



**International Commission on Penicillium and Aspergillus**

**&**



**International Commission on Food Mycology**

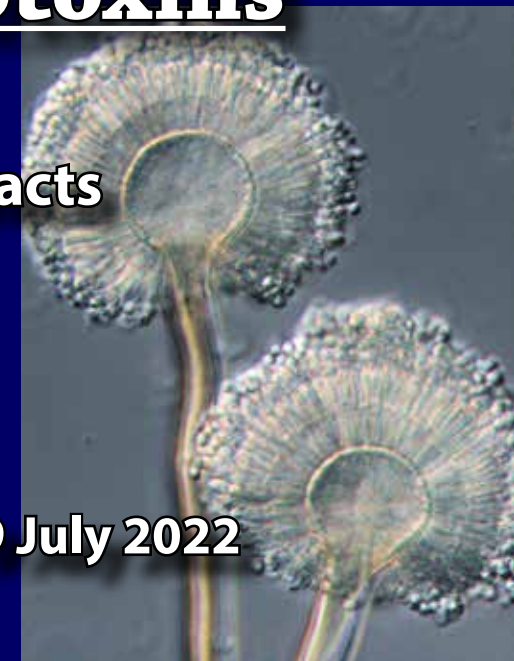
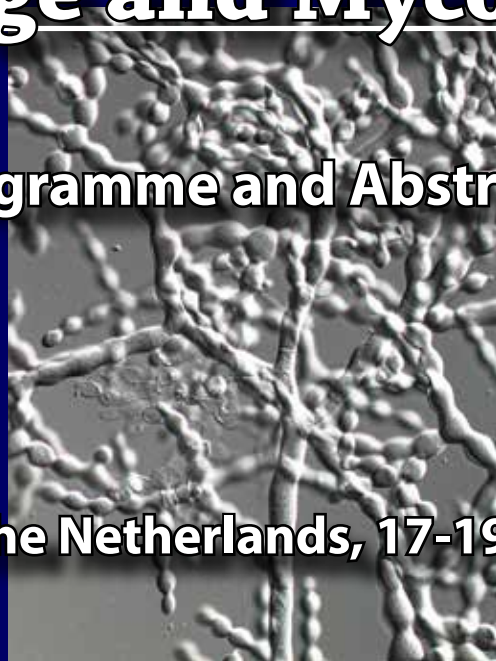
**workshop 2022**

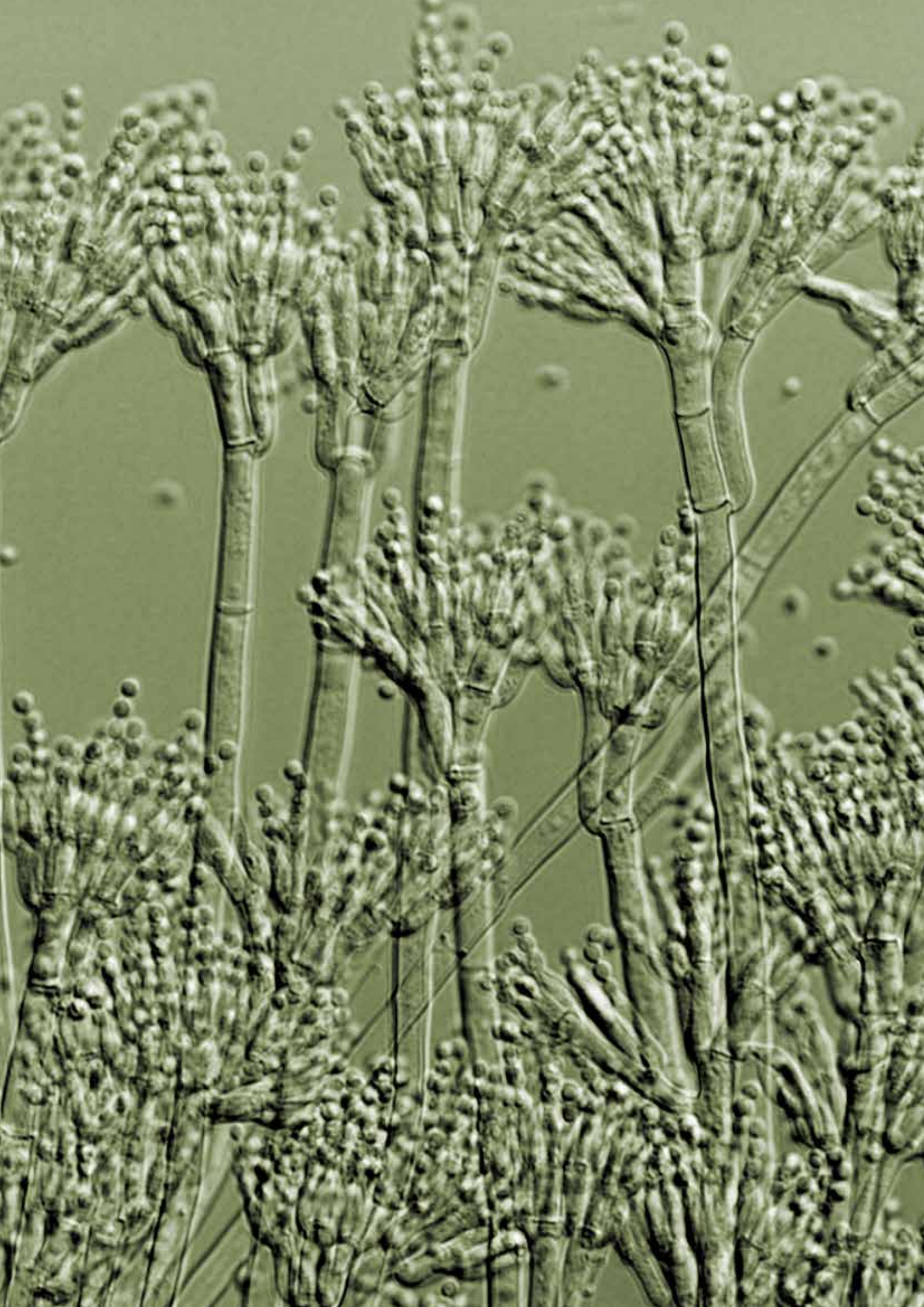
**Food Mycology - Taxonomy,**

**Spoilage and Mycotoxins**

**Programme and Abstracts**

**Utrecht, The Netherlands, 17-19 July 2022**







**ICFM**

International Commission  
on Food Mycology

**International Commission on Penicillium and  
Aspergillus**

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**workshop 2022**

**Food Mycology - Taxonomy,  
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The commission is a COMCOF (Commissions, Committees and Federations) of the International Union of Microbiological Societies (IUMS) and established in 1990.

The aims of the Commission are:

- to improve and standardise methods for isolation, enumeration and identification of fungi in foods;
- to promote studies of the mycological ecology of foods and commodities;
- to interact with regulatory bodies, both national and international, concerning standards for mycological quality in foods and commodities;
- to support regional initiatives in this area. The Commission further aims to extend understanding of the principles and methodology of food mycology in the scientific community by publishing its findings, and by sponsoring meetings, specialist workshops, courses and sessions dealing with aspects of its work.

The first workshop on Methods for Mycological Examination of Food was organised in Boston, USA, in July 1984. After this successful meeting subsequent meetings were held in Baarn (1992), in Copenhagen (1994) near Uppsala (1998), Samsøe (2003), Key West (2007), Freising (2010, 2013, 2016 and 2019).

**Venue:**

Westerdijk Fungal Biodiversity Institute, Uppsalalaan 8, 3584CT Utrecht, The Netherlands

The tenth International ICPA/ICFM workshop is organized by **Jos Houbraken** and **Rob Samson**

**Sponsors**



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**PROGRAMME ICPA/ICFM 2022**


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**Saturday, July 16 2022**

18.30 Get together at Hotel Biltsche Hoek, de Bilt, the Netherlands

**Sunday July 17 2022**

Westerdijk Fungal Biodiversity Institute, Utrecht

08.30 – 09.30 Registration

09.30 Welcome; Ludwig Niessen, Jos Houbraeken, Rob Samson

09.45-10.00 Emilia Rico, Su-lin Hedén and Marta Taniwaki - John Pitt in memoriam and ICFM

**Session: Developments in methodology (chair Jens Frisvad)**

10.05	Emilia Rico	ICFM and the update of the ISO standard enumeration of yeast and moulds
10.25	Christiane Baschien	Advances and limitations of fungal metabarcoding
10.55	Christopher Magro	From orchard to post-harvested <i>Bambinella</i> fruit: a fungal spore assessment through conventional and molecular methods

11:15 **Break**

**Session: Fungal communities and ecology (chair Paul Dyer)**

11.40	Jens C. Frisvad	Different fungal species are associated to different parts of plants
12.00	Grzegorz Ostrowski	Microbial communities of the dry aged beef
12.20	Naresh Magan	Ecology and control of <i>Aspergillus flavus</i> and aflatoxin B1 in chilli powder and whole red chillies using food-grade preservatives
12.40	Marta Taniwaki	Fungal communities and potential for mycotoxin production in Brazilian cassava tubers and food

13.00 **Lunch**

**Session: Mycotoxins (Chair Ludwig Niessen)**

14.00	Esther Garcia-Cela	Characterisation of the emerging mycotoxigenic pathogen <i>Fusarium asiaticum</i> through genome sequencing by Oxford Nanopore technology
14.20	Angel Medina	Impact of environmental conditions and predicted climate change factors on <i>Fusarium asiaticum</i> growth and mycotoxin production in wheat
14.40	Markus Schmidt-Heydt	AflaZ - new safety aspects on aflatoxin producing fungi in maize fields near Nairobi/Kenya
15.00	Endang S. Rahayu	Current food safety status in Indonesia

15.20 **Break**

15.50	Sofía Noemí Chulze*	Biocontrol strategy at pre-harvest stage for reducing AFB1 in maize during storage in Argentina (online)
16.10	María A. Pavicich	Does <i>Alternaria</i> mouldy core infection of apple favour mycotoxin accumulation?
16:30	Jens C. Frisvad	3-Nitropropionic acid is an emerging important mycotoxin

17.00 – 17.45 **Poster session**

18.30 **Dinner** at Hotel De Biltsche Hoek.

**Monday July 18 2022****Session: Taxonomy of food borne fungi (Chair Naresh Magan)**

09.00	Rob Samson	In memoriam: John Pitt and his contribution to the International Commission on Penicillium and Aspergillus
09.20	Jens C. Frisvad	Revitalization of phenotypic taxonomy in Aspergillus, Penicillium and Talaromyces
09.40	Pedro Crous	A taxonomic overview of Fusarium
10.00	Monika Coton	New insights on the unexpected diversity of Nectriaceae species in cheese and a glimpse at their functional diversity
10:20	František Sklenář	Implementation of species delimitation methods in Aspergillus
10.40	Break	
11.00	Roya Vahedi	Aspergillus section Terrei: Taxonomic status and antifungal susceptibility profiles (online)
11.20	Paul Dyer	Relevance of sexual states to modern taxonomy and food mycology, insights from Aspergillus species
11.40	Jos Houbraken	New diversity in <i>Aspergillus</i> and <i>Penicillium</i>

**Session: Biocontrol and processing (Chair Emilia Rico)**

12.05	Elettra Berni	Effect of gaseous ozone and ozonized water against food-spoiling filamentous fungi on stainless steel
12.25	Masja Nierop Groot	Impact of (mild) processing on food spoilage fungi
12.50	Lunch	
13:45	Donato Magistà*	Studies on the efficacy of electrolysed oxidising water to control <i>Aspergillus carbonarius</i> and ochratoxin A contamination on grape ( <b>online</b> )
14:05	Gemma Castellá	Non-ochratoxigenic black aspergilli as biological control agents
14:25	Naresh Magan	Effect of ozone treatment of different commodities: relative control of germination, growth and mycotoxin production by <i>Aspergillus</i> and <i>Fusarium</i> species
14.45	Emmanuel Coton	Biotic and abiotic factors impact the efficacy of antifungal biocides used in the dairy industry
15.05	Break	

**Session: Food fermentation (Chair Marta Taniwaki)**

15.30	Seung-Beom Hong	The role of indigenous meju fungi for fermented soybean products
15.50	Giancarlo Perrone	<i>Penicillium</i> genome project: the case of Italian strains from fermented food
16.10	Vasilis Valdramidis	Optimisation of koji production from a simulant system to rice and its association with flavour enhancement
16.30	Kap-Hoon Han	Fermentation of Korean traditional meju using GRAS fungal strains
16.50	Ewen Crequer	Adaptive differences between <i>Penicillium roqueforti</i> cheese and “non-cheese” populations

**ICFM commission meeting (closed)****Dinner** at Humphries restaurant Utrecht centre

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**Tuesday July 19 2022****Session: Physiology of food spoilage fungi (Chair Emmanuel Coton)**

09.00	Jan Dijksterhuis	The fungal spore; tales of beginnings
09.25	Tom van den Brule	Intraspecific variability in conidial heat resistance of food spoilage fungi
09.50	Miloslava Kavková	The interactions among isolates of <i>Lactiplantibacillus plantarum</i> and dairy yeast contaminants: towards biocontrol applications
10.15	Sjoerd Seekles	Natural variation and the role of Zn2Cys6 transcription factors SdrA, WarA and WarB in sorbic acid resistance of <i>Aspergillus niger</i>
10.40	<b>Break</b>	
11.10	Maarten Punt	High sorbic acid resistance of <i>Penicillium roqueforti</i> is mediated by the SORBUS gene cluster
11.35	Harry Harvey	Preservative resistance of spoilage yeasts at low glucose and relevance for reduced-sugar formulations
12.00	Joe Violet	Inoculum size matters: relationship between preservative MIC and heteroresistance in spoilage yeasts

**Closing of the workshop****13:00 Lunch****POSTERS**

<b>Dana Tančinová</b>	Microscopic fungi causing cherry tomato rot in stores
<b>Gemma Castellá</b>	Diversity of non-ochratoxigenic strains of <i>Aspergillus</i> section <i>Nigri</i> from Spanish grapes
<b>Manuela Zdravec</b>	Diversity of <i>Penicillium</i> and <i>Aspergillus</i> species isolated from traditional meat products of different regions in Croatia
<b>Marina V. Copetti</b>	Biofilm formation by fungi relevant in matured meat production
<b>Massimo Cigarini</b>	<i>Aspergillus montevidensis</i> : how much does its heat-resistance increase in low-aw foods?
<b>Monika Coton</b>	Dynamic changes in Champagne mycobiota diversity from vine to wine
<b>Naresh Magan</b>	Climate change and acclimatization of <i>A. flavus</i> strains influences colonisation, biosynthetic gene expression and aflatoxin B1 production by <i>Aspergillus flavus</i> in vitro and in raw pistachio nuts
<b>Nicolas Nguyen Van Long</b>	Rapid flow cytometry methodology for airborne fungal conidia enumeration
<b>Sjoerd Seekles</b>	The effect of cultivation temperature on the heat resistance of <i>Aspergillus niger</i> conidia
<b>Zuzana Barboráková</b>	Micromycetes responsible for the decay of strawberries and blueberries in Slovak supermarkets
<b>Zuzana Mašková</b>	Fungal agents of table grape spoilage in the retail network and their toxigenic potential
<b>Andika Sidar</b>	A Novel Design of $\alpha$ -Amylase with an N-terminal CBM20 in <i>Aspergillus niger</i> improves binding and processing of raw starch

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**SESSION 1: DEVELOPMENTS IN METHODOLOGY****ICFM AND THE UPDATE OF THE ISO STANDARD “ENUMERATION OF YEAST AND MOULDS”**

Emilia Rico<sup>1</sup>, Jos Houbraken<sup>2</sup>, and members of ISO/TC34/SC9/WG16

<sup>1</sup>*BCN Research Laboratories, Inc., Rockford, Tennessee, USA*, <sup>2</sup>*Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands*

Presenter: [emilia.rico@bcnlabs.com](mailto:emilia.rico@bcnlabs.com)

ISO (the International Organisation for Standardisation) is a worldwide federation of national standards bodies. The work of preparing International Standards is normally carried out through ISO technical committees. In June 2012, SC9 (microbiology) agreed to create the working group “WG16 Yeast and moulds” to revise the standards ISO 21527-1:2008 (colony count technique in products with water activity greater than 0.95) and ISO 21527-2:2008 (colony count technique in products with water activity less than or equal to 0.95). They requested WG16 to merge both standards into one, and use DRBC as general isolation medium in combination with surface plating.

The International Commission on Food Mycology (ICFM) is a COMCOF (Commissions, Committees and Federations) of the International Union of Microbiological Societies (IUMS) and the latter is in liaison with ISO. In 2018, WG16 seek contact with ICFM to participate in the update of the standard on enumeration of yeasts and moulds. At this moment, a draft of the updated standard is evaluated and, in the presentation, an overview of ongoing developments is given.

**ADVANCES AND LIMITATIONS OF FUNGAL METABARCODING**

Christiane Baschien

*DSMZ- Leibniz Institute of Microorganisms and Cell Cultures*

Presenter: [christiane.baschien@dsmz.de](mailto:christiane.baschien@dsmz.de)

Accurate species identification is a requirement to answer the questions of function, resilience, interaction and diversity effects in ecological studies in indoor and food habitats. Furthermore, it is widely accepted that the cultivation bias introduced with isolation strategy and culture media limit the information about the fungal diversity at the time point of sampling.

For more than a decade, the diversity of food and indoor fungi has been estimated using DNA metabarcoding (Amend et al. 2010). For DNA-metabarcoding one or more marker regions in the genome are PCR-amplified and sequenced using a next-generation sequencing (NGS) platform. The partial ITS rDNA operon is the most widely used marker for fungal metabarcoding.

At DSMZ we conducted a range of metabarcoding studies from different habitats. It became evident that fungal metabarcoding has the advantages of overcoming the cultivation bias, increasing the number of possible samples by high-throughput sequencing, and realizing the detection of yet uncultivated fungal taxa. However, the limitations, in particular the lack of taxonomic accuracy when using automated classification, became apparent, too:

Firstly, many widespread taxa are difficult to identify because the phylogenetic resolution of the ITS region is not sufficient for species delimitation, which in turn can lead to underestimates of fungal diversity. On the other hand, the diversity of fungi possessing multiple heterogeneous ribosomal operons is often overestimated during sequence clustering.

During curation of the retrieved dataset, quality of classification i.e. phylogenetic rank and current nomenclature were checked. The manual curation step showed that 20 % of the OTUs represented either chimeric or very short (usually shorter than 80 bp) uninformative sequences despite standard quality filtering and chimera-tests. Re-blasting OTU (Operational Taxonomic Units) resulted in taxonomic correction (including nomenclature) of another 30 % of OTU. About 60% of the insufficient identifications can be

attributed to database shortcomings, i. e. as yet unidentified species or missing reference sequences for taxa not yet sequenced.

Parameters that can be adjusted to improve the classification of species in metabarcoding studies include changing the cut-off threshold for sequence similarity, updating and refining of databases, and aligning and collapsing of OTU.

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### FROM ORCHARD TO POST-HARVESTED BAMBINELLA FRUIT: A FUNGAL SPORE ASSESSMENT THROUGH CONVENTIONAL AND MOLECULAR METHODS

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*Bambinella* is an endemic fruit to the Maltese Islands and is widely sought after both locally and internationally. Ubiquitous airborne fungal spores cause fruit disease and decay, mainly during post-harvest and storage phases, despite the farmer's control efforts. Muscat et al., (2017) have detected the fungi of *Cladosporium ramotenellum*, *Alternaria arborescens*, *Penicillium lanosum*, *Penicillium expansum* and *Aspergillus sydowii* on the *Bambinella* skin. The current work aimed to trace the sources of contamination, their persistence and the levels of occurrence, by performing active air sampling from *Bambinella* orchards, as well as the surface and mass of the post-harvested fruit. Assessments were performed in different geographical regions in Malta. A total of 460 *Bambinella* fruits were collected which were split into 30 batches. Culturable and Next Generation – Amplicon Target Sequencing (NG-ATS) methods were used to identify the fungal pathogens of the *Bambinella* fruit to genus and species level. The results confirmed that common fungal pathogens were present in the air and on the *Bambinella* surface and mass. These included *Cladosporium* spp., *Alternaria* spp., *Penicillium* spp., *Aspergillus* spp. and *Botrytis cinerea* amongst others. The fruits fungal contaminants and diversity were especially highlighted by NG-ATS method. *Cladosporium* spp. and *Alternaria* spp. were prevalent in 100% of air and fruit samples and the latter were reported to cause *Cladosporium* and *Alternaria* rots in pear fruit, respectively. Other fungal pathogens were detected in lower frequencies however they are still known to cause a variety of pre- and post-harvest rots in apple and pear fruit, including blue rot (*Penicillium expansum*), grey rot (*Botrytis cinerea*) and black scab (*Aspergillus carbonarius*). The fruits fungal contaminants and diversity were especially highlighted by NG-ATS method up to species level. Pathogens detected from local airborne mycobiota which colonise *Bambinella* fruit are responsible for fruit's development and maturation, preservation and quality, often leading to their spoilage when given the opportunity to infect.

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## SESSION 2: FUNGAL COMMUNITIES AND ECOLOGY

### DIFFERENT FUNGAL SPECIES ARE ASSOCIATED TO DIFFERENT PARTS OF PLANTS

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There should be a sharp distinction between the spora and funga of different foods. The spora of a certain food item may or may not be indicative for fungal spoilage potential, while the associated funga of a food item is predictive of spoilage and mycotoxin production. While the spora can be determined by traditional dilution plating, surface disinfected food particles and the percentage of infected food items by each species recovered is the preferred method for indicating spoilage and mycotoxin production. The association of filamentous fungi to food plants is depending on the part of the plant. For example apples can be infected by *Monilinia fructigena*, *M. laxa*, *M. fructicola*, *Penicillium expansum*, *P. crustosum*, and *Trichothecium roseum* and mycotoxins may be produced in apples, while the fungi present as endophytes can be species such as *Alternaria tenuissima*, *Epicoccum nigrum*, *Fusarium* spp. etc., in addition to bacteria such as *Bacillus* species, and the apple plant roots (the rhizoplane) have species such as *Penicillium janczewskii* and species in *Penicillium* section *Lanata-Divaricata* associated. Another example is wheat-associated Penicillia. *Penicillium aurantiogriseum*, *P. cyclopium*, *P. melanoconidium*, *P. polonicum*, *P. verrucosum* and similar species are associated to wheat-kernels and may produce mycotoxins such as ochratoxin A, penicillic acid, xanthomegnin in those kernels. The Penicillia associated to wheat rhizoplane and rhizosphere soil represent completely different species such as *Penicillium hordei*, *P. scabrosum*, and *P. canescens* and these species seem to be very important for wheat plant health and for preventing growth of plant-pathogenic *Fusarium* species in wheat.

### MICROBIAL COMMUNITIES OF THE DRY AGED BEEF (DAB)

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Dry aged beef (DAB) is known for its unique flavour. Investigation of the fungal diversity of DAB indicates, that fungi belonging to the *Mucoraceae*, may improve the taste and smell of the final product (Mikami et al., 2021). Traditionally *Thamnidium* was known as the main fungus responsible for meat seasoning. However, based on the metabarcoding study of microorganisms colonising Korean DAB, fungi belonging to *Helicostylum* as well as bacteria belonging to *Lactobacillus*, *Bifidobacterium* and *Streptococcus* genera were reported (Ryu et al., 2020). Our goal was to combine traditional microbiological culture-based isolation techniques with high throughput amplicon sequencing in order to characterise microbiome of DAB samples. Results showed that in culture-based approach fungi representing *Mucor flavus* species complex were detected most often. The same species were also dominant in metabarcoding analyses. The bacteria representing *Pseudomonas* genus were present in all samples, in majority were also present *Brochothrix* and *Carnobacterium*. Comparing these results with previous studies indicates that the bacterial community of DAB is probably highly variable. The community composition may depend on local microbiota inhabiting cattle individuals or other factors. Contrary, the mycobiome of DAB seems to be more stable. *M. flavus* and *Helicostylum* sp. are closely related taxa and can both be found interchangeably on DAB (Mikami et al., 2021). In summary, the core DAB microbial community seems to be composed of fungi belonging to the *Mucor flavus* group (as defined in Walther et al. 2013) and variable composition of bacteria strains. Such microorganisms, due to their enzymatic activity, may have an impact on the process of beef dry ageing.

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## ECOLOGY AND CONTROL OF *A. FLAVUS* AND AFLATOXIN B<sub>1</sub> IN CHILLI POWDER AND WHOLE RED CHILLIES USING FOOD-GRADE PRESERVATIVES

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Chillies and chilli-based products are important spices on a global basis. The production, processing, transport and storage phases of chillies are prone to infection by *Aspergillus* Section *Flavi* and contamination with aflatoxins (AFs), especially aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) for which legislative limits exist in many countries. We have examined the effect of the interacting abiotic factors of water availability (water activity, a<sub>w</sub>; 0.995-0.850 a<sub>w</sub>) and temperature (15-37°C) on growth and AFB<sub>1</sub> production to identify the optimum and boundary conditions for colonisation and toxin production by three *A. flavus* strains on a 10% chilli-based medium. Studies with whole red chillies + *A. flavus* conidial inoculum on AFB<sub>1</sub> contamination during storage for 10-20 days at 30°C were also carried out. This was complimented with studies on the use of different food-grade preservatives for the control of growth and AFB<sub>1</sub> contamination of chilli powder and whole red chillies. Ecologically, there was no statistical difference in growth between the three strains. Optimal growth was at 37°C and 0.982 a<sub>w</sub> with no growth at 0.85 a<sub>w</sub>. Optimal temperature x a<sub>w</sub> conditions for AFB<sub>1</sub> production were at 30°C and 0.982 a<sub>w</sub> with no statistical difference in production between strains. No AFB<sub>1</sub> was produced at 15-20°C at 0.901 and 0.928 a<sub>w</sub> levels, respectively. *In situ* studies with *A. flavus* inoculated whole red chillies at 0.90 and 0.95 a<sub>w</sub> found that this species became the major component of the total fungal populations at 30°C after 10-20 days storage. AFB<sub>1</sub> contamination was above the European legislative limits 5 µg/kg) for spices at 0.90 a<sub>w</sub> after 20 days storage and at 0.95 a<sub>w</sub> after 10 and 20 days. This suggests that storage conditions of ≥0.90 a<sub>w</sub>, especially at ≥25-30°C represents a significant risk of contamination with AFB<sub>1</sub> at levels where rejection might occur, even after only 10-20 days storage. Subsequently seven different food grade preservatives were examined for control of aflatoxins in chillies. Of these compounds, sodium metabisulphite (NaMBS) was the most effective in controlling growth and AFB<sub>1</sub> production by *A. flavus* strains at 0.93, 0.98 and 0.95 a<sub>w</sub>. No growth or production of AFB<sub>1</sub> occurred with 500-2500 mg/L NaMBS. *In situ* studies with chilli powder or whole red chillies (naturally contaminated or + *A. flavus* inoculum) with NaMBS controlled total fungal populations and *A. flavus*. Studies with commercial laminated sheets containing slow-release layers of NaMBS (SO<sub>2</sub>) in stored chillies showed significant reductions in fungal populations and AFB<sub>1</sub> contamination.

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## FUNGAL COMMUNITIES AND POTENTIAL FOR MYCOTOXIN PRODUCTION IN BRAZILIAN CASSAVA TUBERS AND FOOD PRODUCTS

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Cassava (*Manihot esculenta* Crantz) is one of the most widely cultivated foods in the world and is of great socio-economic importance, especially in developing countries. It is mostly consumed in boiled form, but is also used to produce a number of products, including cassava starch, sour starch, cassava flour and tapioca flour (hydrated cassava starch). Fungal spoilage can occur throughout the production chain, impairing both productivity and quality, as well as posing a potential risk of contamination by mycotoxins. The present study determined the mycobiota of 101 samples of cassava (tubers, cassava products and soils from cassava fields), collected in São Paulo, Brazil, using multidisciplinary approaches based on phenotypic and molecular data (*ITS/ BenA/ TEF-1a/ RPB2* loci). In addition, the levels of aflatoxin were analyzed in cassava tubers and products. A total of 20 fungal groups/genera were morphologically characterized, and 37 different species were molecularly identified. The predominant groups/species in cassava tubers were *Fusarium* spp. (*Fusarium fabacearum*, *F. mundagurra* and *F. foetens*), *Penicillium* spp. (*P. citrinum*, *P. javanicum* and *P. brevicompactum*) and *Trichoderma* spp. (*Trichoderma afroharzianum*, *T. peberdyi*, *T. koningiopsis*, *T. arenarium*, *T. pseudoasperelloides* and *T. azevedoi*). In cassava products, the most frequent groups were *Paecilomyces* (*P. saturatus* and *P. formosus*) and *Penicillium* spp. (*P. citrinum*, *P. paneum*, *P. brevicompactum*, *P. chrysogenum*, *P. camponotum*, *P. diatomitis*,

*P. aethiopicum* and *P. coprophilum*). In soil-cultivated cassava samples, the groups found most frequently were *Penicillium* spp., *Cladosporium* spp. and *Fusarium* spp. Some of the species found in cassava tubers and/or product samples were also present in the soil, including *F. mundagurra*, *Neocosmospora solani*, *P. citrinum* and *P. brevicompactum*. Potentially toxigenic species were also found in cassava tubers, *A. flavus*, *A. arachidicola*, *A. novoparasiticus* and *A. parasiticus*. The majority of strains (73.3%) tested for their aflatoxin-producing ability in synthetic media were positive. Despite that, cassava and cassava products were essentially free of aflatoxins, only one sample of cassava flour contained traces of AFB<sub>1</sub> (0.35 µg/kg). The mycobiota of Brazilian cassava proved to be extremely diverse, and the occurrence of several species in cassava tubers and/or products are reported herein for the first time.

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### SESSION 3: MYCOTOXINS

#### CHARACTERISATION OF THE EMERGING MYCOTOXIGENIC PATHOGEN *FUSARIUM ASIATICUM* THROUGH GENOME SEQUENCING BY OXFORD NANOPORE TECHNOLOGY

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*Fusarium graminearum* species complexes (FGSCs) cause serious plant diseases and threaten public health via mycotoxin contamination of the infected crops. Traditional identification based on morphology cannot distinguish between most of the members of the FGSC. Till now *F. asiaticum* has been considered a pathogen confined in Easter regions. However, recently, it has been isolated from small cereals in different parts of the world like the United States of America and Brazil.

The overall purpose of this study was to investigate the potential application of the long-read sequencing provided by Oxford Nanopore technologies to produce a reference genome to promote rapid identification of *F. asiaticum*.

High-quality fungal DNA was extracted from one *F. asiaticum* strain isolated from cereals in China. Library prep and genomic barcoding were prepared according to the 1D Native barcoding genomic DNA (with EXP-NBD104 and SQK-LSK109/EXP-NBD103 and SQK-LSK108) protocol obtained from Oxford Nanopore. Then, the library was loaded to FLO-MN106 Flowcell along with sequencing buffer and loading beads.

A 98x coverage of *F. asiaticum* genome was achieved. The longest read was 200Kb, and more than half of the reads averaged 16.9Kb or longer. These studies have confirmed that the coverage and the N50 obtained in the current study are sufficient to assemble the whole genome.

The results of the first assembly will be presented at the conference.

#### IMPACT OF ENVIRONMENTAL CONDITIONS AND PREDICTED CLIMATE CHANGE FACTORS ON *FUSARIUM ASIATICUM* GROWTH AND MYCOTOXIN PRODUCTION IN WHEAT

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*Fusarium asiaticum* is a predominant fungal pathogen causing Fusarium head blight in wheat and barley in China. This pathogen also produces the EU legislated mycotoxin, deoxynivalenol and zearalenone. These mycotoxins are EU regulated due to their adverse effect on animal and human health and non-compliance results in approximately £201 million in annual economic losses due to grain rejection. Therefore, it is important to characterise this emerging pathogen.

Our objective was to understand how environmental factors and Climate Change conditions impact *F. asiaticum* growth and mycotoxin production. Firstly, we studied the impact of environmental factors  $a_w$  (0.995-0.87 water activity;  $a_w$ ) and different temperature 10-35°C on growth and mycotoxin production by three strains of *F. asiaticum* in wheat-based media. *F. asiaticum* growth was optimum at 25°C/0.98 $a_w$  and the optimum Zearalenone production was at 0.95 $a_w$ /30°C.



Secondly, we studied the impact of 1,000 ppm elevated CO<sub>2</sub> on mycotoxin production in wheat-based media. The first results are showing that Zearalenone production was enhanced at 25°C/0.95 when exposed to elevated CO<sub>2</sub> conditions.

These results are highlighting that Climate Change is likely to increase the Zearalenone production in Chinese wheat unless mitigation techniques are developed.

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## **AFLAZ - NEW SAFETY ASPECTS ON AFLATOXIN PRODUCING FUNGI IN MAIZE FIELDS NEAR NAIROBI/KENYA**

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Aflatoxins are among the most toxic substances produced by fungi and contaminate various foods. In particular, the warm, humid climate of sub-Saharan Africa and other regions of the world provides optimal growth conditions for aflatoxin-producing *Aspergillus* species, which can lead to food and feed heavily contaminated with aflatoxins, resulting in outbreaks of aflatoxicosis with numerous deaths.

AflaZ, which stands for “Zero Aflatoxin”, is a multidisciplinary research project coordinated by the Max Rubner-Institut in Karlsruhe, Germany. Together with scientists from the Friedrich-Loeffler-Institut, the Julius-Kühn-Institut, the University of Koblenz-Landau, and project partners in Kenya, KALRO (Kenya Agricultural and Livestock Research Organization) and EAFF (Eastern Africa Farmers Federation), the formation of aflatoxin on maize and its transfer to milk are being investigated and strategies developed to reduce fungal growth and aflatoxin formation.

During the project period, interesting scientific results have already been achieved by the project partners involved. Among other things, the Department for Safety and Quality of Fruit and Vegetables was able to show that aflatoxin M<sub>1</sub>, a metabolic product of aflatoxin, is not only present in milk, as is often assumed. In addition, laboratory experiments have demonstrated the effectiveness of blue light irradiation in inhibiting the growth of *A. flavus* and *A. parasiticus* on stored maize and the use of the mycoparasitic fungus *Trichoderma afroharzianum* as a biocontrol organism against aflatoxin-producing fungi. On-site support to Kenyan farmers involved in the project throughout its duration, such as regular training in Kenya on the project results in maize cultivation with biocontrol fungi (AflaZ trial fields), harvesting with subsequent drying of the maize, and storage of the harvest using the developed prevention methods, has already resulted in reduced aflatoxin contamination and thus increased food safety for the Kenyan population.

## **CURRENT FOOD SAFETY STATUS IN INDONESIA**

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During last two years, the world, including Indonesia, fought against the COVID-19 pandemic caused by SarCov-2 virus. Long term pandemic has changed most of human culture and civilization. In addition to the COVID-19 pandemic, we are also facing the problem of global warming. Global warming causes erratic climate change, affect in decreasing of the agricultural products quality which indirectly impact the food security, food safety and food availability in Indonesia. According to data of Global Food Security

(2021) for food quality and safety, Indonesia's position has decreased from 91 to 95<sup>th</sup> out of 113 countries in the world. Food security status in Indonesia also went through drastic decline, which was originally ranked 57<sup>th</sup> in 2020, became 69<sup>th</sup> out of 113 countries. Related with dealing for the COVID-19 pandemic, Indonesia had Balanced Nutrition Guidelines suitable for Indonesian diet, which was varied, nutritious and balanced (2014, Ministry of Health). A varied, nutritious, balanced and safe diet can support the immune system to fight COVID-19 pandemic. This statement was supported by our research that diet could helping in the recovery of asymptomatic COVID-19 patients in the Bantul and Sleman shelters, Indonesia. However, it was not in accordance with the availability of food in Indonesia which tends to decrease in every year. Indonesia has various types of natural resources that can be used as food, but there are still a lot of food safety problems caused by mycotoxin contamination. Rice as the staple food in Indonesia, have been proven safe from mycotoxin contamination which were still below the recommended limit. It was because of strictly monitored distribution of rice by the government. In addition to rice as the main source of carbohydrates, soybeans are also a source of protein that is widely consumed by the community which later be processed into *tempe* and tofu. Similar with rice, the average mycotoxin contamination in soybeans were still below the recommendation limit. Indonesia is also famous for its various spices and seasonings. In fact, research data showed that spices and seasonings still high in mycotoxin contamination, although their application still tends to be in small amount. Other commodities such as corn and beans which are widely used as ingredients for making snacks also potential to be exposed to mycotoxin contamination. According to research from Nugraha *et al.* (2018), who conducted research related to risk assessment of Aflatoxin B1 (Afb1) exposure for maize and peanut consumption in Indonesia, stated that risk assessment using Margin of Exposure (MOE) approaches revealed that Afb1 exposure from maize and peanut consumption in Indonesia were generally below 10,000, and for several occurrence data were even below 1000. Meanwhile, based on quantitative liver cancer risk, the estimated number of liver cancer cases associated with Afb1 exposure was generally above the 0.1 cancer cases/100,000 individuals/75 years. From this study, it can be concluded that the frequency of Afb1 contamination cases, tends to be low and is not just the only problem of food safety in Indonesia. Other sources of contamination, such as pathogenic bacteria also need to be considered.

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### DOES *ALTERNARIA* MOULDY CORE INFECTION OF APPLE FAVOUR MYCOTOXIN ACCUMULATION?

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Apple fruits are susceptible to fungal infection during pre- and post-harvest with consequent food spoilage and economic losses. *Alternaria* is able to produce external lesions on fruits and is the main causal agent of mouldy core (MC), a disease whose severity increases during food storage. As MC usually goes unnoticed in the visual inspection performed by industries, the risk of by-products' contamination with *Alternaria* mycotoxins increases. The objective of this study was to evaluate the metabolic capacity and mycotoxins production of *Alternaria* strains isolated from apple fruits *in vitro* and *in vivo* according to their source and under different environmental conditions. A total of 78 *Alternaria* isolates from external lesions and MC were characterized by their *in vitro* secondary metabolite production profiles, determined by ultra-high-performance chromatography (UPLC) coupled with UV detection and high-resolution mass spectrometry (HRMS). Twenty-seven secondary metabolites produced by this genus were identified, many of which are known as mycotoxins. Differences according to the isolate source were observed: those obtained from MC showed greater metabolic capacity than those from external lesions. The mycotoxin production of 3 *Alternaria* strains with high metabolic capacity was evaluated *in vivo*. These strains were inoculated

on both the outside and inside of undamaged fruits, simulating retail (T=25 °C, one month) and storage (T=4 °C, 9 months) conditions. Quantification of alternariol (AOH), alternariol monomethyl ether (AME), tentoxin (TEN), tenuazonic acid (TeA), altenuene, altertoxin-I and -II (ATXs), and modified forms of AOH and AME was done using targeted UPLC-MS/MS. The 3 isolates were able to produce all the described mycotoxins and some modified forms under retail and long-term cold storage conditions in the interior and the exterior of the fruit. Higher levels of AOH, AME, TeA, TEN and ATXs accumulated when *Alternaria* strains colonized the centre of the fruit at 25 °C. Long-term cold storage usually performed by processing industries did not prevent the accumulation of *Alternaria* toxins in apples. The study results imply a risk of the presence of *Alternaria* mycotoxins in fruits, both for retail and destined to processing. Our study suggests the need to assess mycotoxins' natural occurrence in apple fruits and the by-products.

### 3-NITROPROPIONIC ACID IS AN EMERGING IMPORTANT MYCOTOXIN

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Fungi producing the neglected mycotoxin 3-nitropropionic acid (3-BNP) include *Apiospora aurea*, *A. sacchari*, *A. saccharicola*, *A. phaeosperma*, *A. serenensis* and *A. terminalis*, *Aspergillus* species and *Penicillium* species. *Apiospora* species can produce 3-nitropropionic acid in sugarcane and intoxicate children and *Apiospora saccharicola* has also been found growing in coconut milk, in one case causing death in a man. The symptoms of the famous Turkey X disease were thought to be caused by aflatoxins and cyclopiazonic acid, but the symptoms seen in turkeys could also have been caused by 3-BNP, and all three mycotoxins can be produced by *Aspergillus flavus*. There are few studies on the concomitant production of the three mycotoxins in *Aspergillus flavus*, and it is not known whether other aflatoxin producers such as *A. parasiticus*, *A. aflatoxiformans*, *A. austwickii*, *A. cerealis*, *A. mininisclerotigenes*, *A. nomiae*, *A. pseudonomiae*, *A. luteovirescens*, *A. pseudotamarii* and *A. pseudocaelatus* can produce 3-BNP. *Aspergillus oryzae* and *Aspergillus sojae* can also produce 3-BNP, and it has not been determined whether *A. oryzae* can produce 3-BNP in fermented products such as soy sauce. Optimal analytical chemical methods should be developed to quantify 3-BNP in foods and beverages, as well as multi-mycotoxin methods for common species such as *Aspergillus flavus*. *Penicillium atrovenetum* is also reported to produce 3-BNP, but this species has not been found in foods yet. Other species reported to produce 3-BNP including *Aspergillus avenaceus*, *Aspergillus wentii*, *Diaporthe citri*, *Nigrospora oryzae*, *Pestalotia palmarum*, *Phomopsis longicolla*, *Phomopsis oblonga* and *Talaromyces amestolkiae*, but their production of 3-BNP in foods has not been reported.

### BIOCONTROL STRATEGY AT PRE-HARVEST STAGE FOR REDUCING AFB<sub>1</sub> IN MAIZE DURING STORAGE IN ARGENTINA

María Silvina Alaniz Zanon<sup>1</sup>, Marianela Bossa<sup>1</sup>, María Laura Chiotta<sup>1</sup>, Claudio Oddino<sup>1</sup>, Diego Giovanini<sup>1</sup>, Marcelo Leandro Cardoso<sup>2</sup>, Ricardo E. Bartosik<sup>2</sup> and Sofía Noemí Chulze<sup>1\*</sup>.

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Maize (*Zea mays* L.) is an important crop in Argentina. *Aspergillus flavus* can infect maize at growing stage and contaminate kernels with aflatoxins (AFs), whose levels may increase during storage. In Argentina silo

bags are widely used. Biocontrol based on competitive exclusion by atoxigenic *A. flavus* strains is a useful tool for AFs management at pre-harvest stage. In the present study the effect of pre-harvest treatments on AFB<sub>1</sub> accumulation in maize stored in silo bags during 3 and 6 months was evaluated. Three bioformulates were applied at field stage: *A. flavus* AFCHG2 and ARG5/30 strains, as single and mixed inocula. Harvested kernels were stored in non-hermetic and hermetic silo bags. At t<sub>0</sub>, t<sub>3</sub> and t<sub>6</sub> different parameters were evaluated: damage kernels, moisture content, a<sub>w</sub>, *Aspergillus* section *Flavi* presence, relative humidity, O<sub>2</sub> and CO<sub>2</sub> levels into the bags, and AFB<sub>1</sub> levels. The biocontrol strains were able to infect maize kernels during the field trial and displaced toxigenic native isolates. At t<sub>0</sub> control plots showed 10.9 ± 0.4 µg/kg of AFB<sub>1</sub> while no AFB<sub>1</sub> was detected in all the treatments. Along the storage assay AFB<sub>1</sub> levels varied from not detected (<1 µg/kg) to 20.1 ± 0.8 µg/kg. Hermetic bags were better than non-hermetic bags in controlling AFB<sub>1</sub> accumulation. AFB<sub>1</sub> was not detected at t<sub>6</sub> in the treatment with AFCHG2 + ARG5/30 into hermetic bags. Both single and mixed inocula were effective to control AFB<sub>1</sub> accumulation in maize kernels during 3 and 6 months. The application of the biocontrol agents at field stage could be a promissory tool to reduce AFB<sub>1</sub> accumulation under storage in hermetic silo bags.

## SESSION 4: TAXONOMY OF FOOD BORNE FUNGI

### IN MEMORIAM: JOHN PITT AND HIS CONTRIBUTION TO THE INTERNATIONAL COMMISSION ON *PENICILLIUM* AND *ASPERGILLUS*

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*Penicillium* is a large genus with high economic impact, consisting of several hundreds of species. Only a few mycologists have worked on the taxonomy and the first monograph by Raper & Thom appeared in 1949. Thirty years later in 1979 John Pitt published the impressive book *The Genus Penicillium and its teleomorphic states Eupenicillium and Talaromyces*. For identification John introduced a new methodology for identification using keys where each isolate is grown on five Petri dishes with Czapek Yeast Autolysate (CYA), malt extract and 25% glycerol nitrate (G25N) agars. These plates have to be incubated at 5°, 25° and 37°C and colony diameters are measured after 7 days. The new approaches in *Penicillium* taxonomy raised discussions among the mycologists who had to identify *Penicillium* strains. The first International Workshop on *Penicillium* and *Aspergillus* was organized in Baarn in May 1985 to discuss the status of taxonomy and the used methodology. This meeting was very successful and a second one followed at the same venue in 1989. The proceedings were published by Rob Samson & John Pitt: *Advances in Penicillium and Aspergillus systematics* in 1985 and *Modern Concepts in Penicillium and Aspergillus Classification* in 1990. In 1997 the third international workshop was organized and proceedings published as *Integration of modern taxonomic methods for Penicillium and Aspergillus classification* in 2000. The output of the workshops and the published proceedings resulted that the groups of mycologists were establishing a stable classification with generally accepted names. Another important initiative of Pitt and Samson was the production of a list of accepted names in *Penicillium* and *Aspergillus*. This list (Species names in current use (NCU) in the *Trichocomaceae* (Fungi, *Eurotiales*)) was the first in mycology and contributed significantly to the stabilisation of names in both genera.

In April 2012 the International Commission on *Penicillium* and *Aspergillus* which was established in 1989, came together to discuss the One fungus - One name concept in *Aspergillus* and *Penicillium*. The commission unanimously agreed about the generic names *Penicillium* and *Talaromyces* for the biverticillate species. The commission voted to keep the name *Aspergillus* including the sexual states. However, John Pitt decided against this proposal. Unfortunately, this resulted in many debates and publications.

In this presentation the significance of the work by John Pitt for the taxonomy of *Penicillium* and *Aspergillus* will be highlighted.

## REVITALIZATION OF PHENOTYPIC TAXONOMY IN *ASPERGILLUS*, *PENICILLIUM* AND *TALAROMYCES*

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Filamentous fungi are rarely classified (and subsequently identified) using phenotypic methods (taxonomy), but rather cladified (and subsequently identified) using cladistics treatment of nucleotide sequence data of one or more household genes (cladonomy). Combining taxonomy and cladonomy has been suggested under the name polyphasic biosystematics, but data are usually treated using cladistic methods. Numerical taxonomy was introduced for phenotypic data by Peter Sneath and Robert Sokal in the nineteen sixties and seventies, and was based on cluster analysis and/or ordination methods, but these effective methods have rarely been used in modern biosystematics. A reintroduction of numerical taxonomy is proposed, somewhat modified to include only features based on differentiation, that is weighting of data that are selectable, thereby re-introducing selection and not only phylogeny in biosystematics. This will lead to a predictive taxonomy, and features measured could be secondary and tertiary metabolites, secondary proteins, secondary exopolysaccharides, morphological differentiation, ecophysiological and resistance features, and nutritional features. Data treatment will depend on the type of data (binary, quantitative, unimodal), but binary data could be treated by UPGMA clustering of binary data using the Yule coefficient, and quantitative data can be treated by network analysis or ordination analysis such as principal component analysis, non-metric multidimensional scaling, and correspondence analysis. An example will be given on classification of function/family CAZyme data using cluster analysis and the Yule coefficient for species in *Penicillium* and *Aspergillus*, which pointed to the same species sections and series as cladistic data based on nucleotide sequence data.

## REDEFINING *FUSARIUM*

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The *Nectriaceae* (*Hypocreales*, *Sordariomycetes*) includes saprobes, endophytes and numerous important plant and animal pathogens, several of which are used in commercial applications. Members of *Nectriaceae* are circumscribed by having yellow, orange-red to purple uniloculate ascomata, and phialidic asexual morphs. Due to the lack of DNA sequence data for many taxa in the family, species and generic concepts remain poorly defined. To address this issue, we performed a multi-gene phylogenetic analysis using partial nucleotide sequences of the *rpb1*, *rpb2*, and *tef1* and rDNA gene regions for available type and authentic strains representing fusarium-like taxa. Using a polyphasic approach including morphology of the sexual and asexual morphs, these data resolved more than 20 genera in *Fusarium sensu lato*. Following the one fungus = one name initiative, *Fusarium* = *Gibberella*. This genus relates to the F3 clade *sensu* Geiser *et al.* (2013), and not the F1 node eventually chosen by the authors Geiser *et al.* (2021), including an assemblage of different biological genera such as *Albonectria*, *Bisifusarium*, *Cyanonectria*, *Geejayessia*, *Neocosmospora* and *Rectifusarium*. These genera do not only differ in their sexual morphs, but also in their asexual morphology and biology. The fusarium-like morphology, with hyaline, curved macroconidia with basal foot cells, is a synapomorphy that has been lost several times throughout the *Hypocreales*, and does not represent a character of generic value.

## NEW INSIGHTS ON THE UNEXPECTED DIVERSITY OF *NECTRIACEAE* SPECIES IN CHEESE AND A GLIMPSE AT THEIR FUNCTIONAL DIVERSITY

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*Bisifusarium domesticum* is among the main molds used today for cheese making, especially for its “*anticollanti*” property that prevents the sticky smear defect of some cheeses. To create a working collection of *B. domesticum* isolates, numerous cheese rinds were sampled. Doing so, not only did we isolate *B. domesticum* but we observed a completely unexpected diversity of “*Fusarium*-like” fungi belonging to the *Nectriaceae* family. Using a multi-gene phylogenetic approach, four novel cheese-associated species belonging to two genera were described: *Bisifusarium allantoides*, *Bisifusarium penicilloides*, *Longinectria lagenoides*, and *Longinectria verticilliforme*. We then aimed at determining their potential functional impact during cheese-making by evaluating their lipolytic and proteolytic activities as well as their capacity to produce volatile (HS-Trap GC-MS volatilities) and non-volatile extrolites (HPLC & LC-Q-ToF). While all isolates were proteolytic and lipolytic, higher activities were observed at 12°C for several *B. domesticum*, *B. penicilloides* and *L. lagenoides* isolates, which is linked to typical cheese ripening conditions. Using volatilities, we identified multiple cheese-related compounds, especially ketones and alcohols. *B. domesticum* and *B. penicilloides* isolates showed higher aromatic potential although the compounds of interest were also produced by *B. allantoides* and *L. lagenoides*. Finally, an untargeted extrolite analysis suggested a safety status of all these strains as no known mycotoxins were produced. We also pinpointed the production of some potentially novel secondary metabolites that may be linked to antimicrobial properties. This suggests that, in particular, *B. domesticum* may be an interesting candidate for biopreservation applications in the cheese industry in the future as already used for technological properties. We are currently working on a comparative genomics analysis to provide new knowledge on these species.

## IMPLEMENTATION OF SPECIES DELIMITATION METHODS IN *ASPERGILLUS*

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The advent of molecular techniques into the taxonomy and subsequent acquisition of large amounts of sequencing data in recent years is now followed by the spread of species delimitation methods, which are utilizing these molecular (mainly sequence) data. The number of taxonomic studies that employ these methods for many different groups of organisms is growing every year, as well as the amount of software dedicated to this task. The biggest advantage of these methods is that they offer a fresh and unbiased look at the species limits within the specific group of species. We have used these methods to establish the species boundaries in several parts of *Aspergillus*, including sections *Restricti*, *Candidi* and *Flavipedes*, or series *Nigri*, *Versicolores* and *Viridinutantes*. The application of these methods led in some parts of the genus to a description of new species, but also to synonymizing accepted species in other groups (the revision of series *Versicolores* resulted in the reduction of number of species from 17 to 4). The polyphasic approach is currently the golden standard for the description of new species in *Aspergillus*, but we see these methods as a great enhancement of phylogenetic analysis, which is already supposed to be part of the process. The theoretical long-term goal of using these methods is to bring all species in *Aspergillus* (and also other genera) to a phylogenetically similar level, so they can serve their purpose as basic taxonomic rank and fundamental unit of biodiversity.

## **ASPERGILLUS SECTION *TERREI*: TAXONOMIC OVERVIEW AND ANTIFUNGAL SUSCEPTIBILITY PROFILES**

Roya Vahedi-Shahandashti<sup>1</sup>, Martin Meijer<sup>2</sup>, Bart Kraak<sup>2</sup>, Cornelia Lass-Flörl<sup>1</sup>, and Jos Houbraken<sup>2\*</sup>

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Presenter: [Roya Vahedi-Shahandashti](#)

*Aspergillus* section *Terrei* includes species with columnar cinnamon to orange-brown conidial heads and have a worldwide distribution. *Aspergillus terreus* (incl. varieties *floccosus* and *aureus*), *A. carneus* and *A. niveus* were for a long time the only accepted species in the section (as “*Aspergillus terreus* Group”). Nowadays, the section includes three series *Ambigui*, *Nivei*, and *Terrei* and at least 17 accepted species; however, a current comprehensive overview of lacking. The species in section *Terrei* have economic and medical importance. *Aspergillus terreus* is an important opportunistic human pathogen and often causes disseminated infection with increased lethality compared to other *Aspergillus* species. The current study aims to give an overview the section *Terrei* and update the taxonomy using a polyphasic approach. Morphological studies and phylogenetic analyses of partial  $\beta$ -tubulin, calmodulin, and RNA polymerase II subunit 2 sequences resulted in describing new species. In addition, susceptibility profile of tree classes of antifungals was performed, including polyene (amphotericin B), azoles (voriconazole, isavuconazole, and posaconazole), and echinocandins (micafungin, and caspofungin). Micafungin exhibited significant activity in vitro, followed by caspofungin, and posaconazole.

## **RELEVANCE OF SEXUAL STATES TO MODERN TAXONOMY AND FOOD MYCOLOGY, INSIGHTS FROM *ASPERGILLUS* SPECIES**

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Under traditional taxonomic rules fungal species could have two different names, one for the anamorph (mitosporic asexual state) and one for the teleomorph (meiosporic sexual state, if known). For various reasons this dual naming nomenclature system was replaced by the ‘one fungus, one name’ system from 2013. The latter approach has simplified fungal taxonomy and is argued to be more accessible for non-specialists. However, the approach has meant that some biologically important insights of practical importance have potentially been lost by removal of a particular anamorph or teleomorph genus name. The former use of sexual states for classification of *Aspergillus* species will be described in tandem with the most recent nomenclature (including data based on phylogenetic analysis), together with a description of extra information gained by an understanding of the different sexual groupings. New insights of relevance to food mycology will then be discussed, including the reporting of a sexual state of *A. clavatus*, and in particular the importance of consideration of the increased resistance to certain environmental stressors exhibited by ascospores relative to conidia. Results from a recent study looking at possible differential survival to low and high temperature stress in *A. nidulans* will be presented.

## **NEW SPECIES DIVERSITY IN *ASPERGILLUS* AND *PENICILLIUM***

Jos Houbraken

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The family *Aspergillaceae* harbors various economically important genera, such as *Aspergillus*, *Penicillium* and *Monascus*. Infrageneric classifications are commonly used in these genera. These ranks have nomenclatural status, but are also useful in food mycology because members of these ranks share certain physiological characters or ecological niches. Examples relevant for food mycology will be given. Even

though characters can be linked to a section or series, most data is linked to the species. Recently, new insights in the taxonomy of those genera have led to numerous new species and name changes of existing species. Current taxonomic studies indicate that species delimitation in *Penicillium* is rather clear-cut with no intergrading strains. On the other hand, recent studies in *Aspergillus* indicate the opposite: with enriching datasets with more (new) strains and thus variability, species boundaries become more robust and accurate in comparison with the previous studies. Of course, individual characters may be overlapping, such as the size, form and ornamentation of conidia or growth and sporulation as related to temperature, but a large number of characters are non-overlapping. The mycobiota of food and feed is well studied and the species diversity known. However, occasionally new species are isolated and examples will be given.

## SESSION 5: BIOCONTROL AND PROCESSING

### EFFECT OF GASEOUS OZONE AND OZONIZED WATER AGAINST FOOD-SPOILING FILAMENTOUS FUNGI ON STAINLESS STEEL

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The effect of ozone in its gaseous or aqueous form was investigated on three food-spoiling filamentous fungi (*Hyphopichia burtonii*, *Penicillium nordicum* and *Aspergillus brasiliensis* ATCC 16404) inoculated on stainless steel.

**Gaseous ozone:** The tests were carried out under controlled conditions (T=25°C; RH>85%) by positioning the inoculated stainless-steel tiles in Petri dishes within an ozone generator and treating them at concentrations up to 50 ppm and at times up to 300 minutes. Inocula were made as either a mono- or a multi-layer of cells. An effect was observed just at 50 ppm on the single-layered inocula. At these conditions, *A. brasiliensis* proved the most resistant strain to ozone: its 1D-value (134 min) was markedly higher than those registered for both *H. burtonii* (10 min) and *P. nordicum* (17 min).

**Ozonized water:** The tests were carried out at room temperature in static conditions by directly exposing the inoculated stainless-steel tiles to aqueous ozone. At the maximum concentration tested (8.0 ppm) and at times up to 10 minutes, the tested strains greatly varied in their response. *A. brasiliensis* proved the most resistant strain to ozone: within the time considered, its concentration was not significantly reduced, whereas for both *P. nordicum* and *H. burtonii* it was possible to calculate the corresponding 1D-values. The effect of organic acids added to ozonized water was also assessed on the tested strains.

### IMPACT OF (MILD) PROCESSING ON FOOD SPOILAGE FUNGI

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Fungal spoilage forms a widespread challenge for the food industry with respect to crops as fruit and vegetables at the primary part of the chain and processed products including cereals, bakery products, nuts and dried fruit, dairy, and beverages at the end of the chain. Contamination is often initiated by colonization of crop or food by fungal spores. These spores can easily spread via water and air in the processing environment, and remain viable over long periods and thereby form a risk of recontamination.



Elimination of these contaminants can be difficult and depend on the intensity of the (heat) treatment in case of highly heat-resistant ascospores. Mild technologies that rely not (only) on heat for inactivation or surface decontamination, may provide new strategies to control fungal spoilage. For example, high hydrostatic pressure (HHP) treatment can be used to combine moderate heat and high pressure as alternative to thermal treatment. Cold plasma technology provides new opportunities to control fungal spoilage on food products, in particular products that do not tolerate heat. Moreover, elimination of fungal spores from the processing environment can be difficult and this depends on effective sanitizers and knowledge on fungal sensitivity toward different classes of sanitizers.

This presentation will focus on effects of various mild processing technologies as for example HPP, cold plasma, pulsed electric field on food spoilage fungi. Moreover, the survival of drying of conidia on inert surfaces and impact on resistance towards different processing and sanitation methods will be discussed.

## **STUDIES ON THE EFFICACY OF ELECTROLYSED OXIDISING WATER TO CONTROL *ASPERGILLUS CARBONARIUS* AND OCHRATOXIN A CONTAMINATION ON GRAPE.**

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Ochratoxin A (OTA) occurrence in grapes is caused by black Aspergilli (*Aspergillus carbonarius* followed by *A. niger*) vineyards contamination. Climatic conditions, geographical regions, damage by insects, and grape varieties influence at different levels its spreading in vineyards. In general, OTA risk is managed by good agricultural practices, pesticides, and fungicides treatments, but the development of new strategies is always encouraged, especially when an extremely favourable condition occurs in the vineyard. Electrolysed oxidising water (EOW) has become an interesting alternative to chemicals in agriculture, mainly during the post-harvest phase. We tested primarily the efficacy of EOW generated by potassium chloride, *in vitro*, as fungicidal on black Aspergilli conidia, and on detached grape berries infected by *A. carbonarius*. Then, during field trials on Primitivo cv vineyard treated with EOW, *A. carbonarius* contamination, and OTA levels were compared with Switch® fungicide treatment (0.8 g/l). Fungicidal activity on conidia was demonstrated after 2 min of treatment by EOW containing >0.4 g/l of active chlorine. EOW (0.6 g/l active chlorine) treatment reduced the rate of *A. carbonarius* infections *in vitro* of about 87–92% on detached berries and, more than half in the field trials, although Switch® showed better performance. A significant reduction in the OTA concentration was observed for the EOW and Switch® treatments *in vitro* (92% and 96%, respectively), while in the field trials, although the average decrease in OTA was recorded in the treated grapes, it was not statistically significant. These results highlighted that EOW could be considered effective, as a substitute for fungicides, to reduce the contamination of *A. carbonarius* and OTA on grapes.

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## **NON-OCRATOXIGENIC BLACK ASPERGILLI AS BIOLOGICAL CONTROL AGENTS?**

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*Aspergillus carbonarius* is the main source of ochratoxin A in grapes and their derivatives. In previous studies, we isolated and characterized three atypical and unique non-OTA producing strains of *A. carbonarius*. In

this study, we applied the RNA-Seq technology to carry out a global transcriptional analysis on four *A. carbonarius* strains, one OTA producer and the three atypical non-OTA-producing strains, and to analyze the differentially expressed genes related to OTA biosynthetic pathway. Besides, *in vitro* interactions between ochratoxigenic strains of *A. carbonarius* and *A. niger* and non-ochratoxigenic strains of *A. carbonarius* and *A. tubingensis* were assessed to evaluate their potential for controlling OTA production. A total of 696 differentially expressed genes (DEGs) were identified comparing the OTA-producing strain vs. the three non-OTA-producing strains. Among these DEGs, 333 genes were down-regulated in the non-OTA-producing strains. All the genes related with OTA biosynthesis (*AcOTApks*, *AcOTAnrps*, *AcOTAp450*, *AcOTAhal* and the transcription factor *AcOTAbZIP*) were the most down-regulated genes in non-ochratoxigenic strains. We also showed that these strains possess a deleterious mutation in the *AcOTApks* gene that can have a deleterious impact on the biological function of the acyltransferase domain of this gene. The effect of the potential biological agent on OTA production was evaluated using different mixed spore suspensions of OTA-producing strain:non-OTA-producing strain ratios. The non-OTA-producing strain of *A. carbonarius* gave the best control, resulting in practically complete inhibition of OTA production in co-inoculation with an ochratoxigenic strain of *A. niger* and high percentages of OTA reduction with an ochratoxigenic strain of *A. carbonarius*. This study opens new possibilities for using non-ochratoxigenic strains of *A. carbonarius* as biocontrol agents in grapes. Thereby, other non-ochratoxigenic black aspergilli isolated from grapes and recently characterized in our laboratory (*A. uvarum*, *A. japonicus*, *A. niger*, *A. welwitschiae*, *A. brasiliensis*, and *A. tubingensis*) are susceptible to be assessed as potential BCAs against *A. carbonarius*.

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## **EFFECT OF OZONE TREATMENT OF DIFFERENT COMMODITIES: RELATIVE CONTROL OF GERMINATION, GROWTH AND MYCOTOXIN PRODUCTION BY *ASPERGILLUS* AND *FUSARIUM* SPECIES**

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There has been a lot of interest in the use of gaseous ozone (O<sub>3</sub>) as a method for controlling the life cycle of mycotoxigenic spoilage fungi. However, the concentrations needed for different species and in different commodities may vary profoundly. We have examined the exposure of *A. flavus* (aflatoxins in peanuts, pistachio nuts, chilli powder/whole chillies), *A. carbonarius* and *A. westerdijkiae* (ochratoxin A in coffee) and *Fusarium verticillioides* (fumonisins in maize). *In vitro* studies examined the effect of O<sub>3</sub> at various concentrations on germination, growth and mycotoxin production on relevant commodity-based media. In addition, naturally contaminated commodities or that inoculated with specific mycotoxigenic species were exposed to O<sub>3</sub> for short periods of time and then stored under different water activity x temperature conditions. Subsequently, the changes in fungal populations and mycotoxin contamination were quantified. Conidia of these species appeared to be quite sensitive to up to 200-300 ppm gaseous O<sub>3</sub>. However, in some cases the spore germination recovered subsequently. Pigmentation may assist these fungi to overcome exposure to this gaseous treatment. Mycelial growth was often relative unaffected by exposure to these concentrations for up to 60 mins. *In situ* studies suggested that while temporary reductions in fungal populations occurred it was difficult to control mycotoxin contamination in some of these commodities. We believe that gaseous O<sub>3</sub> is not a very user-friendly approach to control mycotoxigenic spoilage moulds. It may be necessary to use very low concentrations for an extended time period, to obtain the target efficacy required. For some commodities, especially lipid rich ones, the flavour or palatability may be affected.

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**BIOTIC AND ABIOTIC FACTORS IMPACT THE EFFICACY OF ANTIFUNGAL BIOCIDES USED IN THE DAIRY INDUSTRY**

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Among methods used to control fungal contaminations in the dairy industry, disinfection procedures are crucial tools to avoid contamination by airborne conidia. Antifungal activity is evaluated according to international standards (i.e. EN 1275, 1650, 13697/+A1 and 17272); however, the actual biocide efficacy in food industry conditions is questionable as it can depend on the fungal species, intraspecific variability, physiological state of the fungal species but also how biocides are applied. In this framework, we evaluated the antifungal activity of ethanol, sodium hypochlorite and hydrogen peroxide against the main fungal contaminants (i.e. *Penicillium commune*, *Aspergillus flavus*, *Cladosporium cladosporioides* and *Mucor circinelloides*) using conidia with different physiological states. The obtained results showed that biocidal activity of a given molecule depended on the tested species but also strain. *P. commune* was the most resistant species and ethanol was the most efficient molecule. Conidia produced in conditions simulating airborne spores in food plant conditions (production at 0.95  $a_w$  and dry-harvesting) were more resistant than those produced at 0.99  $a_w$  and harvested using an aqueous solution (norm conditions). We also quantified the impact of concentration and temperature on the efficacy of four commercial sanitizers to inactivate conidia produced at 0.95  $a_w$  and dry-harvested. The obtained results showed that low temperature strongly impacted biocide efficacy. The contact time needed to inactivate 4 log of the fungal population was increased by 3 to 20 fold when temperature decreased from 20 to 8°C. For concentration, product dilution mainly impacted biocides that are supposed to be used in pure form. Overall, the obtained data highlighted that disinfection efficacy is dependent on various biotic and abiotic factors and that this multifactorial aspect should be taken into account in the food industry.

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## SESSION 6: FOOD FERMENTATION

## THE ROLE OF INDIGENOUS MEJU FUNGI FOR FERMENTED SOYBEAN PRODUCTS

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A fungus, *Aspergillus oryzae* is used alone for the production of soybean products such as soy sauce and soy paste, in factories. However, many fungi occur in naturally fermented meju, which is a traditional Korean fermented soybean cake for soy sauce and paste. The role of fungi commonly isolated from traditional meju for production of soybean product (traditional meju fungi) was investigated. Manufacture of soybean fermentation products (Soy sauce and paste) is divided into two stages. First, the fungus grows in soybeans or meju, and produces enzymes necessary for fermentation. This is called 'meju fermentation'. The next step is the 'aging fermentation' in which soybean proteins are decomposed by the enzymes made in meju fermentation. In this experiment, fermenting enzymes after meju fermentation by 20 strains of traditional meju fungi and fermented metabolites after aging fermentation by 20 strains were investigated. Amount of 7 enzymes which is important for soybean fermentation [total protease, Leucine aminopeptidase (LAP), Carboxy peptidase (CaP), Glutaminase, Gamma Glutamyl transferase (GGT), Aspartyl aminopeptidase (DAP), X-Prolyl aminopeptidase (XPAP)] were compared after meju fermentation. Although, *Scopulariopsis* strains produced high amounts of total protease and CaP, and *Penicillium* strains produced high amounts of GGT, *Aspergillus oryzae* strain which is used for commercial soy sauce and paste production, showed excellent enzyme production overall. Fermentation metabolites were analyzed after aging fermentation of 20 traditional meju fungi. When the total free amino acids including aspartic acid and glutamic acid, which give umami taste, were analyzed, *Scopulariopsis* and *Penicillium* strains produced high amounts of total amino acids, but it did not reach the production of the commercial *Aspergillus oryzae* strain. However, in the production of  $\gamma$ -glutamyl peptides, strains of *Penicillium* were superior to commercial *A. oryzae* strain.  $\gamma$ -Glutamyl peptides are known as substances with Kokumi taste (Kipenmat in Korean), which is explained as continuous, mouthful and thick flavor that cannot be explained by five basic tastes alone. In particular, strains of *Penicillium* produced highest contents of  $\gamma$ -glu-val-gly ( $\gamma$ -EVG), the most potent kokumi peptide among  $\gamma$ -Glutamyl peptides. Further research is needed on kokumi peptides produced by traditional meju fungi.

### **PENICILLIUM GENOME PROJECT: THE CASE OF ITALIAN STRAINS FROM FERMENTED FOOD**

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*Penicillium* is one of the largest and ubiquitous fungal genera, currently accounting for more than 500 accepted species occurring worldwide in a diverse range of habitats, from soil to vegetation, air, indoor environments, and various food products [1]. It plays significant and varied roles, such as the important production of antibiotic penicillin, special cheeses by *P. camemberti* and *P. roqueforti*, and fermented sausages by *P. nalgiovense* and *P. salamii* [2]. Other *Penicillia* can have negative effects: allergies in humans, causes of food spoilage, mycotoxin contamination of foods. *Penicillium* species are also screened for the production of novel enzymes and other extrolites for a wide range of applications [3].

In particular, *Penicillium* species could be relevant for food processes, and play an important role in the development of peculiar sensorial characteristics of some traditional fermented foods, participating on metabolic processes through production of many enzymes and metabolites during the ripening period. However, in many cases they are only present spontaneously and without a driven selection of possible strains carrying desirable functional traits to improving/standardize the fermentation processes, thus raising questions about quality and safety of foodstuffs. In this respect, investigating genomic diversity of *Penicillium* species associated with some traditional Italian foods draws attention to bridge the gap among *Penicillium* genome variability and the related enzymatic and functional peculiarities. To this aim and as a pilot for a large “whole genus” *Penicillium* genome sequencing project aimed at discovering new enzymes for biomass degradation and novel biochemistry for synthetic biology applications, a *Penicillium* sequencing project targeting 26 *Penicillium* strains, retrieved from Italian ITEM collection, was launched in a collaboration between the CNR-ISPA fungal genomic research group and the JGI-DOE. The selected strains, included as representative isolate from different Italian food matrices (cheese, salami, bread, olive brines, wheat), belong to the following species: *P. bialowiezense*, *P. bifforme*, *P. bilaiae*, *P. brevicompactum*, *P. caseifulvum*, *P. chrysogenum*, *P. commune*, *P. crustosum*, *P. cvjetkovicii*, *P. discolor*, *P. expansum*, *P. flavigenum*, *P. fructuariae-cellae*, *P. gravinicasei*, *P. jugoslavicum*, *P. nalgiovense*, *P. paneum*, *P. polonicum*, *P. roqueforti*, *P. salamii*, *P. solitum*. The sequencing of the *Penicillium* species and the subsequent comparative and functional analyses will help unravel their peculiar genomic traits and diversity, the insight into the enzymatic activities responsible for fermentation processes, the assessment of the safety aspects for their use and the potential for further biotechnological applications.

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## OPTIMISATION OF KOJI PRODUCTION FROM A SIMULANT SYSTEM TO RICE AND ITS ASSOCIATION WITH FLAVOUR ENHANCEMENT

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*Aspergillus oryzae* is a filamentous fungus that can be used for rice fermentation to produce Asiatic food products like koji. During this process macromolecules such as carbohydrates, fats, sugars and proteins breakdown into smaller metabolites by enzymes, the activity of which can be controlled by applied environmental conditions; such as temperature and water activity. Different expression levels of key enzymatic genes result in different metabolites and their respective quantities, which can be translated into a variety of flavours and aromas. By fermenting rice in different conditions an array of koji with a variety of flavours and aromas is expected to be formed (Lee et al. 2016). This study aimed at identifying based on predictive mycology tools the optimal environmental conditions for the growth of *Aspergillus oryzae* and the expression of key enzymatic genes by performing the fermentation process in different temperatures and water activity. The highest growth rates for *A. oryzae* were found to be at temperatures varying from 32°C to 37°C and at a *aw* of 0.970 in a rice agar model. The highest germination rates for *A. oryzae* are achieved by the same range of temperatures at a *aw* of 0.990 using rice agar plates as a medium. RNA extraction of the koji product and gene expression assessment of flavour related-genes were also optimised, providing a tool that can be used by manufacturers to produce a bespoke product of enhanced flavour.

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**FERMENTATION OF KOREAN TRADITIONAL MEJU USING GRAS FUNGAL STRAINS**

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Korean traditional soybean brick, Meju, has been a good sauce for making many traditional fermented foods since ancient times in Korea. However, since the microbial community in the Meju is naturally inoculated, sometimes it's easy to be contaminated with mycotoxigenic fungal strains. In this study, we tried to devise a method that prevents aflatoxin contamination in commercially manufactured fermented Meju and produces various beneficial effects by mixing several fungi like the traditional fermented Meju. Total of 260 fungal strains were isolated through rRNA ITS sequencing from the Meju in a traditional way and commercial yellow koji and identified some of them. Among the isolates, strain *Aspergillus oryzae* 3222 had high peptidase activity and was confirmed to be suitable as a starter for soybean fermentation. In addition, this strain was confirmed through whole genome analysis that aflatoxin could not be produced due to mutations in the aflatoxin gene cluster. *Mucor racemosus* 324 and *Rhizopus oryzae* 3115 strains were also fungal strains isolated from the traditional fermented Meju, and they did not produce mycotoxins. Since traditional Meju contains variety of microbial species, we mixed the three strains for using them as a starter for Meju fermentation and analyzed the fungal distribution during the fermentation process. We plan to investigate the inoculation ratio for optimal fermentation in Meju mixed inoculated with three types of fungi and confirm the fermentation pattern through metagenome analysis. This research was supported by a grant (21162MFDS028) from Ministry of Food and Drug Safety in 2022.

**ADAPTIVE DIFFERENCES BETWEEN *PENICILLIUM ROQUEFORTI* CHEESE AND "NON-CHEESE" POPULATIONS**

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Some fungal species have been domesticated, especially for fermented food production (e.g., *Aspergillus oryzae* for soy sauce, *Penicillium camemberti* for moldy-rind cheese). Artificial selection along with a shift from natural and complex environments to the food environment is thought to foster specialized adaptation. In *Penicillium roqueforti*, population genomics revealed five differentiated genetic populations. Populations were specific to different environments, including three clusters corresponding to distinct blue cheese types, named "Roquefort", "Termignon" and "non-Roquefort", for all other blue cheeses. The other populations correspond to "lumber/spoiled food" and "silage/spoiled food" populations. The fact that the differentiated populations are associated with different habitats, exhibiting contrasted environmental conditions and compositions, raises questions about their adaptation to these environments.

To evaluate possible adaptive differences to abiotic conditions, we compared growth kinetics of isolates belonging to the five populations under various conditions. High throughput growth monitoring was performed using laser nephelometry to model the impact of the following abiotic conditions individually: pH, temperature, salinity and lactic acid concentration. Growth rates were measured for each condition and secondary modeling was used to determine cardinal values. Growth rates were also compared between populations either in minimal medium supplemented with different carbon sources or in rich

medium supplemented with fungal growth inhibitors (natamycin used cheese or tebuconazol used in plant culture). Overall, cardinal values differentiated the “non-Roquefort” population from all the other populations, with higher salt and lactic acid tolerances, and a lower optimal growth temperature. These differences are in agreement with adaptation of the “non-Roquefort” blue cheese population to the typical cheese environment. Our study also showed differences between cheese and “non-cheese” populations in their ability to grow in the presence of various carbon sources.

Mycotoxin production profiles were also determined using a newly developed miniaturized extraction method and LC-QTOF. We looked in genomes for specific features associated with the different observed production profiles for the different populations. Isolates from environmental populations tended to produce the highest levels of the target extrolites in comparison to cheese isolates. Our preliminary results also confirmed that no mycophenolic acid was produced by isolates belonging to the “non-Roquefort” population which is linked to a deletion in the *mpaC* gene in the biosynthesis pathway.

Overall, our study sheds new light on how *P. roqueforti* populations adapted to their respective environments but also how domestication impacted their physiological and metabolic traits.

## SESSION 7: PHYSIOLOGY OF FOOD SPOILAGE FUNGI

### THE FUNGAL SPORE; TALES OF BEGINNINGS

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Many stories of spoilage and post-harvest infection start with a fungal spore. However, when we take an average air sample, if this is possible, not all available spoilage fungi are found. The story of spoilage outbreaks is of interest here. Do fungi, and fungal spores, occur mostly in relation or close to the product or crop they spoil? Most spores deposit closely to the place where they are produced. Alternatively, there is indirect evidence that they can travel for thousands of kilometres. Conidia, ascospores and other spores are highly variable, also between strains.

Preventing spores to be in the air is helping, high spore concentrations are correlated with increased spoilage. Even when spores make it on the product there might be ways to eradicate their germination. This I would like to discuss two aspects, the variability of spore resistance between species. Secondly, the breaking of dormancy and formation of the germ tube as possible targets.

### INTRASPECIFIC VARIABILITY IN CONIDIAL HEAT RESISTANCE OF FOOD SPOILAGE FUNGI

Tom van den Brule<sup>1,2\*</sup>, Maarten Punt<sup>1,3</sup>, Sjoerd Seekles<sup>1,4</sup>, Frank Segers<sup>1</sup>, Jos Houbraken<sup>1,2</sup>, Wilma Hazeleger<sup>5</sup>, Marcel Zwietering<sup>1,5</sup>, Heidy den Besten<sup>1,5</sup>, Han Wösten<sup>1,3</sup>, Jan Dijksterhuis<sup>1,2</sup>

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Fungal food spoilage often begins with contamination by spores. To prevent spoilage of processed foods and drinks, industry often challenges their products with the worst-case spoilage scenario. As microorganisms are inherently variable, interspecies and intraspecies variability of food spoilage fungi should be considered when defining the worst case presented by fungal spores. Among other types of spores, filamentous fungi in the order Eurotiales produce airborne conidia which are ubiquitous in

the environment. These asexual spores vary in stress resistance among species, but also within species. This presentation focusses on quantification of variability in conidial heat resistance of the food spoilage fungi *Aspergillus niger*, *Penicillium roqueforti* and *Paecilomyces variotii*. Comparison with three spore-forming bacteria and two non-spore-forming bacteria revealed that the intraspecific variability of the different species was in the same order of magnitude, which hints to a microbial signature of variation that exceeds kingdom boundaries. In addition, phenotypic characterization of *P. variotii* conidia revealed that internal trehalose concentration and conidia size correlated significantly with heat resistance. This work emphasizes the importance of quantifying variability in predictive food microbiology to realistically predict heat inactivation of fungal spores.

## THE INTERACTIONS AMONG ISOLATES OF *LACTIPLANTIBACILLUS PLANTARUM* AND DAIRY YEAST CONTAMINANTS: TOWARDS BIOCONTROL APPLICATIONS

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Yeast diversity in the cheese manufacturing process and in the cheeses, themselves includes indispensable species for the production of specific cheeses and undesired species that cause cheese defects and spoilage. The control of yeast contaminants is problematic due to limitations in sanitation methods and chemicals used in the food industry. The utilisation of lactic acid bacteria and their antifungal products is intensively studied. *Lactiplantibacillus plantarum* is one of the most frequently studied species producing a wide spectrum of bioactive by-products. In the present study, twenty strains of *L. plantarum* from four sources were tested against 25 species of yeast isolated from cheeses, brines, and dairy environments. The functional traits of *L. plantarum* strains, such as the presence of class 2a bacteriocin and chitinase genes and *in vitro* production of organic acids, were evaluated. The extracellular production of bioactive peptides and proteins was tested using proteomic methods. Antifungal activity against yeast was screened using *in vitro* tests. Testing of antifungal activity on artificial media and reconstituted milk showed significant variability within the strains of *L. plantarum* and its group of origin. Strains from sourdoughs (CCDM 3018, K19-3), raw cheese (L12, L24, L32) strongly inhibited the highest number of yeast strains on medium with reconstituted milk. These strains showed a consistent spectrum of genes belonging to class 2a bacteriocins, the gene of chitinase and its extracellular product 9 LACO Chitin-binding protein. Strain CCDM 3018 with the spectrum of class 2a bacteriocin gene, chitinase and significant production of lactic acid in all media performed significant antifungal effect in artificial and reconstituted milk-based media.

## NATURAL VARIATION AND THE ROLE OF ZN<sub>2</sub>CYS<sub>6</sub> TRANSCRIPTION FACTORS SDRA, WARA AND WARB IN SORBIC ACID RESISTANCE OF *ASPERGILLUS NIGER*

Sjoerd J. Seekles<sup>1,2\*</sup>, Jisca van Dam<sup>2</sup>, Mark Arentshorst<sup>2</sup> and Arthur F.J. Ram<sup>1,2</sup>

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Weak acids, such as sorbic acid, are used as chemical food preservatives by the industry. Fungi overcome this weak-acid stress by inducing cellular responses mediated by transcription factors. In our research, a large-scale sorbic acid resistance screening was performed on 100 *A. niger sensu stricto* strains isolated from various sources to study strain variability in sorbic acid resistance. The minimal inhibitory concentration of undissociated (MIC<sub>u</sub>) sorbic acid at pH = 4 in the MEB of the *A. niger* strains varies between 4.0 mM and 7.0 mM, with the average out of 100 strains being 4.8 ± 0.8 mM, when scored after 28 days. MIC<sub>u</sub> values were roughly 1 mM lower when tested in commercial ice tea. Genome sequencing of the most sorbic-acid-sensitive strain among the isolates revealed a premature stop codon inside the sorbic acid response regulator encoding gene *sdrA*. Repairing this missense mutation increased the sorbic acid resistance, showing that the



sorbic-acid-sensitive phenotype of this strain is caused by the loss of SdrA function. To identify additional transcription factors involved in weak-acid resistance, a transcription factor knock-out library consisting of 240 *A. niger* deletion strains was screened. The screen identified a novel transcription factor, WarB, which contributes to the resistance against a broad range of weak acids, including sorbic acid. The roles of SdrA, WarA and WarB in weak-acid resistance, including sorbic acid, were compared by creating single, double and the triple knock-out strains. All three transcription factors were found to have an additive effect on the sorbic acid stress response.

## HIGH SORBIC ACID RESISTANCE OF *PENICILLIUM ROQUEFORTI* IS MEDIATED BY THE SORBUS GENE CLUSTER

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*Penicillium roqueforti* is a major food-spoilage fungus known for its high resistance to the food preservative sorbic acid. Here, we demonstrate that the minimum inhibitory concentration of undissociated sorbic acid (MIC<sub>u</sub>) ranges between 4.2 and 21.2 mM when 34 *P. roqueforti* strains were grown on malt extract broth. A genome-wide association study revealed that the six most resistant strains contained the 180 kbp gene cluster SORBUS, which was absent in the other 28 strains. In addition, a SNP analysis revealed five genes outside the SORBUS cluster that may be linked to sorbic acid resistance. A partial SORBUS knock-out (>100 of 180 kbp) in a resistant strain reduced sorbic acid resistance to similar levels as observed in the sensitive strains. Whole genome transcriptome analysis revealed a small set of genes present in both resistant and sensitive *P. roqueforti* strains that were differentially expressed in the presence of the weak acid. These genes could explain why *P. roqueforti* is more resistant to sorbic acid when compared to other fungi, even in the absence of the SORBUS cluster. Together, the MIC<sub>u</sub> of 21.2 mM makes *P. roqueforti* among the most sorbic acid-resistant fungi, if not the most resistant fungus, which is mediated by the SORBUS gene cluster.

## PRESERVATIVE RESISTANCE OF SPOILAGE YEASTS AT LOW GLUCOSE AND RELEVANCE FOR REDUCED-SUGAR FORMULATIONS

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Several governments have been introducing taxes on soft drinks that contain high sugar, e.g., >5% (w/v) sugar. The impacts of these reduced-sugar contents for preservation from microbial spoilage are, as yet, barely characterized. Sorbic acid (2,4-hexadienoic acid), which is a commonly used preservative permitted in soft drinks at up to 300ppm (2.7mM), prevents growth of most yeast species. Recently, sorbic acid has been shown to target respiratory metabolism which, for many yeasts, is the dominant glucose catabolic process at low glucose concentrations. Fermentation is usually more important at high glucose. This suggests that sorbic acid could be more effective in low glucose conditions. This hypothesis was challenged in this study, through tests with different spoilage yeast species in low versus high glucose conditions. We have further characterised resistance effects at low glucose using other respiration-targeting weak acids and present evidence that a metabolic shift at low-glucose in the presence of these acids may facilitate spoilage-yeast resistance and growth.

**INOCULUM SIZE MATTERS: RELATIONSHIP BETWEEN PRESERVATIVE MIC AND HETERORESISTANCE IN SPOILAGE YEASTS**

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A wide range of condiments and soft drinks are preserved with weak organic acids such as sorbic acid, however specialised yeasts such as *Zygosaccharomyces bailii* can grow in sorbic acid concentrations above the legal limit. While many mechanisms of resistance have been proposed, including metabolism of sorbic acid and maintenance of a more fermentative metabolism, *Z. bailii* has additionally been shown to exhibit high variation in cell-cell resistance to sorbic acid, suggesting a possible bet hedging strategy to produce hyper-resistant sub populations (heteroresistance). While examples of heteroresistance in contexts such as antibiotic resistance are numerous, the relative contributions of heteroresistance and population-average resistance ( $IC_{50}$ ) to the observed Minimum Inhibitory Concentration (MIC) in food preservation has not been assessed. Here we use a novel heteroresistance assay across a panel of *Z. bailii* and its interspecies hybrids *Z. parabailii* and *Z. pseudobailii*, to assess the degree to which heteroresistance and  $IC_{50}$  contribute towards MIC. This approach revealed considerable variation in these parameters, both between individual isolates and subspecies. Our results suggest differences in  $IC_{50}$  are quite closely related to those in MIC, with heteroresistance also being correlated with MIC in particular contexts, e.g., subspecies where  $IC_{50}$  variation is low.

## ABSTRACT POSTERS

### 01. DIVERSITY OF NON-ochratoxigenic STRAINS OF *ASPERGILLUS* SECTION *NIGRI* FROM SPANISH GRAPES

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Ochratoxin A (OTA) is produced during the infection of grapes in vineyards by toxigenic strains of species belonging to *Aspergillus* section *Nigri*. While *A. carbonarius* is the main responsible source of OTA in wine, grapes, and raisings from main viticultural regions worldwide, OTA production consistency varies in other species from this section. Many studies have shown that biological control using microbial antagonists has emerged as a promising approach for control of mycotoxins in both pre- and post-harvested crops. As an example, one of the more promising strategies for control of aflatoxins in crops involves the use of atoxic strains of *A. flavus* as biological control agents (BCAs). Nowadays, prevention of the growth of OTA-producing fungi is the most effective strategy for controlling the entry of OTA in grapes. Despite the use of preventive strategies or chemical controls, the use of BCAs remains the most eco-friendly approach. An effective strategy for reducing OTA in grape could be the application of non ochratoxigenic strains of *Aspergillus* section *Nigri* in the vineyards to outcompete naturally toxigenic *A. carbonarius* strains in the field. In this study, a total of 95 strains of *Aspergillus* section *Nigri* isolated from wine and liquor grapes were studied. OTA production was analyzed in all strains using a previously described HPLC screening method designed in our laboratory. Identification of strains was made using macroscopic and microscopic morphological criteria and all strains were molecularly identified by sequencing of the calmodulin gene. Twenty-three strains were morphologically identified as belonging to the uniseriate species and 72 as *A. niger* aggregate. Based on the calmodulin sequences, among the uniseriate group, *A. japonicus*, and *A. uvarum* were found. Within the *A. niger* aggregate, the following species were found: *A. niger*, *A. welwitschiae*, *A. brasiliensis*, and *A. tubingensis*. None of the strains were able to produce OTA, so these strains of *Aspergillus* section *Nigri* are susceptible to be assessed as potential BCAs against *A. carbonarius*.

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### 02. RAPID FLOW CYTOMETRY METHODOLOGY FOR AIRBORNE FUNGAL CONIDIA ENUMERATION

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In food manufacturing facility, fungal conidia can contaminate food products during accidental exposition to contaminated air flows and lead to significant food losses. Current techniques for airborne fungi enumeration mainly rely on cultural approach with a time-to-response closely dependent on the growth kinetics of the collected conidia. The objective of the present work was to develop a non-cultural methodology to enumerate airborne conidia. Linearity, quantification limit, inclusivity and robustness of the method were evaluated using up to 21 fungal isolates from *Cladosporium*, *Penicillium*, *Aspergillus* and other fungal genera. The optimized methodology was based on vortex air sampling, conidia staining and flow-cytometry enumeration with a time-to-result shorter than 1 hour. Calcofluor White (CFW) was selected to stain the chitin-based cell wall of conidia. The staining protocol was optimized regarding the stain/conidia ratio and settle time before analysis. The result can be obtained within less than 1 hour if the fungal contamination is higher or equivalent to  $2 \cdot 10^2$  conidia/m<sup>3</sup>. Indeed, the mean quantification limit was  $3.9 \cdot 10^2$  conidia/mL which can be achieved with a 40 min air sampling (50L/min) at this contam-

ination level. However, higher contamination levels can be analyzed with shorter sampling time, e.g. 20 min for  $4 \cdot 10^2$  conidia/m<sup>3</sup> or 8 min at  $1 \cdot 10^3$  conidia/m<sup>3</sup>. The robustness of the method is satisfying regarding the potential presence of insect debris containing chitin. Interestingly neither the age of conidia nor the duration of dry storage impacted the performance of the rapid method whereas 12 weeks dry conidia had a longer latency for growth on potato dextrose agar than conidia stored only 3 weeks. Overall, the method is an interesting alternative or complement to culture-based methods to enumerate airborne conidia when the results need to be obtained rapidly or in situations where the relevant culture medium is unknown.

### 03. DIVERSITY OF *PENICILLIUM* AND *ASPERGILLUS* SPECIES ISOLATED FROM TRADITIONAL MEAT PRODUCTS OF DIFFERENT REGIONS IN CROATIA

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Mycoflora's growth on the surface of traditional meat products (TMP) gives them a specific texture, flavour and colour. The composition of mycoflora depends on climatic conditions, mostly on temperature and humidity. The climate in Croatia is very heterogeneous. The characteristics of Croatia's climate is a cold and moist winter and a hot summer in the continental part of Croatia; a moist and mild winter, and very hot and dry summer in the coast. The aim of this study was to obtain an insight of the moulds species diversity of mycoflora isolated from 130 samples of TMP from the continental (n=68) and coastal (n=62) part of Croatia. Isolated mould species were identified using a combination of classical and molecular methods by beta-tubulin and calmodulin loci sequencing. In the continental region 158 isolates were obtained, of which 65 % *Penicillium* species, and 35% *Aspergillus* species, while in the coastal region 160 isolates were obtained, of which 53% *Penicillium* and 47% *Aspergillus* species. In total, 12 different *Penicillium* and 15 *Aspergillus* species, mostly teleomorphs, were determined. Among the determinate species it has to be emphasized that the potential mycotoxigenic species, *A. flavus* (6%) as aflatoxin and cyclopiazonic acid producer, *P. commune* (47%) as cyclopiazonic acid producer; *P. citrinum* (22%) as citrinin producer, and *P. nordicum* (9%) as ochratoxin producer, were isolated. It can be concluded that *Penicillium* species, as the species which prefers the more continental climate, were isolated more often in the continental region than the *Aspergillus* species, which prefers the hotter and dry coastal climate, and vice versa. Furthermore, the isolated micotoxigenic species could represent a potential issue for public health.

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### 04. MICROMYCETES RESPONSIBLE FOR THE DECAY OF STRAWBERRIES AND BLUEBERRIES IN SLOVAK SUPERMARKETS

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Strawberries and raspberries belong to the "soft fruit" group. They have a high content of antioxidants and are effective in preventing the occurrence of chronic and degenerative diseases associated with oxidative damage, such as e.g. cancer and cardiovascular diseases. Fresh fruits are prone to fungal contamination in the field, during harvesting, transport and during the sale. It is important to know, which fungal

contaminants are responsible for decay of these products, because some micromycetes can grow and produce mycotoxins on these commodities. The yeasts and moulds can cause infections and allergies, as well. The fruit contains a lot of sugar and other nutrients and has an ideal water activity for microbial growth. In this study was monitored the occurrence of micromycetes in 25 samples of strawberries and in 17 samples of blueberries obtained from Slovak supermarkets. All of the samples were contaminated by micromycetes. Fungal mycelium obtained from strawberries and blueberries was inoculated on MEA (Malt extract agar) and cultivated ( $25\pm 1$  °C, 7 d). A total of 90 isolates of micromycetes belonging to 9 genera (*Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Mucor*, *Penicillium* and *Rhizopus*) were isolated. Samples of strawberries (25) contained representatives of the genera *Botrytis* (isolation frequency 80.0%), *Penicillium* (44.0%: *P. atramentosum*, *P. aurantiogriseum*, *P. brevicompactum*, *P. expansum*, *P. fellutanum*, *P. hordei* and *P. olsonii*), *Rhizopus* (28.0%), *Cladosporium* (28.0%), *Mucor* (4.0%) and *Fusarium* (4.0%). Samples of blueberries contained representatives of the genera *Botrytis* (88.2%), *Cladosporium* (35.3%), *Penicillium* (35.3%: *P. pulvis*, *P. expansum* and *P. crustosum*), *Alternaria* (29.4%), *Aspergillus* (11.8%), *Fusarium* (5.9%) and *Epicoccum* (5.9%). Nine isolates of potentially productive strains of the genus *Penicillium* associated with fruit degradation were tested for their ability to produce selected mycotoxins by using the thin layer chromatography. All tested isolates of *P. expansum* (7) produced patulin, citrinin and roquefortine C. Tested isolate of *P. crustosum* produced roquefortine C and penitrem A, and tested isolate of *P. hordei* did not produce roquefortine C.

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## 05. MICROSCOPIC FUNGI CAUSING CHERRY TOMATOE ROT IN STORES

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The aim of the research was to identify microscopic fungi causing cherry tomatoe rot during sale. In case of potentially toxicogenic identified species, analysis of their ability to produce selected mycotoxins *in vitro* were done. Samples of rotting cherry tomatoes were taken directly from stores. The fungi growing on the samples were inoculated on the malt extract agar and cultivated at  $25\pm 1$  °C for 7 days. Of the 26 rotten samples of cherry tomatoes, the following genera were identified as the causative agents of their degradation: *Penicillium* (80.8% of the samples), *Alternaria* and *Botrytis* (26.9%), *Dipodascus* (23.1%), *Cladosporium* (11.5%), *Rhizopus* (11.5%), *Mucor* and *Aspergillus* (7.7%). A total of 8 samples were degraded by only one genus. In the other samples, more genera (from 2 to 4) were identified. *Penicillium olsonii* was the most common factor in the degradation of cherry tomatoes. This species was identified as the causative agent of 15 samples (57.7%). The ability to produce selected mycotoxins *in vitro* was determined as follows: *P. expansum* (1 tested strain) produced patulin and citrinin; *P. griseofulvum* (5 strains tested) produced patulin (4 strains), griseofulvin (5 strains), and cyclopiazonic acid (2 strains); *P. camemberti* (1 tested strain) produced cyclopiazonic acid; *Aspergillus* section *Flavi* (1 tested strain) produced aflatoxin B1, aflatoxin G1 and cyclopiazonic acid. Tested strain of *Aspergillus ochraceus* did not produce ochratoxin A.

Acknowledgements: This research was carried out with the financial support of the project VEGA/0517/21.

## 06. *ASPERGILLUS MONTEVIDENSIS*: HOW MUCH DOES ITS HEAT-RESISTANCE INCREASE IN LOW-AW FOODS?

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Spoilage of pasteurized vegetable low-aw foods can occur due to an accidental re-contamination by osmophilic yeasts or xerophilic moulds, but also due to the presence of fungal ascospores in the raw materials used. With the present study, we assessed the heat-resistance of an ascospore-forming fungus, *Aspergillus montevidensis* SSICA 28219, isolated from a spreadable honey-based cream (0.71 aw), in both a high-aw and a low-aw medium. For the inactivation tests, the spore suspension obtained was diluted 1:10 with a glucose solution (12.5°Bx, pH 3.60) or with a spreadable honey-based cream (82.3°Bx, pH 5.80). Polythene bags containing about 5.0 mL of the diluted suspension were sealed and plunged into a water bath at temperatures ranging from 70 to 85°C up to 180 minutes. Ascospores suspended in a glucose solution showed significantly lower  $D_T$  values (28.8, 3.7, and 0.4 minutes, respectively at 70, 75, and 80°C) than those calculated for ascospores suspended in the honey-based cream (30.9, 6.7, and 1.0 minutes, respectively at 75, 80, and 85°C). The z-values calculated from the decimal reduction time curves did not prove influenced by the heating medium, being equal to 5.4°C in the glucose solution and to 6.7°C in the honey-based cream. Based on these results, *A. montevidensis* heat-resistance could allow the surviving of their ascospores in pasteurized foods where high sugar concentrations exert a protective effect.

## 07. FUNGAL AGENTS OF TABLE GRAPE SPOILAGE IN THE RETAIL NETWORK AND THEIR TOXINOGENIC POTENTIAL

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The aim of the present study was to perform mycological analyzes of grape samples with visible microbial damage and to test potential toxinogenic isolates for the ability to produce selected mycotoxins using thin-layer chromatography. A total of 31 samples from various retail chains in Slovakia were analysed, grape spoilage fungi were inoculated on the malt extract agar and cultivated at 25±1°C for 7 days. The following fungal genera were identified as the spoilage agents: *Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, *Dipodascus*, *Fusarium*, *Isatchenka*, *Penicillium*, *Rhizopus* and *Stemphylium*. *Penicillium* spp. (61.3%) and *Botrytis cinerea* (54.8%) were recorded with the highest isolation frequency (Fr). Detected *Penicillium* strains were classified into 10 species (*P. bialowiezense*, *P. brevicompactum*, *P. citrinum*, *P. corylophilum*, *P. chrysogenum*, *P. expansum*, *P. glabrum* clade, *P. italicum*, *P. olsonii*, *P. palitans*). *P. expansum* strain demonstrated the ability to produce citrinin, patulin and roquefortine C, the *P. chrysogenum* strain was characterized by a significant production of roquefortine C and the *P. palitans* strains were tested for cyclopiazonic acid production, but the ability was not confirmed. Within the genus *Alternaria* (Fr 25.8%), representatives of 3 species-groups were identified (*A. alternata*, *A. arborescens* and *A. tenuissima*). Isolates of this genus were tested for the ability to produce altenuene, alternariol and alternariol monomethylether and 29% of the isolates produced all three toxins and 71% of strains produced at least one of them. Genus *Aspergillus* was detected with Fr 17.4% and strains were divided into *Nigri* (85.7%) and *Flavi* (14.3%) sections. Isolated strains were tested for the ability to synthesize cyclopiazonic acid, ochratoxin A and aflatoxins B<sub>1</sub>, G<sub>1</sub> and G<sub>2</sub>. Of these metabolites, only the production of cyclopiazonic acid by the strain from the *Flavi* section was recorded.

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**08. BIOFILM FORMATION BY FUNGI RELEVANT IN MATURED MEAT PRODUCTION**

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The establishment of fungal biofilms in difficult-to-access areas and surfaces of matured meat production facilities could influence their mycobiota. Biofilms from spoilage and/or toxigenic species may lead to the persistence of these undesirable moulds in the industrial environment, while the establishment of beneficial species, including those generally used as starter cultures, may provide sensory advantages and some protection against the undesirable ones. This study evaluated the capability of biofilm formation by *Aspergillus westerdijkiae*, *Aspergillus ochraceus* and *Penicillium nalgiovense*, species relevant in the matured meat industry. Eight different strains were cultivated in 96 Well Microplate containing Czapek Yeast Extract Broth (CYB), Malt Extract (MEB) and Sabouraud Broth (SAB) and were incubated at 10°C and 25°C for 24, 48 and 72 h. The presence of extracellular matrix, indicating biofilm presence, was checked after three successive washes with Phosphate Saline Buffer (PBS) and staining with a 0.5% safranin solution. Both media and temperature influenced the biofilm production by the tested species. Strains of *A. westerdijkiae* showed to be strong producers of biofilm in all the tested conditions, *A. ochraceus* produced variable amounts of extracellular matrix according the media but only at 25°C, and *P. nalgiovense* strains were not capable of produce biofilm in any of the evaluated conditions.

**09. DYNAMIC CHANGES IN CHAMPAGNE MYCOBIOTA DIVERSITY FROM VINE TO WINE**

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The Champagne wine-growing region is located in Northern France and benefits from both oceanic and continental climatic influences. The grapes in these vineyards grow in both wet and extreme (-5°C at +40°C) temperature conditions. The main grape varieties are Pinot Noir, Pinot Meunier and Chardonnay. This varietal diversity, together with the diversified terroirs encountered, contributes to the typicity of Champagne wines. Like for other vineyards in France, wine quality is highly linked to the characteristics of the raw materials. The influence of pedoclimatic conditions is already well documented. However, the Champagne grape microbiota, especially its fungal component, has yet to be fully characterized and described.

In this context, the first objective of this study was to decipher fungal diversity and dynamics at five key stages from the vine to wine, namely, fruit set, veraison, harvest, must and still wine. To do so, we sampled grape berries from 33 Champagne vineyards, located in different geographical areas. At harvest, grapes were crushed to obtain must samples, then micro-vinifications were performed to obtain still wines. For each sample, culture-dependent and -independent (metagenetics) approaches were applied.

Fifteen fungal isolates were selected per sample and purified to create a vast working collection (n=1248 fungal isolates) before isolate dereplication using Maldi-Tof for molds and FTIR for yeasts. Species level identifications are currently being performed for representative isolates. Overall, fungal counts increased during berry ripening by ~1-2 log, reaching on average about 5 log TFU/g at the harvest stage concomitant to a decrease in acidity and increase in total sugars, as reported in the literature. Grape berries from fruit set to veraison were mainly dominated by *Cladosporium* and *Aureobasidium* spp. while *Penicillium* spp. populations increased during harvest stage. After grape crushing, fungal counts decreased by up to ~4 log TFU/ml in musts and micro-vinified wines. *Penicillium* spp. were the most dominant molds in musts before counts decreased during fermentation, once starter yeasts were added. For metagenetic analyses, we are using Illumina sequencing technology to target both bacterial and fungal taxa (V3-V4 region of the 16S RNA gene and ITS2 region, respectively). Global data analyses will then be carried out by

combining the obtained results with all available data related to climatic conditions, and vine and grape growing practices to determine which factors might impact the microbial ecosystem. In parallel, microbial co-occurrence networks and patterns will be explored using metagenetics data. This should provide deeper understanding about species interactions within the different vineyards and potentially linked to specific traits of interest in wines. Overall, this study will provide novel data on the diversity and specificities of Champagne vineyards microbiota.

## 10. CLIMATE CHANGE AND ACCLIMATIZATION OF *A. FLAVUS* STRAINS INFLUENCES COLONISATION, BIOSYNTHETIC GENE EXPRESSION AND AFLATOXIN B<sub>1</sub> PRODUCTION BY *ASPERGILLUS FLAVUS* IN VITRO AND IN RAW PISTACHIO NUTS

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Pistachio nuts are an important economic tree nut crop which is used directly or processed for many food-related activities. They are colonized by mycotoxigenic spoilage fungi, especially *Aspergillus flavus*, resulting in contamination with aflatoxins (AFs), especially aflatoxin B<sub>1</sub> (AFB<sub>1</sub>). The prevailing climate in which these crops are grown changes as temperature and atmospheric CO<sub>2</sub> levels increase, and episodes of extreme wet/dry cycles occur due to human industrial activity. In addition, such fungal pathogens may evolve resilience when acclimatized for several generation in elevated CO<sub>2</sub>. The objectives of this study were to evaluate the effect of interacting Climate Change-related abiotic factors of temperature (35 vs. 37°C), CO<sub>2</sub> (400 vs. 1000 ppm) and water stress (0.98–0.93 water activity, a<sub>w</sub>) on (a) colonization, (b) *aflD* and *aflR* biosynthetic gene expression, (c) AFB<sub>1</sub> production by strains *A. flavus* (AB3, AB10) and (d) acclimatization for 5 generations in elevated CO<sub>2</sub> on colonization of raw pistachio nuts and AFB<sub>1</sub> contamination. The *A. flavus* strains were very resilient in terms of colonization of pistachio nuts with no significant difference when exposed to the interacting three-way climate-related abiotic factors. The relative expression of the structural *aflD* gene involved in AFB<sub>1</sub> biosynthesis was decreased or only slightly increased, relative to the control conditions at elevated CO<sub>2</sub>, regardless of the a<sub>w</sub> level examined. For the regulatory *aflR* gene expression, there was a significant (p < 0.05) increase in 1000 ppm CO<sub>2</sub> and 37°C for both strains, especially at 0.95 a<sub>w</sub>. There was a significant (p < 0.05) stimulation of AFB<sub>1</sub> production at 35°C and 1000 ppm CO<sub>2</sub> for both strains, especially at 0.98 a<sub>w</sub>. At 37 °C, AFB<sub>1</sub> production was either decreased, or remained similar depending on the strain when exposed to 1000 ppm CO<sub>2</sub>. Acclimatized strains of *A. flavus* (5 generations) showed changes in colonization patterns and some stimulation in AFB<sub>1</sub> production in pistachio nuts. This suggests that *A. flavus* strains are very resilient to climate change factors, with differential effects on AFB<sub>1</sub> production that may be strain dependent. This will impact on the relative toxin risks during processing of this tree nut under future climate-related abiotic factors and the development of appropriate control strategies.

## 11. THE EFFECT OF CULTIVATION TEMPERATURE ON THE HEAT RESISTANCE OF *ASPERGILLUS NIGER* CONIDIA

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Preventing food spoilage is a major challenge in the food industry. Preservation techniques such as heat inactivation are widely used, albeit with the cost of consequently altering food profiles. In order to know at which temperatures food companies should treat their products to inactivate spores (conidia) from food spoilage fungus *Aspergillus niger*, additional knowledge is required on the intrinsic properties that contribute



to heat resistance of these conidia. Our research is focused on the effect of cultivation temperature during conidiation and the resulting heat resistance of conidia from *Aspergillus niger*. We show that different cultivation temperatures have a major impact on the heat resistance of the resulting *Aspergillus niger* conidia. Cultivation of *Aspergillus niger* at 37 °C instead of 28 °C results in conidia with increased heat resistance. Furthermore, this heat resistance increase correlates with an increase in trehalose concentration in the conidia. To determine the role of trehalose accumulation in spores at higher temperature, a trehalose null knock-out mutant was made using CRISPR/Cas9. The trehalose null knock-out mutant produces conidia that lack any trehalose and were indeed more sensitive to heat. However, when cultivating this trehalose null knock-out mutant at 37 °C the conidia were still increased in heat resistance when compared to the conidia of the trehalose mutant grown at 28 °C. This suggests that perhaps other factors such as heat shock proteins play a role in the heat resistance of *Aspergillus niger* conidia.

## **12. A NOVEL DESIGN OF $\alpha$ -AMYLASE WITH AN N-TERMINAL CBM20 IN *ASPERGILLUS NIGER* IMPROVES BINDING AND PROCESSING OF RAW STARCH**

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In the starch processing industry including food and medicine industry,  $\alpha$ -amylase is one of the most important enzymes that are utilized to hydrolyze the  $\alpha$ -1,4 glycosidic bond in starch producing shorter maltooligosaccharides. Structurally, starch molecules are organized in the granules that are very compact and rigid. The level of starch granule rigidity affects the resistance towards enzymatic hydrolysis, resulting in inefficient starch degradation by industrially available alpha-amylase. In an attempt to enhance the starch hydrolysis, the domain architecture of a Glycoside hydrolase (GH) family 13  $\alpha$ -amylase from *Aspergillus niger* was engineered. In all fungal GH13  $\alpha$ -amylases that carry a carbohydrate binding domain (CBM), these modules are of the CBM20 family and always located at the C-terminus of  $\alpha$ -amylase. Therefore, in this research, a new GH13 gene encoding a N-terminal CBM20 domain was designed and found to be fully functional. The starch binding capacity of the N-terminal CBM20 version of  $\alpha$ -Amylase was found to be better than that of the C-terminal CBM20 version and much better than  $\alpha$ -Amylase without a CBM20. The purified N-terminal CBM20\_GH13 displayed higher specific activity toward various starch substrates including starch from rice, corn, wheat, and raw potatoes compared to the GH13\_CBM20. Furthermore, the GH13CBM20 has the lowest activity especially toward raw potato starch. The enzyme activity on starchy substrates from CBM20\_GH13 was at least 15% higher than that of GH13\_CBM20 based on the Km values. This observation suggests that the new design of CBM20\_GH13 alpha amylase has great potential for the raw starch degradation.





