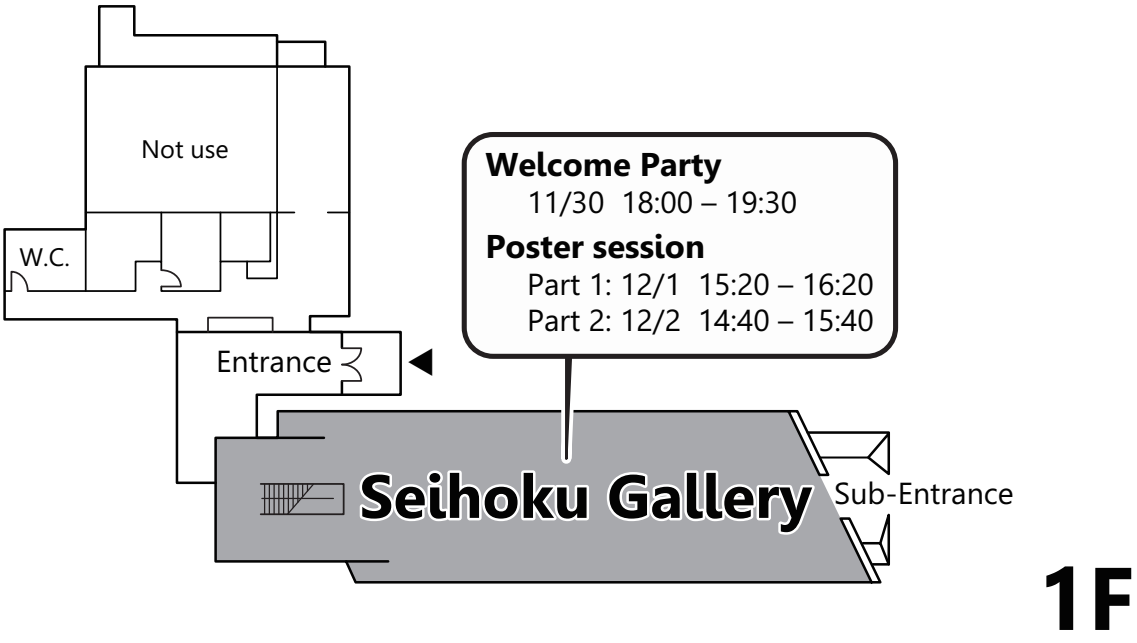


# Yayoi Auditorium, Ichijo Hall




*Eating and drinking are prohibited inside the Ichijo Hall.

# Yayoi Auditorium, Annex



# NOTIFICATION FOR PARTICIPANTS

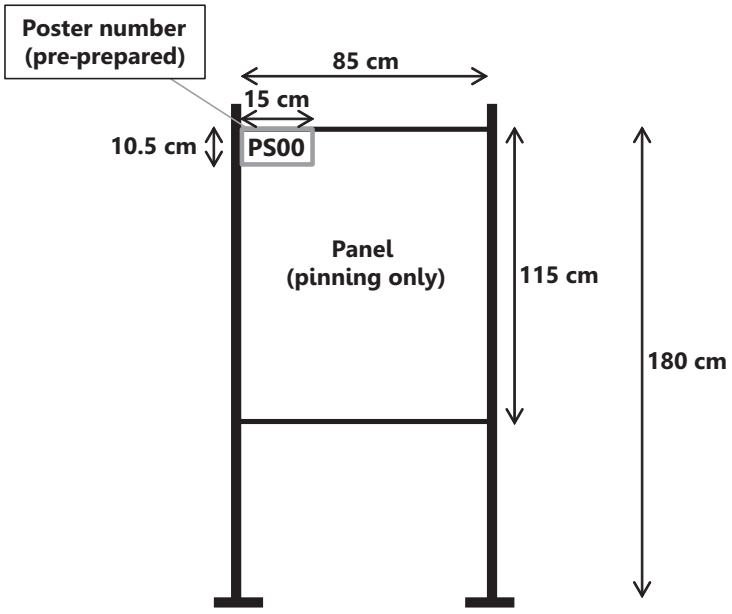
! Eating and drinking are prohibited inside the Ichijo Hall (the place for oral presentations).

- Wireless LAN is available around Yayoi Auditorium during the conference.
  You can get information on SSDI, WAP2-key, username and password at the reception.
  Please ask staff at the reception how to set it when you need some help.
- Cloak room is open during 12:00-18:00 on Nov. 30th, 08:00-18:00 on Dec. 1st, and 08:00-18:00 on Dec. 2nd.
- All participants are welcome to attend "Welcome Party" held at Yayoi Auditorium Annex during 18:00-19:30 on Nov. 30th.
  You can enjoy "Sushi Demae" and beer on tap at the party.
- Please have lunch (lunch box) at two rooms (1F 121 and 1F 106) of Fac. of Agri. Bldg. 2 during 12:10-13:00 on Dec. 1st and at 1F 106 during 11:30-12:30 on Dec. 2nd.
  We also have a luncheon meeting at 1F 121 during 11:30-13:10 on Dec. 2nd. Talks on mycotoxin research will be kindly provided by invited speakers from Asian countries. Participants with interests in the meeting are welcome to attend the luncheon meeting.
  Lunch box for vegetarian is available at lunch.
- The place of special dinner for speakers and chairpersons on Dec. 1st (18:30-20:30) is the restaurant "Abreuvoir" located in the same campus (Yayoi Campus).
- The "Farewell Party" on Dec. 2nd (18:30-20:30) is held at Sanjo Conference Hall located in the neighboring campus (Hongo campus). All invited participants and participants who have paid party fee in advance or on-site are welcome to enjoy the farewell party.
- Participants of "Excursion" on Dec. 3rd are kindly requested to contact staff at the reception on Nov. 30th or Dec. 1st to confirm your attendance. We will meet up at the "No-seimon gate on Dec. 3rd, 09:30. Sorry that new application to the excursion on-site is not possible due to riding capacity of bus.

# INSTRUCTIONS FOR PRESENTATIONS

## POSTER PRESENTATION

- Poster panel (see the Image).
- Size of usable area (including the poster number): 85 cm wide × 115 cm high.
- Attach your poster during 8:00 - 9:00 on Dec. 1st (Thu) and detach it during 16:00 - 17:00 on Dec. 2nd (Fri).
- Use pinning for poster attachment (pins are available at the reception desk in the poster presentation place).
- Times of poster presentations are 15:20 - 16:20 on Dec. 1st for odd number posters and 14:40 - 15:40 on Dec. 2nd for even number posters. Wear a mark showing the poster presenter during your poster presentation (mark is available at the reception desk).




## ORAL PRESENTATION

Bring PowerPoint and PDF files (Option 1) or your PC (Option 2) on the day.

**Option 1:** Contact staffs at the "Desk for speakers" at the reception more than 30 min before your session starts. Load both of PowerPoint and PDF files into PC (Windows 7, PowerPoint 2010, 2013) provided at the desk, and check your slides with the PC at the desk. (PDF file is very important when PowerPoint file can not be used.)
If it is necessary to preview your slides using PC (Windows 7, PowerPoint 2010, 2013) provided at the conference hall, ask the preview to staffs at the hall more than 10 min before your session starts.

**Option 2:** Contact staffs at the "Desk for speakers" more than 15 min before your session starts. They take you to the conference hall for previewing your slides. Staffs at the hall keep your PC for your presentation. DSub-15 pin (mini) and HDMI connectors are available.

Your presentation (12 min, 15 min, 20 min, or 30 min) includes 2 min discussion time.

# ISMYCO 2016 Program at a Glance

## Wed, November 30

12:00~
Registration

13:30~13:35 **Welcome Speech**

13:35~14:35
**Selected Oral Session**
**SS01** Motoichiro KODAMA
**SS02** Jinap SELAMAT
**SS03** Michael ROUTLEDGE
**SS04** Ryuichi UEGAKI
**SS05** Giuseppina MULÈ

14:35~15:00
**Coffee Break**

15:00~17:36
**Young Researcher Session**
**YS01** Wasana CHAISRI
**YS02** Norlia MAHROR
**YS03** Wantanwa MONGKON
**YS04** Saranya POAPOLATHEP
**YS05** Chayma RAGOUBI
**YS06** Sharmin SULTANA
**YS07** Tram TH LE
**YS08** Tomoya YOSHINARI
**YS09** Tigran HARUTYUNYAN
**YS10** Diana AVETYAN
**YS11** Tomohiro FURUKAWA
**YS12** Kurin IIMURA
**YS13** Francis M.C. Sigit SETYABUDI

18:00~19:30
**Welcome Party**
(Yayoi Auditorium Annex)

## Thu, December 1

8:00~9:00 Registration

9:00~9:10 **Opening Remark**

9:10~10:10
**Keynote**
**Keynote 01** Susumu KUMAGAI
**Keynote 02** Antonio F. LOGRIECO

10:10~10:30
**Coffee Break**

10:30~11:50
**Session 1**
**S1-1** Maiko WATANABE
**S1-2** Zakaria LATIFFAH
**S1-3** Victor LIMAY-RIOS
**S1-4** Endang S. RAHAYU

11:50~12:00 **Group Photo**

12:00~13:00
**Lunch**
(Fac. of Agri. Bldg. 2, 1F 106, 121)

13:00~15:20
**Analytical Session**
**AS01** Kurt BRUNNER
**AS02** Ingrid HUNTLEY
**AS03** Michael FISCHER
**AS04** Danrey TOTH
**AS05** Yuichi YOTSUYANAGI
**AS06** Thu HUYNH
**AS07** Monika PLOTAN

15:20~16:20
**Poster Session (odd numbers)**
and
**Coffee Break**

16:20~17:50
**Session 2**
**S2-1** Anthony C. SALES
**S2-2** Oyunchimeg BATKHUU
**S2-3** Ei Ei CHAW
**S2-4** Hasitha WEERATUNGE
**S2-5** Alka MEHTA
**S2-6** Hiroyuki NAKAGAWA

## Fri, December 2

9:00~10:00
**Keynote**
**Keynote 03** Rudolf KRSKA
**Keynote 04** Yin-Won LEE

10:00~10:20
**Coffee Break**

10:20~11:20
**Session 3**
**S3-1** Yun Yun GONG
**S3-2** Koji AOYAMA
**S3-3** Shigeru MIYAZAKI

11:30~12:30
**Lunch**
(Fac. of Agri. Bldg. 2, 1F 106)

11:30~13:10
**Lunch Meeting for strengthening network in Asia**
(Fac. of Agri. Bldg. 2, 1F 121)

13:20~14:40
**Session 4**
**S4-1** Isabelle P. OSWALD
**S4-2** Amnart POAPOLATHEP
**S4-3** Kenneth A. VOSS
**S4-4** Takashi UMEMURA

14:40~15:40
**Poster Session (even numbers)**
and
**Coffee Break**

15:40~17:20
**Session 5**
**S5-1** Yang LIU
**S5-2** Chris MARAGOS
**S5-3** Wulf-Dieter MOLL
**S5-4** Masayo KUSHIRO
**S5-5** Haruhisa SUGA

17:20~17:30 **Award Ceremony**

17:30~17:35 **Closing Remark**

18:30~ **Farewell Party**
(Lounge of Sanjo Conference Hall)

## Sat, December 3

10:00~  **Excursion** (Asakusa & Tokyo Skytree)

# ISMYCO 2016 Program

## WEDNESDAY, NOVEMBER 30

12:00 – **Registration**

13:30 – 13:35 **Welcome Speech** Haruo Takahashi (Japan)

13:35 – 14:35 **Selected Oral Session**

**Chairs:** Kiminori Shimizu, Amnart Poapolathep

**13:35-**

SS01 **The evolution of mycotoxin/phytotoxin biosynthesis and virulence in the plant pathogenic fungus *Alternaria alternata***

Yasunori AKAGI[1)], Takashi TSUGE[2)], Motoichiro KODAMA[1)]

[1)] Laboratory of Plant Pathology, United Graduate School of Agricultural Sciences, Tottori University, Tottori, Japan.
[2)] Graduate School of Bioagricultural Sciences, Nagoya University, Aichi, Japan.

**13:47-**

SS02 **Development of magnetic graphene oxide-chitosan adsorbant for the reduction of multi-mycotoxins in animal feed and toxicity evaluation of the resultant feeds**

Atena ABBASI PIROUZ[1)], Jinap SELAMAT[1,2)], Mirhosseini HAMED[3)], Rosa ABEDI-KARJIBAN[4)]

[1)] Institute of Tropical Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.
[2)] Food Safety Research Centre (FOSREC), Universiti Putra Malaysia, Serdang Selangor, Malaysia.
[3)] Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, Serdang Selangor, Malaysia,
[4)] Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

**13:59-**

SS03 **Aflatoxin exposure measured by aflatoxin albumin adduct biomarker in six african countries**

Michael ROUTLEDGE[1)], Yun Yun GONG[2)]

[1)] School of Medicine, University of Leeds, Leeds, UK
[2)] School of Food Science & Nutrition, University of Leeds, Leeds, UK

**14:11-**

SS04 **Cultivation methods of forage corn for reducing fumonisin: new "Kimimaru" variety and pestiside application**

Ryuichi UEGAKI[1)], Sunao UOZUMI[2)], Hiroshi UCHINO[2)]

[1)] National Institute of Animal Health, National Agriculture and Food Research Organization (NARO), Ibaraki, Japan
[2)] Tohoku Agricultural Research Center, NARO, Ibaraki, Japan

**14:23-**

SS05 **Mycotoxin degradation by laccase enzymes from *Pleurotus* spp**

Martina LOI[1,2], Francesca FANELLI[1], Vania C. LIUZZI[1], Miriam HAIDUKOVSKI[1], Maria Teresa CIMMARUSTI[1,2], Antonio F. LOGRIECO[1], Giuseppina MULÈ[1]*

[1] Institute of Sciences of Food Production, CNR, via Amendola 122/O, 70126 Bari, Italy.
[2] Department of Economics, University of Foggia, via Napoli 25, 71122, Foggia, Italy

14:35 – 15:00 **Coffee Break**

15:00 – 17:36 **Young Researcher Session**

**Chairs:** Makoto Kimura, Takahito Toyotome, Motoichiro Kodama, Osamu Kawamura

**15:00-**

YS01 **Feed and feed management factors in relationship to Aflatoxin M1 contamination in bulk milk of small holder dairy farms**

Wasana CHAISRI[1], Yoshiko SUGITA-KONISHI[2], Kanokwan NOIKREU[1], Anon KITIMA[1], Dirk van DAM[3], Ingrid HUNTLEY[3], Witaya SURIYASATHAPORN[1]*

[1] Department of Food animal Clinics, Faculty of Veterinary Medicine, Chiang Mai University, Muang, Chiang Mai 50100, Thailand
[2] Department of Food and Life Sciences, The Graduate School of Life and Environmental Sciences, Azabu University, 1-17-71, Fuchinobe Chuo-ku, Sagamihara
[3] Charm Sciences Inc., 659 Andover Street, Lawrence, MA 01843, USA

**15:12-**

YS02 **Critical point of aflatoxins contamination in peanut along the supply chain**

Norlia MAHROR[1,2], Jinap SELAMAT[1,3], Nor Khaizura MAHMUD @ AB RASHID[1], Cheow Keat CHIN[4]

[1] Food Safety Research Centre (FOSREC), Universiti Putra Malaysia, Serdang Selangor, Malaysia.
[2] School of Industrial Technology, Universiti Sains Malaysia, Minden, Penang, Malaysia.
[3] Institute of Tropical Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.
[4] Food Safety and Quality Division, Ministry of Health, Malaysia.

**15:24-**

YS03 **Feed characteristic associated with Aflatoxin B1 contamination in concentrate dairy cow feed**

Wantanwa MONGKON[1], Wasana CHAISRI[1], Yoshiko SUGITA-KONISHI[2], Witaya SURIYASATHAPORN[1]

[1] Department of Food Animal Clinics, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai Province, Thailand.
[2] Department of Food and Life Sciences, The Graduate School of Life and Environmental Sciences, Azabu University, Kanagawa, Japan.

**15:36-**

YS04 **Health risks of mycotoxin contamination in imported wine and beer consumed in Thailand**

Saranya POAPOLATHEP, Somsakul PHUANGKHAM, Usuma JERMNAK, Amnart POAPOLATHEP

Department of Pharmacology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand.

**15:48-**

YS05 **Implementation of ISO 22000 food safety management system in Tunisian "Pasta Warda" company and hazard analysis of mycotoxins type ochratoxin A**

Imed MAATOUK[1)], Chayma RAGOUBI[1)], Yassine ELLOUM[2)], Amel MEHREZ[1)], Aya BEN AMARA[1)], Ahmed LANDOULSI[1)]

[1)] Biochemistry of Lipids Unit and Interaction of Macromolecules in Biology, Laboratory of Biochemistry and Molecular Biology, Faculty of Sciences of Bizerte, Tunisia.
[2)] The company "Pâtes Warda" specialized in the production of pastas and couscous, Tunisia.

**16:00-**

YS06 **Fumonisin production recovery in a *Fusarium fujikuroi* strain by complementation of three fum genes**

Sharmin SULTANA[1)], Hironori KOBAYASHI[1)], Ryuou YAMAGUCHI[1)], Masafumi SHIMIZU[1)], Koji KAGEYAMA[2)], Haruhisa SUGA[3)]

[1)] Faculty of Applied Biological Science, Gifu University, Gifu, Japan.
[2)] River Basin Research Center, Gifu University, Gifu, Japan.
[3)] Life Science Research Center, Gifu University, Gifu, Japan.

**16:12-**

YS07 **Protein *O*-mannosyltransferases are involeved in sterigmatocystin production and fungal development**

Tram TH LE[1,2)], Kiminori SHIMIZU[1)]

[1)] Department of Biological Science and Technology, Tokyo University of Science, Tokyo, Japan.
[2)] Division of Microbial Biotechnology, Biotechnology Center of Ho Chi Minh City, Viet Nam.

**16:24-**

YS08 **Studies on the inhibotry activity of blasticidin S toward aflatoxin production**

Tomoya YOSHINARI, Jun TERAJIMA

Division of Microbiology, National Institute of Health Sciences, Tokyo, Japan.

**16:36-**

YS09 **Protective effects of schiff base cyclic amino acid derivatives against mycotoxins geno- and cytotoxicity**

Tigran HARUTYUNYAN[1)], Galina HOVHANNISYAN[1)], Nelli BABAYAN[1,2)], Thomas LIEHR[3)], Arsen ARAKELYAN[2)], Rouben AROUTIOUNIAN[1)], Margarita MALAKYAN[2)]

[1)] Department of Genetics and Cytology, Yerevan State University, Yerevan, Armenia.
[2)] Institute of Molecular Biology, National Academy of Sciences, Yerevan, Armenia.
[3)] Institute of Human Genetics, Jena University Hospital, Friedrich Schiller University, Jena, Germany.

**16:48-**

YS10 **Protective role of schiff base derivatives of amino acids against aflatoxin B1-induced apoptosis in rats**

Diana AVETYAN[1)], Gohar MKRTCHYAN[1)], Roksana ZAKHARYAN[1)], Svetlana KIRAKOSYAN[1)], Hakob DEVEJYAN[1)], Violeta AYVAZYAN[1)], Elina ARAKELOVA[1)], Margarita MALAKYAN[1,2)], Arsen ARAKELYAN[1)]

[1)] Institute of Molecular Biology NAS RA, Yerevan, Armenia.
[2)] Scientific Centre of Radiation Medicine and Burns, Ministry of Health, Yerevan, Armenia.

**17:00-**

YS11 **Studies on the mode of action of dioctatin that inhibits aflatoxin production of *Aspergillus* species.**

Tomohiro FURUKAWA[1)], Hidekazu KATAYAMA[2)], Takuma ICHIKAWA[2)], Tomoya YOSHINARI[3)], Lumi NEGISHI[4)], Michio SUZUKI[1)], Shohei SAKUDA[1)]

[1)] Department of Applied Biological Chemistry, The University of Tokyo, Tokyo, Japan.
[2)] Department of Applied Biochemistry, Tokai University, Kanagawa, Japan.
[3)] National Institute of Health Sciences, Tokyo, Japan.
[4)] The Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan.

**17:12-**

YS12 **Study on the target molecule of cyclo($_{L}$-Ala-$_{L}$-Pro), a specific aflatoxin production inhibitor**

Kurin IIMURA[1)], Tomohiro FURUKAWA[1)], Lumi NEGISHI[2)], Toshiyoshi YAMAMOTO[1)], Michio SUZUKI[1)], Shohei SAKUDA[1)]

[1)] Department of Applied Biological Chemistry, The University of Tokyo, Tokyo, Japan.
[2)] The Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan.

**17:24-**

YS13 **The synergy of CEMycoS network in overcoming mycotoxins problems**

Francis M.C. Sigit SETYABUDI

Department of Food and Agricultural Product Technology, Faculty of Agriculture Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia.

18:00 – 19:30 **Welcome Party**
[ Yayoi Auditorium Annex ]

# THURSDAY, DECEMBER 1

8:00 – 9:00 **Registration**

9:00 – 9:10 **Opening Remark** Yoshiko Sugita-Konishi (Japan)

## 9:10 – 10:10 Keynote

**Chairs:** Yoshiko Sugita-Konishi, Haruo Takahashi

**9:10-**

Keynote 01 **Food safety risk assessment on mycotoxins**

Susumu KUMAGAI
Food Safety Commission, Cabinet Office, Japan.

**9:40-**

Keynote 02 **Integrated and innovative actions for mycotoxin management and MycoKey contribution**

Antonio F. LOGRIECO[1)], Paola BATTILANI[2)], Cees WAALWIJK[3)], Sarah DE SAEGER[4)], Susanne VOGELGSANG[5)], Arja LAITILA[6)], Michelangelo PASCALE[1)], Theo VAN DER LEE[3)], Antonio MORETTI[1)], Giuseppina AVANTAGGIATO[1)], Giuseppina MULÈ[1)], Nunzia CITO[1)]

[1)] Research National Council, ISPA, Bari, Italy.
[2)] Università Cattolica del Sacro Cuore, Piacenza. Italy.
[3)] Stichting Dienst Landbouwkunding Onderzoek –DLO Wageningen, The Netherlands.
[4)] Ghent University, Ghent, Belgium.
[5)] Technical Research Centre of Finland-VTT, Otaniemi, Finland.
[6)] Agroscope-ISS, Zurich, Switzerland.

10:10 – 10:30 **Coffee Break**

## 10:30 – 11:50 Session 1:

Distribution of mycotoxigenic fungi and molecular genetics

**Chairs:** Endang S Rahayu, Maiko Watanabe

**10:30-**

S1-1 **Evaluation of molecular markers for identification of *Aspergillus* and *Fusarium* spp.**

Maiko WATANABE
Division of Microbiology, National Institute of Health Sciences, Tokyo, Japan.

**10:50-**

S1-2 **Aflatoxins and fumonisins from rice grains in Malaysia**

Zakaria LATIFFAH, Idris FARHANA NAZIRA, Salleh BAHARUDDIN

School of Biological, Sciences, Universiti Sains Malaysia, Penang, Malaysia.

**11:10-**

S1-3 **Critical point sources of ochratoxin A contamination in on-farm stored winter wheat**

Victor LIMAY-RIOS[1)], J. David MILLER[2)], Art SCHAAFSMA[1)]

[1)] Department of Plant Agriculture, University of Guelph, Ridgetown, Canada.
[2)] Department of Chemistry, Carleton University, Ottawa, Canada.

**11:30-**

S1-4 **Indonesian mycotoxigenic fungi from agricultural products**

Endang S. RAHAYU

Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia.

11:50 – 12:00 **Group Photo**

12:00 – 13:00 **Lunch**
[ Fac. of Agri. Bldg. 2, 1F 106, 121 ]

13:00 – 15:20 **Analytical Session**

**Chairs:** Setsuko Tabata, Tomoya Yoshinari

**13:00-**

AS01 **Mycotoxin analysis of food and feed: from rapid on-site tests to highly accurate mass spectrometry methods**

Kurt BRUNNER[1)], Lilian KUSTER[2)], Mabel NG[3)]

[1)] Romer Labs Division Holding GmbH, Tulln, Austria.
[2)] Romer Labs Division Holding GmbH Getzersdorf, Austria.
[3)] Romer Labs Singapore Pte Ltd, Singapore.

**13:20-**

AS02 **Charm Science's mycotoxin testing solutions**

Ingrid HUNTLEY[1)], Atsuko HASEGAWA[2)], Yasuhiro MARUYAMA[2)], Robert MARKOVSKY[1)]

[1)] Charm Sciences, Inc., MA, USA
[2)] Laboratory for Analytical Technologies Ltd., Tokyo, Japan

**13:40-**

AS03 **Mycotoxin testing in your hand – on-site testing with smartphone-based test evaluation**

Michael FISCHER, David STEINMANN, Verena ZIMMERMANN, Walter LÜBBE, Michael MÄTTNER

R-Biopharm AG, Darmstadt, Germany.

**14:00-**

AS04 **Validation of Afla-V AQUA™ lateral flow test kit for detection aflatoxins in corn using a water -based extraction**

Danrey TOTH, Chris BORBONE, Katie JOHNSON, Heather RICHARDS, Nancy ZABE

VICAM—A Waters Business, Milford, Massachusetts, USA.

**14:20-**

AS05 **Investigation to build simultaneous analysis methods of mycotoxins using HPLC and LC-MS**

Azusa UCHIDA[1)], Keiko MATSUMOTO[1)], Yuichi YOTSUYANAGI[1)], Minori NAKASHIMA[1)], Makoto OGAITO[1)], Tadayuki YAMAGUCHI[1)], Jun WATANABE[1)], Naoki MOCHIZUKI[2)]

[1)] Shimadzu Corporation, Kyoto, Japan.
[2)] Yokohama University of Pharmacy, Kanagawa, Japan.

**14:40-**

AS06 **Overcoming Matrix Interference in the Detection of Mycotoxins in Foods**

Thu HUYNH

Department of Research and Development, Helica Biosystems Inc., Santa Ana, CA, USA

**15:00-**

AS07 **The use of biochip array technology for rapid multimycotoxin screening**

Monika PLOTAN, Raymond DEVLIN, Jonathan PORTER, M. El Ouard BENCHIKH, María Luz RODRÍGUEZ, R. IVAN MCCONNELL, S. Peter FITZGERALD

Randox Food Diagnostics, 55 Diamond Rd, Crumlin, County Antrim, BT29 4QY, United Kingdom

15:20 – 16:20 **Poster Session (odd numbers)** and **Coffee Break**

16:20 – 17:50 **Session 2:**
Mycotoxin analysis and survey of mycotoxin contamination
**Chairs:** Hiroyuki Nakagawa, Anthony Sales

**16:20-**

S2-1 **Mycotoxin research in the Philippines: the last two decades**

Anthony C. SALES

Department of Science and Technology, Metro Manila, Philippines.

**16:35-**

S2-2 **Current situation of action on mycotoxin and surveillance of mycotoxin contimination in Mongolia**

Oyunchimeg BATKHUU, Sainjargal DORJGOTOV

National Reference Laboratory for Food Safety, General Agency for Specialized Inspection, Ulaanbaatar, Mongolia.

**16:50-**

S2-3 **Analysis of aflatoxin contamination in Myanmar agricultural commodities**

Ei Ei CHAW

Commodity Testing and Quality Management Center, Department of Consumer Affairs, Ministry of Commerce, Yangon, Myanmar

**17:05-**

S2-4 **Analysis of aflatoxins in spices by validated methodologies in Sri Lanka**

Hasitha WEERATUNGE[1)], Selvaluxmy KATHIRGAMANATHAR[1)], Sudharaka WEERAKOON[2)]

[1)] Industrial Technology Institute, 363, Bauddhaloka Mawatha, Colombo 7, Sri Lanka.
[2)] Institute of Chemistry Ceylon, Adamantane House, 341/22, Kotte Road, Welikada, Rajagiriya, Sri Lanka.

**17:20-**

S2-5 **Mycotoxin problem in India and its prevention by natural products**

Alka MEHTA

School of Biosciences and Technology, VIT University, Vellore, India.

**17:35-**

S2-6 **Screening for modified mycotoxins by high-resolution LC-MS**

Hiroyuki NAKAGAWA

National Agriculture and Food Research Organization (NARO), Food Research Institute, Ibaraki, Japan

# FRIDAY, DECEMBER 2

## 9:00 – 10:00 Keynote

**Chairs:** Masahiro Nakajima, Shohei Sakuda

**9:00-**

Keynote 03 **On the impact of climate change and reduction efforts on the occurrence and detection of mycotoxins**

Rudolf KRSKA, Christoph BÜSCHL, Michael SULYOK, Franz BERTHILLER, Rainer SCHUHMACHER

University of Natural Resources and Life Sciences, Vienna (BOKU), Dep. IFA-Tulln.

**9:30-**

Keynote 04 **Biosynthesis and regulation of zearalenone production in *Fusarium graminearum***

Yin-Won LEE

Department of Agricultural Biotechnology, Seoul National University, Seoul, Korea

## 10:00 – 10:20 Coffee Break

## 10:20 – 11:20 Session 3: Exposure to mycotoxins and risk assessment

**Chairs:** Shigeru Miyazaki, Yun Yun Gong

**10:20-**

S3-1 **Measuring human mycotoxin exposure using biomarkers**

Yun Yun GONG[1)], Gaoyun CHEN[2)], Michael N ROUTLEDGE[1)]

[1)] School of Food Science and Nutrition, University of Leeds, Leeds, UK.
[2)] Institute for Global Food Security, Queen's University of Belfast, Belfast, UK.

**10:40-**

S3-2 **Mycotoxin co-occurrence in feedstuffs in Japan**

Koji AOYAMA

Feed Analysis I Division, Food and Agricultural Materials Inspection Center, Saitama, Japan.

**11:00-**

S3-3 **Risk assessmet of mycotoxins by Food Safety Commision, Japan**

Shigeru MIYAZAKI

Research Institute for Animal Science in Biochemistry and Toxicology, Kanagawa, Japan.

11:30 – 12:30 **Lunch**
[ Fac. of Agri. Bldg. 2, 1F 106 ]

11:30 – 13:10 **Lunch Meeting for strengthening network in Asia**
[ Fac. of Agri. Bldg. 2, 1F 121 ]

13:20 – 14:40 **Session 4:** Toxicity and action mechanism of mycotoxins
**Chairs:** Ken Voss, Hitoshi Nagashima

**13:20-**

S4-1 **Mycotoxins and intestine**

Isabelle P. OSWALD[1)], Imourana ALASSANE-KPEMBI[1)], Alix PIERRON[1,2)], Philippe PINTON[1)]

[1)] Toxalim (Research Centre in Food Toxicology), Université de Toulouse, INRA, ENVT, INP-Purpan, UPS, Toulouse, France.
[2)] BIOMIN Research Center, Technopark 1, 3430 Tulln, Austria.

**13:40-**

S4-2 **Toxicity and Toxicokinetics of Fusarenon-X in Animals**

Amnart POAPOLATHEP

Department of Pharmacology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand

**14:00-**

S4-3 **Evidence for inhibition of ceramide synthase by fumonisins in maize-based foods: studies in high- and low-exposure communities in Guatemala**

Ronald T. RILEY[1)], Olga TORRES[2)], Jorge MATUTE[3)], Simon G. GREGORY[4)], Allison E. ASHLEY-KOCH[4)], Jency L. SHOWKER[1)], Trevor MITCHELL[1)], Joyce R. MADDOX[5)], Janee B. GELINEAU-VAN WAES[5)], Kenneth A. VOSS[1)]

[1)] Toxicology & Mycotoxin Research Unit, USDA-ARS-USNPRC, Athens, Georgia,USA.
[2)] Laboratorio Diagnóstico Molecular S. A., Guatemala City, Guatemala.
[3)] Centro de Investigationes en Nutrition y Salud, Guatemala City, Guatemala.
[4)] Department of Medicine Duke University, Durham, North Carolina, USA.
[5)] Department of Pharmacology, Creighton University, Omaha, Nebraska, USA.

**14:20-**

S4-4 **Molecular mechanisms underlying ochratoxin A-induced mutagenesis**

Takashi UMEMURA

Division of Pathology, National Institute of Health Sciences, Tokyo, Japan.

14:40 – 15:40 **Poster Session (even numbers)** and **Coffee Break**

15:40 – 17:20 **Session 5:** Mechanism and regulation of mycotoxin production and control of mycotoxin contamination in food and feed

**Chairs:** Haruhisa Suga, Masayo Kushiro

**15:40-**

S5-1 **Studies on *Aspergillus* species for the control of mycotoxins**

Yang LIU

Institute of Food Science & Technology, Chinese Academy of Agricultural Sciences / Key Laboratory of Agro-products processing comprehensive laboratory, Ministry of Agriculture, Beijing, China

**16:00-**

S5-2 **Detection of mycotoxins using imaging surface plasmon resonance (iSPR)**

Chris MARAGOS, Zakir HOSSAIN

Mycotoxin Prevention and Applied Microbiology Research Unit, U.S. Department of Agriculture, Agricultural Research Service, Peoria, Illinois, U.S.A.

**16:20-**

S5-3 **Novel recombinant enzymes for deactivating *Fusarium* mycotoxins**

Wulf-Dieter MOLL[1)], Sebastian FRUHAUF[1)], Michaela THAMHESL[1)], Andreas HÖBARTNER[1)], Patricia FAJTL[1)], Markus ALESCHKO[1)], Elisavet KUNZ-VEKIRU[2,3)], Heidi E. SCHWARTZ-ZIMMERMANN[2,3)], Rudolf KRSKA[3)], Franz BERTHILLER[2,3)], Gerd SCHATZMAYR[1)]

[1)] BIOMIN Research Center, Tulln, Austria.
[2)] Christian Doppler Laboratory for Mycotoxin Metabolism.
[3)] Center for Analytical Chemistry, Department of Agrobiotechnology IFA-Tulln, University of Natural Resources and Life Sciences Vienna, Tulln, Austria.

**16:40-**

S5-4 **Retention of major *Fusarium* mycotoxins during Japanese soft wheat processing**

Masayo KUSHIRO[1)], Yazhi ZHENG[1)], Megumi YOSHIDA[2)], Hiroyuki NAKAGAWA[1)], Hitoshi NAGASHIMA[1)], Hiroshi OKADOME[1)], Takashi NAKAJIMA[3)]

[1)] Food Research Institute, National Agriculture and Food Research Organization (NARO), Tsukuba, Japan.
[2)] Tohoku Agricultural Research Center, NARO, Morioka, Japan.
[3)] Kyushu Okinawa Agricultural Research Center, NARO, Kumamoto, Japan.

**17:00-**

S5-5 **The gene polymorphisms involving fumonisin producibility in *Fusarium fujikuroi***

Haruhisa SUGA

Life Science Research Center, Gifu University, Gifu, Japan.

17:20 – 17:30 **Award Ceremony**

17:30 – 17:35 **Closing Remark** Shohei Sakuda (Japan)

| | |
|---|---|
| 18:30 – | **Farewell Party**<br>[ Lounge of Sanjo Conference Hall ] |

## SATURDAY, DECEMBER 3

| | |
|---|---|
| 10:00 – 15:00 | **Excursion:** Asakusa and Tokyo Skytree (by bus) |

# POSTER SESSION

## PS01 Genetic population structure of *Fusarium graminearum* species complex in Korean cereals

Theresa LEE[1)], Todd J. WARD[2)], Jung-Hye CHOI[1)], Hyeonheui HAM[1)], Soohyung LEE[1)], Sung Kee HONG[1)], Jae-Gee RYU[1)]

[1)] Microbial Safety Team, National Institute of Agricultural Sciences, Rural Development Administration, Wanju, Korea.
[2)] Mycotoxin Prevention and Applied Microbiology, National Center for Agricultural Utilization Research, USDA-ARS, Illinois, USA.

## PS02 Inoculation of fumonisin-producing *Fusarium fujikuroi* isolates to grape berries and the fumonisin production on the berries

Ruiko HASHIMOTO[1)], Hiroyuki NAKAGAWA[2)], Kazuhiro HASHIMOTO[3)], Hisayuki ODA[3)], Yuji KAWAKAMI[3)], Yoshiki ONJI[4)], Youhei KITAOKA[4)], Maiko WATANABE[5)], Haruo TAKAHASHI[5)]

[1)] Chiba prefectural Institute of Public Health, Chiba, Japan.
[2)] National Agriculture and Food Research Organization, Food Research Institute, Ibaraki, Japan.
[3)] Laboratory of Environmental Science, FCG Research Institute, Inc., Tokyo, Japan.
[4)] Nara prefectural Institute of Health, Nara, Japan.
[5)] Division of Microbiology, National Institute of Health Sciences, Tokyo, Japan.

## PS03 Risk potential of rice grains contaminated with an endophytic fungus *Penicillium brocae*

Nozomi SHIRATORI[1)], Masahiko TAKINO[2)], Osamu ENDO[3)], Phitsanu TULAYAKUL[4)], Naoki KOBAYASHI[1,3)], Yoshiko SUGITA-KONISHI[1,3)]

[1)] Graduate school of Life and Environmental Sciences, Azabu University, Kanagawa, Japan.
[2)] Agilent Technologies, Japan, Ltd., Tokyo, Japan.
[3)] Faculty of Life and Environmental Sciences, Azabu University, Kanagawa, Japan.
[4)] Faculty of Veterinary Medicine, Kasetsart University, Nakorn-Pathom,Thailand.

## PS04 Transcriptional regulation of *Tri6* in *Fusarium graminearum*

Yuichi NAKAJIMA, Qi JIN, Takuya SHIOBARA, Kazuyuki MAEDA, Kyoko KANAMARU, Tetsuo KOBAYASHI, Makoto KIMURA

Department of Biological Mechanisms and Functions, Sciences, Graduate School of Bioagricultural Sciences, Nagoya University, Aichi, Japan.

## PS05 Improvement of the method for detection of aflatoxin-producing fungi

Tadahiro SUZUKI[1)], Yumiko IWAHASHI[1)]

[1)] Division of Food Biotechnology, Food Research Institute, NARO, Ibaraki, Japan.
[2)] Division of Food Safety, Food Research Institute, NARO, Ibaraki, Japan.

## PS06 Production of aflatoxin and the biosynthetic cluster in clinical isolates of *Aspergillus flavus*

Satoe YAMAGUCHI[1)], Masahiko TAKINO[2)], Katsuhiko KAMEI[3)], Takahito TOYOTOME[1,3)]

[1)] Diagnostic Center of Animal Health and Food Safety, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan.
[2)] Agilent Technology, Tokyo, Japan.
[3)] Medical Mycology Research Center, Chiba University, Chiba, Japan.

## PS07 An amino acid substitution of an aminotransferase involved in AAL-toxin biosynthesis alters the structure of mycotoxin AAL-toxin in pathogenic fungus *Alternaria alternata tomato pathotype*

Yasunori AKAGI[1)], Takashi TSUGE[2)], Atsushi ISHIHARA[3)], Motoichiro KODAMA[1)]

[1)] Laboratory of Plant Pathology, United Graduate School of Agricultural Sciences, Tottori University, Tottori, Japan.
[2)] Graduate School of Bioagricultural Sciences, Nagoya University, Aichi, Japan.
[3)] Faculty of Agriculture, Tottori Univesity, Tottori, Japan.

PS08 **Phylogenic studies on saccharifying activity and fumonisin production in the strains of Kuro-koji molds and their relatives isolated from fermented foods**

Yuna SUZUKI[1)], Haruo TAKAHASHI[2)], Tomoya YOSHINARI[2)], Naoki KOBAYASHI[3)], Yoshiko SUGITA-KONISHI[3)], Jun TERAJIMA[2)], Keiichi GOTO[1)], Maiko WATANABE[2)]

[1)] School of Marine Science and Technology, Tokai University, Shizuoka, Japan.
[2)] Division of Microbiology, National Institute of Health Sciences, Tokyo, Japan.
[3)] Department of Food and Life Science, Azabu University, Kanagawa, Japan.

PS09 **Spread and change in stress resistance of Shiga toxin-producing *Escherichia coli* O157 on food-related fungal colonies**

Ken-ichi LEE[1)], Naoki KOBAYASHI[2)], Maiko WATANABE[2)], Yoshiko SUGITA-KONISHI[2)], Hirokazu TSUBONE[1)], Susumu KUMAGAI[1)], Yukiko HARA-KUDO[2)]

[1)] Graduate School of Agricultural and Life Sciences, the University of Tokyo.
[2)] Division of Microbiology, National Institute of Health Sciences.

PS10 **Biochip arrays for the flexible multi-mycotoxin semi-quantitative screening of animal feed samples**

Monika PLOTAN, Raymond DEVLIN, Jonathan PORTER, M. El Ouard BENCHIKH, Evan WILCOX, Maria Luz RODRIGUEZ, R. Ivan McCONNELL, S. Peter FITZGERALD

Randox Food Diagnostics, Crumlin, United Kingdom.

PS11 **Trace analysis of aflatoxins in spices by HPLC coupled with solid-phase dispersive extraction followed by fluorescence derivatization, and its accuracy management for method validation**

Koichi SAITO, Junki ISHII, Saya NOBUMOTO, Rie ITO

Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Hoshi University, Tokyo, Japan.

PS12 **Occrrence of ochratoxin A in Japanese, Thai, and Chinese instant noodls (ramen)**

Osamu KAWAMURA, Fang XIAYAN, Mai NAGASAWA

Faculty of Agriculture, Kagawa University, Kagawa, Japan.

PS13 **Multiple detection of sugar derivatives of fumonisins in corn by LC-Orbitrap MS**

Yosuke MATSUO[1)], Kentaro TAKAHARA[2)], Hidemi HATABAYASHI[1)], Masayo KUSHIRO[1)], Hiroyuki NAKAGAWA[1)]

[1)] National Agriculture and Food Research Organization (NARO), Food Research Institute, Ibaraki, Japan.
[2)] ThermoFisher Scientific, Yokohama, Japan.

PS14 ***In vitro* decontamination of aflatoxin B1 by different clays**

Chaima RAGOUBI, Imed MAATOUK, Amel MEHREZ, Aya BEN AMARA, Ahmed LANDOULSI

Biochemistry of Lipids Unit and Interaction of Macromolecules in Biology, Laboratory of Biochemistry and Molecular Biology, Faculty of Sciences of Bizerte, Sousse, Tunisia.

PS15 **Determination of multi-class mycotoxins in varieties of rice by liquid chromatography-tandem mass spectrometry**

Piyaluk SINPHITHAKKUL[1,2)], Amnart POAPOLATHEP[1,2)], Kanjana IMSILP[1,2)], Saranya POAPOLATHEP[1,2)]

[1)] Department of Pharmacology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand.
[2)] Unit of Pharmacology and Toxicology, Center for Advanced Studies for Agriculture and Food, Kasetsart University for Advanced Studies, Kasetsart University (CASAF; NRU-KU), Bangkok, Thailand.

PS16 **Monitoring for mycotoxin contamination of food in Korea, 2015**

Seong-ju KIM, Young-Hyun KIM, Miok EOM, Seong-Wan SON, Sun-Hee PARK

Livestock Product Standard Division Food standard planning office, Ministry of Food and Drug Safety, Chungcheongbuk-do, Korea

PS17 **Screening analysis of 10 mycotoxins using HPLC**

Azusa UCHIDA[1)], Minori NAKASHIMA[2)], Makoto OGAITO[1)], Tadayuki YAMAGUCHI[2)]

[1)] Global Application Development Center, Analytical & Measuring Instruments Division, Shimadzu Corporation, Kanagawa, Japan.
[2)] LC Business Unit, Analytical & Measuring Instruments Division, Shimadzu Corporation, Kyoto, Japan.

PS18 **Optimization of extraction and IAC purification for ELISA to detect aflatoxin in interfering food matrices**

Noriko MATSUURA, Eri HOJO, Naoki MORISHITA, Yukihiro SUGAWARA

R&D Center, NH Foods Ltd, Tsukuba, Ibaraki, Japan.

PS19 **Simultaneous determination of multi-mycotoxins in sesame seed by liquid chromatography-tandem mass spectrometry**

Nitwarat RUENNARONG, Saranya POAPOLATHEP, Phanwimol TANHAN, Usuma JERMNAK, Amnart POAPOLATHEP

Department of Pharmacology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand.

PS20 **Global survey of aflatoxin $M_1$ concentration in powder milk and powder whey products**

Ingrid HUNTLEY[1)], Miyuki HOJO[2)], Atsuko HASEGAWA[3)], Yasuhiro MARUYAMA[3)], Robert MARKOVSKY[1)], Naoki KOBAYASHI[2)], Yoshiko SUGITA-KONISHI[2)]

[1)] Charm Sciences, Inc., MA, USA.
[2)] Azabu University, Kanagawa, Japan.
[3)] Laboratory for Analytical Technologies Ltd., Tokyo, Japan.

PS21 **An analytical method using a qualitative kit for aflatoxins in crude drug product and kampo extract products**

Hisako SAKUMA, Kentaro SEKI, Takashi KOTSUKA

Separation & Refining Business Group, Specialty Chemicals Department,Functional Chemicals Division, SHOWA DENKO K.K., Tokyo, Japan.

PS22 **Rapid tests for mycotoxins: requirements and challenges for rapid strip tests**

Kurt BRUNNER[1)], Philipp GRUBER[2)], Mabel NG[3)]

[1)] Romer Labs Division Holding GmbH, Tulln, Austria.
[2)] Romer Labs Division Holding GmbH Getzersdorf, Austria.
[3)] Romer Labs Singapore Pte Ltd, Singapore.

PS23 **Aflatoxin M1 in dairy products: the low regulatory limits require ultra-sensitive test kits**

Kurt BRUNNER[1)], Philipp GRUBER[2)], Mabel NG[3)]

[1)] Romer Labs Division Holding GmbH, Tulln, Austria.
[2)] Romer Labs Division Holding GmbH Getzersdorf, Austria.
[3)] Romer Labs Singapore Pte Ltd, Singapore.

PS24 **Aflatoxin $M_1$ in human breast milk and aflatoxin $B_1$ in peanut from processing industry, Brazil**

Angélica Tieme ISHIKAWA[1)], André Ribeiro DA SILVA[2)],
Cassia Reika YAMASHITA-TAKABAYASHI[2)], Lívia Montanheiro Médici ZANIN[2)],
Gervásio Hitoshi SAITO[2)], Artur Kikuchi BAGATIN[6)], Fabiana Felipin RIGOBELLO[1)],
Felipe Pedoti FRACALOSSI[2)], Marie OSHIIWA[4)], Carlos KMMEIEMER[5)],
Elisabete Yurie Sataque ONO[3)], Osamu KAWAMURA[6)], Eiko Nakagawa ITANO[1)],
Elisa Yoko HIROOKA[2)]

[1)] Department of Pathological Sciences, State University of Londrina, Londrina, Brazil.
[2)] Department of Food Science and Technology, State university of Londrina, Londrina, Brazil.
[3)] Department of Biochemistry and Biotechnology, State University of Londrina, Londrina, Brazil.
[4)] FATEC- Faculty of Food Technoloy, Marília, São Paulo, Brazil.
[5)] State University of Maringá, Paraná, Brazil.
[6)] Food Hygiene Laboratory, Faculty of Agriculture, Kagawa University, Kagawa, Japan.

PS25 **Low-cost rapid immunoassay for aflatoxin screening in chicken liver and eggs**

Lívia Montanheiro Médici ZANIN[1)], Thaís Marques Amorim UMBELINO[1)],
Angélica Tieme ISHIKAWA[2)], Cassia Reika Takabayashi YAMASHITA[1)],
Fernanda Ramos de Pádua SALLES[1)], Mariana Ribeiro BENFATTI[1)], Gervásio H. SAITO[1)],
José Carlos RIBEIRO JÚNIOR[3)], Kerlei Cristina MÉDICI[3)], Geraldo Masahiro HAYASHI[4)],
Carlos KMMELMEIER[5)], Eiko Nakagawa ITANO[2)], Osamu KAWAMURA[6)],
Elisa Yoko HIROOKA[1)]

[1)] Department of Food Science and Technology, State University of Londrina, Paraná, Brazil.
[2)] Department of Pathological Sciences, State University of Londrina, Paraná, Brazil.
[3)] Department of Preventive Veterinary Medicine, State University of Londrina, Paraná, Brazil.
[4)] Agari Assessory LTDA, Londrina, Paraná, Brazil.
[5)] State University of Maringá, Paraná, Brazil.
[6)] Food Hygiene Laboratory, Faculty of Agriculture, Kagawa University, Kagawa, Japan.

PS26 **Analysis of sterigmatocystin and aflatoxins with immunoaffinity column using an organic solvent-tolerant monoclonal antibody**

Mikiko UCHIGASHIMA

HORIBA, Ltd., Kyoto, Japan.

PS27 **Dietary exposure of aflatoxins and ochratoxin a in Thai cuisine from dried chili and selected spices**

Kanithaporn VANGNAI, Warapa MAHAKARNCHANAKUL, Kullanart TONGKHAO

Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University. Bangkok, Thailand.

PS28 **Effect of administration of single oral subclinical doses of aflatoxin $B_1$ in the liver and gut microbiota in C57Bl/6 mice**

Angélica T. ISHIKAWA[1)], Marcio C. COSTA[2)], Paula L-A SILVA[1)], João Paulo ASSOLINI[1)],
Gervásio H. SAITO[3)], Lívia M. M. ZANIN[3)], Bruna P. SANTOS[2)], Gabriela G. OLIVEIRA[4)],
J Scott WEESE[5)], Ana Paula F. R. L. BRACARENSE[2)], Amauri A. ALFIERI[2)], João S. SILVA[6)],
Osamu KAWAMURA[7)], Elisa Y. HIROOKA[3)], Eiko N. ITANO[1)]

[1)] Department of Pathological Sciences, State University of Londrina, Londrina, Brazil.
[2)] Department of Preventive Veterinary Medicine, State University of Londrina, Paraná, Brazil.
[3)] Department of Food Science and Technology, State university of Londrina, Londrina, Brazil.
[4)] Federal University of Fronteira Sul, Chapecó, Brazil.
[5)] Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Canada.
[6)] Department of Immunology, University of São Paulo, Ribeirão Preto, Brazil.
[7)] Food Hygiene Laboratory, Faculty of Agriculture, Kagawa University, Kagawa, Japan.

PS29 **Issues of list of fungi designated by the biosafety level (BSL) and Japanese regulation laws**

Sayaka BAN, Shoko OJI, Koji SAKAMOTO, Yuko KITAHASHI, Masumi FUJITA, Hiroko KAWASAKI

Biological Resource Center, National Institute of Technology and Evaluation, Chiba, Japan.

PS30 **Degradation of ochratoxin A in aqueous solutions by gamma irradiation**

Amel MEHREZ[1)], Chayma RAGOUBI[1)], Roberto ROMERO-GONZALEZ[2)], Aya BEN AMARA[1)], Mokhtar KRAIEM[3)], Antonia GARRIDO FRENICH[2)], Ahmed LANDOULSI[1)], Imed MAATOUK[1)]

[1)] Laboratory of Biochemistry and Molecular Biology, Zarzouna, Tunisia.
[2)] Department of Analytical Chemistry, Almeria University, Almeria, Spain.
[3)] National Center for Nuclear Sciences and Technologies (CNSTN), Tunis cedex, 2020, Tunisia.

PS31 **Fates and tissue depletion of nivalenol in broilers**

Jutamart KONGKAPAN[1,2)], Mario GIORGI[4)], Saranya POAPOLATHEP[3)], Supaporn ISARIYODOM[5)], Amnart POAPOLATHEP[1,2,3)]

[1)] Interdisciplinary Graduate Program in Agricultural Biotechnology, Graduate School, Kasetsart University, KamphaengSaen Campus, Nakhon Pathom 73140, Thailand
[2)] Center of Excellence on Agricultural Biotechnology: (AG-BIO/PERDO-CHE), Bangkok 10900, Thailand
[3)] Department of Pharmacology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand
[4)] Department of Veterinary Sciences, University of Pisa, Via Livornese, (lato monte), San Piero a Grado, 56122 Pisa, Italy
[5)] Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand

PS32 **Fusarenon-X induced apoptosis in lymphoid tissues of mice after 14 days oral exposure**

Sawinee AUPANUN[1)], Saranya POAPOLATHEP[2)], Kanjana IMSILP[2)], Amnart POAPOLATHEP[1,2)]

[1)] Interdisciplinary Graduate Program in Genetic Engineering, The Graduate School, Kasetsart University, Thailand.
[2)] Department of Pharmacology, Faculty of Veterinary Medicine, Kasetsart University, Thailand.

PS33 **The effects of eushearilide, a novel macrolide antifungalantibiotic, on mitochondrial structure and respiratoryfunction as a mechanism for the antifungal activity**

Akira KITAGAWA[1)], Tomoo HOSOE[2)], Ken-ichi KAWAI[2)], Kiyoshi KAWAI[1)]

[1)] Department of Nutrition, Shigakkan University, Ohbu, Japan
[2)] Faculty of Pharmaceutical Sciences, Hoshi University, Tokyo, Japan

PS34 **Anorexic action of fusarenon X in hypothalamus and intestine**

Kazuo KOBAYASHI-HATTORI, Misa TOMINAGA, Saori ICHIKAWA, Fumiko SAKASHITA, Miki TADAISHI

Department of Nutritional Science, Tokyo University of Agriculture, Tokyo, Japan.

PS35 **Contamination of acetylated deoxynivalenol in feeds and their toxicokinetics in pigs**

Yosuke UCHIYAMA[1)], Kaori TOYA[2)], Seigo ITO[2)], Tomoyuki KADOTA[3)], Arisa IWANUMA[2)], Yoshiko SUGITA-KONISHI[4)]

[1)] Meat Inspection Station, Kanagawa Prefectural Government, Kanagawa, Japan.
[2)] Department of Veterinary Sciences, Azabu University, Kanagawa, Japan.
[3)] Central Labratories for Key Technologies, Research and Development Division, Kirin Company Limited, Kanagawa, Japan.
[4)] The graduate School of Life and Environmental Sciences, Department of Food and Life Sciences, Azabu University, Kanagawa, Japan.

PS36 **Reduction of aflatoxin $B_1$ toxicity by using food compounds and CYP3A4 gene in human**

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PS37 **Changes in mycotoxin concentrations during ensiling**

Chie TOZUKA[1)], Ryo OOSAWA[2)], Kazuo KAWANO[3)], Toshiyuki KIMURA[4)], Ryuichi UEGAKI[5)]

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PS38 **Studies on mode of action of blasticidin A, an aflatoxin production inhibitor**

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PS39 **Studies on aflatoxin production inhibitors produced by microbes**

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PS40 ***In vitro* metabolism of *Fusarium* mycotoxin by bovine rumen microorganisms**

Miyako YOSHIOKA[1)], Ryuichi UEGAKI[1)], Chie TOZUKA[2)], Yuji TAKAHASHI[1)], Shozo ARAI[1)], Noriko YAMANAKA[1)]

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PS41 **Profiling the microbial degradation process of fumonisin B1 through liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC/Q-TOF MS)**

Jun WANG, Nanxi WANG, Songling WU, Changpo SUN

Group of Grain and oil microbes, Academy of State Administration of Grain, P.R.China.

PS42 **Effect of fluoropyrimidines on aflatoxin production**

Toshiyoshi YAMAMOTO[1)], Takahiro KAGEMOTO[1)], Kurin IIMURA[1)], Yasumitsu KONDOH[2)], Kaori HONDA[2)], Hiroyuki OSADA[2)], Shohei SAKUDA[1)]

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PS43 **Evaluation of aflatoxin reduction by near infrared rays sorting in highly contaminated peanut lot**

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# Abstracts

# Keynote 01

## FOOD SAFETY RISK ASSESSMENT ON MYCOTOXINS

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The oldest human mycotoxicosis which has been recognized in the literature is ergotism caused by the fungus *Claviceps* on crops. Epidemics occurred in the middle age in Europe are known as St.Anthony's Fire.

Alimentary toxic aleukia (ATA) is known as a human mycotoxicosis occurred in Orenburg region of Russia from 1942 to 1947 due to over-wintered crops contaminated with *Fusarium*, which produced T-2 toxin, HT-2 toxin or neosolaniol. The same disease possibly occurred in other regions before the occurrence of ATA.

Acute cardiac beriberi was a serious human disease frequently seen in Japan before 1950. A Japanese researcher suspected that moldy rice could be a cause of this disease, and reported the toxic effect of the mold extracts in experimental animals in 1892. Although thiamine became generally accepted as a causal agent of beriberi around 1925, a number of mycotoxins such as luteoskylin, islanditoxin, citreoviridin and citrinin were isolated from moldy rice from 1940 to 1960, leading to the prevention of human diseases by moldy rice.

Noodles and breads made from *Fusarium*-infected scabby wheat occasionally caused food poisoning in Japan before 1960. The largest outbreak occurred in 1946 involved 2404 reported cases. Trichothecenes including deoxynivalenol, nivalenol and fusarenon X were subsequently isolated from scabby crops.

Thus, human mycotoxicoses have occurred in various regions of the world since ancient times. However, the problem became worldwide in 1960 when Turkey X disease occurred in England due to aflatoxin-contaminated peanut meal imported from Brazil. JECFA and IARC have conducted risk assessments of mycotoxins since the first report by each organization was published in 1987 and 1972, respectively.

Worldwide risk assessments on mycotoxins to ensure food safety seem to be more important than ever in the face of global climate change. In view of the historical human mycotoxicoses, the assessments may need to consider not only direct health effects of mycotoxins, but also possible indirect effects including modulation of the protective function of the human body against pathogens and hazardous chemicals.

# Keynote 02

## INTEGRATED AND INNOVATIVE ACTIONS FOR MYCOTOXIN MANAGEMENT AND MYCOKEY CONTRIBUTION

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Among the emerging issues in food safety, the increase of plant diseases associated to the occurrence of mycotoxigenic fungal species is of major importance. As a result of their secondary metabolism, these fungi can produce mycotoxins, which are low-molecular-weight toxic compounds that represent a serious risk for human and animal health worldwide. The management of good agricultural practices in the pre-harvest is a key issue for minimizing the risk of mycotoxin accumulation in the crops before the harvest. Such practices can involve crop rotation, tillage, proper fertilization and fungicide or biological control distribution, variety selection, timely planting and harvests and the control of the insects which often act as vectors of toxigenic fungi spores. On the other hand, the reduction of mycotoxins along the agro-food chains is also highly depending from a correct post-harvest management that must aim firstly at the separation of the infected crop products from the healthy material. Therefore, the use of different tools such as manual sorting or optical sensors is also a crucial point for reducing the level of mycotoxin contamination of a given crop. Moreover, it is extremely important to prevent post-harvest contamination during the storage by obtaining low temperature and humidity conditions, in order to limit the development of toxigenic fungal genera. An update review of an integrated management of pre-and post harvest practices aiming at the minimizing the risk of mycotoxin contamination of the main crops of agro-food importance and main effective solutions proposed by EU project MycoKey (http://www.mycokey.eu/) will be provided in the presentation. The project will contribute to reduce mycotoxin contamination at global level with particular attention in Europe and China, where frequent and severe mycotoxiun contaminations occur in crops, and where international trade of commodities and contaminated batches are increasing. The project will finally integrate key information and practical solutions for mycotoxin management into a smart ICT tool (MycoKey App).

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# Keynote 03

## ON THE IMPACT OF CLIMATE CHANGE AND REDUCTION EFFORTS ON THE OCCURRENCE AND DETECTION OF MYCOTOXINS

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The Intergovernmental Panel on Climate Change [1] found that increasing temperatures, frequencies and duration of extreme weather events, and elevated $CO_2$ and ozone concentrations in the atmosphere likely impacts also the quality of food. As a result of global warming, Bebber and colleagues forecasted a pole-wards shift of plant pathogens and pests at about 3-5 km per year [2]. The EU's Rapid Alert System for Food and Feed (RASFF) showed that of the total border rejections in 2015, 18.3% were due to mycotoxin contamination exceeding the EU legislative limits, accounting for the most frequently reported chemical hazard. In a recent multi-mycotoxin survey, 81% of all samples were contaminated with at least one mycotoxin and 45% contained more than one secondary metabolite of fungi [3]. Climate change adds to the complexity of new challenges regarding the occurrence and metabolization of mycotoxins. This is illustrated by the Serbian maize scandal of 2013 among others: Due to extended periods of drought, with a 30-year record low of mean precipitation and overall 2°C increase of mean temperature during the previous year's growing period, *Aspergillus* spp. found an optimum environment for growth and production of aflatoxins.

These challenges increase the requirement of LC-MS/MS-based methods in analytics to examine a broad spectrum of potential toxic compounds in the food-production chain. It is expected that resistance breeding methods and/or the impacts of climate change will cause new metabolites, or the change of the metabolite spectrum. Forecasting models that link weather conditions at critical development stages of a crop may support such monitoring schemes. High performing metabolomics based methods will play an increasingly important role regarding plant-fungi-interactions, and thus for the detection of new metabolites.

1. Evaluation of Climate Models. *In: Climate Change*, (2013). https://www.ipcc.ch/pdf/assessment-report/ar5/wg1/WG1AR5_Chapter09_FINAL.pdf
2. Bebber et al., *Nature Climate Change*, 3, 985–988 (2013).
3. Kovalsky et al., *Mycotoxins*, (2014). http://www.allaboutfeed.net/Mycotoxins/Keep-up-to-date-on-mycotoxins

# Keynote 04

## BIOSYNTHESIS AND REGULATION OF ZEARALENONE PRODUCTION IN *FUSARIUM GRAMINEARUM*

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Zearalenone (ZEA) is a causative agent of hyperestrogenic syndrome in mammals and is mainly produced by *Fusarium graminearum* and *F. culmorum* [1]. In *F. graminearum*, the ZEA biosynthetic cluster is composed of four genes, *PKS4*, *PKS13*, *ZEB1*, and *ZEB2*, which encode a reducing polyketide synthase, a nonreducing polyketide synthase, an isoamyl alcohol oxidase, and a transcription factor, respectively [2]. Although it is known that ZEB2 primarily acts as a regulator of ZEA biosynthetic cluster genes, the mechanism underlying this regulation remains undetermined. Recently, two isoforms (ZEB2L and ZEB2S) from the *ZEB2* gene in *F. graminearum* were characterized. It was revealed that ZEB2L contains a basic leucine zipper (bZIP) DNA-binding domain at the N-terminus, whereas ZEB2S is an N-terminally truncated form of ZEB2L that lacks the bZIP domain. Interestingly, ZEA triggered the induction of both *ZEB2L* and *ZEB2S* transcription. In ZEA producing condition, the expression of *ZEB2S* transcripts via alternative promoter usage was directly or indirectly initiated by ZEA. Physical interaction between ZEB2L and ZEB2L as well as between ZEB2L and ZEB2S was observed in the nucleus. The ZEB2SZEB2S interaction was detected in both the cytosol and the nucleus. ZEB2LZEB2L oligomers activated ZEA biosynthetic cluster genes, including *ZEB2L*. ZEB2S inhibited *ZEB2L* transcription by forming ZEB2L-ZEB2S heterodimers, which reduced the DNA-binding activity of ZEB2L [3]. This study provides insight into the autoregulation of *ZEB2* expression by alternative promoter usage and a feedback loop during ZEA production.

1. Leslie and Summerell. *The Fusarium laboratory manual,* 388p (2006).
2. Kim et al., *Mol. Microbiol*., 58, 1102-1113 (2005).
3. Park et al., *Mol. Microbiol*., 97, 942-956 (2015).

# S1-1

## EVALUATION OF MOLECULAR MARKERS FOR IDENTIFICATION OF *ASPERGILLUS* AND *FUSARIUM* SPP.

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To control fungal hazard of plants, animals and humans, and rapidly evaluate a risk for foods contaminated by fungi, there is a need for a rapid, easy and accurate identification system. To date, researchers have developed many strategies for rapid and easy molecular identification of fungal isolates, such as a search by the nucleotide sequences homology with reference sequences in database, construction of phylogenetic tree with reference sequences and PCR-based assays with species-specific regions of genes. However, there are also some problems in the field of molecular identification of molds, especially *Fusarium* and *Aspergillus* species. Because, some genetic markers commonly used for identification of these genera have few species-specific diagnoses in sequences among the closely related species. Therefore, it is necessary to identify a gene that has a high evolutionary rate and have the species-specific diagnosis.

We evaluated the 18S rRNA gene (rDNA), internal transcribed spacer region 1, 5.8S rDNA, 28S rDNA, β-tubulin gene (*β-tub*), and aminoadipate reductase gene (*lys2*), using calculation of the nucleotide sequence homology with pairwise comparison of all tested strains, inference the ratio of the nucleotide substitution rates of each gene, and construction and comparison among the phylogenetic trees of each gene for *Fusarium* identification [1]. The result indicated that the 18S rDNA, 5.8S rDNA, 28S rDNA and ITS1 sequences leaded to the accurate identification of only several isolates, because of perfect matches to sequences of more than two species of reference strains, or mis-identification. The lys2 sequences leaded to the accurate identification of several isolates not identified by above-mentioned four regions. However, other several isolates could not be identified because of non-amplification of *lys2* by PCR. The *β-tub* sequences leaded to the accurate identification of all tested-isolates. Thus, the *β-tub* is more useful genetic marker for identifying *Fusarium* isolates in a wide range than other five loci including *lys2*. Then, we evaluated the *β-tub* and mitochondrial cytochrome b gene (*cyt-b*) [2] for *Aspergillus* identification. It was indicated that the *cyt-b* is the genetic marker as useful as, or more useful than the *β-tub* for identification of species in several *Aspergillus* sections.

1. Watanabe et al., *J. Sci. Food Agric*., 91, 2500-2504 (2011).
2. Yokoyama et al., *FEMS Microbiol. Lett*., 200, 241-246 (2001).

# S1-2

## AFLATOXINS AND FUMONISINS FROM RICE GRAINS IN MALAYSIA

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Like any other storage products, rice is also commonly contaminated with toxigenic fungi. In the present study, 96 rice samples, namely basmati (n = 2), parboiled rice (n = 3), paddy (n = 4), black glutinous rice (n = 5), rice products (n = 7), brown rice (n = 7), white glutinous rice (n = 11), fragrant rice (n = 12) and white rice (n = 45) were collected randomly from sundry shops and supermarkets in Peninsular Malaysia to determine the occurrence of toxigenic fungi and to detect aflatoxins (AFB1, AFB2, AFG1 and AFG2) and fumonisins (FB1 and FB2). Based on morphological characteristics, five genera of fungi were identified as *Aspergillus, Penicillium, Fusarium, Alternaria* and *Curvularia*. *Aspergillus* spp. were the most common toxigenic fungi isolated from different types of rice and rice products. The presence of AFB1, AFB2, AFG1, AFG2, FB1 and FB2 in rice samples were detected using High Pressure Liquid Chromatography with a fluorescent detector. AFB1 (0.39-24.09 μg/kg) was detected from two samples of black glutinous rice, two white rice, and one rice product (emping); and AFB2 (0.04 to 2.45 μg/kg) was detected from one sample of black glutinous rice and rice product (emping). None of the samples tested exceeded the Malaysian permissible limits of AF (35 μg/kg total aflatoxins). Fumonisins B1 and B2 (total 10 to 120 μg/kg) were detected in 22 rice samples comprising paddy, black glutinous rice, fragrant rice, rice products (emping and rice cake) and white rice. Co-occurrence of both aflatoxins (AFB1= 1.59 μg/kg; AFB2 = 0.04 μg/kg) and fumonsins (FB1= 30 μg/kg; FB2 = 100 μg/kg) were detected in two rice products (emping and rice cake). High humidity and temperature could contribute to the occurrence of toxigenic fungi and mycotoxins production in addition to inappropriate storage conditions.

# S1-3

## CRITICAL POINT SOURCES OF OCHRATOXIN A CONTAMINATION IN ON-FARM STORED WINTER WHEAT

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Winter wheat has become an important crop for Ontario concentrating 73% of Canadian production. Maintaining grain quality and preventing mycotoxin formation in storage are crucial for individual farmers and the grain industry in general. A multi-year annual survey from 2011 to 2014 of the occurrence of *P. verrucosum* and OTA targeting on-farm stored winter wheat was conducted in 6 counties where approximately 50% of the total grain is produced. Five grain samples tested positive for OTA or 2.2% of samples and three samples, or 1.3% of the total grain samples taken, had OTA concentrations exceeding the EU regulation of 5 ng/g for raw cereal grains. A robust database was generated to identify critical points to minimize OTA accumulation by adding in the mycology data. Further, bins containing samples contaminated with OTA were characterized in detail. Risk factors for the accumulation of OTA in decreasing order of likelihood included: 1) grain clumps accumulated around or directly under manhole openings; 2) debris and residue of old grain or grain clumps from the bin walls or left on storage floor and augers; 3) grain clumps accumulated around side doors; 4) grain samples from untouched surface affected by high moisture migration to the central top part of grain bulk; 6) samples from the first or last loads. Samples collected from disturbed surfaces and intermediate loads were negative for OTA. Even when grain enters storage below the 14% threshold of moisture, condensation and moisture migration occurs in hotspots in modern corrugated steel storage bins. This often resulted in grain crusting, caking and clumps due to inadequate aeration and exposure to moisture from precipitation. A summary of best management practices will be presented.

# S1-4

## INDONESIAN MYCOTOXIGENIC FUNGI FROM AGRICULTURAL PRODUCTS

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Fungi and mycotoxin contamination of agricultural products is a major problem in Indonesia as well as in other tropical countries. This is due to the high temperature and humidity, which favor the growth of fungi, followed by producing their toxins. Several studies have been conducted to survey mycotoxin contamination in several commodities, such as peanut, corn, cocoa, coffee, dried cassava, dried fish, and chili. Several fungi have been isolated and identified based on morphological and molecular characteristics. Key genes responsible for mycotoxin metabolisms have also been used in this study to conform the potency of strains in mycotoxin metabolisms. Black aspergilli, the potential ochratoxin A (OTA) producers, were predominant fungi in cocoa bean, coffee bean, and dried cassava. OTA producing-strains were identified as *A. carbonarius* and *A. niger*, meanwhile *A. tubingensis* and *A aculeatus* were found as non OTA producing-strains [1]. Ochratoxin A producer *Penicillium* have been also detected in these commodities. This study shows that most species *Penicillium* found in cocoa and coffee beans are belong to *P. citrinum* which likely have a capability in the production of OTA [2]. Other study was to detect patulin producer *Aspergillus* isolated from dried cassava based on isoepoxydon dehydrogenase (IDH) gene of the patulin metabolic pathway. From this study 5 isolates identified as *Aspergillus niger, Aspergillus wentii, Aspergillus oryzae, Aspergillus flavus,* and *Aspergillus tamari* were detected IDH genes that have the potential to produce patulin. While fumonisin producer *Fusarium* have been identified in maize, i.e., *Fusarium verticillioides*, *Fusarium temperatum*, *Fusarium globosum*, *Fusarium proliferatum* and *Fusarium subglutinans* [3]. Our preliminary study show that aflatoxigenic and ochratoxigenic fungi were also detected in dried salted fish and dried chili sold in the market.

1. Apriyanto D Nugroho et al., *Journal of Food Science and Engineering*, 3, 472-480 (2013).
2. *Mona Nur Moulia et al., Indonesian Food and Nutrition Progress* 13 (1), 1-10 (2014).
3. Petrus A Nugroho et al., *Journal of Food Science and Engineering*, 3, 534-540 (2013).

# S2-1

## MYCOTOXIN RESEARCH IN THE PHILIPPINES: THE LAST TWO DECADES

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Researches on mycotoxins including aflatoxins (AF) in the Philippines in the last two decades have been few and far between. This situation is quite disturbing considering that prior to 2010, a number of researches have shown significant levels of AF and other mycotoxins in Philippine rice and its byproducts as well as other commodities.

Levels of AF reported in 2004-2011 in Philippine commodities ranged from <5 ug/kg in banana chips to <5 to 16,000 ug/kg in peanuts and peanut products. Occurrence of AF (% of samples >20 ug/kg) in these commodities ranged from 17.14 in peanuts and peanut products to 29.63 in corn products.

The total AF levels in 78 samples of polished and brown rice, determined by an immunoaffinity column clean-up method coupled with HPLC (detection limit: 25 ng/kg), ranged from <0.025-2.7 μg/kg (mean of positive samples: 0.37 μg/kg) and 0.03-8.7 μg/kg (mean of positive samples: 2.7 μg/kg), respectively. The incidence (percentage of positive samples) of AF in polished and brown rice were 94% and 100%, respectively.

The estimated potential daily intake of $AFB_1$ from rice is between 0.1 to 7.5 ng/kg of body weight/day, the mean of which is 1.0 ng representing 9.1-5.3 times the estimated tolerable daily intake for $AFB_1$ reported to date for Asia. Average natural concentration of AF in rice dust was 25 μg/kg. Estimates of the amount of inhaled AF by workers in an 8-h shift in a rice milling facility ranged from 0.06 to 114 ng. Thus, strategies should be developed and implemented to address both modes of exposure to AF. Public and private investments for research on the surveillance, epidemiology and control of AF and other mycotoxins is urgently needed to provide basis for short and long term interventions.

# S2-2

## CURRENT SITUATION OF ACTION ON MYCOTOXIN AND SURVEILLANCE OF MYCOTOXIN CONTIMINATION IN MONGOLIA

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A mycotoxin is a toxic secondary metabolite produced by fungi, also known as molds and usually produced by fungi of genus *Aspergillus, Penicilium* and *Fusarium*. Such mycotoxins contaminate food products, raw materials for food production and animal feeds and are highly dangerous to human health with risk of causing cancer, immune deficiency and mutation.

National Reference Laboratory for Food Safety (NRLFS), GASI, Mongolia approved the national standardized screening method ELISA for 7 types of mycotoxins in import and export goods in 2007 and set national standard for maximum permissible residue levels of mycotoxins contained in food products and animal feed and have been ensuring these regulatory compliances in Mongolia.

Around 70% of Mongolia's food imports are from China, Russia and South Korea. Basic food commodities imported in 2009-2012 that might have the risk of containing mycotoxins are grains (4.1-114.7 thousand tons), flour (50.1-105.7 thousand tons), flour products (9.2-14.8 thousand tons), rice (12-31.6 thousand tons) and millet (1.3-1.9 thousand tons) [1].

Studies done by National Cancer Center of Mongolia shows that accounted cancer incidences in population of 10000 people in 2011 and 2012 are considerably higher compared to that in 2009 and 2011. Percentage of patients with liver cancer is 6.6%, uterine cancer is 2.4%, lung cancer is 1.2%, oesophageal cancer is 1%, other diseases are 3.9% and other cancers are 4.2% in the population of 10000 people [2].

Center for Research and Risk Assessment; Chemical and Toxicological Laboratory and Microbiology Laboratory at NRLFS together with Ministry of Health initiated the first study of risk assessment for mycotoxins in food products in Mongolia. However, the result obtained from this study is not reliable as the numbers of samples are few and the ELISA method used is not confirmation method.

1. Custom House of Mongolia
2. National Statistical Commission

# S2-3

## ANALYSIS OF AFLATOXIN CONTAMINATION IN MYANMAR AGRICULTURAL COMMODITIES

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This paper provides the analyzing data of aflatoxins (AFs) contamination in Myanmar agricultural commodities which were intended to export and domestic consumption during 2008 to 2015 [1]. Most of the samples were white rice, broken rice, parboiled rice, green mung bean, black sesame seed, white sesame seed, black matpe, butter bean, toor whole, peyin bean (bamboo bean) and yellow maize. The total AFs concentration of these samples was quantitatively analyzed by the Romer method using thin layer chromatography with visual estimation [2]. The contamination of $AFB_1$ was commonly detected in all contaminated samples, however, $AFG_1$ and $AFG_2$ contamination with no AFB group was found in only one sample of broken rice in 2014. In addition, some samples were contaminated with not only $AFB_1$ but also $AFB_2$ and $AFG_1$. The sample which contaminated with four kinds of AF was not occurred. In 2008, one of the yellow maize samples was found with the highest concentration of $AFB_1$ (30.35 μg/kg). Generally, total AFs levels of the most contaminated samples were below the permissible limit of total AFs level regulated by European Union and Codex Alimentarius Commission.

1. Data source: Mycotoxin Laboratory from CTQM (HLEGU)
2. Romer, T. R., *J. Assoc. Off. Anal. Chem*., **58**, 500-506 (1975)

# S2-4

## ANALYSIS OF AFLATOXINS IN SPICES BY VALIDATED METHODOLOGIES IN SRI LANKA

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As an improvement in food security, more regulations continue to develop until acceptable levels of aflatoxins (AFs) obtain globally, especially for spices in Europe [1]. These regulations are important in developing countries as AFs occurrence is significantly high and affecting international trade and the economy. These spices are more susceptible for AFs contamination from the time of harvest, processing, transportation and product development [2]. Therefore, validated methodologies for the analysis of AFs in spices including pepper, nutmeg and chilli using HPLC-FLD is important. The extraction solvent and immunoaffinity column cleanup method were optimized to analyze $AFB_1$, $B_2$, $G_1$ and $G_2$ in nutmeg, pepper and chilli by reverse-phase HPLC and florescence detector (FLD) with TFA derivatization. The samples used for recovery study were spiked with 3 different concentrations of $AFG_1$, $AFB_1$ (30.0, 10.0 and 3.0 μg/kg) and $AFG_2$, $AFB_2$ (12.0, 3.0 and 1.2 μg/kg). The recovery percentage of $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ ranged from 65% to 105% for nutmeg, whereas from 70% to 95% for pepper. However the chilli showed comparatively low recoveries ranged from 60% to 85%. The RSD of repeatability for the determination of $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$ in all spices ranged from 1% to 18% whereas the LOD was 0.1 μg/kg for $AFB_1$/$AFG_1$ and 0.03 μg/kg for $AFG_2$/$AFB_2$. The LOQ was 1.0 μg/kg for $AFG_1$/$AFB_1$ and 0.3 μg/kg for $AFB_2$/$AFG_2$. The results indicated that the method developed for the analysis of spices is suitable for the routine laboratory analysis with much better LOD, LOQ, recovery, reproducibility, repeatability and accuracy. In this study, AFs in fifteen representative spices samples (five samples from each spice) were analyzed by above developed method. The analysis data showed that 60% of chilli were contaminated with total AFs level in the range of 0.4 to 8.7 μg/kg and pepper at 40%, the range of 1.2 to 10 μg/kg. However, nutmeg showed the highest contamination, which is 80% with the total AFs level in the range of 0.6 to 13.5 μg/kg.

1. Commission Regulation (EC) No 472/2002 of 12 March 2002 amending regulation (EC) No 466/2001 setting maximum levels for certain contaminant in foodstuffs. Official Journal of the European Communities, L75/18, Luxembourg.
2. Fatih Ozbey, Bulent Kabak, *Food Control*, 28, 354-361 (2012).

# S2-5

## MYCOTOXIN PROBLEM IN INDIA AND ITS PREVENTION BY NATURAL PRODUCTS

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Mycotoxins are secondary metabolites produced by toxigenic strains of Aspergillus, Penicillium and Fusarium. These are unavoidable contaminants of food and feed reported from all over the world. The tropical weather condition in India is conducive for the fungal infestation and subsequent mycotoxin production. Several common mycotoxins like Aflatoxins, Fumonisins, Zearalenone, T2 toxins are reported from Maize, Wheat, Sorgham, Rice, Parboiled rice, groundnut mainly due to unsatisfactory storage conditions. Red yeast rice, which is a dietary supplement and preservative, has been found to be often contaminated with citrinin. Consumption of such contaminated food causes adverse health effects. Several outbreaks of aflatoxicoses in poultry and farm animals have been reported from various parts of India in years 1966, 1985, 1994. Rare, but the aflatoxicoses outbreaks claiming human lives have been reported from India in1974 and Kenya in 2004. From these incidences, it is evident that mycotoxin contamination in food and feed is a major concern from food safety point of view. Development of effective degradation and detoxification methods is an important area of research as no suitable, economical and practical method has been established for wide variety of contaminated grains. Methods developed in our laboratory for degrading aflatoxin $B_1$ and citrinin include *physical* (heat with moisture), *chemical* (hydrogen peroxide, alkali, and lactic acid), *botanical* (spices, plant extracts) and *microbial* (*Pseudomonas putida*) treatments. At present India has 30 μg/kg aflatoxin as the permissible limit. This is much higher than the regulatory limits of Europe and America and needs to be revised in the wake of economic growth and knowledge about the serious effects of mycotoxins present in very small amounts.

# S2-6

## SCREENING FOR MODIFIED MYCOTOXINS BY HIGH-RESOLUTION LC-MS

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*Fusarium* fungi are plant pathogens infecting cereals known to cause disease called Fusarium head blight or scab. Some of them also produce mycotoxins such as trichothecenes and zearalenone. For these *Fusarium* mycotoxins, several glucoside derivatives are reported and known as "masked mycotoxins" or "modified mycotoxins." Since the hydrolysis of these glucosides releasing their aglycons was reported, they are considered to present an additional potential risk for mycotoxins. Considering the structural similarity among *Fusarium* mycotoxins (especially trichothecenes) and their simultaneous prevalence in cereals, screening of new modified mycotoxins was performed. For the detection of modified mycotoxins whose presence are uncertain, high resolution LC-MS (LC-Orbitrap MS) was used. With the accurate mass and high-resolution measurement, the detection of compounds whose chemical standards were not available was achieved. The identification was carried out on the basis of characteristic ions and fragmentation patterns observed with LC-Orbitrap MS. We detected masked mycotoxins derived from type B trichothecenes (fusarenon-X and nivalenol) in wheat grain that was artificially infected with *Fusarium* fungi. We further detected those derived from type A trichothecenes (T-2 toxin, HT-2 toxin, neosolaniol, di-acetoxyscirpenol, and mono-acetoxyscirpenol) in commercially available corn powder reference material. Although the absolute structures were not clarified except T-2 toxin-3-glucoside and di-acetoxyscirpenol-3-glucoside, glucose conjugation at the C-3 position in the trichothecene moiety seemed to be dominant based on the fragment profiles and concomitant detection of deoxynivalenol-3-glucoside (DON3Glc) in the identical samples. These findings indicate that not only type B, but also type A trichothecenes are possibly glucosylated in plants such as wheat and corn. Further, the presence of modified mycotoxins was searched for another group of *Fusarium* mycotoxins (fumonisins), and some new glucose conjugates were detected in our recent studies. A part of this work was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan.

1. Nakagawa et al., *JSM Mycotoxins*, 66, 21-25 (2016).
2. Matsuo et al, *Toxins*, 7, 3700-3714 (2015).

# S3-1

## MEASURING HUMAN MYCOTOXIN EXPOSURE USING BIOMARKERS

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**Background:** Aflatoxin exposure is prevalent in many parts of the world due to the consumption of contaminated food. Aflatoxin exposure has been associated with liver cancer, child stunting and immune suppression. Exposure biomarkers, for example aflatoxin albumin adducts (AF-alb) in blood and aflatoxin M1 (AFM1) in urine, have been developed and applied in epidemiological studies. It is known that urinary AFM1 reflects recent exposure (24 hours) but AF-alb has a longer half-life and can reflect the previous month's exposure. The use of these biomarkers in human health epidemiological studies will be briefly reviewed [1].

Following on, we will present a comparative study of the two biomarkers conducted in Tanzanian children. AF-alb levels were measured using an indirect competitive ELISA method following albumin extraction and purification. Urinary AFM1 was measured directly using a simple commercial ELISA kit. The limit of detection (LOD) of AF-alb and AFM1 was 3 pg/mg albumin and 7.5 pg/ml, respectively. A significant correlation between the two biomarkers was observed ($r=0.4865$, $p<0.001$). AF-alb and AFM1 showed a similar trend when either villages or seasons are compared. AFM1 was not correlated with age and maize intake of the child, whilst AF-alb was. The study suggests that AFM1 is compatible to AF-alb in many ways and is advantageous due to its easy and non-invasive sampling, as well as versatile detection by either ELISA (as a single biomarker) or by LC-MS as part of multiple biomarker analysis. However, AFM1 appears to be less reliable for indicating chronic exposure levels when compared with AF-alb.

1. M.N.Routledge and Y.Y Gong., Developing biomarkers of human exposure to mycotoxins. In: Determining mycotoxins and mycotoxigenic fungi in food and feed. Edited by S. De Saeger. pp225-244 (2011).

# S3-2

## MYCOTOXIN CO-OCCURRENCE IN FEEDSTUFFS IN JAPAN

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For the steady production of safe animal products, it is important to ensure the safety of feedstuffs. The Food and Agricultural Materials Inspection Center (FAMIC) conducts inspections of feedstuff samples in Japan, and analyzes various contaminants including mycotoxins. Mycotoxins in feedstuffs are mainly analyzed by the simultaneous determination of 16 mycotoxins using LC-MS/MS, which is the official Japanese method for determining contaminants in feedstuffs. This work reports the results of mycotoxin co-occurrence in feedstuffs in Japan.

Several compounds related to zearalenone (ZEN), a mycotoxin with estrogenic activity, have been identified. Most of these compounds have stronger estrogenic activity than ZEN [1]. Zearalanone, $\alpha$- and $\beta$-zearalenol were detected in maize, which is a main ingredient of compound feed in Japan, and in sorghum, which is an important source of ZEN contamination [2]. In relation to the estrogenic activity of ZEN, the activities of zearalanone and $\alpha$-zearalenol cannot be ignored, because both compounds possess higher estrogenic activities than ZEN.

Sterigmatocystin (STC), a mycotoxin, is known to be a precursor in aflatoxin biosynthesis. Analysis of the presence of STC in both maize and compound feed indicated that ingredients other than maize might be the main source of STC contamination in compound feed. On the other hand, the occurrence of STC and aflatoxin $B_1$ ($AFB_1$) showed a weak negative correlation in maize and compound feed; i.e., high STC concentrations caused a low $AFB_1$ concentration and *vice versa*.

The paired correlations between six mycotoxins (deoxynivalenol (DON), nivalenol (NIV), T-2 toxin (T-2), ZEN, $AFB_1$, and STC) in maize were investigated. Maize was mainly imported from USA, Brazil, Argentina, and Ukraine. NIV was detected mostly in Brazilian maize and had a strong positive correlation with DON. A high T-2 level was detected in Ukrainian maize, which had a weak positive correlation with DON.

Additionally, the paired correlations between the aforementioned six mycotoxins in compound feed were investigated. It found that compound feeds in Japan did not present high levels of mycotoxin contamination.

1. Shier et al., *Toxicon*, 39, 1435-1438 (2001).

2. Aoyama et al., *J. Food Hyg. Soc. Jpn*., 50, 47-51 (2009).

# S3-3

## RISK ASSESSMET OF MYCOTOXINS BY FOOD SAFETY COMMISION, JAPAN

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In Japan, the Food Safety Commission (FSC) is an organization conducting the risk assessment on food safety in a scientific, independent and fair manner. FSC has 12 Expert Committees that operate to either implement risk assessments on individual hazards such as food additives, pesticides and so on. Expert Committee for natural toxins and mycotoxins is one of those committees, which is in charge on risk assessments of mycotoxins in foods. Until now, this committee has published the risk assessment reports on patulin, total aflatoxins, deoxynivalenol and nivalenol, ochratoxin A, aflatoxin M1 in milk and aflatoxin B1 in feeds, and ochratoxin A, and now the Committee is discussing the risk of fumonisins.

In this lecture, discussions on the risk assessments of deoxynivalenol, nivalenol, aflatoxin M1 in milk, aflatoxin B1 in feeds and ochratoxin A will be reviewed.

Full report or summary of the results of the risk assessments of those mycotoxins written in English are available on the website of FSC as shown below.

1. Ochratoxin A
   (https://www.fsc.go.jp/english/evaluationreports/nm_toxins/ochratoxina_tx_fs94_2013.pdf)
2. Aflatoxin M1 in milk and aflatoxin B1 in feeds
   (https://www.fsc.go.jp/english/evaluationreports/nm_toxins/aflatoxin_m1_b1_fs526.pdf)
3. Deoxynivalenol and nivalenol
   (https://www.fsc.go.jp/english/evaluationreports/nm_toxins/toxs_fullreport_fs872_2010.pdf)

# S4-1

## MYCOTOXINS AND INTESTINE

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As the most extensively exposed surface in the body, the intestinal mucosa has to face important chemical and biological challenges. The intestinal mucosa has three main physiological functions. It establishes a physical barrier between the internal milieu and the luminal content. The intestinal mucosa is also responsible for luminal nutrients digestion and their subsequent absorption. The mucosal epithelium is at the interface of immune system and luminal contents, including dietary antigens and microbial products. This implies a local defence mechanisms regulation that involves integrating all the signals that come from the external and internal world to preserve immune homeostasis steady-state conditions. Either of these intestinal physiological functions may be targeted by feed contaminants such as mycotoxins. Mycotoxins are produced on a wide variety of raw materials before, during and after harvest. Very resistant to technological treatments, mycotoxins can be present in food. Among the "main" mycotoxins, DON and FB have been studied especially for their toxicity on the intestine [1-3]. They are not only locally toxic for this organ, but also dysregulate many intestinal functions and impair the immune response. This results in systemic toxicity leading to many symptoms and impairment of zootechnical parameters. Feed contamination with mycotoxins also increases translocation of bacteria across the intestine and thus intestinal and systemic infections. For AF, ZEN and OTA, less data are available concerning their intestinal toxicity [4,5]. The increased performance of analytical methods reveals new toxins, especially emerging ones, as well as "masked" or "modified" forms; it still needs to be determined if they represent a new for the intestine [6,7]. Global surveys indicate that co-contamination occus frequently but the health risk from exposure to a combination of mycotoxins is incompletely understood [8].

1. Pinton and Oswald. *Toxins*. 6, 1615-1643 (2014).
2. Payros et al., *Arch Toxicol*. In press (doi 10.1007/s00204-016-1826-4).
3. Bouhet and Oswald. *Mol. Nutr. Food Res*., 51, 925-931 (2007).
4. Grenier and Applegate. *Toxins*, 5, 396-430 (2013).
5. Akbari et al., *Arch Toxicol*., In press (doi 10.1007/s00204-016-1794-8).
6. Pierron et al., *Arch. Toxicol*., 90, 2037–2046 (2016).
7. Pierron et al., *Sci Rep*., 6, 29105 (2016).
8. Broekaert et al., *Food Chem Toxicol*., 80, 17-31 (2015).
9. Alassane-Kpembi et al., *Crit. Rev. Food Sci. Nutr*., In press (doi 10.1080/10408398. 2016.1140632).

# S4-2

## TOXICITY AND TOXICOKINETICS OF FUSARENON-X IN ANIMALS

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Trichothecene mycotoxins are a group of sesquiterpenoid mycotoxins that are synthesized by *Fusarium* fungi into their direct environment. Recent epidemiologic investigations indicate a significant increase in the global presence of fungi species. More than 180 derivatives of trichothecenes have been identified and divided into four types-A, B, C, and D-depending on their functional groups. Fusarenon-X (FX), a type B trichothecene mycotoxin, has been frequently observed along with deoxynivalenol and nivalenol (NIV) in agricultural commodities. The major toxicity of FX occurs through inhibition of protein synthesis, followed by the disruption of DNA synthesis. FX has also been shown to induce apoptosis *in vitro* (Jurkat human T cells) and *vivo* studies [1,2]. The targets of FX are organs containing actively proliferating cells including, thymus, Peyer's patches, spleen, small intestine, hematopoietic tissues, and embryonic brains [1,6]. We found that FX can induce apoptosis in lymphocytes in mice and developing mouse brain, and through an effect on *Bax*, *Bid*, *Trp53*, and *Caspase-9* which are involved in the intrinsic apoptotic pathway [2,6]. Based on the toxicokinetic information, FX is efficiently absorbed from the gastrointestinal tract of mice, broilers, ducks and piglets. The elimination half-life of FX is 1-2 hours after intravenous injection in broilers, ducks and piglets [3-5]. The liver and kidney are the organs responsible for the FX-to-NIV metabolism [3-5]. The results obtained from our study, which contributed to knowledge of the toxicity and toxicokinetic characteristics, particularly, FX, in mice and food-producing animals. However, adequate information about the fate and disposition of mycotoxins is also needed to establish the regulatory limit for human consumption and the ecological impact. We recommended that the toxicokinetics and residues of other trichothecenes should also be investigated to assess consumer health risk.

1. Aupanun et al., *Res. Vet. Sci*., 102, 217-222 (2015).
2. Aupanun et al., *Toxicon*, 123, 15-24 (2016).
3. Poapolathep et al., *Toxicon*, 41, 1047-1054 (2003).
4. Poapolathep et al., *Poult. Sci.,* 87, 1510-1515 (2008).
5. Saengtienchai et al., *Food Chem. Toxicol.,* 66, 307-312 (2014).
6. Sujarit et al., *Toxicol. Lett.,* 229, 292-302 (2014).

# S4-3

## EVIDENCE FOR INHIBITION OF CERAMIDE SYNTHASE BY FUMONISINS IN MAIZE-BASED FOODS: STUDIES IN HIGH- AND LOW-EXPOSURE COMMUNITIES IN GUATEMALA

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Fumonisins (FB) are found predominantly in corn and corn-based foods. Fumonisin $B_1$ ($FB_1$) is the most common FB and causes species-specific diseases in animals including hepto- and nephrotoxicity in rodents and neural tube defects (NTDs; serious birth defects) in mice. The critical event in the mechanism of FB toxicity in animals is inhibition of ceramide synthase, which leads to disruption of sphingolipid metabolism and increased tissue concentrations of sphinganine, sphingosine and their sphinganine-1-phosphate (Sa1P) and sphingosine-1-phosphate (So1P) metabolites. The human health implications of FBs are not known although they are a suspected risk factor for cancer and NTDs in populations regularly consuming large amounts of foods containing FBs. To further study human FB exposure patterns, $FB_1$ intakes in Guatemalan women (n=1240) from two low- and one high-FB exposure communities were compared using urinary $FB_1$ ($UFB_1$) as a putative biomarker of exposure. Sa1P, So1P, and the Sa1P/So1P ratio were also determined from whole blood samples collected from the women at the same time: samples were collected every three months from March 2011 to February 2012. LC-MS/MS analysis revealed that UFB1, Sa1P/ml blood, and blood Sa1P/So1P ratio were significantly higher in women living in the high-FB exposure community compared to those residing in the two low-FB exposure communities. These findings were corroborated in a second investigation (samples collected in February 2013) involving 300 women from a third low-FB exposure and two additional high-FB exposure communities. The correlations found between high FB1 intake and changes in blood Sa1P level and Sa1P/So1P ratio in these studies are consistent with those found in animals experimentally exposed to FBs, are evidence that FBs inhibit ceramide synthase in humans, and form a basis for further biomarker-based studies on FBs as potential risk factors for human disease.

# S4-4

## MOLECULAR MECHANISMS UNDERLYING OCHRATOXIN A-INDUCED MUTAGENESIS

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Ochratoxin A (OTA) is a renal carcinogen primarily affecting the S3 segment of proximal tubules in rodents. However, the results of the conventional mutagenicity tests have caused controversy regarding the role of genotoxic mechanisms in the carcinogenesis. Accordingly, site-specific *in vivo* mutagenicity in the kidney of *gpt* delta rats given a carcinogenic dose of OTA was performed. As a result, Spi$^-$ (*red*/*gam* gene) mutant frequencies (MFs) but not *gpt* MFs in the outer stripe of the outer medulla (OM) mainly composed of S3 segment were significantly higher than in controls despite the absence of cortical changes. Spectrum analysis in Spi$^-$ mutants induced by OTA treatment revealed large deletion (>1kb) in the red/gam gene. Further studies using *in vivo* comet assay, and analysis of DNA damage/repair related mRNA and/or protein expression demonstrated that OTA was capable of inducing double-strand breaks (DSBs) of DNA at the target sites. It is likely that DSBs induced by OTA were repaired predominantly by homologous recombination repair, possibly due to OTA-specific cell cycle regulation, consequently producing large deletion mutations at the carcinogenic target site. Additionally, global gene expression analysis showed that the expression of various genes transcribed by p53 fluctuated specifically at the target site. Therefore, effects of *p53* knockout on OTA-induced genotoxicity were performed in *p53*-deficient *gpt* delta mice. As a result, significant increases in Spi$^-$ MFs were observed in the kidney of *p53*-deficient, but not in *p53*-proficient *gpt* delta mice. G1/S arrest due to activation of the p53/p21 pathway may contribute to the prevention of DSBs in *p53*-proficient mice. Single base deletions/insertions/substitutions were predominant in mice. Overall, it is probable that OTA induced DSBs at the carcinogenic target site in rats and mice, and that p53/p21-mediated cell cycle control prevented an increase in the formation of DSBs, leading to gene mutations.

# S5-1

## STUDIES ON *ASPERGILLUS* SPECIES FOR THE CONTROL OF MYCOTOXINS

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Mycotoxins are toxic secondary metabolites produced by different fungal species. Aflatoxins are the key mycotoxins mainly produced by *Aspergillus flavus* and *A. parasiticus* and aflatoxins are known for their highly toxic and carcinogenic nature. Preventing the aflatoxin producing *Aspergillus* sp growth in contaminated food or feed willl in-turn reduces the aflatoxin contamination in it. Several strategies have been developed for control and prevent the aflatoxin producing *Aspergillus* strains. Use of atoxigenic *A. flavus* strains plays a better promising role in controlling aflatoxin producing *A. flavus* strains in the field especially in peanut fields. Several *Aspergillus* strains isolated from peanuts, corn, and peanut fields from different regions of China were tested for their atoxigenic nature by phenotypic and molecular approaches. Of them, many *A. flavus* strains did not produce aflatoxins and cyclopiazonic acids (CPA) which was confirmed by HPLC detection. Amplification of aflatoxin producing genes in the aflatoxin gene cluster of atoxigenic strains showed nearly 24 deletion patterns (B-Y), with 22 deletion patterns identified for the first time. At least one of these four genes, i.e. *aflT*, *nor-1*, *aflR* and *hypB* was found deleted. Several CPA gene deletion patterns were observed in atoxigenic strains. Atoxigenic *A. flavus* strains isolated from the peanut-cropped field showed deletion in twelve aflatoxin biosynthesis genes (*aflT*, *pksA*, *nor-1*, *fas-2*, *fas-1*, *aflR*, *aflJ*, *adhA*, *estA*, *norA*, *ver-1* and *verA*). Co-inoculation of the atoxigenic strains with the toxigenic strain GD-1, at different ratios could significantly reduce the aflatoxin production in PDA medium, peanuts, or maize. Application of atoxigenic *Aspergillus* strains in the field has proved to be highly effective in preventing the aflatoxin contamination. Several *Aspergillus* strains isolated from different source also proved effective in detoxifying aflatoxins and removal of its contamination in feed.

# S5-2

## DETECTION OF MYCOTOXINS USING IMAGING SURFACE PLASMON RESONANCE (iSPR)

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Significant progress has been made in the development of biosensors that can be used to detect mycotoxins. One technology that has been extensively tested is surface plasmon resonance (SPR). In 2003 a multi-toxin method was reported that detected aflatoxin $B_1$ ($AFB_1$), zearalenone (ZEA), fumonisin $B_1$ ($FB_1$), and deoxynivalenol (DON) in four separate flow cells [1]. Despite this early promise, there were no further reports describing application of SPR to multi-mycotoxin detection in foodstuffs until Kadota et al. developed a method for the simultaneous detection of nivalenol (NIV) and DON [2]. Meneely et al. detected DON and HT-2/T-2 toxins in parallel channels [3]. The instruments used individual channels for each toxin, often with a reference channel to control for matrix effects. More recently, SPR has been combined with imaging technologies that allow multiple sites within a flow cell to be rapidly interrogated. The result is imaging SPR (iSPR), where assays are performed at multiple locations in arrays. The technique was applied to DON and ZEA in maize and wheat [4]. Recent modifications include using polymer "brushes" and secondary antibodies labeled with gold nanoparticles (AuNP) to improve sensitivity [5]. The "traditional" and iSPR techniques have also been compared for a set of six mycotoxins [6]. The detection ranges of DON, ZEA, T-2 toxin, and $FB_1$ in assays using two chips in the more traditional format allowed measurement at the EU regulatory limits in barley. Also reported was the design of a prototype iSPR instrument that was portable but less sensitive. In our research we have been investigating the potential application of iSPR for the detection of DON, T-2 toxin, ZEA, and $FB_1$ in wheat. The selection of appropriate pairs of antibodies and immobilized antigens are critical. While affinity of antibodies for immobilized antigens is essential, regeneration of the sensor surface requires antibodies that are not of such high affinity that they cannot be removed during the regeneration steps. Thus the development of sensitive assays requires antibodies with a balance of affinities for the toxins and the antigens. The practical aspects of sensor design and function will be discussed, in particular the effects of solvent and matrix upon assay performance.

1. van der Gaag et al., *Food Control*, 14, 251-254 (2003).
2. Kadota, et al., *Anal. Chim. Acta*, 673, 173-178 (2010).
3. Meneely, et al., *World Mycotoxin J.*, 5, 117-126 (2012).
4. Dorokhin, et al. *Anal. Bioanal. Chem*., 400, 3005-3011 (2011).
5. Hu, et al., *J. Colloid Interface Sci*., 431, 71-76 (2014).
6. Joshi, et al., *Analyst*, (*DOI10.1039/c5an02512e*) (2016).

# S5-3

## NOVEL RECOMBINANT ENZYMES FOR DEACTIVATING *FUSARIUM* MYCOTOXINS

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Contamination of animal feed with mycotoxins is common. Even at concentrations below maximum values or guidance values, mycotoxins may affect animal health and productivity. Therefore, we are working on recombinant enzymes for use as feed additives for gastrointestinal degradation and detoxification of mycotoxins. FUM*zyme*, a fumonisin esterase which is registered in the European Union as fumonisin degrading feed additive, is an example.

Zearalenone (ZEN) is a mycotoxin produced by *Fusarium graminearum* and related species. The toxicity of ZEN is caused by its affinity for estrogen receptors, and it is a common natural contaminant of maize, wheat and other grains. We screened soil samples for ZEN degradation activity, enriched active cultures, and isolated a strain of *Rhodococcus erythropolis*, designated PFA D8-1, which hydrolysed ZEN with a lactonase. We isolated the previously elusive metabolite hydrolysed zearalenone (HZEN), and confirmed lack of estrogenic activity for HZEN and the spontaneously generated decarboxylated hydrolysed zearalenone DHZEN. The gene for zearalenone lactonase, *zenA*, was found by cloning a genomic library of strain PFA D8-1 in another *Rhodococcus erythropolis* strain and screening of pooled and then single clones for HZEN formation. The gene mapped to a megaplasmid designated pSFRL1. We cloned and expressed *zenA* in *E. coli*, purified the recombinant enzyme ZenA, and characterised it with a photometric assay and an HPLC method for quantification of ZEN, HZEN and DHZEN. Related sequences were found in GenBank, and we produced the recombinant enzymes in *E. coli*, characterised them, and determined atomic coordinates by X-ray crystallography for one of them. Determination of ZEN, HZEN and DHZEN in urine of piglets verified activity of ZenA in the gastrointestinal tract, and is allowing to compare engineered enzyme variants and enzyme concentrations. Development of ZenA, under the trade name ZEN*zyme*, as feed enzyme for gastrointestinal hydrolysis of ZEN is continued.

# S5-4

## RETENTION OF MAJOR *FUSARIUM* MYCOTOXINS DURING JAPANESE SOFT WHEAT PROCESSING

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Fusarium head blight (FHB) caused by several *Fusarium* species, which are commonly found in field, is a widespread and destructive disease of wheat, barley, and other small-grain cereals. In Japan, the precipitation during wheat flowering can result in FHB of wheat and causes two forms of agricultural damage: a reduction in yield and a contamination of grains by mycotoxins, such as deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA). Mycotoxins are commonly considered to be stable during processing and can contaminate finished processed foods. Different values of regulatory limits have been set for DON in wheat depending on the processed stage in many countries. In Japan, the regulatory limit for DON in unprocessed wheat grain was set as 1.1 μg/g in 2002 tentatively, according to the previous data obtained in foreign countries. Most of studies of the effect of processing on mycotoxin content in grains are focused on DON [1,2]. ZEA is another major *Fusarium* toxin and known as an estrogenic mycotoxin. For the settlement of the regulation level for ZEA, the retention of ZEA through processing should be clarified, however; studies using Japanese wheat cultivars were very limited and aimed for milling [3,4]. Since most of Japanese wheat cultivars are for noodle making, to assess the retention of ZEA in the final product, it is required to analyze the retention of ZEA after noodle cooking. In this study, a patent flour sample prepared from *Fusarium*-damaged Japanese soft wheat was subjected to noodle cooking, and the contents of ZEA in noodles and broth were determined using an in-house validated analytical method. In this sample, the substantial retention of ZEA in the boiled noodle (*udon*) was over 50%. Different effects of processing on DON and ZEA were also observed.

1. Kushiro, *Int. J. Mol. Sci*., 9, 2127-2145 (2008).
2. Kushiro, *JSM Mycotoxins*, 63, 117-131 (2013).
3. Zheng et al., *Food Control*, 40, 193-197 (2014).
4. Kushiro et al., *Jpn. J. Food Chem. Safety*, 21, 173-178 (2016).

# S5-5

## THE GENE POLYMORPHISMS INVOLVING FUMONISIN PRODUCIBILITY IN *FUSARIUM FUJIKUROI*

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*Fusarium fujikuroi*, rice bakanae pathogen, is known to produce fumonisins but some strains do not produce detectable level of fumonisins (non-producers). Investigation of Japanese *F. fujikuroi* indicated that 43 out of 93 strains were fumonisin non-producers [1] and phylogenetic analyses showed that non-producers are genetically different from the producers. A fumonisin biosynthetic gene (FUM) cluster was detected not only in fumonisin producers but also in non-producers. Therefore, genetic elucidation of fumonisin non-production is required for clarification of fumonisin non-producibility in *F. fujikuroi*. First, we performed PCR detection of a FUM cluster in non-producers. The results indicated that some non-producers retain an entire FUM cluster but some non-producers lack latter half of the cluster. Strain Gfc0801001 (G1) and Gfc0825009 (G9) were chosen for further analyses as a non-producer with an entire FUM cluster and a producer, respectively. Except for inessential *FUM17*, decisive aberrance was not detected in ORF of G1's FUM genes by sequencing. However, unsuccessful detection of the RT-PCR products of many FUM genes in G1 and a genetic mapping suggested that the cause of fumonisin non-production in G1 is attribute to the FUM cluster. Therefore, portions of the FUM cluster of G9 were transformed into G1. Fumonisin production was recovered by simultaneous complementation of the *FUM21* and *FUM6/7/8/3/10/11/2/13* regions. But the recovery was not observed by their independent complementation. These results suggested that the causative mutations are distributed in these regions. *FUM21* encodes a transcription factor and g.2551G>T substitution in G1 truncates C-terminal 11 amino acids. Complementation by *FUM21* with g.2551G successfully recovered fumonisin production in G1, in that the *FUM6/7/8/3/10/11/2/13* region had previously been complemented, while it was failed by *FUM21* with g.2551T. The results indicated that g.2551G>T in *FUM21* is one of the causative mutations of fumonisin non-production in *F. fujikuroi*.

1. Suga et al., *Fungal Biology*, 118, 402-412 (2014).

# AS01

## MYCOTOXIN ANALYSIS OF FOOD AND FEED: FROM RAPID ON-SITE TESTS TO HIGHLY ACCURATE MASS SPECTROMETRY METHODS

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Many countries all over the world introduced regulatory limits for the most important mycotoxins, including aflatoxin, deoxinivalenol, zearaleneone, fumonisin and ochratoxin. Different analytical approaches were developed and each method has its particular benefits and drawbacks. In general, two main strategies can be observed: rapid and simple screening tests and highly accurate reference methods. Commercially available rapid tests rely on the ability of antibodies to recognize certain toxins with high sensitivity and high specificity. These screening tests can be designed as ELISA, which allows the quantitative analysis of numerous samples in parallel or as lateral flow tests which allow the analysis of samples in a very short time, usually in a few minutes. Rapid tests are only able to analyze one particular mycotoxin in a sample but often one sample must be analyzed for several – usually the regulated – mycotoxins. Recently, mass spectrometry methods have been developed and published which are able to determine more than 200 mycotoxins in a single run. However, the development of LC-MS/MS methods is often impeded by the chemical diversity of mycotoxins and the compromises that therefore have to be made during sample preparation. Unfortunately, co-eluting matrix components influence the ionization efficiency of the analyte positively or negatively, impairing the repeatability and accuracy of the method. External matrix calibration for each commodity to be tested is too tedious for routine analysis.

The most recent developments using 13C labelled internal standards allow the efficient compensation of the influence of the matrix. Additionally, these stable isotope standards are often be combined with simple clean-up columns, which remove many undesired co-extracted compounds. The combination of spin columns with 13C standards opens a broad field of applications and improvement in mycotoxin analysis.

# AS02

## CHARM SCIENCE'S MYCOTOXIN TESTING SOLUTIONS

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Mycotoxins are produced by fungi ubiquitous in raw grains and finished feeds as a result of environmental conditions that occur during harvest or storage, often exacerbated by severe weather conditions. Mycotoxin contaminations lead to serious health issues for both humans and animals.

The Charm EZ®-M System is an all-in-one incubator and reader that automatically selects the test, then reads and displays the quantitative results. Immediate risk analysis is accomplished using real-time data integration to track contamination back to the specific farm or storage silo. A portable option includes GPS to track the location from where the test was performed. It supports a comprehensive mycotoxin control program by allowing testing to occur either at the time of collection/ harvest location, at the time of delivery, or in the quality control laboratory.

Charm Sciences manufactures complete testing solutions to detect mycotoxins in raw materials including Aflatoxins, Deoxynivalenol, Fumonisin, Zearalenone, Ochratoxin A, and T2-HT2. These rapid, easy-to-use, and economical tests can be implemented to screen and prevent mycotoxins from entering the feed or food supply.

Charm's Water Extraction Technology (WET) represents the first commercial application to eliminate methanol and ethanol to extract mycotoxins. This economic alternative reduces hazardous chemical shipping and storage, and allows negative results to be disposed of with normal waste. WET tests were initially developed for Aflatoxin and, based on feedback from industry partnerships, have grown to include Deoxynivalenol, Fumonisin, Zearalenone and Ochratoxin that is under development.

The United States Department of Agriculture Grain Inspection, Packers & Stockyards Administration (USDA GIPSA) offers a verification program for the performance of rapid tests for mycotoxins in grains. Charm Sciences currently holds the USDA contract to screen official grain samples for Aflatoxin and Deoxynivalenol.

Charm Sciences recently received GIPSA approval for a WET Fumonisin test. Charm currently has the fastest approved water-based test for both Aflatoxin in corn (3-minute test) and Deoxynivalenol (2-minute test). Charm also offers the only GIPSA approved Ochratoxin test. It is through growing partnerships that Charm's continuing innovation in mycotoxin testing solutions is possible.

# AS03

## MYCOTOXIN TESTING IN YOUR HAND – ON-SITE TESTING WITH SMARTPHONE-BASED TEST EVALUATION

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Monitoring mycotoxin contaminations by testing is necessary to verify that the products will meet national and international regulations and guidelines. Yet, instead of testing large numbers of end-products, a more pro-active approach would have many benefits. During the entire process from field to food or feed critical steps can be identified to monitor mycotoxins. For this approach a mobile, easy to use and quick tool for on-site mycotoxin testing is the optimal solution. Lateral flow-based tests are well accepted methods for this. However, for a quantitative result, a lab environment was still required. R-Biopharm now presents the next generation in rapid on-site mycotoxin testing, the RIDA®SMART APP. This software application allows you to use a smartphone as a powerful and easy to handle reader for a flexible and fast mycotoxin testing with RIDA®QUICK lateral flow tests - anytime, anywhere. It scans the test strip and evaluates it immediately. Quantitative results are generated within seconds by analyzing the line pattern on the taken picture. With this innovative and highly flexible tool R-Biopharm RIDA®QUICK mycotoxin tests can be evaluated reliably and fast.

The innovative evaluation tool RIDA®SMART APP gives accurate and precise results under controlled light conditions by showing very good recoveries with Trilogy® reference material. The RIDA®SMART APP software installed on validated smartphones generates reliable results in combination with all quantitative R-Biopharm RIDA®QUICK mycotoxin tests available. Additional to that further powerful features are included which turns this software into a fast, valuable and cost-effective alternative to conventional lateral flow readers. This makes it an optimal tool for testing in combination with RIDA®QUICK lateral flow mycotoxin tests - mobile, accurate, reliable.

# AS04

## VALIDATION OF AFLA-V AQUA™ LATERAL FLOW TEST KIT FOR DETECTION AFLATOXINS IN CORN USING A WATER -BASED EXTRACTION

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Aflatoxins (AFs) are a family of toxins produced by fungi, mainly Aspergillus flavus and Aspergillus parasiticus. The four major aflatoxins are AFB1, AFB2, AFG1 and AFG2. They are found in agricultural food crops such as corn and grow abundantly in warm, humid regions. Consumption by humans and livestock of AFs can cause mild to severe illnesses such as cirrhosis (liver damage) and result in development of liver cancer. AFB1 is the most potent known natural carcinogen. Due to this known fact, many developed countries have set regulations on allowable AFs consumption. The USFDA allowable consumption for humans is less than 20ppb and for livestock is less than 300ppb. These regulations cause a need for grain mills to test raw food crops. Many commercial test kits that are available use hazardous organic solvents to extract AFs this causes costly hazardous waste disposal fees. Vicam's Afla-V AQUA™ is a rapid, quantitative, water-based lateral flow strip test kit used for screening raw food crops such as corn. The Afla-V AQUA™ test kit were validated using four levels (5ppb, 20ppb, 100ppb and 300ppb) of naturally contaminated corn samples. At each level seven subsamples were analyzed by three separate analysts each using a different lot of test strips. The % RSD at 5, 20, 100 and 300 ppb were 13%, 9.7%, 13% and 15% respectively. The means of the determinations were 5.9, 16.5, 113 and 304 ppb respectively. The test kit values are within the test performance specifications for quantitative aflatoxin test kits published by GIPSA. The rapid, water-based Afla-V AQUA™ test kit will aid grain mills and regulatory agencies in detecting and monitoring aflatoxins without the use of hazardous and flammable organic solvents.

# AS05

## INVESTIGATION TO BUILD SIMULTANEOUS ANALYSIS METHODS OF MYCOTOXINS USING HPLC AND LC-MS

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Mycotoxins mainly aflatoxins in food are inspected to control harmful substances on regulations. Regulations on mycotoxins have been strengthened and control subjects have been widened lately. Since demands for simultaneous analysis of mycotoxins are increasing from such a background, we investigated to build simultaneous analytical methods of multi-mycotoxins for efficient inspections. We intended to build a screening method with high-usability using HPLC and a confirmation method with high-accuracy and sensitivity using LC-MS. In this session, we introduce the results of our investigations regarding simultaneous analysis of mycotoxins.

• Analysis of mycotoxins using HPLC

We built an analytical method of 10 mycotoxins in 14 minutes, which has high-sensitivity adjusted to strict EU regulations. And we optimized sample pretreatment procedures for grain, milk and apple samples. We packaged the analytical method including information about analysis conditions, sample pretreatment and some know-hows as Mycotoxin Screening Kit. And we've released Mycotoxin Screening System consisting of the Mycotoxin Screening Kit and Shimadzu Nexera-i HPLC system.

• Analysis of mycotoxins using LC-MS

We built an analytical method for 23 mycotoxins simultaneously with high-sensitivity, which is performed by the ultra-fast performance of our LC-MS mainly 5 msec polarity switching and 30,000 u/sec scanning speed. Even 3-ADON and 15-ADON could be separated completely. And we've succeeded to eliminate carryover of FMs under LOQs (1μg/L) by unique rinse methods using multi-solvents and a metal-free column.

# AS06

## OVERCOMING MATRIX INTERFERENCE IN THE DETECTION OF MYCOTOXINS IN FOODS

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Aflatoxin $B_1$ is a well-known potent human carcinogen produced by toxigenic fungi. Given its ubiquitous presence in a wide variety of foods and beverages, aflatoxin $B_1$ levels must be measured and monitored to prevent contaminated food from reaching the consumer. Enzyme linked immunosorbent assays (ELISAs) are frequently employed as a rapid and inexpensive method to screen samples that may contain aflatoxin $B_1$ concentrations above the legal permissible limit. To date, many available ELISAs are limited in the range of commodities that can be tested because they are subject to matrix interferences and require an additional clean-up step. The aim of this study was to evaluate if a single ELISA kit can accurately detect aflatoxin $B_1$ in commodities that typically pose matrix interferences, including nuts, spices, and other common cooking ingredients. The various comestibles were purchased from local markets and evaluated for matrix interferences. A Student's *t*-test was performed to determine any significant differences between the extracted commodity and extraction solvent alone. Samples were then fortified with aflatoxin $B_1$ at various concentrations ranging from 2.5-20ppb and measured by a previously developed competitive inhibition ELISA. The % recovery and %CVs were calculated. All food items exhibited minimal matrix interferences. The mean signal of the commodities did not demonstrate a statistically significant differences compared to the mean signal of the extraction solvent alone ($p<0.05$). Most commodities showed excellent recoveries of 82-111% with %CVs of less than 13%. The data demonstrates that a single ELISA kit can be used to successfully quantify aflatoxin $B_1$ in most commodities without the need for special extraction methods or clean-up procedures.

# AS07

## THE USE OF BIOCHIP ARRAY TECHNOLOGY FOR RAPID MULTIMYCOTOXIN SCREENING

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The main known groups of mycotoxins are aflatoxins, fumonisins, ochratoxins, type A trichothecenes (T-2 toxin and HT-2 toxin), type B trichothecenes (deoxynivalenol), and zearalenones. They are harmful to humans, domestic animals, and livestock. In Europe, maximum permitted limits for aflatoxin B1 are set, and guidance levels are recommended for the other mycotoxins. This study applied biochip array technology to semiquantitative multimycotoxin screening at different levels to facilitate the verification of the compliance of feed material with acceptable safety standards. This application was developed and validated based on European Commission Decision No. 2002/657/EC. After a single generic sample–preparation method, simultaneous competitive chemiluminescent immunoassays were used and applied to the Evidence Investigator analyzer. The r and within-laboratory R values showed low overall CVs (10.6 and 11.6%, respectively). Low matrix effect and, consequently, low decision limits and detection capabilities proved the high sensitivity of the technology. The overall average recovery was 104%. Samples ($n$ = 16) investigated within the Food Analysis Performance Assessment Scheme (FAPAS) program showed excellent correlation to assigned values. FAPAS proficiency-testing feed samples ($n$ = 10) were within the schemes' $z$-score ±2 range. The authentic feed samples survey showed excellent correlation with LC–MS/MS. This application is, therefore, reliable and represents an innovative, cost-effective, and multianalytical tool for mycotoxin screening [1].

1. Plotan et al., *J. AOAC Int*., 99, no. 4, (2016).

# YS01

## FEED AND FEED MANAGEMENT FACTORS IN RELATIONSHIP TO AFLATOXIN M1 CONTAMINATION IN BULK MILK OF SMALL HOLDER DAIRY FARMS

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The present study was aimed to identify feed and feed management factors that were associated AFM1 contamination in bulk milk of farms. The study was performed in May to July, 2016 using all small holder dairy farms. Data on feed and feed management were collected. In most farms, roughages originated from postharvest agricultural plants including grasses, hays and maize and by-product from local corn factories including dry grind outer layer of corn seed (corn dust) and dry boiled corn cobs with husks (Factory-corn silage). Commercial concentrates were mostly purchased from the cooperative. Feed management factors included characteristic of commercial concentrate (with or without cracked particles), keeping as a roughage pile within cow barn (yes or no), having mold in the surface area of roughage pile. AFM1 from the milk samples were determined by the Charm ROSA MRLAFMQ (Aflatoxin M1) Test using the Charm EZ system (Charm sciences Inc., USA). Fisher's exact chi-square tests were used to determine the association of feed and feed management factors with AFM1 contamination. From the total of 67 farms, 50 farms were included in the final analysis. Results showed that 46% of samples were contaminated with AFM1. Farms using factory-corn silage (62.5%) had significantly more percentage of AFM1 contamination than farms without (30.8%). The AFM1 contamination in farms using concentrates with cracked particles (64.3%) was higher percentage than that of without cracked particle (22.7%) at $P<0.01$. For feed management, roughages kept as piles within the cow barn (61.5% compared to 29.2%) had significantly higher percentages of AFM1 contamination. In addition, the roughage pile having mold at the surface area (60% vs 25%) had a higher percentage of AFM1 contamination ($P=0.02$). In conclusion, both the type of feed and the feed storage management factors were associated with AFM1 contamination in bulk milk.

# YS02

## CRITICAL POINT OF AFLATOXINS CONTAMINATION IN PEANUT ALONG THE SUPPLY CHAIN

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Peanut marketed in Malaysia is mostly imported from other countries and Malaysian Regulations established a limit of 15 ppb and 10 ppb for total aflatoxins in raw peanut and peanut based products, respectively. In this study, the level of aflatoxins and *Aspergillus* sp. contamination in peanut along the supply chain were determined by targeting the main peanut stakeholders (importer, manufacturer, and retailer). A total of 178 samples, consisted of raw peanut (peanut kernel and peanut in shell) and peanut based products (peanut snack, roasted peanut, roasted peanut in shell, peanut cookies, peanut sauce, and peanut butter) were collected from each stakeholder. Results show that raw peanut kernel samples from retailer were the most contaminated (0.25 – 1021.4 μg/kg) followed by manufacturer (2.07 – 181.9 μg/kg), which were 38% and 22% exceeded the limit, respectively. No aflatoxins were detected in samples from importers. On the other hand, 15% and 5.9% of peanut based products from retailer and manufacturer exceeded the limit, respectively. Fungal contamination (2.52 – 3.39 log CFU/g) was significantly higher in raw peanut samples compared to peanut based products (<2 – 2.32 log CFU/g) and *A. flavus* was detected in almost all raw peanut samples. A total of 128 isolates of *A. flavus* were recovered from both types of peanut and aflatoxigenicity test confirmed that those species were toxigenic. There was no significant different ($P>0.05$) of aflatoxins and *Aspergillus* contamination in raw peanut and peanut based products from different stakeholders. This study concluded that manufacturer and retailer were the critical points for aflatoxins contamination. However, fungal contamination was more critical in raw peanut sample compared to peanut based product for all stakeholder. Therefore, all stakeholders need to improve their practice and handling especially by controlling the temperature and humidity in storage room of peanut in order to keep the peanut safe for consumer.

# YS03

## FEED CHARACTERISTIC ASSOCIATED WITH AFLATOXIN B1 CONTAMINATION IN CONCENTRATE DAIRY COW FEED

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Aflatoxin B1 (AFB1) is the toxic metabolite of fungi produced by Aspergillus species. The contamination of this toxin has been widely reported in various animal feeds [1]. In dairy farm, AFB1 contamination in feedstuff is a major limitation of feed sources and improper feed management problems [2]. Although AFB1 contamination in dairy feed was continuous monitoring [3], however the problem still remain and affect to farm production efficiency. Therefore, this study was investigated AFB1 contamination in dairy cow feeds and investigated the association between AFB1 contamination and feed characteristic in small holder dairy farms. This study was conducted in 82 small holder dairy farms which located in the same area in Chiang Mai province, Thailand during March to May 2013. Ninety seven concentrate feed samples were collected for feed characteristic determination. The AFB1 concentration was measured by DOA-Aflatoxin ELISA Test Kit. Fisher's exact chi-square test was used to determine the association of feed characteristic with AFB1 contamination. Results demonstrated that 40.21% of concentrate feed samples was contaminated with AFB1. Cracked particle (43.3%), positive in blue greenish fluorescence test (44.33%) and rancid odor (19.59%) associated with AFB1 contamination in concentrate feed. In conclusion, characteristic of concentrate dairy cow feed was associated with AFB1 contamination.

1. Steinmeyer et al., *Toxicology*, 299, 69-79 (2012).
2. Abera, ***FSQM***, 50, 56-64 (2016).
3. Bertin et al., *Nutritional and Foraging Ecology of Sheep and Goats*, 85, 205-224 (2009).

# YS04

## HEALTH RISKS OF MYCOTOXIN CONTAMINATION IN IMPORTED WINE AND BEER CONSUMED IN THAILAND

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Mycotoxins are toxic metabolites which produced naturally by a group of common fungi such as *Aspergillus*, *Penicillium* and *Fusarium* [1]. The presence of mycotoxins has been reported in a wide range of commodities, including wine and beer [2,3]. Due to the widespread consumption of these beverages, assessment of toxins contamination and their residue level are required for consumer health. The aim of the present study was to evaluate the incidence of multiple mycotoxins in imported wine and beer being commercialized in Thailand, and to assess consumer health risk. A total of two hundred samples (one hundred of red wine samples and one hundred of beer samples) were collected randomly from supermarkets and retail shops in Bangkok, Thailand. Sample extraction was based on the dispersive liquid-liquid microextraction procedure and liquid chromatography tandem mass spectrometry (LC–MS/MS) was employed for the quantification and confirmation of 24 mycotoxins. The method was validated in terms of linearity, accuracy, precision, recovery, limits of detection (LOD) and quantification (LOQ). The results demonstrate that red wine samples and beer samples were contaminated with mycotoxins at 51% and 35%, respectively. The most prominent mycotoxins found in red wine samples were alternaria mycotoxins, ochratoxin A and fumonisins whereas deoxynivalenol, sterigmatocystine and zearalenone were predominantly found in beer samples. However, all toxin contaminant levels were far below the maximum limits established by the European Commission under EC regulation [4] . The results from this study indicated that the risk of mycotoxin exposure via imported wines and beers appears very low in urban areas of Thailand.

1. Frenich et al., *J. Chromatogr. A*. 1218, 4349-4356 (2011).
2. Mateo et al., *Int. J. Food Microbiol*. 119, 79-83 (2007).
3. Al-Taher et al., *J. Agri. Food Chem*. 61, 2378-2384 (2013).
4. Official Journal of the European Union L25, 3-5 (2005).

# YS05

## IMPLEMENTATION OF ISO 22000 FOOD SAFETY MANAGEMENT SYSTEM IN TUNISIAN "PASTA WARDA" COMPANY AND HAZARD ANALYSIS OF MYCOTOXINS TYPE OCHRATOXIN A

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The processing of cereals products (flour and semolina) faces many failures on the quality assurance of their products. The application of ISO 22000: 2005 (FSMM) allow, among others, some manufacturers to respond to the satisfaction of its customers and to contribute to the assessment of critical risks related to production processes in order to control them. The important consumption of cereal products in the Tunisian diet as a protein source and the presence of various contaminants in these products are strong motivators to implement a FSMM according to ISO 22000: 2005.

In this context, the Tunisian company "Pasta Warda" is engaged in the application of ISO 22000:2005 in the implementation of the pasta production line. In this work, various methods and procedures have been developed to monitor pasta safety issues at an early stage, including early detection of risks, warning systems, vulnerability assessment and corrective actions. Firstly, the implementation of the prerequisite programs (PRPs) allowed to control the probability of impact physical, chemical and microbiological risks. Moreover, the implementation of the HACCP principals allowed monitoring some hazards related to five critical control points: metal shrapnel, the presence of foreign bodies, the presence and proliferation of mycotoxins type ochratoxin for transfer and storage of raw materials, semi-finished and finished products, and, finally, the presence of foreign bodies during packaging. The "Pasta Warda" company appears to meet all the chapters of the ISO22000 standard and many positive aspects were found in his favor. Thus, the analytical monitoring by TLC and HPLC revealed the absence of ochratoxin A in raw materials and finished products, showing value the efforts employed by the "Pasta Warda" company in controlling this type of hazard.

# YS06

## FUMONISIN PRODUCTION RECOVERY IN A *FUSARIUM FUJIKUROI* STRAIN BY COMPLEMENTATION OF THREE FUM GENES

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Bakanae is an important rice disease in Asia caused by *Fusarium fujikuroi*. Not all but some *F. fujikuroi* strains are known to produce a carcinogenic mycotoxin, fumonisin. Sixteen clustered genes associated with fumonisin biosynthesis (FUM cluster) are revealed in the *F. fujikuroi* genome [1]. A FUM cluster was detected not only in fumonisin producers but also in non-producers (the strains do not produce detectable level of fumonisins). Therefore, genetic elucidation of fumonisin non-production is required for clarification of a fumonisin non-producibility in *F. fujikuroi*. The causative mutations of fumonisin non-production was investigated with a non-producer Gfc0801001 (G1) in this study. Previously, the g.2551G>T substitution in *FUM21* that truncate C-terminal 11 amino acids had been revealed as a causative mutation of fumonisin non-production in G1. However, a complementation by *FUM21* with g.2551G from a fumonisin producer Gfc0825009 (G9) failed recovery of fumonisin production in G1 and it suggested that G1 has an additional mutation(s) in the FUM cluster. In order to identify the location of the mutation(s), simultaneous complementation of *FUM21* and *FUM6*/*FUM7* regions was conducted in G1. The *FUM21* region from G9 was introduced into FfT67FUMKOD-1 that was previously created with complementation by *FUM6*/*FUM7* region from G9 but fumonisin production was not recovered in it. Twenty *FUM21* complementary transformants of FfT67FUMKOD-1 were newly created and *in vitro* culture indicated that fumonisin production was positive in their 19 transformants. Previous studies showed that fumonisin production recovery was failed by independent complementation of *FUM21* and *FUM6*/*FUM7* regions in G1. However, fumonisin production recovery was succeeded by simultaneous complementation of the *FUM21* and *FUM6*/*FUM7* regions in this study. These indicates that cause of fumonisin non-production in G1 is not a single mutation and the mutations are distributed in these regions.

1. Wiemann et al., *PLoS Pathogens*, 9, e1003475 (2013).

# YS07

## PROTEIN *O*-MANNOSYLTRANSFERASES ARE INVOLEVED IN STERIGMATOCYSTIN PRODUCTION AND FUNGAL DEVELOPMENT

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*Aspergillus nidulans* produces sterigmatocystin (ST), a precursor of a carcinogenic secondary metabolite aflatoxin, during its developmental process. ST biosynthetic pathway has been shown to be affected by various regulatory factors. In this study, we investigated the involvement of protein *O*-mannosyltransferases (Pmts), consisting of PmtA, PmtB and PmtC, in ST production and morphological development. Deletion of *pmtA* (*pmtA*Δ), *pmtB* (*pmtB*Δ) or *pmtC* (*pmtC*Δ) caused no spore production and a significant decline of vegetative growth. The growth morphology of these deletants were also different from each other. Influences of *PmtB* (*pmtB*::*ptrA*) or *pmtC* (*pmtC*::*ptrA*) disruption on fungal development were different from *pmtB*Δ and *pmtC*Δ deletants, respectively. Addition of 0.6 M KCl restored the wild-type level of radial growth in *pmtB*::*ptrA* and *pmtC*::*ptrA* disruptants and sporulation in *pmtB*::*ptrA* strain, but not in *pmtB*Δ or *pmtC*Δ. These results suggest that N-terminal portions of the PmtB and PmtC proteins have some unknown partial activity required for normal development. When a *pmt* gene was deleted, the expression levels of the remaining two *pmt* genes in the mutants were higher than the wild-type, implying that a loss of *pmt* gene may be compensated by increased expression of the remaining *pmt* genes. All *pmt*Δ lost their ability to produce wild-type level of ST. At 72 h time point after inoculation, ST production of *pmtA*Δ, *pmtB*Δ and *pmtC*Δ were 14.59 ng/μl, 2.62 ng/μl and 1.93 ng/μl, respectively; which were much lower than that in the wild-type, 185.72 ng/μl. Expression level of *aflR* gene was also down-regulated in all three mutants. As ST biosynthesis is regulated by *aflR*, our data suggests that Pmts affect ST production through regulating expression of *aflR*.

# YS08

## STUDIES ON THE INHIBOTRY ACTIVITY OF BLASTICIDIN S TOWARD AFLATOXIN PRODUCTION

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Aflatoxins are a group of structurally related metabolites produced by some *Aspergillus* species including *A. flavus* and *A. parasiticus*, and are found in a wide variety of food and feed commodities. Because they are potent mutagens and are liver carcinogens in humans and a wide range of animal species, the contamination of aflatoxins in agricultural products causes huge economic loss and is a serious health threat. A practical use of aflatoxin production inhibitors is expected to be an effective method to prevent aflatoxin contamination in agricultural products. Blasticidin S (BcS) is a metabolite of *Streptomyces griseochromogenes*, and is known to block protein synthesis of both bacteria and eukaryotes by inhibiting peptidyl transfer in their ribosomes. BcS shows strong growth inhibitory activity against a number of microorganisms. However, BcS inhibited aflatoxin production by *A. flavus* ($IC_{50}$ 27μM) without affecting its growth. A compound which can inhibit aflatoxin production strongly and specifically is desirable in practical application. In this study, we prepared several BcS derivatives. Their inhibitory activity against aflatoxin production by *A. flavus* and growth inhibitory activity against some microorganisms were evaluated, and the specificity for aflatoxin production was compared.

Among six kinds of BcS derivatives, blasticidin S methyl ester (MeBcS), deaminohydroxy blasticidin S and pyrimidinoblasticidin S inhibited aflatoxin production by *A. flavus* in potato dextrose broth and their $IC_{50}$ values were 5, 110 and 610μM, respectively. BcS and MeBcS inhibited the growth of *Aspergillus niger*, *Saccharomyces cerevisiae and Escherichia coli*, and the minimum inhibitory concentrations of the two compounds were same levels. MeBcS inhibited aflatoxin production by *A. flavus* in YES medium ($IC_{50}$ 80μM), but BcS and its derivatives did not. These results indicated that the inhibitory activity of BcS against aflatoxin production was enhanced by esterification of its carboxyl group and MeBcS might be more practical than BcS because the specificity of MeBcS for inhibition of aflatoxin production is higher than that of BcS.

# YS09

## PROTECTIVE EFFECTS OF SCHIFF BASE CYCLIC AMINO ACID DERIVATIVES AGAINST MYCOTOXINS GENO- AND CYTOTOXICITY

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The objective of this study was to investigate the protective effects of Schiff base cyclic amino acid derivatives picolinyl-L-phenylalanine (PLP), picolinyl-L-tryptophan (PLT) and nicotinyl-L-tryptophan (NLT) against genotoxicity and cytotoxicity of mycotoxins aflatoxin B1 (AFB1) and ochratoxin A (OTA) in rat blood and bone marrow using comet assay and trypan blue exclusion test in vivo. Additionally molecular-cytogenetic effects of AFB1 on copy number variants (CNVs) was studied in vitro in human leukocytes. Oral administration of AFB1 and OTA at concentration of 25 μg/kg/day over 21 days leads to increased levels of DNA damage but does not show the cytotoxic effect. PLP, NLT and PLT (5 and 10 mg/kg/day, 10 days of administration) demonstrated weak genotoxic activity, with exception for PLT which was non-genotoxic at concentration 5 mg/kg/day. PLP, PLT and NLT had no impact on cell viability. AFB1-induced DNA damage was reduced by 50% after treatment with PLT (10 mg/kg/day). OTA-induced DNA damage was reduced by 34% after treatment with NLT (5 mg/kg/day). It was revealed that genotoxic and protective activities of substances investigated are tissue-dependent. Thus, the PLT demonstrated the lowest genotoxicity and highest protective activity and requires further surveys. Additionally the influence of AFB1 on CNVs was studied in vitro in human leukocytes using parental origin determination fluorescence in situ hybridization. The obtained results indicate that AFB1 can induce CNVs instability in 8p21.2 and 15q11.2 regions which was a consequence of deletions. This first study on influence of AFB1 on CNVs requires further systematic trials in future.

# YS10

## PROTECTIVE ROLE OF SCHIFF BASE DERIVATIVES OF AMINO ACIDS AGAINST AFLATOXIN B1-INDUCED APOPTOSIS IN RATS

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**Background:** Mycotoxins contaminate the diet of a large proportion of the world's population. In many low-income countries mycotoxins affect staple foods, including groundnuts, corn, other cereals and nuts, such that exposure is continuous and often at high levels. They are currently a major health threat in human population. The aim of this study was to evaluate the potential ability of picolinyl-L-phenylalaninate (PLP), picolinyl-L- tryptophanate (PLT), and nicotinyl-L-tryptophanate (NLT) Schiff base amino acid derivatives to act against damaging effects of aflatoxin B1 (AFB1) mycotoxin using animal models of mycotoxicosis. For this purpose, the rate of apoptosis marker proteins - annexin V (ANX-V), B-cell lymphoma 2 (Bcl-2) proper and Bcl-2 associated X protein (Bax) were determined in blood plasma samples.

**Methods:** White pubescent rats (with 180-200 b/w) were used in all experiments. Animal care, handling and use in research were performed according to the international regulations. Concentration of the annexin V, Bax and Bcl-2 proteins were measured in plasma by ELISA using commercially available kits according to manufacturer's instruction.

**Results:** According to the results obtained, PLP, PLT and NLT caused significant increase of ANX-V levels both compared with control and mycotoxicosis group. In aflatoxicosis bearing rats treated with the tested Schiff bases the ANX-V levels were higher compared to AFB1 group. AFB1 caused significant increase of Bax level. According to the data obtained, no differences of Bcl-2 levels were observed in all studied groups compared to control.

**Conclusions:** Overall the results of the assessment indicated that AFB1 causes massive increase of the levels of pro-apoptotic marker protein Bax in the blood of rats. Meanwhile, it has become apparent that Schiff bases are capable to initiate increase of ANX-V levels in blood. However, this elevation probably is not linked to apoptotic processes.

# YS11

## STUDIES ON THE MODE OF ACTION OF DIOCTATIN THAT INHIBITS AFLATOXIN PRODUCTION OF *ASPERGILLUS* SPECIES.

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Aflatoxin contamination is a serious problem worldwide. Effective methods to control the contamination are desired urgently. We have been studying specific inhibitors of aflatoxin production, because such inhibitors are expected to be useful for prevention of aflatoxin contamination without incurring a rapid spread of resistant strains, as well as for investigation of the basic regulatory mechanisms of aflatoxin production in fungi.

Dioctatin A, a metabolite of *Streptomyces* sp., has a strong aflatoxin production inhibitory activity against *Aspergillus parasiticus* [1] and *Aspergillus flavus*. To determine the molecular mechanism of the dioctatin activity, we first synthesized dioctatin-immobilized magnetic nanobeads (Dot-beads) by click reaction of ethynyl-conjugated dioctatin and azide-conjugated beads. Dot-beads were incubated with *A. flavus* protein extracts. After extensive washing, a dioctatin-binding protein was eluted from Dot-beads by a dioctatin solution and subjected to SDS-PAGE. On the gel, a band was observed around 27 kDa and LC/MS/MS analysis revealed that the band contains mostly Clp protease proteolytic subunit (ClpP), a mitochondrial localized protease. Bacterially expressed *A. flavus* ClpP also binds to Dot-beads and eluted by the dioctatin solution, confirming the specific binding of dioctatin to ClpP. Dioctatin promoted casein degradation by recombinant ClpP in the concentration-dependent manner. These results suggested that dioctatin causes a functional disorder of ClpP, which might cause mitochondrial dysfunction and metabolic disorder, leading to inhibit aflatoxin production.

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1. Yoshinari et al., *Microbiology*, 153, 2774-2780 (2007).

# YS12

## STUDY ON THE TARGET MOLECULE OF CYCLO($_L$-Ala-$_L$-Pro), A SPECIFIC AFLATOXIN PRODUCTION INHIBITOR

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Aflatoxin is a carcinogenic mycotoxin which is produced by some *Aspergillus* species. Aflatoxin contamination in crops occurs worldwide and related economic losses are speculated to reach million dollars per year. Therefore, effective methods to control the contamination have been urgently required. Usage of specific aflatoxin inhibitors has been considered to be an effective solution for this problem. Cyclo($_L$-Ala-$_L$-Pro), a metabolite isolated from *Stenotrophomonas* strain No. 27, can inhibit aflatoxin production without inhibiting the growth of aflatoxin-producing fungi [1]. Thus, cyclo($_L$-Ala-$_L$-Pro) and its producing strain may be used for controlling aflatoxin contamination by *Aspergillus* species without incurring a rapid spread of resistant strains. However, the mode of action of this compound has not been clarified yet. In this study, as a first step for understanding the mode of action, we identified the target molecule of cyclo($_L$-Ala-$_L$-Pro).

Cyclo($_L$-Ala-$_L$-Pro)-binding proteins were screened against proteins extracted from *A. flavus* by using cyclo($_L$-Ala-$_L$-Pro)-immobilized sepharose beads, and glutathione *S*-transferase (AfGST) was identified as a candidate for the target molecule. Immuno-blot analysis with His-tagged recombinant AfGST (His-AfGST) confirmed the binding of cyclo($_L$-Ala-$_L$-Pro) to AfGST. In addition, the binding was inhibited under the competitive condition suggesting that cyclo($_L$-Ala-$_L$-Pro) binds to AfGST specifically. The expression analysis showed that cyclo($_L$-Ala-$_L$-Pro) did not affect AfGST expression. Enzymological analysis showed that cyclo($_L$-Ala-$_L$-Pro) inhibited the GST activity of AfGST. On the other hand, cyclo($_L$-Ala-$_L$-Pro) did not affect the GST activity of *Schistosoma japonicum* GST. Thus, cyclo($_L$-Ala-$_L$-Pro) is supposed to inhibit GST activity of AfGST specifically. From these results, cyclo($_L$-Ala-$_L$-Pro) is considered to inhibit aflatoxin production through inhibiting the GST activity of AfGST. Now, we are preparing the AfGST deletion mutant and will analyze AfGST's function in aflatoxin production with the mutant.

This work is supported by Research Project for Improving Food Safety and Animal Health.

1. Jermnak et al., *Microbiology*, 159, 902-912 (2013).

# YS13

## THE SYNERGY OF CEMycoS NETWORK IN OVERCOMING MYCOTOXINS PROBLEMS

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A melting pot of activities engaged in education, research, and public services, concerning the prevention and reduction of fungal infestation and mycotoxin contamination in university, have delivered to initiate a group study of mycotoxin consortium. An experience of this emerging consortium, consisted of university, research institute, and community, will be encouraged to the dissemination of research output and community service in overcoming mycotoxigenic fungi and mycotoxins in food chains. The effort, strategy and approach as well as implementation of research output should be able to answer the community challenges through enhancement of utilization endogenous resources. The role of endogenous filamentous fungi has been initiated for reducing and detoxifying aflatoxin $B_1$ under solid state fermentation in feed and food. Moreover, the limited trial of its detoxified product has also been performed and compared to mycotoxin binder in broiler feeding study. In case of agricultural waste materials, the utilization of rice hull ash for potential source of silica in solid support of immunoaffinity column for aflatoxins clean-up have also been studied. The effort and contribution in overcoming mycotoxins problems will be accelerated if understanding of mycotoxin problems and strengthening Asia network are encouraged to enhance the role of researcher as facilitator agent of culture, knowledge and science in community empowerment. This role could open many opportunities to enhance any network in Asia countries for mutual collaboration of overcoming mycotoxins problems based on lesson learning in specific countries.

# SS01

## THE EVOLUTION OF MYCOTOXIN/PHYTOTOXIN BIOSYNTHESIS AND VIRULENCE IN THE PLANT PATHOGENIC FUNGUS *ALTERNARIA ALTERNATA*

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The tomato pathotype of *Alternaria alternata* (*A. arborescens*), the causal agent of the stem canker of tomato, produces host-specific AAL-toxin and induces apoptotic cell death on tomato cells. The chemical structure of the AAL-toxin resembles to that of a mycotoxin fumonisin produced by *Fusarium* (*Gibberella*) spp., and both toxins are classified as sphinganine-analog mycotoxins due to their structural similarity to sphinganine. The AAL-toxin biosynthetic (*ALT*) gene cluster consists of at least 13 genes homologous to the fumonisin biosynthetic (*FUM*) genes in *F. verticillioides*. The *FUM* (*ALT*) cluster homologues are found in *Fusarium* spp., *Aspergillus niger* and *Cochliobolus heterostrophus*. On the other hand, only the tomato pathotype of *A. alternata* has the cluster among *Alternaria* spp.. The *ALT* cluster in the tomato pathotype might be acquired by horizontal transfer of the entire cluster genes from those pathogenic fungi. The *ALT* cluster locates only on the 1.0 Mb small chromosome found in the tomato pathotype. Based on biological and pathological observations, the small chromosome was termed conditionally dispensable chromosome (CDC) and pathogenicity chromosome. The CDC in the tomato pathotype strains from different geographical origins was identical although the genetic backgrounds of the strains differed. The results imply that the CDC has a different evolutionary history from the essential or core chromosomes in the same genome. A hybrid strain between two different pathotypes was shown to harbor the CDCs from both parental strains and had an expanded pathogenicity range, indicating that CDCs could be transmitted from one strain to another and stably maintained in the new genome. We propose a hypothesis whereby the ability to produce a toxin and to infect a plant is distributed among *A. alternata* strains by horizontal transfer of an entire pathogenicity chromosome. This could provide a possible mechanism by which new mycotoxin-producers (plant pathogens) arise in nature.

# SS02

## DEVELOPMENT OF MAGNETIC GRAPHENE OXIDE-CHITOSAN ADSORBANT FOR THE REDUCTION OF MULTI-MYCOTOXINS IN ANIMAL FEED AND TOXICITY EVALUATION OF THE RESULTANT FEEDS

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A novel magnetic graphene oxide modified with chitosan beads (MGO-CTS) was synthesized as an adsorbent in order to investigate the simultaneous removal of multi-mycotoxin. The composite was characterized by various techniques FTIR, SEM and XRD measurements. The adsorption evaluation was studied with the effect of pH, initial multi-mycotoxin concentration, adsorption time and temperature. The maximum adsorption capacity according to the Freundlich isotherm model was 68.58, 251.48 and 420.93 ng/g for $AFB_1$, OTA and ZEA, respectively. Thermodynamic results involving the changes in free energy ($\Delta G^o$), enthalpy ($\Delta H^o$) and entropy ($\Delta S^o$) indicated that the adsorption of multi-mycotoxin was spontaneous, exothermic and favorable. The modified adsorbent was applied for the reduction of multi-mycotoxins in contaminated palm kernel cake (PKC) in animal feed. PKC at 0, 10, 20 and 30.0% was combined with corn, soybean and palm oil as a new formula for animal feed. The results revealed that MGO-CTS could reduce mycotoxins in feed (≥20% PKC). Multi-mycotoxin (1, 5, 10, 50, 125, 250, 500, 1000 and 2000 mg/L) was added to zebrafish in order to assess the effects of MGO-CTS after their reduction in animal feed. Development of zebrafish embryos, developmental abnormalities including edemas and malformations were observed at 72 hours post fertilization (hpf) and 3 days post fertilization (dpf). No significant developmental abnormalities including edemas and malformations were associated with embryonic exposure mycotoxins. The effect of multi-mycotoxin exposure was concentration-dependent with $LD_{50}$ value of 360 mg/kg. All embryos died after exposure to 1000 mg/L. Embryo did not show any detrimental effect on liver, kidney, fertility, growth rate and feed intake when it was exposed to the sub-lethal concentrations of 75, 150 and 300 mg/l of multi-mycotoxin. Therefore, multi-mycotoxin of the mentioned doses had no apparent toxicological signs on zebrafish embryos.

# SS03

## AFLATOXIN EXPOSURE MEASURED BY AFLATOXIN ALBUMIN ADDUCT BIOMARKER IN SIX AFRICAN COUNTRIES

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We have applied a validated ELISA method to assess aflatoxin exposure using the aflatoxin albumin adduct (AF-alb) biomarker in children and/or adults in six African countries; Gambia, Guinea, Kenya, Senegal, Tanzania and Uganda. In children in Tanzania mean AF-alb levels increased with age from 4.7 pg/mg albumin at 6-14 months old to 12.9 pg/mg albumin at 12-20 months old and 23.5 pg/mg albumin at 18-26 months old [1]. There was a correlation between food intake of aflatoxin and AF-alb [2]. In The Gambia AF-alb levels also increased in children with age from a mean of 11 pg/mg at 6 months, to 55 pg/mg at 12 months and 98 pg/mg at 18 months. Also in The Gambia, AF-alb levels were higher in pregnant women in the dry season (when stored groundnuts are consumed) compared to the rainy (harvest) season; geometric mean = 52.8 vs 29.6 pg/mg albumin [3]. In Guinea AF-alb levels were also found to be higher in 288 children (mean age 29 months) during the storage season compared to the harvest season (mean = 16.3 vs 12.7 pg/mg) [4]. Higher levels of AF-alb were measured in adults in Senegal (15-80 pg/mg), whilst AF-alb levels in Ugandan children (0-3 years) and adults (18-89 years) were lower and not very different between these two groups (mean = 9.7 and 11.5 pg/mg, respectively) [5,6]. The highest levels of AF-alb were measured in samples taken from Kenyan children (mean age 12 years) in 2002 (mean = 114.5 pg/mg) and 2004 (mean = 539.7 pg/mg) [7], a year of outbreaks of acute aflatoxicosis in Kenya. These studies have measured widespread exposure to aflatoxin in children and adults at levels for which chronic exposure has been associated with deleterious health effects, highlighting the pressing need for action to reduce aflatoxin exposure in sub-Saharan Africa.

1. Shirima et al. *Environ. Health Persp.,* 123, 173-178 (2015).
2. Routledge et al. *Biomarkers*, 19, 430-435 (2014).
3. Castelino et al., *Trop. Med. Int. Health,* 19, 348-354 (2014).
4. Watson et al., *Mol. Nutr. Food Res.,* 60, 511-518 (2016).
5. Watson et al., *World Mycooxin J*., 8, 525-531 (2015).
6. Asiki et al., *Trop. Med. Int. Health*, 19, 592-599 (2014).
7. Gong et al., *Environ. Health Persp.,* 120, 893-896 (2012).

# SS04

## CULTIVATION METHODS OF FORAGE CORN FOR REDUCING FUMONISIN: NEW "KIMIMARU" VARIETY AND PESTISIDE APPLICATION

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It was recently reported that *Fusarium* mycotoxins, especially fumonisin, have been detected in domestic forage corn (*Zea mays* L.). Thus, it is essential to develop effective and realistic measures to control *Fusarium* mycotoxins in forage corn. In this report, we introduce a new variety of corn, "Kimimaru," that has a low concentration of *Fusarium* mycotoxins, and present a cultivation method for reducing concentrations of *Fusarium* mycotoxins by using pesticide.

Comparison of mycotoxin concentrations in Kimimaru and other varieties: Corn varieties 36B08, 34N84, and X7M657 (Pioneer EcoScience, Co., Ltd.), and Kimimaru (NARO) were cultured in 2010. They were cultivated in the following six locations in Tohoku district in Japan. In the yellow-ripe stage, three individuals from each variety were harvested 10 cm above, and subjected to mycotoxin quantitative analysis. The fumonisin ($B_1$+$B_2$+$B_3$) concentration of X7M657 was found to be the highest, at 4800-5600 μg/kg, and the Kimimaru variety showed the lowest concentration, at under 10-150 μg/kg. Other *Fusarium* mycotoxins (nivalenol, deoxynivalenol and zearalenone) were also found to have low concentrations in the Kimimaru variety [1]. Accordingly, we believe that Kimimaru is a low *Fusarium* mycotoxin content variety.

Mycotoxin concentration reduction by pesticide application: Corn varieties LG3490 (Snow Brand Seed Co., Ltd.) and Kimimaru were cultured in Iwate in 2015. Pesticides propiconazole and azoxystrobin were then applied following conventional methods. At the full-ripe stage, kernels were sampled and subjected to fumonisin quantitative analysis. The fumonisin concentrations in control samples (no pesticide) were 4610 μg/kg and 13300 μg/kg for Kimimaru and LG3490, respectively. Under propiconazol application, Kimimaru showed a fumonisin concentration of 2030 μg/kg, while LG3490 showed only 420 μg/kg. Under azoxystrobin application, Kimimaru and LG3490 showed concentrations of 2490 μg/kg and 1490 μg/kg, respectively. Thus, both pesticides reduced fumonisin. Used effectively, these pesticides can successfully reduce fumonisin concentrations.

1. Uozumi et al., *Jpn. Soc. Grassl. Sci*., 59, 198-104 (2013).

# SS05

## MYCOTOXIN DEGRADATION BY LACCASE ENZYMES FROM *PLEUROTUS* SPP

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Mycotoxin occurrence in staple food commodities represents a great concern worldwide. These fungal metabolites constitute an important food safety hazard due to their demonstrated toxic, carcinogenic, teratogenic and mutagenic activity towards humans and animals (International Agency on Research on Cancer, 2012). Mycotoxin contamination greatly affects global economy and international trade, accounting for billions of dollars lost every year all along the food and feed supply chains (Wu, 2015). The development of new methods for mycotoxin reduction is a crucial and concrete struggle, especially with regards to food and feed supply chains. With this aim, we evaluated the degrading activity of two pure laccase enzymes from *Pleurotus* spp towards aflatoxin $B_1$ ($AFB_1$), aflatoxin $M_1$ ($AFM_1$) and zearalenone. (ZEA) Laccases were produced and purified to homogeneity and tested in *in vitro* degradation assays, performed in buffer solution (sodium acetate pH5 1mM).

After 72h of static incubation at 25°C, chemical analyses revealed that both laccase enzymes were able to degrade $AFB_1$ (up to 90%), $AFM_1$ (up to 100%) and ZEA (up to 100%). Laccase degrading activity was also tested in real matrices, such as skimmed UHT milk artificially contaminated with $AFM_1$ and maize flour naturally contaminated with zearalenone. $AFM_1$ was completely degraded within 72h, while ZEA was reduced by 40% within 5 days of incubation at 25°C.

The green feature, effectiveness and multi mycotoxin degrading capability of laccase enzymes are the key attributes of a potential biotransforming agent. These results opened new perspective for laccase application in the food and feed supply chains.

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1. International Agency on Research on Cancer, 2012.
2. Wu. World *Mycotoxin Journal* 8, 137-142, 2015

# PS01

## GENETIC POPULATION STRUCTURE OF *FUSARIUM GRAMINEARUM* SPECIES COMPLEX IN KOREAN CEREALS

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Small grain cereals are frequently contaminated with toxigenic *Fusarium* species. Members of the *Fusarium graminearum* species complex (FGSC) are known as a head blight pathogens and mycotoxin producers. In order to characterize the FGSC populations associated with cereals in Korea, barley, corn, maize, and rice grains were collected from fields at harvest or during storage from 2009 to 2011. The fungal colonies recovered from grain samples were identified based on morphological characteristics and analyses of *TEF* sequences, and trichothecene chemotype was analyzed using a *TRI12* PCR assay. Among 517 FGSC isolates, *Fusarium asiaticum* accounted for more than 70% of all isolates, and was especially common in barley, wheat and rice. Most *F. asiaticum* had the nivalenol chemotype (~85%), while 3-acetyl deoxynivalenol (3-ADON, ~15%) and 15-ADON (<1%) chemotypes were also detected. In corn, however, *F. graminearum*, was dominant (56%), followed by *F. asiaticum* (38%). Among *F. graminearum* isolates from corn, the 15-ADON chemotype was predominant (72%) and 21% had the 3-ADON type. *F. vorosii* and *F. boothii* appeared at very low frequency during the test period, and were excluded from further analysis. Analyses of diversity at VNTR loci using STRUCTURE confirmed the separation between *F. graminearum* and *F. asiaticum*. Individual analyses of each species suggested that *F. graminearum* had a different and stronger genetic substructure than *F. asiaticum*. Population analyses using Arlequin revealed that *F. asiaticum* clusters displayed a low level of differentiation (Fst= 0.08), whereas *F. graminearum* clusters exhibited a much higher level of genetic differentiation (Fst= 0.23).

# PS02

## INOCULATION OF FUMONISIN-PRODUCING *FUSARIUM FUJIKUROI* ISOLATES TO GRAPE BERRIES AND THE FUMONISIN PRODUCTION ON THE BERRIES

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Recently [1] we found fumonisin$B_1$ ($FB_1$) and fumonisin$B_2$ ($FB_2$) in wine produced by a Japanese winery whereas ochratoxin and $FB_2$ were recognized as mycotoxin contaminants in wine as previously reported. $FB_1$ and $FB_2$ have been commonly discovered in corn and corn products contaminated with *Fusarium* species including *F. verticillioides* and *F. fujikuori*. Then, we isolated $FB_1$ and $FB_2$ producing strains of *F. fujikuori* and *F. proliferatum*, from grape berries and other sources in the winery as previously reported [2]. In this study we observed fungal invasion by inoculating of the *F. fujikuori* to the berries and production of the fumonisins (FBs) to know the causative fungus of the mycotoxin contamination.

**Materials and Methods:** *F. fujikuori,* isolated from the berries, was inoculated to intact and differently pericarp-damaged berries. After the inoculation, the berries were incubated for 14 days at 25C. Then fungal invasion of the fungus in the berries was observed microscopically and contents of FBs in the berries were determined by LC/MS/MS as previously reported.

**Results and Discussions:** *F. fujikuori* cannot enter into the berries without damage of the pericarp. In the berries with slightly damaged pericarp the fungal mycelium scarcely invaded into the inner part of berry, maybe due to phenolic compounds, antifungal agents, relatively concentrated in hypodermis under the pericarp. Fungal invasion or development in the berries and subsequent FBs production were likely to be affected by the damage of pericarp.

These results clearly showed that *F. fujikuori* occurred as an opportunistic pathogen to grape berry and also the causative fungus responsible for FBs contamination in the vineyard of Japan.

1. Nakagawa et al. In preparation.
2. Hashimoto et al. In preparation.

# PS03

## RISK POTENTIAL OF RICE GRAINS CONTAMINATED WITH AN ENDOPHYTIC FUNGUS *PENICILLIUM BROCAE*

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*Penicillium brocae* is an endophytic fungus, which was isolated from the tissue of insects collected at coffee plantations, or the tissue of marine organisms. At present, we found it for the first time on rice grains in Thailand, indicating a possible contaminant of rice. Among mycotoxigenic *Penicillium*, *P. islandicum*, *P. citreonigrum*, and *P. citrinum* are well known to be causative species responsible for toxic yellowed rice. The metabolites of these species had been reported to be harmful for human health. Until now, some researchers have documented limited kinds of secondary metabolites of *P. brocae,* but those were little toxic. Due to no reports on *P. brocae*-damaged rice grains, we conducted mutagenicity test. Test samples were methanol extracts of mouldy rice grains inoculated with two isolates of *P. brocae* in rice medium. As tester strains, *Salmonella typhimurium* TA98, TA100, TA1537, and YG7108, and *Escherichia coli WP2/uvrA/*pKM101 were used for the estimation of base-pair substitution and/or frameshift mutations. According to the official guidelines, the assay was performed both with and without S9 mix conditions. As the results, the crude extracts of two isolates were positive for *S. typhimurium* TA100 and YG7108, suggesting the mutation of base-pair substitution. In TA100, one extract showed the mutagenicity in the absence of S9 mix, and another one showed it in either of S9 mix conditions. On the other hand, the mutagenicity of two extracts in YG7108 were observed in both with and without S9 mix conditions. The mutagenic activity for YG7108 was slightly stronger than that for TA100. As YG7108 lacks $O^6$-methylguanine methyltransferase enzyme, it is highly sensitive to alkylating agents. Our data appeared that *P. brocae* possessed the ability to produce mutagenic chemicals. In conclusion, rice grains contaminated with *P. brocae* are capable of being a new risk factor for human health.

# PS04

## TRANSCRIPTIONAL REGULATION OF *TRI6* IN *FUSARIUM GRAMINEARUM*

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A $Cys_2His_2$ zinc finger transcription factor encoded by *Tri6* regulates expression of trichothecene biosynthesis genes (*Tri* genes) in *Fusarium* species. While *Fusarium sporotrichioides* produces trichothecenes in axenic liquid culture without sucrose, *Fusarium graminearum* requires sucrose for the mycotoxin production. To understand the regulatory mechanisms of *Tri6* expression, we generated transgenic *F. graminearum* strains, in which promoter and coding region of *Tri6* were replaced with those of *F. sporotrichioides*, respectively. In contrast to *F. sporotrichioides*, neither of the transgenic stains produced trichothecene in the absence of sucrose, suggesting that the promoter and coding sequences of *Tri6* are not responsible for the inability of *F. graminearum* to produce the mycotoxin in the absence of sucrose. When *Tri6* was constitutively expressed in *F. graminearum,* the transgenic strain produced an extremely limited amount of trichothecenes in the absence of sucrose. By adding a small amount of sucrose (100 μM) that is insufficient for inducing trichothecene production for the wild-type strain, the transgenic strain was stimulated to produce the mycotoxin to a remarkably greater extent. Mutations of the putative TRI6p-binding consensus sequence on the *Tri4-Tri6* divergent promoter (AGGCCT → AGGgCT) abolished the trichothecene-producing activity of *F. graminearum* even in the presence of a high level of sucrose (175 mM), but heterologous expression of *Tri4* outside from the trichothecene gene cluster restored the toxin production by the mutant to some extents. The effect of nitrogen sources on transcriptional regulation of *Tri6* was also examined. Based on the results, we propose a model in which a limited amount of *Tri6* mRNA, initially transcribed in a TRI6-independent manner, culminates in subsequent self-activation step of *Tri6* transcription with the help of post-translationally functionalized TRI6p.

# PS05

## IMPROVEMENT OF THE METHOD FOR DETECTION OF AFLATOXIN-PRODUCING FUNGI

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Methods for detection of aflatoxin (AF)-producing fungi, e.g., plate culture methods, are continually modified. Nevertheless, plate culture methods require refinement because they produce unclear data occasionally. In this study, activated charcoal powder (carbon) was added to culture media containing cyclodextrin (CD) to enhance the contrast of fluorescence and to improve the efficiency of detection of AF. Three culture media—potato-dextrose-agar (PDA), yeast extract-sucrose-agar (YESA), and rice extract-agar (REA)—were evaluated using plate culture conditions. The final concentration of CD in the media was set to 0.3 mg/mL, and that of carbon was set to 0.1–0.5 mg/mL. According to the comparison of three types of CDs, alpha CD (αCD), which is composed of a 6-membered ring, yielded the highest fluorescence intensity. In combination with >0.3 mg/ml carbon, the CD plate enhanced AF-derived fluorescence intensity. Several fungi that could not be detected with CD alone in the medium were detected after the addition of carbon. Meanwhile, high-contrast fluorescence was not observed on the YESA plate although formation of colonies was faster on YESA than on PDA. Additionally, the REA plate, which provides oligotrophic culture conditions, did not yield high-contrast fluorescence as compared to the other plates. By examining the change in optical characteristics, we found that addition of carbon improved visibility of the fluorescence by attenuating light scatter by ~30% in the PDA plate. Besides, a reflected-light setup was more suitable for fluorescence analysis than a transmitted-light setting was. These results suggest that addition of carbon to PDA containing αCD improves the efficiency of detection of AF-producing fungi.

# PS06

## PRODUCTION OF AFLATOXIN AND THE BIOSYNTHETIC CLUSTER IN CLINICAL ISOLATES OF *ASPERGILLUS FLAVUS*

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*Aspergillus flavus* is an important human and animal pathogen. Moreover, the fungus is well-known as an aflatoxin producer. Previous studies show that ratio of aflatoxin productivity in *A. flavus* is small in the environment of Japan. However, the aflatoxin productivity of clinical isolates of *A. flavus* in Japan has not been investigated. In this work, we examined the productivity of aflatoxin and the structure of aflatoxin biosynthesis clusters of 11 clinical isolates in A. *flavus*.

Our sequencing analysis indicates that aflatoxin biosynthesis clusters in three of 11 strains are partially deleted. Aflatoxin biosynthesis clusters in five strains indicate high homology with the cluster of *A. oryzae* RIB40, and the clusters in the remaining three strains indicate high homology with the cluster in *A. flavus* NRRL3357 strain, an aflatoxin producer. We analyzed aflatoxin productivity of 11 strains with ELISA and LC-MS/MS. Aflatoxin production was confirmed in two strains among 11 strains by LC-MS/MS. These two strains possess aflatoxin clusters similar to the cluster in *A. flavus* NRRL3357 strain.

These data suggest that in Japan the number of aflatoxin producers among clinical isolates of *A. flavus* is small as well as environmental isolates. Although further investigate is required, aflatoxin productivity might be dispensable as a pathogen.

# PS07

## AN AMINO ACID SUBSTITUTION OF AN AMINOTRANSFERASE INVOLVED IN AAL-TOXIN BIOSYNTHESIS ALTERS THE STRUCTURE OF MYCOTOXIN AAL-TOXIN IN PATHOGENIC FUNGUS *ALTERNARIA ALTERNATA TOMATO PATHOTYPE*

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AAL-toxins are produced by the tomato pathotype of *Alternaria alternata* (*Alternaria arborescens*), which causes stem canker of tomato disease on certain susceptible cultivars. The toxins structurally resemble to mycotoxin fumonisins produced by several *Fusarium* spp., and they are referred to as sphinganine-analog mycotoxins (SAMs). AAL-toxins and fumonisins contain several derivatives, which show different bioactivity in plants and mammalian cells. For example, fumonisin B (FB) and C (FC) series are mainly produced by *F. verticillioides* etc. and rare strains of *F. oxysporum*, respectively, the bioactivities of them are higher than that of fumonisin A and P. The structural change of the amino group in these toxins drastically alters the bioactivity. The amino group of AAL-toxin is derived from glycine as well as that of FC series, while that of FB series is derived from alanine. The gene, *ALT4* and *FUM8*, involved in addition of the amino group to each polyketide that forms backbone structure of each toxin have been identified in *A. alternata* tomato pathotype and *F. verticillioides*, respectively. Comparative analysis of the amino acid sequence of the deduced protein encoded by *ALT4* and *FUM8* showed that an amino acid is different between FvFum8 and FoFum8 derived from FB and FC producing strain, respectively, and the corresponding amino acid of Alt4 is identical to that of FoFum8. Introduction of the construct including amino acid substitution at the corresponding site of Alt4 into *ALT4* deleted mutant generated three transformants. LC-MS analysis of the metabolites in three transformants indicated that all transformants produce novel derivatives of AAL-toxins, while normal AAL-toxin were significantly reduced. The toxicity of the metabolites and pathogenicity were reduced on host plants. These results suggested that an amino acid of the Alt4 is involved in the structure of amino group in AAL-toxins.

# PS08

## PHYLOGENIC STUDIES ON SACCHARIFYING ACTIVITY AND FUMONISIN PRODUCTION IN THE STRAINS OF KURO-KOJI MOLDS AND THEIR RELATIVES ISOLATED FROM FERMENTED FOODS

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In Japan, Kuro-koji molds have been used in brewing shouchu, a spirit, because they highly produced organic acid to prevent undesired bacterial growth and also have higher saccharifying activity. They also have been used in processing of Pu-erh tea in China for post-fermentation. Previous reports showed *Aspergillus niger* and Kuro-koji molds were closely related species whereas recent reports demonstrated that *A. niger* produced mycotoxins, fumonisin B2 (FB2) and others. In this study we examined fumonisin production, saccharifying activity, and phylogenic relationships in the isolates of Kuro-koji molds and their relatives to establish the safeness and to know the property as the fermenting starter.

Seventy four strains were isolated from Kuro-koji for shouchu, fermented tea leaves and other foods which were made in Japan or other Asian countries. The strains were identified by morphology and molecular analyses including sequence analysis of β-tubulin gene. Potential for fumonisin production of the strain was examined by the presence of PCR-amplified fum8 gene as a predictor of FB2 production. Saccharifying activity was estimated by detecting halo formation on the incubated starch agar plate after starch-iodine reaction and also by the dinitoro salicylate assay.

Phylogenic analysis proved that the strains tested were classified into three lineages of *Aspergillus luchuensis*, *A. niger* and *Aspergillus tubingensis*. All of the strains from Kuro-koji for producing shouchu belonged to *A. luchuensis*. PCR-amplifications of *fum8* gene was detected only in *A. niger* strains. Strong and weak saccharifying activities were found in *A. luchuensis* strains from environment and in several strains of *A. niger*, respectively, whereas other strains including from tea leaves did not form distinct halo even though the they were *A. luchuensis*. Present study was correspondent to those results from several previous studies about FB2 productions, and they indicated that any strains of *A. luchuensis* never produced FB2 while our result indicated that saccharifying activity of them was different depending on the source, suggesting they are the good starter.

# PS09

## SPREAD AND CHANGE IN STRESS RESISTANCE OF SHIGA TOXIN-PRODUCING *Escherichia coli* O157 ON FOOD-RELATED FUNGAL COLONIES

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Various kinds of microbial interaction on food are known and would affect food safety. To elucidate the physical effect of fungal hyphae on the foodborne bacterial pathogen, the spread and change in stress resistance of Shiga toxin-producing *Escherichia coli* (STEC) O157 were evaluated after coculture with 11 species of food-related fungi including fermentation starters. Spread distances of STEC O157 varied depending on the co-cultured fungal species, and the motile bacterial strain spread for longer distances than the non-motile strain. The population of STEC O157 increased when co-cultured on colonies of nine fungal species but decreased on colonies of *Emericella nidulans* and *Aspergillus ochraceus*. Confocal scanning microscopy visualization of green fluorescent protein-tagged STEC O157 on fungal hyphae revealed that the bacterium colonized in the water film that existed on and between hyphae. To investigate the physiological changes in STEC O157 caused by co-culturing with fungi, the bacterium was harvested after 7 days of co-culturing and tested for acid resistance. After co-culture with eight fungal species, STEC O157 showed greater acid resistance compared to those cultured without fungi. Our results indicate that fungal hyphae can spread the contamination of STEC O157 and can also enhance the stress resistance of the bacteria. In addition to chemical effects that enhance growth of bacterial pathogen [1,2], physical microbial interaction on food should be further investigated.

1. Lee et al., *J. Food Sci*., 77, M102-M107 (2012).
2. Cibelli et al., *J. Food Prot*., 71, 2129-2132 (2008).

# PS10

## BIOCHIP ARRAYS FOR THE FLEXIBLE MULTI-MYCOTOXIN SEMI-QUANTITATIVE SCREENING OF ANIMAL FEED SAMPLES

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**Introduction.** Biochip Array Technology (BAT) allows the simultaneous semi-quantitative determination of a broad range of mycotoxins from a single sample to accommodate the lowest established global guidance limits for a particular feed type. The main known groups of mycotoxins are aflatoxins, fumonisins, ochratoxins, trichothecenes A (T-2 toxin, HT-2 toxin), trichothecenes B (deoxynivalenol), and zearalenones. Co-occurrence of mycotoxins in a wide range of samples has been previously reported globally. For food and feed safety the determination of these mycotoxins is extremely important. BAT enables the screening of any combination of mycotoxins with minimum of three from a list of ten mycotoxins assays available to suit the requirements of testing laboratories.

This study presents applicability of the technology to the simultaneous detection of the most prevalent mycotoxins in animal feed samples.

**Methodology.** Simultaneous competitive chemiluminescent immunoassays, defining discrete test sites on the biochip surface and applied to the Evidence Investigator analyser were employed. Assay validation was based on 2002/657/EC. All the mycotoxins were extracted from each feed sample using a single generic liquid/liquid extraction developed in-house.

**Results.** Aflatoxins, ochratoxin A, fumonisins, trichothecenes A (T-2 toxin, HT-2 toxin), trichothecenes B (deoxynivalenol) and zearalenone including metabolites were simultaneously detected with the Myco 7 biochip array. The screening decision levels ranged from 0.25ppb (ochratoxin A and aflatoxin B1 assays) to 100ppb (deoxynivalenol assay) for sensitive detection and from 3.12ppb (ochratoxin A and aflatoxin B1 assays) to 1250ppb (deoxynivalenol assay) for monitory level. The analysis of ten animal feed samples from the Food Analysis Performance Assessment Scheme (FAPAS) and thirteen FAPAS QC showed values within the range assigned by FAPAS for samples presenting single or multi-mycotoxin contamination.

**Conclusions.** The results indicate applicability of BAT to the reliable multi-mycotoxin screening in animal feed samples and also the capacity of this technology to detect co-occurrence of different mycotoxins.

# PS11

## TRACE ANALYSIS OF AFLATOXINS IN SPICES BY HPLC COUPLED WITH SOLID-PHASE DISPERSIVE EXTRACTION FOLLOWED BY FLUORESCENCE DERIVATIZATION, AND ITS ACCURACY MANAGEMENT FOR METHOD VALIDATION

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Aflatoxins $B_1$, $B_2$, $G_1$, and $G_2$ (AFs) are mycotoxins that display strong carcinogenicity in nature. There are anecdotal reports that AFs are present in imported food, such as beans and spices, at levels exceeding regulatory limits. In this study, a simple, rapid, and effective cleanup method was developed for the determination of AFs in spices by high-performance liquid chromatography (HPLC) with fluorescence detection (FL). The developed method features two pretreatment steps: solid-phase dispersive extraction (SPDE) [1,2] and solid-phase *in situ* fluorescence derivatization using immunoaffinity (IA) gel. In addition, accuracy management for method validation, such as internal and external quality control, was performed to determine the applicability of the method to spices, which are generally difficult to analyze.

White pepper selected as the representative spice was extracted with 80% aqueous methanol. The extract was diluted with an aqueous surfactant and cleaned up by SPDE using IA gel. After removal of the liquid phase from the solid phase (IA gel) by centrifugation, AFs retained on the IA gel were *in situ* derivatized into fluorophores by adding trifluoroacetic acid directly to the IA gel. The fluorophores were eluted with water, and the eluate was subjected to HPLC-FL. The combination of SPDE and solid-phase *in situ* fluorescence derivatization offered a simple and rapid means for sample preparation for AF measurement by HPLC-FL. As another merit, SPDE and solid-phase *in situ* fluorescence derivatization could be sequentially performed in a closed system, thereby minimizing exposure to the hazardous chemicals, AFs. Intra-day, inter-day, and inter-laboratory assay data for method validation suggested that the proposed method has sufficient precision for the determination of residual AFs in spices.

1. Sakamoto et al., *Anal. Sci*., 30, 271-275 (2014).
2. Saito et al., *J. Pharm. Biomed. Anal*., 100, 28-32 (2014).

# PS12

## OCCRRENCE OF OCHRATOXIN A IN JAPANESE, THAI, AND CHINESE INSTANT NOODLS (RAMEN)

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Ochratoxin A (OTA), which is a nephrotoxin and possible human carcinogen (Group 2B) by IARC, occurs in various cereals and their products, beans, wine, and coffee. Wheat is an important intake source of OTA in Japan, but most of the contamination in wheat related foods was not reported. Therefore, we collected commercially available instant noodles (ramen) from Japan (22 samples), China (29), and Thailand (28) in 2015-16, and analyzed OTA and OTB by immmunoaffinity column (IAC)-HPLC method using OTB.2 monoclonal antibody, which was bispecific to OTA and OTB.

Powdered instant ramen (20 g) was extracted with 60 mL of methanol : 1% $NaHCO_3$ (7+3) using high speed blender (10,000 rpm, for 3 min). The filtrate was diluted 4 times with PBS. After filtration with glass fiber filter (GS-25), 10 mL of the filtrate was applied to the IAC. The eluate was analyzed by HPLC.

The limit of determination of OTA or OTB was 0.016 ppb. Sixty eight percent (15/22) of Japanese instant ramen were contaminated with OTA (the overall average was 0.108 ppb. The maximum was 0.469 ppb). Fifty seven percent (16/28) of Thai instant ramen were contaminated with OTA (the overall average was 0.096 ppb. The maximum was 0.288 ppb). Twenty eight percent (7/29) of Chinese samples were contaminated with OTA (the overall average was 0.038 ppb, which was significantly lower ($p<0.05$) than Japanese and Thai. The maximum was 0.207 ppb). OTB was not detected in any samples.

When a person eats 2 bags of maximum contaminated instant ramen everyday, the intake of OTA was 93.8 ng/day, which was 12.5% of TDI. OTA, which is not high concentration, was frequently found in Japanese and Thai instant ramen.

# PS13

## MULTIPLE DETECTION OF SUGAR DERIVATIVES OF FUMONISINS IN CORN BY LC-ORBITRAP MS

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Fumonisins are a group of mycotoxins that are typically produced by *Fusarium verticillioides* and *F. proliferatum*. The European Committee concluded the establishment of a group PMTDI of 2 μg $kg^{-1}$ bw $day^{-1}$ for fumonisin $B_1$ ($FB_1$), $FB_2$, and $FB_3$, combined. A part of fumonisin was known to be trapped to matrix macro-constituents such as a starch, and it is called hidden (bound) fumonisin. In order to inquire amount of total fumonisin in food and feed, the hydrolyzed fumonisin (HFB) is used for quantification of fumonisin. Although this method is able to determine free and hidden (bound) fumonisins at the same time, it is not able to determine *N*-substituted fumonisins such as *N*-(1-deoxy-D-fructos-1-yl) fumonisin $B_1$ (NDfrc-$FB_1$). NDfrc-$FB_1$ is constructed with $FB_1$ and the carbonyl group of reducing D-glucose. For evaluating the fumonisin's risk correctly, FB derivatives should also be contained to mycotoxin analysis as with other modified mycotoxins. The aim of this study was to screen for the FB derivatives other than NDfrc-$FB_1$. Mycotoxin reference material corn powder was extracted with methanol/water (75:25, v/v). A portion of the supernatant was purified through a strong anion exchange column. The purified sample was subjected to LC-Orbitrap MS analysis. For the confirmation of the binding position of sugar residue in the fumonisin structures, treatment with the *o*-phthalaldehyde reagent was performed. The result demonstrated that the FB derivatives with di-saccharide as well as mono-saccharide were contaminated. Such FB derivatives were detected in other reference materials. In order to confirm the structure of the detected sugar derivatives of fumonisins, some possible candidates were synthesized, and analyzed. Consequently, the presence of glucose conjugates of $FB_2$ and $FB_3$ were detected for the first time [1]. A part of this work was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan.

1. Matsuo et al., *Toxins*., 7, 3700-3714 (2015).

# PS14

## *IN VITRO* DECONTAMINATION OF AFLATOXIN B1 BY DIFFERENT CLAYS

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Because of its hot and humid climate, Tunisia is known for the growth of mold in particular in coastal areas, which promotes the remarkable observation of certain mycotoxins (aflatoxins, ochratoxin A, zearalenone and citrinin) and the associated health risks. In addition, the eating habits of the Tunisian people and the conditions of storage and handling of food and feed are also other factors that promote Tunisian food contamination by these toxins. Against The risks related to mycotoxins, several studies have been proposed to seek preventive and curative methods limiting mycotoxin contamination and prevent their production by toxigenic molds. In this context, we study *in vitro* the efficacy of different types of clays in adequate adsorption of aflatoxin B1 (AFB1). The adsorption was achieved in a batch system of standard AFB1 solution on adsorbents. Some factors such as contact time, concentration of AFB1 and particle size of adsorbent were evaluated. The amount of AFB1 adsorbed AFB1 on adsorbents of different types of clays is very fast. This chemical means seems to be efficacy in reducing AFB1 amounts was calculated based on the difference of AFB1 concentration before and after adsorption determined by high performance liquid chromatography (HPLC) analysis. Result shows that adsorption of after interaction in an aqueous medium. A small dose of one of these clays (5 mg) is sufficient to eliminate AFB1.

# PS15

## DETERMINATION OF MULTI-CLASS MYCOTOXINS IN VARIETIES OF RICE BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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The prevalence of mycotoxins occurrence is often increased by climatic conditions prevailing in tropical regions. Therefore, countries located in tropical areas such as Thailand certainly encounter high risk of mycotoxins exposure. There are existing reports that revealed contamination of mycotoxins, including aflatoxins, zearalenone, deoxynivalenol, ochratoxin A and fumonisins in rice [1-3]. Thus, this study was conducted to determine the occurrence of multi-class mycotoxins in 10 different types of rice being commercialized in Thailand for assessing consumer health risk. A total of 300 rice samples were analyzed for the presence of 18 mycotoxins using QuEChERS-based procedure and determined by a triple quadrupole mass spectrometer equipped with electrospray ionization source. The results showed that 119 of the total 300 samples (39.7%) were contaminated with at least one mycotoxin, among which 29.3% of mycotoxin-positive samples were simultaneously contaminated with more than one toxin. The incidences of mycotoxins contamination varied among different types of rice samples. Beauvericin, diacetoxyscirpenol, zearalenone and aflatoxins are the most frequently found in all contaminated samples. However, all the contamination levels were below the regulatory limits.

1. Sugita-Konishi et al., *J. Food Prot*., 69, 1365-1370 (2006).
2. Bansal et al., *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess*, 28, 767-774 (2011).
3. Lim et al., *J. Agri. Food Chem*., 63, 3104-3113 (2015).

# PS16

## MONITORING FOR MYCOTOXIN CONTAMINATION OF FOOD IN KOREA, 2015

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Mycotoxins are toxic metabolites produced by filamentous fungi, such as Aspergillus, Penicillium and Fusarium. They are a source of grave concern regarding food contamination, and can cause adverse consequences on human and animal health. Typically, toxin production is influenced by moisture, time, temperature, and food substrates. For this reason, the impact of climate change accompanied by global warming has been identified as an emerging issue for food safety. Also, the safety management for food is required due to the annual growth in volume of food imports in Korea. Therefore, we have collected a total of 11,622 analytical results for mycotoxin in domestic and imported food samples in Korea (2015) for the evaluation of mycotoxin contamination. Our results for all mycotoxins (total aflatoxin, aflatoxin B1, M1, total fumonisin, deoxynivalenol, ochratoxin A, patulin, and zearalenone) showed that the detection rates were 2.2, 0.4, 50.6, 7.1, 7.2, 1.1, 0.6, and 3.1%, respectively, and all of them have not exceeded the maximum limit set by Ministry of Food and Drug Safety in Korea. The data generated from this study can contribute greatly to the safety management for food in Korea.

# PS17

## SCREENING ANALYSIS OF 10 MYCOTOXINS USING HPLC

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Recently, more and more companies involved in imported and processed food trading voluntarily check for components subject to regulation contained in raw materials including mycotoxins to secure the safety of foods. As part of food safety inspections, exported foods must be inspected quickly for the presence of differently regulated mycotoxins for each destination country.

In consideration of this fact, we developed the mycotoxin screening system, which is based on the i-Series of integrated high performance liquid chromatograph (HPLC). This system is designed for rapid screening for 10 mycotoxin components in grain products, such as wheat and rice flours, apples, and milk at high sensitivity in a mere 14 minutes.

There are three key features of the mycotoxin screening system.

- Detects mycotoxins with high sensitivity at criteria concentrations specified by EU standards, which are the strictest standards in the world. The combination of PDA detector on i-Series and fluorescence detector RF-20Axs that provides the world-highest sensitivity enables to detect the mycotoxin at the EU directive level(*) without fluorescence derivatization. (*)Excluding foods intended for infants.
- By using the provided kit, which includes a column and CD-ROM, containing optimized pretreatment methods that minimize effects from contaminant components and analytical parameters for regulated components, sample measurements can be started immediately.
- Screening results can be checked at a glance as soon as measurements are completed, and the results can be checked quickly with analysis software. Measurement results for each sample can be automatically included in individual quantitative reports prepared for each sample or in a summary report or output in PDF format. Pass/fail results for multiple samples can be output in a table, so that samples that fail the criteria can be identified at a glance.

# PS18

## OPTIMIZATION OF EXTRACTION AND IAC PURIFICATION FOR ELISA TO DETECT AFLATOXIN IN INTERFERING FOOD MATRICES

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Aflatoxins (AF)-contaminated foods, even with low concentration, pose a serious health risk, e.g. carcinogenesis, to human. We have developed an ELISA (enzyme-linked immunosorbent assay) combined with IAC (Immunoaffinity Column) purification, i.e. IAC-ELISA for rapid detection of AF in various foods. Although the method was rapid and reliable, several food matrices were revealed to be interfering. In this study, we have optimized extraction and IAC purification for several interfering food matrices to broaden applicability of IAC-ELISA.

IAC and ELISA kit used in this study was MycoCatch Total Aflatoxin (NH Foods) and MycoJudge Total Aflatoxin (NH Foods), respectively. Fourteen interfering food matrices including spices, herbs, processed foods were spiked with AF at 10 μg/kg. Spiked samples were extracted and IAC-purified. Purified extracts were subjected to AF quantification by ELISA and HPLC analysis.

Fourteen non-contaminated food samples were assayed by ELISA, and twelve (85.7%) samples were tested positive (≥2 ppb). IAC-ELISA resulted in one (7.1%) false positive. With optimized extraction and IAC purification, all false-positive samples were tested negative. In spiked recovery test with ELISA, one matrix (7.1%) met the recovery criteria mentioned in the notification by Japanese Ministry of Health, Labour and Welfare (70-120%). IAC-ELISA improved recoveries, and eight matrices (57.1%) met the criteria. Optimization of extraction and IAC purification further improved recoveries, the criteria was met for all matrices tested. Improved flow in optimized IAC purification enables shorter hands-on time. Optimized IAC-ELISA is suitable for AF screening in various foods.

# PS19

## SIMULTANEOUS DETERMINATION OF MULTI-MYCOTOXINS IN SESAME SEED BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Sesame seed is well known as a health food with high nutritional value. It is commonly used in a wide variety of food especially in vegetarian dishes and bakery products. However, reports of mycotoxins contamination in sesame seed are still limited [1,2]. Therefore, the purpose of this study is to obtain information on occurrence of mycotoxins contamination in sesame seed. A total of 200 hundred samples including 100 black sesame seed and 100 white sesame seed were randomly collected from supermarkets located in Bangkok, Thailand from May to July, 2016. Target toxins were extracted and purified by a modified QuEChERS method [3]. The quantitative analysis was performed using a liquid chromatography coupled with an electrospray ionization tandem mass spectrometer. The results showed that beauvericin was the most frequently detected toxin in both white and black sesame samples at a percentage of 42% and 51%, respectively. Sterigmatocystin, deoxynivalenol and aflatoxin B1 were also detected in white sesame seed with percentages of 19%, 5% and 3%, respectively, whereas aflatoxin G1, aflatoxin B1, Sterigmatocystin, aflatoxin B2 and aflatoxin G2 were found in black sesame seed at 18%, 8%, 7%, 2% and 1%, respectively. Although the toxins levels found in all of the positive samples were lower than the EU standard values [4], a long-term consumption of sesame seed which contain toxins might be able to exert some adverse health effects. With regards to consumer health, our finding suggested that continued monitoring mycotoxins contamination in sesame seed concomitant with risk assessment is recommended.

1. Hosseininia et al, *Mycotoxin Res*., 30, 43-46 (2014).
2. Yentür et al., *Eur. Food Res. Technol*., 224, 167-170 (2006).
3. Yogendrarajah et al., *J. Chromatogr. A*, 1297, 1-11 (2013).
4. Commission Regulation (EC) No 1881/2006, *Off. J. Eur. Commun*. L364 (2006).

# PS20

## GLOBAL SURVEY OF AFLATOXIN $M_1$ CONCENTRATION IN POWDER MILK AND POWDER WHEY PRODUCTS

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Aflatoxin $M_1$ ($AFM_1$) is the metabolite of Aflatoxin $B_1$ that, upon ingestion by dairy cattle, is secreted in milk and can enter the human food supply. It is generally acknowledged that feed management practices influence the prevalence of $AFM_1$ at the farm especially when corn silage and other factors are present. Due to the global nature of the dairy industry even when harvest and storage conditions are well controlled there is still a risk for the production of mycotoxins in imported products. Since $AFM_1$ is classified by the International Agency for Research on Cancer as a Group 1 human carcinogen control of and screening for this mycotoxin is critical.

This study analyzed powder milk and powder whey samples from a random selection of countries in Asia, the Pacific Islands, North America, South America, and Europe. These samples were collected both directly from participating dairies and indirectly purchased from grocery stores, then blind labeled to maintain sample anonymity. $AFM_1$ concentrations in the powder milk samples were determined using the Charm ROSA MRLAFMQ (Aflatoxin $M_1$) Test using the Charm EZ system (Charm sciences Inc., USA). The quantitation range of the test was between 15-100 ppt. $AFM_1$ concentration higher than 40 ppt indicated the sample was contaminated with $AFM_1$ in accordance with the test manual. This 40 ppt threshold for a positive $AFM_1$ result is designed for compliance with the European Union 50 ppt MRL for $AFM_1$ in raw milk, pasteurized milk, and milk used in the manufacture of dairy products. Fisher's exact chi-square tests were used to determine if there was an statistically significant difference between the number of positive results for $AFM_1$ and either a geographic area or between powder milk and powder whey. The significant value was defined at $P<0.05$.

There were a total of 82 powder dairy samples tested in this survey. Results showed that while there was a slightly higher rate in positive powder dairy products in Europe (24%) it was not significant compared to the results from the other four regions. There was a statistically significant difference in the percentage of positive results in whey powder compared to powdered milk (45% compared to 11%, respectfully). In this study the whey samples obtained came from Europe, North America, and South America and totaled 16 compared to 66 powder milk samples.

# PS21

## AN ANALYTICAL METHOD USING A QUALITATIVE KIT FOR AFLATOXINS IN CRUDE DRUG PRODUCT AND KAMPO EXTRACT PRODUCTS

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Controlling impurities in the production of medical plants has been demanded with Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme GMP guide Annex 7. Analytical methods for aflatoxins in Kampo extracts have already established with the qualitative immunochromatography kit (AgraStrip™, Romer Labs) [1]. The method doesn't involve the use of advanced equipment like HPLC instrument. Samples are clean-upped by immunoaffinity column (IAC) and tested for aflatoxins using the kit. Analytical methods using a qualitative kit for aflatoxins in crude drug and crude drug preparations were published in the Japanese Pharmacopoeia 17th edition as the general information. Using this screening method, we tried the screening of aflatoxin in crude drug product and Kampo extract products. One g of sample was weighted in a centrifugal tube, and extracted with 4 mL of acetonitrile: water: methanol = 6:4:1. The tube was shaken for 30 minutes and centrifuged at 2000 rpm for 10 minutes. Two mL of sample extract was diluted to 50 mL with PBS containing 4% Tween 20, after filtration by a glass filter, loaded on IAC (AflaStar™R, Romer Labs). After washing the column, aflatoxins eluted with acetonitrile were evaporated, and resolved in 70% methanol solution as equivalent to 1 g sample/mL. Finally, the sample solution was checked by the kit. Total aflatoxins were spiked into 1 g sample as equivalent to spiked 10 μg per 1 kg sample. Pigments were removed and aflatoxins were clean-upped by the pretreatment with IAC. Using a clean-upped sample solution, screening aflatoxins in crude drug product and Kampo extract products were simply achievable. In addition, we could confirm the results by HPLC with the same clean-upped sample solution.

1. Sakuma et al., *Shoyakugaku Zasshi*, 68, 53-57 (2014).

# PS22

## RAPID TESTS FOR MYCOTOXINS: REQUIREMENTS AND CHALLENGES FOR RAPID STRIP TESTS

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Rapid screening methods such as Lateral Flow Devices (LFDs) are gaining more and more acceptance for monitoring the mycotoxin contamination on-site. Within the last years, the expectations of the users on these tests highly increased. As these simple devices are used in completely different (non-laboratory) environments with varying ambient temperature and humidity conditions, the robustness of the tests can now be considered as one of the major criteria to obtain accurate results. A big design improvement has recently been made by replacing organic extraction solvents with water-based buffer systems for extraction. However, until now only limited data are available which demonstrate the reliability and robustness of these novel systems. Therefore, we have challenged our in-house developed LFDs for mycotoxin detection regarding extraction, temperature sensitivity, compliance of test time as well as accuracy of results gained by different analysts in extended detection ranges. The water-based extraction was compared to standard analytical laboratory reference methods. Furthermore, the influence of the ambient temperature on the test performance was investigated by running the tests at temperatures very different from 20°C. Furthermore, the incubation time or the runtime of the strip was varied to evaluate the influence of these steps. Overall accuracy was ensured by comparing test results gained by three different analysts using naturally contaminated samples only and testing highly contaminated samples up to 30 mg toxin per kg corn as example. This presentation demonstrates the robustness of rapid strip tests for mycotoxins and shows how that even major deviations from the standard procedure still lead to acceptable results. The performance of rapid tests under conditions that vary significantly from the procedure described in the respective manual will be highlighted.

# PS23

## AFLATOXIN M1 IN DAIRY PRODUCTS: THE LOW REGULATORY LIMITS REQUIRE ULTRA-SENSITIVE TEST KITS

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Aflatoxins are toxic metabolites produced by a variety of molds such as *Aspergillus flavus* or *Aspergillus parasiticus*. They are extremely carcinogenic and can be present in grains, nuts, cottonseed and other commodities associated with human food or animal feed. Animals are exposed to aflatoxins by consumption of contaminated crops. When cows are fed contaminated feed, the aflatoxin B1 is then converted by hydroxylation to aflatoxin M1, which is subsequently secreted in the milk of lactating cows. Aflatoxin M1 is a stable molecule and is not destroyed by normal milk processing methods such as pasteurization and therefore may persist into final products for human consumption. Most countries worldwide have introduced regulations regarding the amount of aflatoxins allowable in human and animal foodstuffs and many countries have declared limits for the presence of aflatoxin M1 in milk and milk products. In the European Union the limit for the presence of M1 in milk has been set as low as 0.05 µg/L (50 ppt), whereas most other countries introduced a limit of 500 ppt. These low limits represent a challenge for test kit developers as extremely sensitive antibodies are required to detect 50 ppt of aflatoxin M1. This talk will describe the development of an aflatoxin M1 high sensitive ELISA test within an LOQ of only 5 ppt.

# PS24

## AFLATOXIN $M_1$ IN HUMAN BREAST MILK AND AFLATOXIN $B_1$ IN PEANUT FROM PROCESSING INDUSTRY, BRAZIL

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The $AFM_1$ levels were determined in breast milk samples from lactating mothers who live in Northern Paraná State, and the $AFB_1$ was analyzed in 60 peanut lot samplings (each lot represents five tons) provided by processing industry in the peanut region of Western São Paulo State, Brazil. $AFB_1$ in peanut was analyzed by indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) using monoclonal antibody (mAb, 555 μg $mL^{-1}$ diluted to 1: 30,000) produced by hybridoma strain AF.4. The assay showed low matrix interference (9.6%), good linearity ($r^2$ of 0.9993), detection (LOD) and quantification (LOQ) limits of 2.35 ng $g^{-1}$ and 4.95 ng $g^{-1}$, respectively, with mean aflatoxin recovery rates of 96.8; 101.7 and 107.0% for 4.0; 10.0 and 20.0 ng $g^{-1}$, respectively, and good precision (repeatability CV of 5.0% and intermediate precision CV of 5.0%). Two lots (3.3% of 60 lots) showed AF level >20 μg $kg^{-1}$, whereas 22 (36.7%) samples were <LOQ (4.95 ng $g^{-1}$), suggesting that the icELISA is an easy tool for rapid peanut monitoring, and it can contribute for lowering the risk through detecting such contaminated sections. When the $AFM_1$ levels were analyzed in breast milk samples using a homemade immunoaffinity column, followed by HPLC coupled with fluorescence detection (LOD: 0.004 ng $g^{-1}$; LOQ: 0.021 ng $g^{-1}$), $AFM_1$ was detected in 5.3% (n = 5) of the breast milk samples, with levels in positive samples ranging from 0.013 to 0.025 ng $g^{-1}$ (mean, 0.018±0.005 ng $g^{-1}$). All samples were within the maximum limit established by European guidelines for $AFM_1$ (0.025 ng $g^{-1}$). Low $AFM_1$ level detected in breast milk indicated that lactating mothers in Northern Paraná had low exposure to this mycotoxin and $AFB_1$.

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# PS25

## LOW-COST RAPID IMMUNOASSAY FOR AFLATOXIN SCREENING IN CHICKEN LIVER AND EGGS

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Indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) based on anti-aflatoxin $B_1$ monoclonal antibody (mAb) was standardized and validated for aflatoxin detection in chicken liver and eggs. The mAb was produced by *in vitro* culture of hybridoma strain AF.4 (high cross-reactivity with aflatoxicol) in RPMI medium with fetal bovine serum (FBS), followed by gradual adaptation in H-SFM medium. The culture supernatant was filtered, precipitated with $(NH_4)_2SO_4$, and concentrated IgG was dialyzed. The ic-ELISA for egg analysis was standardized: $AFB_1$-BSA (250 ng $mL^{-1}$), AcM (1.47 mg $mL^{-1}$) at a titer of 1:30,000, and anti-IgG-HRP at 1:5,000. The limit of detection (LOD) and quantification (LOQ) were 0.35 ng $g^{-1}$ and 0.57 ng $g^{-1}$, respectively, with $r^2$ of 0.992. Egg matrix showed low interference (5.4%), with mean recovery rates of 95.5, 98.3 and 99.2%, when $AFB_1$ was spiked at 1.0, 2.0 and 5.0 ng $g^{-1}$, respectively, in homogenized eggs. The ic-ELISA for liver analysis was validated with mAb (555 µg $mL^{-1}$) at a titer of 1: 10,000, and it showed low matrix interference (9.8%), good linearity ($r^2$ = 0.9917), (LOD and LOQ limits of 1.2 ng $g^{-1}$ and 1.5 ng $g^{-1}$, respectively, with mean aflatoxin recovery rates of 90.5, 91.3 and 81.3% for 1.5, 3.0 and 5.0 ng $g^{-1}$, respectively. This ic-ELISA showed good precision expressed by repeatability (CV = 4.1%) and intermediate precision (CV = 5.2%), and it could reduce the costly factor in approximate 160-fold when compared with commercial kits. Such advantage would be crucial to provide a promising rapid tracking for meat safety in a country ranked as the first in broiler supply in globalized world. Regarding the egg analysis by ic-ELISA, it was applied comparing two egg production setups in local farm. Such a rapid tool could contribute in value aggregation by integrating egg as an important ingredient in food industry.

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# PS26

## ANALYSIS OF STERIGMATOCYSTIN AND AFLATOXINS WITH IMMUNOAFFINITY COLUMN USING AN ORGANIC SOLVENT-TOLERANT MONOCLONAL ANTIBODY

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Sterigmatocystin (STC) is a mycotoxin produced by several *Asprgillus* and *Penicillium* species, and known as an intermediate in the aflatoxin (AF) synthesis pathway. It is commonly known as a contaminant of grain products. A monoclonal antibody specific to AF was prepared. In an indirect competitive enzyme-linked immunosorbent assay, the half maximal inhibitory concentration values were 0.45, 0.45, 0.72, 1.02, and 5.26 ng/mL for $AFB_1$, $AFB_2$, $AFG_1$, $AFG_2$, and STC, respectively. This antibody showed reactivity with STC and AFs. Next, an immunoaffinity column (IAC) was prepared using the antibody by bounding it with agarose gel beads. The recovery of STC with this IAC was over 80% when STC was purified in a solvent of up to 20% methanol concentration or up to 10% acetonitrile concentration. The results show that the IAC using this antibody has high organic solvent tolerance. The IAC using the organic solvent-tolerant monoclonal antibody can be used to much wider range of samples.

# PS27

## DIETARY EXPOSURE OF AFLATOXINS AND OCHRATOXIN A IN THAI CUISINE FROM DRIED CHILI AND SELECTED SPICES

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The present study aims to evaluate the amount of contamination and the dietary risk associated with Ochratoxin A (OTA) in dried chili and Aflatoxins (AFs) in selected spices (including garlic, ground peppers, ground turmeric and coriander seeds). Quantitative analysis of OTA was performed in 90 dried chili products collected from the retail stores and markets during June-July 2016. OTA was detected by 28% in the range of 1.6-807.8 μg/kg while AFB1 was detected in only one sample, ground turmeric (4 μg/kg). There were 7 dried chili samples contained OTA amounts exceed the EU regulation limit (30 μg/kg). The dietary exposures to OTA and AFs contaminated in dried chili and spices were estimated based on the national food consumption data (2016) and the occurrence data from this study. %Risk of the consumption of food contaminated with OTA was calculated based on the consumption of chili powder and 12 Thai dishes made from red curry, which were 0.2-49% and 0.03-15.3%, respectively, considered as the very low risk in overall. %Risk of the population at the age of 18-35 years was the highest. One dish (Nam-Ya) appeared to be the highest number of %Risk among other dishes. Risk assessment of the AFs in spices were estimated to be 0.0027, 0.000136, 0.0004923 and 0.000067 person/year/100,000 people for the consumption of garlic, ground peppers, ground turmeric (calculated based on pork satay) and coriander seed (calculated based on red curry dishes) respectively, considered as the very low risk in overall as well. Although the risk of OTA and AFs via consumption of dried red chili and spices are very low, however, one sample contained OTA at >800 ug/kg which still pointed out the problems of OTA contamination in dried chili. Routinely monitoring is needed to minimize the risk from the contamination of OTA in dried spices.

# PS28

## EFFECT OF ADMINISTRATION OF SINGLE ORAL SUBCLINICAL DOSES OF AFLATOXIN $B_1$ IN THE LIVER AND GUT MICROBIOTA IN C57Bl/6 MICE

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Aflatoxin $B_1$ ($AFB_1$) is a secondary metabolite from fungi that present toxic, mutagenic, and carcinogenic effect. It is found in both food and feed resulting in human and animal risk. The purpose of the present study was to investigate the effects of single oral administration of a subclinical dose of $AFB_1$ in the liver and gut microbiota in mice. A total of 25 male C57Bl/6 mice were allocated in five groups. Three groups were treated with a single oral dose of $AFB_1$ (44 μg of $AFB_1$/kg of body weight, b.w., 442 μg of $AFB_1$/kg b. w., and 663 μg of $AFB_1$/kg of b. w.) on the first day and euthanized on the 5th day. Two control groups were treated only with water and vehicle (saline:ethanol, 95:5). For liver, it was included determination of cytokines (IL-4, IFN-γ, IL-17) levels and histopathology analysis was performed. Sequencing of the V4 region of the 16S rRNA was performed with an Illumina MiSeq sequencer to evaluate the gut microbiota composition. The animals treated with 663 μg of $AFB_1$/kg of b. w. had liver lesions such as necrosis, infiltrate inflammatory, megalocytosis, cell and nuclear vacuolation; and a significant upregulation of IL-4 and IFN-γ levels in the liver ($p < 0.05$). In addition, this group presented increased family of Lachnospiraceae on the gut microbiota. In conclusion, single oral subclinical dose of $AFB_1$ exposure can induce liver tissue lesions, liver cytokine modulation and possible change in gut microbiota in C57Bl/6 mice.

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# PS29

## ISSUES OF LIST OF FUNGI DESIGNATED BY THE BIOSAFETY LEVEL (BSL) AND JAPANESE REGULATION LAWS

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According to the World Health Organization [1], the assignment of an agent to a biosafety level (BSL) must be based on a risk assessment considered by which occupation category and where the laboratory work. In the case of fungi, risk classifications of human pathogens were published by; the National Institute of Infectious Diseases (NIID), the Japanese Society for Medical Mycology, that assumed for use in laboratories and hospitals [2], and Miyaji & Nishimura [3] that assumed for living environment. Companies using microorganisms in Japan usually refer to the list by NIID. Additionally, microorganism utilizations are regulated by the multiple domestic laws and global treaties that are demarcated by competent authorities and their purposes. Thus, those are not easy to refer for companies.

To simplify those regulations and facilitate compliance management at industrial companies, we combined above biosafety groupings and four Japanese laws; prevention of infectious diseases (human pathogen), animal protection, the foreign trade law (prohibit bioterrorism), and the relevant low of Cartagena protocol (living modified organisms), except for the Plant Protection Act. (plant pathogen). As a result, about 500 species of fungi and yeasts were integrated into one table. This table revealed present issues should be improved as bellows;

(1) Mycotoxin producing fungi defined as BSL2 were disconnected cause by genus-, section- or species-level in each organizations and laws.

(2) It was not mentioned the definition of “mycotoxin”.

(3) Confusion from nomenclature of fungi (1F=1N). Even though mycological taxonomists published new names, but they were not common.

Our work settled the matter of names expediently. The further genotype studies and more data federation among each jurisdiction organizations were needed to resolve others.

1. WHO: Laboratory biosafety manual. World Health Organization, Geneva, (2004).
2. Miyaji, M. et al., *Jpn. J. Med. Mycol*., 34, 220-229 (1993).
3. Miyaji, M. & Nishimura, K., *“Sumai-to-kabi-to-byogensei” published by Yasaka Shobo, Tokyo*, (2009). in Japanese.

# PS30

## DEGRADATION OF OCHRATOXIN A IN AQUEOUS SOLUTIONS BY GAMMA IRRADIATION

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The mycotoxin ochratoxin A (OTA) is a widespread contaminant in a wide range of human food and animal feed [1]. OTA is a well-known nephrotoxic agent and has been classified as a possible (category 2B) human carcinogen [2]. Moreover, it exhibits a wide range of toxic effects including teratogenicity, immunotoxicity, neurotoxicity, genotoxicity and mutagenicity [3]. The present study was undertaken to assess the efficacy of gamma irradiation as a method of decontamination. Therefore, we have conducted systematic studies to determine the fate of OTA following gamma irradiation (GI) under various conditions. The effects of the initial OTA concentration, irradiation dose as well as dose rate on OTA radiostability were investigated. Residual OTA levels were analysed using ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS). Results showed that gamma irradiation of aqueous OTA solutions caused a marked reduction of initial OTA levels and the degradation rate varied from 87 to 94.5%, depending on the applied irradiation dose and dose rate. The observed OTA reduction in an aqueous system is likely due to the presence of reactive radicals generated from radiolysis of water that attacked its chemical structure. Furthermore, the degradation products were preliminary identified, indicating chemical degradation of the toxin. It can be concluded that that gamma irradiation treatment could prove to be a promising method for reducing OTA contamination levels.

1. Duarte et al.,, *Vet. J*., 192(3): 286-292 (2012).
2. IARC (International Agency for Research on Cancer), 56 (1993)
3. Duarte et al., *Vet. Microbiol*., 154(1-2), 1-13 (2011).

# PS31

## FATES AND TISSUE DEPLETION OF NIVALENOL IN BROILERS

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Nivalenol (NIV), a type B trichothecene mycotoxin, is mainly produced by the fungi of *Fusarium* genus, which naturally occurs in agricultural commodities [1]. NIV has been recently shown to possess one of the more potent toxicities among mycotoxins of this group [2]. Regarding our knowledge, the toxicokinetics of NIV is incompletely understood [3]. Limited data are available and toxicokinetics profile of NIV has been reported in pigs and mice only [4,5]. Consumers are particularly concerned over the toxicity and safety of NIV in food animal products. To evaluate the toxicokinetics and persistence of residues of NIV, NIV was administered intravenously (iv) or orally (po) to broiler chickens at a dosage of 0.8 mg/kg body weight. The concentration of NIV in the plasma and various tissues was detected using liquid chromatography tandem-mass spectrometry. The plasma concentration of NIV in broilers could be measured up to 24 h and 12 h after iv and po administration, respectively. The value of elimination half-life of NIV was 5.27 ± 0.82 h and 2.51 ± 0.88 h after iv and po administration, respectively. The absolute oral bioavailability was 3.98 ± 0.08%. NIV was detected in the intestines, kidneys, muscle, heart and liver after po administration. Regarding tissue residues, largest quantities of NIV were found in the small intestine. These results suggest that NIV is absorbed from the gastrointestinal tract with low bioavailability and it has the ability to diffuse into various tissues of broilers.

1. Placinta et.al., *Anim. Feed Sci*., 78, 21-37 (1999).
2. Cheat et.al., *Toxin*, 7, 1945-1961 (2015).
3. Kongkapan et.al., *Isr. J. Vet. Med*., 71, 3-9 (2016).
4. Hedman et.al., *Arch. Anim. Nutr*., 50, 13-24 (1997).
5. Poapolathep et.al., *Toxicon*, 41, 1047-1054 (2003).

# PS32

## FUSARENON-X INDUCED APOPTOSIS IN LYMPHOID TISSUES OF MICE AFTER 14 DAYS ORAL EXPOSURE

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Fusarenon-X (FX) is one of the members of type B trichothecene mycotoxin which mainly produced by *Fusarium* species [1]. It has been observed with other type B trichothecenes such as deoxynivalenol (DON) and nivalenol (NIV) [1] as a contaminant in cereals [2]. DON induces apoptosis in mouse thymic epithelial cells [3] and orally administered with NIV is able to induce apoptosis in lymphoid tissues of mice [4]. FX has been reported to induce apoptosis *in vitro* particularly at high dose exposure. However, the toxicological study of subacute exposure of FX is limited. To investigate subacute treatment with FX can induce apoptosis particularly in lymphoid tissues of mice, eighty 3-week-old male ICR mice were orally administered once daily with FX at 0, 0.1, 0.3, and 0.5 mg/kg body weight for 14 days, and examined at 3, 6, 12, 24, and 48 hr after last treatment (HAT) on Day 14. FX did not affect body and organ weights, nevertheless, at the higher doses FX caused nuclear condensation and fragmentation of lymphocytes in the cortical thymus and germinal center of Peyer's patches. Apoptotic lymphocytes evaluated by modified TUNEL method showed dose-dependency and peaked at 12 HAT in the Peyer's patches and thymus of 0.3 and 0.5 mg/kg FX-treated mice, whereas apoptotic bodies engulfed by macrophage were clearly observed by electron microscopy in 0.5 mg/kg FX-treated mice. Regarding RT-PCR, some apoptosis-related genes were up-regulated in thymus of mice. The results suggested that repeated exposure to low doses of FX induces apoptosis in the lymphoid tissues of mice.

1. IARC monographs on the evaluation of carcinogenic risks to humans, 56, 394-444 (1993).
2. Juan et al., *Food Chem.* 141, 1747-1755 (2013).
3. Li et al., *Environ. Toxicol. Pharmacol*. 38, 163-171 (2014).
4. Poapolathep et al., *Exp. Toxic. Pathol*. 53, 441-446 (2002).

# PS33

## THE EFFECTS OF EUSHEARILIDE, A NOVEL MACROLIDE ANTIFUNGALANTIBIOTIC, ON MITOCHONDRIAL STRUCTURE AND RESPIRATORYFUNCTION AS A MECHANISM FOR THE ANTIFUNGAL ACTIVITY

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Eusherilide, a newly isolated macrolide antibiotic isolated from *Eupenicillium shearii*, has been examined for the toxic effects on mitochondrial structure and respiration to gain insight into the molecular mechanism for the antifungal activity, by using freshly isolated rat liver mitochondria. Eushearide inhibited both respirations oxidizing L-glutamate and succinate. The inhibition was not reversed by TMPD, which generated an electron transport shunt over the inhibition sites of rotenone and antimycin A, and was not restored by ascorbate, an artificial substrate for the cytochrome *c* oxidase. The all of cytochromes *a*, *b* and *c* were kept reduced in the presence of eushearilide in the reduced-minus oxidized difference spectrum of cytochromes, suggesting that eushearilide inhibited the electrontransport and the inhibition site is located at cytochrome *c* oxidase (complex IV) in the respiratory chain. Eusherilide induced large amplitude swelling in mitochondria suspended in the isotonic KCl solution, which was prevented by cyclosporine A, indicating the generation of the permeability pore in the inner membranes. From these results it was proposed as a mechanism that eushearilide exerted the impairing effects on both mitochondrial structure and respiratory function, resulting in inhibiting the fungi growth.

# PS34

## ANOREXIC ACTION OF FUSARENON X IN HYPOTHALAMUS AND INTESTINE

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Fusarenon X (FX) is one of type B trichothecene mycotoxins produced by *Fusarium* species, contaminating cereals including wheat, barley, and corn. FX has been reported to have adverse effects such as emesis, anorexia, immunotoxicity, and developmental toxicity. However, the mechanism of anorexia induced by FX ingestion has not been fully clarified. Thus, we investigated the anorexic actions of FX in the hypothalamus and intestine. When female B6C3F1 mice were orally exposed to different doses of FX (0.5, 1.0, and 2.5 mg/kg bw), a significant anorexic action was observed at a dose of 2.5 mg/kg bw from 3 to 6 h after administration. Exposure to FX (2.5 mg/kg bw) for 3 h significantly increased the hypothalamic mRNA levels of anorexic pro-opiomelanocortin (POMC) and its downstream targets, including melanocortin 4 receptor, brain-derived neurotrophic factor, and tyrosine kinase receptor B; at the same time, orexigenic hormones were not affected. In addition, the exposure to FX significantly elevated the hypothalamic mRNA levels of proinflammatory cytokines (IL-1β and TNF-α) and activated nuclear factor-kappa B (NF-κB), an upstream factor of POMC. These results suggest that FX-induced proinflammatory cytokines increased the POMC level via NF-κB activation. Moreover, the exposure to FX significantly enhanced the gastrointestinal mRNA levels of anorexic cholecystokinin (CCK), but not those of anorexic peptide YY and orexigenic ghrelin. In addition, the exposure to FX significantly enhanced the gastrointestinal mRNA level of transient receptor potential ankyrin-1 channel (TRPA1), which is associated with CCK production by deoxynivalenol (another type B trichothecene mycotoxin) [1,2]. Taken together, these results suggest that FX induces anorexic POMC via NF-κB activation by increasing proinflammatory cytokines in the hypothalamus and brings about CCK production, possibly through increasing intestinal TRPA1 expression, leading to anorexic actions.

1. Wu et al., *Arch. Toxicol*., published online:15 March 2016.
2. Tominaga et al., *Toxicon*, 118, 54-60 (2016).

# PS35

## CONTAMINATION OF ACETYLATED DEOXYNIVALENOL IN FEEDS AND THEIR TOXICOKINETICS IN PIGS

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In Japan, as feed for pigs, the formula and liquid feed are used generally. The formula feed means solid feed and its main content is corn. The liquid feed is made of the food debris including the wheat flour products such as bread and noodle. Although DON and its derivatives are contaminants in grain, the information of contamination of DONs is limited.

To survey of acetylated trichothecene mycotoxin deoxynivalenols (DONs), 15-acetyl DON (15ADON) and 3-acetyl DON (3ADON) in feed, they were analyzed by liquid chromatography-tandem mass spectrometry and enzyme-linked immunosorbent assay. In both formula and liquid feed, DON and 15ADON were detected, while 3ADON was detected in only the formula feed.

Furthermore, to evaluate their toxicokinetics in pig via oral, a toxicokinetic experiment using the diet administration route with DON and acetylated DONs. This study revealed that the acetylated DONs were immediately converted to DON in the blood. The maximal DON serum concentration (Cmax) values of 15ADON and 3ADON were 115% and 52% of that of DON, respectively. The time of Cmax (Tmax) showed that the acetylated DONs were absorbed earlier that DON.

These results demonstrated that 15ADON could achieve a similar concentration to DON in the blood earlier than DON and contaminated both formula and liquid feeds, thus suggesting it may be a newly recognized risk for swine health.

# PS36

## REDUCTION OF AFLATOXIN $B_1$ TOXICITY BY USING FOOD COMPOUNDS AND CYP3A4 GENE IN HUMAN

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Aflatoxin $B_1$ ($AFB_1$) is one of the most important mycotoxins due to its mutagenic effect in human. *Aspergillus flavus* (*A. flavus*) is the fungi responsible for the production of $AFB_1$. In order to detoxification of $AFB_1$ in foods, heat treatments, solvent extraction and ozonization were carried out. However, these methods are partially effective for the detoxification of $AFB_1$.

In this study, we tried to reduce the toxicity of $AFB_1$ by using two kinds of approach.

(1) *A. flavus* having an $AFB_1$ was reacted with recombinant *E. coli* cells expressing human CYP3A4. As a result, the LC-MS peak of main metabolite was consistent with $AFQ_1$. It is known that the $AFQ_1$ is a metabolite of $AFB_1$ in human and the toxicity of $AFQ_1$ was much weaker than original $AFB_1$. It was also shown that $AFQ_1$ lost their own mutagenicity. Therefore, it is suggested that the technique for metabolism of $AFB_1$ by using CYP3A4 expressed *E. coli* may become an effective tool for the detoxification of $AFB_1$.

(2) In order to decrease the metabolic activation of $AFB_1$ *in vivo*, we investigated the effect of food compounds on the enzyme activity of CYP3A4. After the screening of fourteen food compounds, curcumin, myricetin and resveratrol inhibited the metabolism of CYP3A4 effectively. Moreover, we evaluated the effect of SNPs in CYP3A4 for the metabolic activation of $AFB_1$. When we investigated the metabolic activation of $AFB_1$ with ten kinds of CYP3A4 SNPs, it was observed that l metabolic activation by CYP3A4*5 was lower than that of wild type CYP3A4.

These two results suggested that detoxification of $AFB_1$ by CYP3A4 *in vitro* and inhibition of CYP3A4 activity *in vivo* are promising methods for the reduction of $AFB_1$ toxicity in foods.

# PS37

## CHANGES IN MYCOTOXIN CONCENTRATIONS DURING ENSILING

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In Japan, *Fusarium* fungus-producing mycotoxins (FTs) pose serious concerns. It has been reported that fumonisin does not increase in rice grain silage under ordinary silage conditions [1]. It is thought that almost all contaminants in forage crops are produced during the pre-harvest period in the field, and it is rare that FTs increase post-harvest. However, we have encountered a case in which one of the FTs in forage rice grain increased during ensilage. We report that case in this paper.

Forage rice was used as silage material. After preparing silage, samples were taken from the upper and lower portions of 9 silage bags, respectively.

Concentrations of fumonisin, nivalenol and deoxynivalenol showed little change in samples taken before and after silage storage. On the other hand, the concentrations of zearalenone in upper portion of two bags of the 9 silage bags showed a significant increase. Zearalenone concentrations were 21 μg/kg dry matter (DM) in material, and 41,300 and 5460 μg/kg DM in two bags. In the sample that showed the highest concentration, pH was high (5.14), and the number of living fungi was also high. Therefore, it was thought that the silage fermentation had not progressed favorably. On the other hand, the zearalenone concentration in all lower portion samples showed no change and no significant differences.

Therefore, we conclude that it is rare that the concentrations of mycotoxins increase during ensiling, but it may occasionally increase if conditions are right. Under good conditions for silage, pH levels decrease and lactic acid bacteria inhibits the growth by lactic fermentation of other micro-organisms. When fermentation is not good or anaerobic conditions are not maintained during ensiling, fungi growth is encouraged. Maintaining an anaerobic environment is the essential condition for improving silage quality, and it is essential to do this in order to prevent mycotoxins.

1. Uegaki et al., *Animal Science Journal*, 84, 48-53 (2012).

# PS38

## STUDIES ON MODE OF ACTION OF BLASTICIDIN A, AN AFLATOXIN PRODUCTION INHIBITOR

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Aflatoxins are a group of mycotoxins with potent toxicity and carcinogenicity. Some *Aspergillus* species produce aflatoxins and its contamination in agricultural products is a serious problem from the viewpoint of food safety and stable supply. However, it is difficult to resolve the problem due to lack of an effective method for control of aflatoxin production. Specific inhibitors of aflatoxin production would be effective drugs for prevention of aflatoxin contamination without incurring a rapid spread of resistant strains. Blasticidin A (BcA), a metabolite of *Streptomyces griseochromogenes*, selectively inhibited aflatoxin production of *Aspergillus parasiticus*. BcA inhibits protein synthesis [1], but its target molecule in aflatoxigenic fungi and molecular mechanism for inhibition of aflatoxin production are still unclear. In this study, we investigated the mode of action of BcA, which may contribute to clarify the regulatory mechanism for aflatoxin production in fungi and to develop an effective method for prevention of mycotoxin contamination.

An alkyne derivative of BcA was prepared by the reaction of the hemiacetal moiety of BcA and triethylene glycol mono(2-propynyl) ether. The alkyne derivative was coupled with magnetic beads through azide group by click reaction, to prepare BcA-immobilized magnetic beads. Protein extracts were prepared from protoplasts of *Aspergillus flavus* and the extracts were mixed with the BcA-immobilized magnetic beads. After washing the beads with water, a BcA solution was used to elute beads-binding proteins. The elution was subjected to SDS-PAGE and several protein bands were observed. Experiments to determine which band on the gel contains a protein that can specifically bind to BcA and to identify the BcA-binding protein are in progress.

1. Yoshinari, T. et al., *J. Antibiotics*., 63, 309-314 (2010).

# PS39

## STUDIES ON AFLATOXIN PRODUCTION INHIBITORS PRODUCED BY MICROBES

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Aflatoxins are poisonous and cancer-causing chemicals that are produced by some *Aspergillus* species, mainly by *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin contamination frequently occurs in improperly stored staple commodities such as corn, peanuts, rice, wheat, and a variety of spices. Since aflatoxin contamination is a serious problem worldwide, development of effective methods to control is urgently required.

We have been studying specific inhibitors of aflatoxin production by aflatoxigenic fungi. Specific aflatoxin production inhibitors are useful not only to prevent foods and feeds from aflatoxin contamination without incurring rapid spread of resistant strains, but also to know the regulatory mechanism of aflatoxin production by fungi at a molecular level. As a resource for inhibitors, metabolites of soil microorganisms are attractive because natural products are environmentally kind and plant-friendly microbes producing inhibitors are useful as biocontrol agents for aflatoxin control.

We screened about 100 soil microorganisms and found that culture broths of the two strains, *Paenibacillus* sp. PS1 and *Delftia* sp. H16, showed strong inhibitory activities against aflatoxin production by *A. flavus* and *A. parasiticus*. To isolate active compounds produced by the strains, we are conducting bioassay-guided fractionation. In the case of strain PS1, methanol mycelial extracts showed strong activity. When the extracts of strain PS1 were fractionated with ethyl acetate extraction, inhibitory activity was observed in the neutral fraction. On the other hand, the culture filtrate of strain H16 showed strong activity. When the culture filtrate was extracted with chloroform, strong inhibitory activity was detected in the basic fraction. Works to isolate and identify the active compounds produced by the strains are now in progress.

# PS40

## *IN VITRO* METABOLISM OF *FUSARIUM* MYCOTOXIN BY BOVINE RUMEN MICROORGANISMS

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The *Fusarium* mycotoxins deoxynivalenol (DON), zearalenone (ZEA) and fumonisin (FM) occur worldwide in foods such as cereals and animal forages, and they have been associated with animal food poisoning. Ruminants are regarded as relatively resistant to DON because their rumen microorganisms are able to detoxify it. We investigated cows' ability to metabolize *Fusarium* toxins by using an *in vitro* ruminal incubation technique to examine the cows' mycotoxin resistance. We divided rumen fluid (RF) obtained from a fistulated dairy cow into three groups: RF alone (100% RF), artificial saliva buffer alone (BF), and a 1:1 mixture of RF and BF (50% RF). The metabolism of three mycotoxins (DON, ZEA and FM-B1) was evaluated following incubation (1 μM) in RF for up to 24 h, using an *in vitro* rumen system (sealed flask, anaerobic condition, gentle agitation, 39 °C). The pH and the total volatile fatty acid amount, which are indicators of ruminal fermentation, were not changed after 24-h incubation. The depletions of DON and ZEA in the 100% RF samples were approx. 60% and 40% respectively after 24-h incubation, whereas the concentrations of these mycotoxins in the BF samples were unchanged. The rates of decrease of DON and ZEA in the 50% RF samples were each approx. 20%. RF that was microorganism-inactivated by a freeze-thaw cycle (F-RF), as well as BF, had no ability to degrade DON or ZEA in a 24-h incubation. HPLC analyses of 100% RF after 24-h incubation indicated that DON was partially converted to de-epoxy-DON. However, LC-MS/MS analysis revealed that the concentration of FM-B1 in 100% RF was not changed after 24-h incubation, thus confirming that FM-B1 is poorly metabolized in the rumen of cattle, as reported previously. These results indicate that the *in vitro* ruminal incubation technique described here will be useful for evaluations of ruminants' ability to degrade *Fusarium* mycotoxins.

# PS41

## PROFILING THE MICROBIAL DEGRADATION PROCESS OF FUMONISIN B1 THROUGH LIQUID CHROMATOGRAPHY COUPLED WITH QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY (LC/Q-TOF MS)

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In this study fumonisin B1 (FB1) was degraded by a self-separate fungi stain through time-course experiments of 0 to 72 hours. LC/Q-TOF MS profiles showed that three new metabolites were formed after microbial degradation. The $[M+H]^+$ of these ions are 564.3742, 406.3527, 288.2096. Through in-depth analysis, interesting results were gained that the degradation process has two obvious segments. In the first step, ions of 564.3742 and 406.3527 were formed and this reacted from 0 to 18 hours. Then ions in step one turned to the second step, continuously transformed to 288.2096 in time segment 24 to 48 hours. The first degradation step has been already reported [1], mainly induced by carboxylesterases, which hydrolyse the two side chains, tricarballylic acid, and formed hydrolyzed FB1. For the second step, product ions 288.2096 ($C_{15}H_{30}NO_4$) has not been reported. It has a shorter carbon chain compare to FB1 and HFB1, but still with one amino group. Through tandem ms/ms analysis, acetylation may react on the amino gruoup, yet it need to be confirmed by nuclear magnetic resonance analysis in future. This work separated a new fungi that could high-efficiency degraded FB1 and profiled the degrading process using LC/Q-TOF MS. It provide a good prospect for FB1 degradation treatment.

1. Barbara A et al., *Nat. Toxins*. 7, 31-38 (1999).

# PS42

## EFFECT OF FLUOROPYRIMIDINES ON AFLATOXIN PRODUCTION

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Aflatoxin (AF) is one of the major strong carcinogenic and toxic secondary metabolites produced by some *Aspergillus* species such as *A. parasiticus* and *A. flavus*. AF contamination in crops causes serious economic losses in agriculture. However, definitive countermeasure for controlling aflatoxin contamination has not been developed yet.

Application of antifungal agents has a problem of resistant strains. As AF is not essential to growth of aflatoxigenic fungi, we focus on drugs which suppress only AF production, not the fungal growth. Such drugs are expected to become effective AF control agents.

Our screening revealed that 5-fluorouracil (FU), a well-known anticancer agent, suppressed AF production of *A. flavus* with weak growth inhibition. Among FU colleagues, 5-fluorodeoxyuridine (FUDR) inhibited AF production more specifically than FU, and 5-fluorouridine (FUrd) inhibited both of AF production and fungal growth more potently than FU. FUDR, which is converted from FU or FUrd, is a strong inhibitor of thymidine synthase (TS). Competition of FUDR with thymidine, however, did not rescue AF production, although uridine or deoxyuridine could. Moreover, FUDR and FUrd reduced expression level of *aflR*, an intrinsic transcription factor of clustered genes responsible for AF biosynthesis, to less than one-fifth of that of control, but FU did not suppress *aflR* expression. Comprehensive analytical works on gene expression in fluoropyrimidine treated mammalian cells showed possibility that fluoropyrimidine targeted expression of specific genes. Therefore, our results suggested that fluoropyrimidines inhibit AF production through a directed gene expression control mechanisms. Studies on the suppression mechanism of fluoropyrimidine against AF production may be important for identifying target genes useful for AF control.

# PS43

## EVALUATION OF AFLATOXIN REDUCTION BY NEAR INFRARED RAYS SORTING IN HIGHLY CONTAMINATED PEANUT LOT

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Sorting to reduce the mycotoxin depending on appearance of peanut kernel is effective but not completely because an apparently healthy but highly contaminated (internally molded) kernel commonly occurs in the toxigenic lot. Previous study showed near infrared rays was transmutable and able to reduce the contents of aflatoxin in low levels of contaminated lots by detecting internal changes such as fungal digestions within a moldy kernel [1]. In this study we evaluated the spectrometric sorter, Q-Sorter, to reduce level of aflatoxins in the highly contaminated lot.

**Materials and Methods:** Shelled peanut lot (278 kg) contaminated with aflatoxins (212 μg/kg, in total) was sorted twice by Q-Sorter (MK-06, ANZAI MFG CO., LTD) into passed (PC) and rejected categories (RC) after hand picking. Peanut kernels after the sorting were opened to inspect the internal changes visually before the aflatoxin analysis.

**Results and Discussions:** In the first trial of sorting, 11.7 kg of peanut (4.2 % wt. basis) was segregated as RC. Most of aflatoxins (approx. 80%) originally contained in the toxigenic lot could be removed, indicating that level of aflatoxins, 212 μg/kg in initially present, decreased to 45.1 μg/kg after the sorting. By the second trial, the level of aflatoxins further became smaller to 13.0 μg/kg after removing of 3.9 % peanut to RC. Approx. 92 % of peanut in the initial lot was finally partitioned to PC after the repeated sorting.

Visual observation showed that the internally molded kernels and discolored kernels occurred at higher incidence in RC, suggesting that both of the damaged kernels were segregated to RC efficiently. These results clearly demonstrated that Q-sorter was applicable to reduce aflatoxins also in the highly aflatoxin contaminated lot by removing the molded kernels.

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1. Hirano et al., *Biosci. Biotechnol. Biochem*., 62, 102-107 (1998).

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