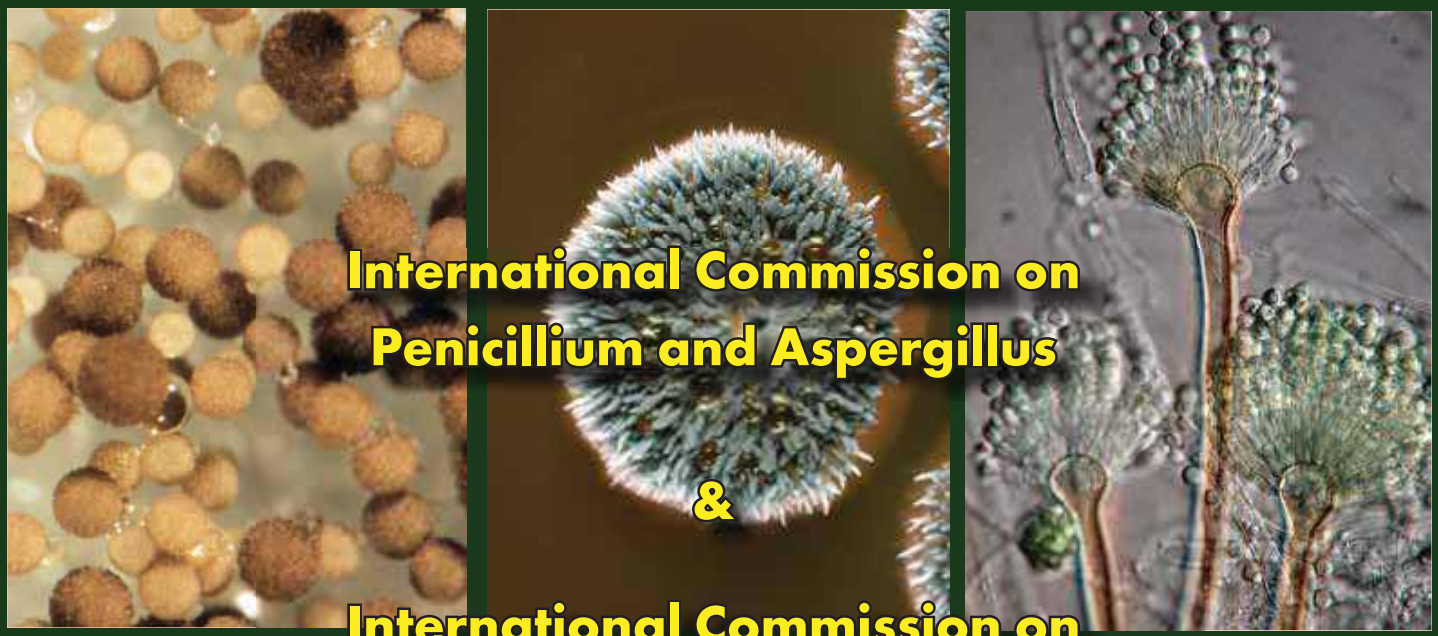


International Commission on Penicillium and Aspergillus

&

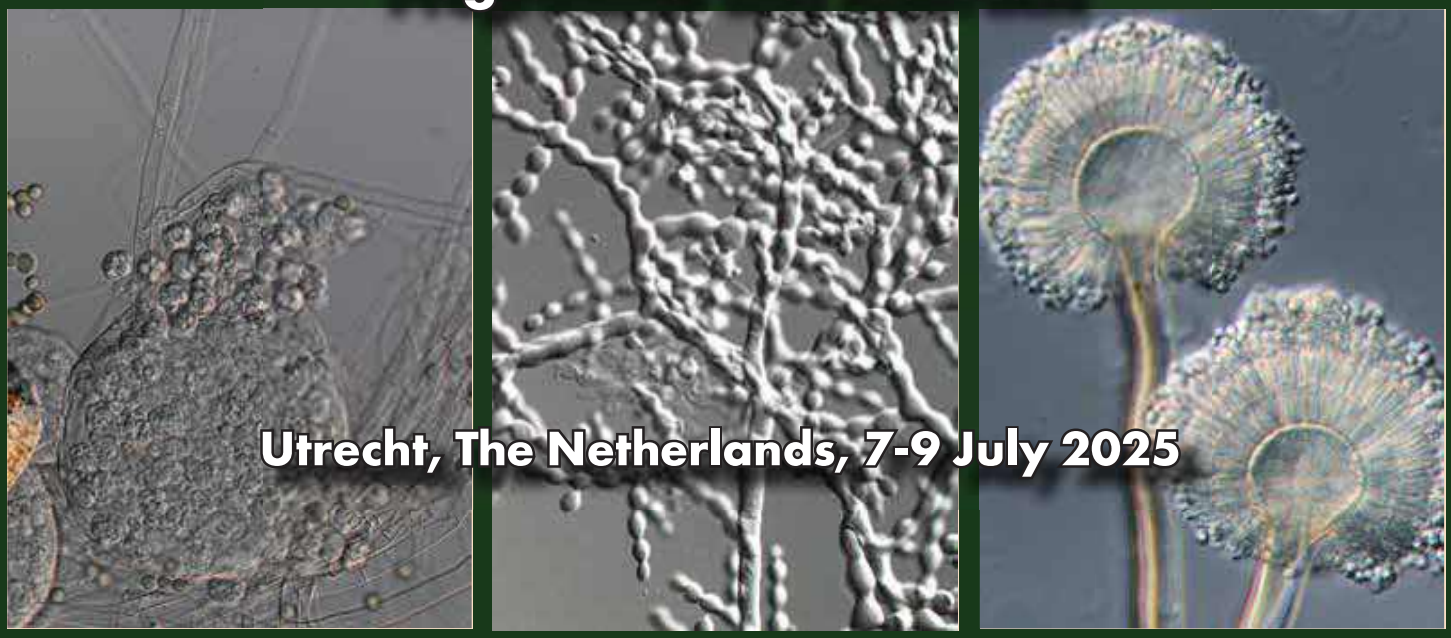
International Commission on Food Mycology

workshop 2025

Future challenges in Food Mycology –

food spoilage, safety and security

Programme and Abstracts



Utrecht, The Netherlands, 7-9 July 2025

INTERNATIONAL COMMISSION ON FOOD MYCOLOGY

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 (1990), in Copenhagen (1994) near Uppsala (1998), Samsøe (2003), Key West (2007), Freising (2010, 2013, 2016 and 2019) and in Utrecht (2022).

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

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

Sponsors

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British Mycological Society promoting fungal science

PROGRAMME ICPA/ICFM 2025

Sunday 6 July 2025

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

Monday 7 July 2025
Westerdijk Fungal Biodiversity Institute, Utrecht

08.30 – 09.30 Registration

09.30 Welcome; Jos Houbraken & Rob Samson

09.45 Rob Samson Westerdijk Fungal Biodiversity Institute, the Netherlands

The International Commission on Food mycology (ICFM). Past, present and future.

Session 1: Food Spoilage Reduction – Preservatives. Chair Emilia Rico.

10.05 **Jan Dijksterhuis**, Westerdijk Fungal Biodiversity Institute, the Netherlands
Rethinking used and novel strategies to prevent food spoilage.
10.25 **Frank Segers**, Corbion, the Netherlands
The combined impact of organic acids and modified atmosphere on fungi resistant to modified atmosphere packaging.
10.45 **Mélanie Cadoret**, Univ Brest, France
Impact of UV and/or biocides on the inactivation of *Aspergillus brasiliensis* ATCC 16404.

11.05 Break

11.35 **Alex Grum-Grzhimaylo**, Westerdijk Fungal Biodiversity Institute, the Netherlands
Genetic basis and evolution of resistance to the polyene preservative natamycin.
11.55 **Petter Melin**, RISE Research Institutes of Sweden, Sweden
Practical use of weak acid preservatives in meat-analogues and other products.
12.15 **Roya Choupannejad**, Westerdijk Fungal Biodiversity Institute, the Netherlands
Natural antimicrobials for enhanced food bio-preservation.
12.35 **Siavash Atashgahi**, AB Mauri, the Netherlands
Natural preservation of bakery products.

13.00 Lunch

Session 2: Mycotoxin Contamination and Exposure Risk in Food. Chair Paul Dyer.

13.50 **Ana-Rosa Ballester**, Institute of Agrochemistry and Food Technology, Spain
Deciphering ochratoxin A biosynthesis and degradation in *Aspergillus niger*: functional insights from halogenase and ochratoxinase mutants.
14.10 **Andika Sidar**, Gadjah Mada University, Indonesia
Mycotoxins on Indonesian agricultural commodities: Challenges and mitigation approaches.
14.30 **Angel Medina-Vaya**, Cranfield University, UK
Towards climate change resilient biocontrol to avoid OTA contamination in Robusta coffee production.
14.50 **Monika Coton**, Univ Brest, France
How to evaluate mycotoxin exposure due to mouldy foods at the consumer level. A case study on *Alternaria* mycotoxins in tomatoes.

15.10 **Break**

15.30 **Myrsini Kakagianni**, Department of Food Science and Nutrition, School of Agriculture Sciences, University of Thessaly, 43100, Karditsa, Greece – [online]
Probabilistic assessment of deoxynivalenol (DON) exposure from pita bread consumption: A Greek population study.

15.50 **Paula Cristina Azevedo Rodrigues**, Instituto Politécnico de Braganca, Portugal
Toxigenic fungi from Mozambican maize, peanuts and rice: what is the associated risk?
16.10 **Sylvia Kalli,** Wageningen University & Research, the Netherlands
Expanding the mycotoxin horizon: Analytical approaches for fungal metabolites in lupins and forage grasses.
16.30 **Sofia Noemi Chulze**, CONICET-UNRC, Argentina
An increasing risk driven by climate change: Aflatoxins and the urgent need for biocontrol.

16.50 Posters

18.00 Dinner at Biltsche Hoek Hotel

Tuesday 8 July 2025

Session 3: Food Spoilage Reduction – Biocontrol and Processing. Chair Monika Coton

09.00 **Maodo Malick Cissé**, Cheikh Ahmadoul University of Touba, Senegal [online]
Evaluation of the antagonistic activity of indigenous *Trichoderma* species against *Colletotrichum gloeosporioides*, the fungal pathogen causing mango anthracnose in Senegal.
09.20 **Emilia Rico**, BCN Research Laboratories, USA
Heat-resistant moulds (HRM) spoilage of thermal-processed beverages: has anything changed in the last 35 years?
09.40 **Muhammad Ahmed Ihsan**, University of Malta, Malta
Antifungal properties of lactic acid bacteria isolated from Maltese sheep milk and cheese.
10.00 **Alicia Rodríguez**, University of Extremadura, Spain
Discovering the effect of two antagonistic yeasts on metabolites involved in aflatoxin biosynthesis of *Aspergillus flavus* in a dried fig-based medium

10.20 Break

11.00 **Diana Sousa**, CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal [online]
Comparative heat activation and inactivation of *Talaromyces trachyspermus* ascospores inside and outside ascocarps.
11.20 **Miloslava Kavková**, Dairy Research Institute Ltd., Czech Republic
The antifungal activity of lactobacilli against spoilage fungi in milk, bakery and vegetable matrices.

Session 4: Fungi for Alternative Proteins and Food Fermentation. Chair Sofia Chulze.

11.40 **Alex James Pate**, University of Nottingham, UK
Meddling with mycoprotein - novel strain development of *Fusarium venenatum*.
12.00 **Eleni Kollia**, National and Kapodistrian University of Athens, Greece
Mycological fermentation of plant-based substrates for blue cheese analogue production.
12.20 **Asaph Kuria**, University of Nottingham, UK
Unravelling the enzymatic dynamics of mould-ripened Camembert and Brie cheese.
12.40 **Emmanuel Coton**, Univ Brest, France
Metabolite profile variability in *Penicillium roqueforti* populations: a footprint of ecological niche specialisation and domestication.

13.00 Lunch

Session 5: Ecological Insights into Fungal Communities and Mycotoxin Formation in Food. Chair Vasilis Valdramidis.

13.50 **Maria Laura Ramirez**, Instituto de Investigación en Micología y Micotoxicología, Argentina [online]
Aspergillus section *Nigri* and ochratoxin A accumulation in raisins: A comparative study of drying systems.
14.10 **Andrea Patriarca**, Cranfield University, UK
Ecophysiology of *Alternaria* strains from tomato producing AAL toxins.

14.30 **Marta Taniwaki**, Food Technology Institute (ITAL), Brazil.
Beyond the flavor: Assessing the risks and rewards of Brazilian artisanal cheese.
14.50 **Júlia Marquès**, Veterinary Faculty, Universitat Autònoma de Barcelona, Spain
Competitiveness study among black aspergilli strains.

15.10 Break

15.40 **Mahshid Saedi**, Westerdijk Fungal Biodiversity Institute, the Netherlands
Exploring the mycobiota and mycotoxin contamination in traditional Iranian foods.
16.00 **Marie Belair**, Univ Brest, France
Ecological niche shapes fungal communities from vine to wine and impacts FMA detection in wine.
16.20 **Su-lin Hedén (Leong)**, Swedish University of Agricultural Sciences, Sweden
Mycotoxin production by Penicillium species during refrigerated storage of plant-based analogues of cheese, fraiche and pâté.

17.00 ICFM commission board meeting (only for ICFM committee members)

Dinner at Stadskasteel Oudaen restaurant Utrecht centre (Oudegracht 99, 3511 AE Utrecht)

Wednesday 9 July 2025

Session 6: Guidelines and New Insights in the Identification of Mycotoxigenic Fungi. Chair Su-lin Hedén (Leong).

09.00 **Nazik Hussain**, Institute of Plant Sciences University of Sindh Jamshoro, Pakistan [online]
Morphological and molecular characterisation of *Alternaria alternata* from tomato Lycopersicon esculentum fruit
09.20 **Jens Christian Frisvad**, DTU - Bioengineering, Denmark
Chemistry and morphology are excellent for separating *Aspergillus oryzae* and *Aspergillus flavus*, but difficult to achieve using genome sequencing.
09.40 **Jos Houbraken**, Westerdijk Fungal Biodiversity Institute, the Netherlands
An update on *Aspergillus*, *Penicillium* and *Talaromyces* taxonomy.
10.00 **Ya Bin Zhou**, Westerdijk Fungal Biodiversity Institute, the Netherlands
Barcoding *Aspergillus*, *Penicillium* and *Talaromyces* strains from the CBS biobank.
10.20 **Ioanna Pyrri**, National and Kapodistrian University of Athens, Greece
Penicillium section *Brevicompacta*: new insights in taxonomy.

10.40 Break

Session 7: Methodology Development. Chair Angel Medina Vaya.

11.10 **Laura García Calvo**, Nofima AS, Norway
Whole Genome Sequencing of *Penicillium* spoilage mould from food producers.
11.30 **Kaitlyn Parra**, Veterinary Faculty, Universitat Autònoma de Barcelona, Spain
Development of a droplet digital PCR assay for population study of ochratoxigenic and non-ochratoxigenic *Aspergillus carbonarius* strains.
11.50 **Manuela Zadravec**, Croatian Veterinary Institute, Croatia
Challenges in sample preparation of *Alternaria*, *Cladosporium* and *Fusarium* species for MALDI TOF analyses.
12.10 **María A. Pavicich**, Ghent University, Belgium
Hyperspectral imaging for early fungal detection and prediction of mycotoxins in apples.

12.30 Closing of the workshop

13.00 Lunch

POSTERS

Alberto Martín, University of Extremadura, Spain
Study of *Alternaria alternata* on tomato agar by VOCs, mycotoxin and metabolomic analysis.

Bruna Sepúlveda, University of Minho, Braga, Portugal [online]
Isolation of filamentous fungi from beans, maize and peanuts from Cuanza Sul, Angola.

Dana Tančinová, Slovak University of Agriculture in Nitra, Slovakia
Ability of selected plant essential oils to inhibit cyclopiazonic acid production by *Penicillium commune* strains.

Elettra Berni, Stazione Sperimentale per l'Industria delle Conserve Alimentari-Fondazione di Ricerca – SSICA, Italy
Influence of reduced water activity on *Monascus ruber* heat- and sorbate-resistance.

Frank Segers, Corbion, the Netherlands
Predictive modeling for bread spoilage prevention: simplifying complex data.

Inês Mendonça, National Institute for Agrarian and Veterinary Research, Portugal [online]
Effectiveness of encapsulated lemon thyme and prince herb essential oils against *Stemphylium vesicarium* and *Alternaria* spp. isolated from Portuguese "Rocha" pear orchards.

Linda Mezule, Riga Technical University, Latvia
Enzymes from wood-decaying fungi as tools for waste hydrolysis.

Santiago Ruiz-Moyano, Universidad de Extremadura, Spain
Optimization of a HPLC-fluorescence method for quantification of fumonisins FB1 and FB2 in food matrices and synthetic culture media.

Simas Borkertas, Lithuanian Research Centre for Agriculture and Forestry, Lithuania
Fungal strains of industrial food by-products fermentation and its techniques for mycelium and food production.

Teresa Vale Dias, University of Minho, Braga, Portugal [online]
Fungal ecology along the production line of Portuguese goat cheese.

Zuzana Barboráková, Slovak University of Agriculture in Nitra, Slovakia
Ochratoxin A producers in green coffee beans.

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The International Commission on Food Mycology (ICFM). Past, present and future.

Robert A. Samson

Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands

The concept of a specialist food mycology workshop was borne during conversations at the Gordon Research Conference on "Microbiological Safety of Foods" in Plymouth, New Hampshire, in July 1982. The necessity, for standardization of methods for the examination of foods for contaminant and spoilage mycoflora was apparent for some time. Discussions at that time resulted in an proposal for a workshop with a unique format: all attendees would be expected to contribute and papers in nearly all sessions would be presented as a set of data on a single topic, not as a complete research paper. Each session should be followed by general discussion, and then a panel would formulate recommendations for approval by a final plenary session.

This series of workshops commenced in Boston, USA, in July 1984, from which the proceedings were published as *Methods for Mycological Examination of Food* (edited by A. D. King et al., published by Plenum Press, New York, 1986). The second meeting was held in Baarn, the Netherlands in August 1990. John Pitt and Rob Samson proposed ICFM as a commission under the IUMS (International Union of Microbiological Societies). IUMS has three divisions: Virology, Bacteriology and Applied Microbiology and Mycology (BAM) and Eukaryotic microbiology (MEM). ICFM is a commission under MEM. After the successful meeting in Baarn (1990), subsequent meetings in Copenhagen (1994), Uppsala (1998), Samsøe (2003), Key West (2007), Freising (2010, 2013, 2016 and 2019) and in Utrecht (2022).

Managing an international commission, however, requires much administration. Since 2018 ICFM has been accommodated as a legal entity as a foundation requiring reports to the Dutch fiscal authorities and chambre of commerce. Maintaining a website for our commission is important but also this needs time. Nevertheless ICFM is an important commission serving many food microbiologists.

The ICFM workshops have proven a successful platform for food mycologists to present their data. Although data on methods for mycological examination of food are still be presented, topics such as preservation, mycotoxin contamination, biocontrol, fermentation, fungal ecology and taxonomy, modern methodology are now important topics. ICFM and the workshops have a great future and it is expected that many meetings will take place.

SESSION 1: DEVELOPMENTS IN METHODOLOGY

Food spoilage and possible prevention strategies

Jan Dijksterhuis

Food and Indoor Mycology, Westerdijk Fungal Biodiversity Institute, Utrecht, NL

The amount of food lost for human consumption each year is immense and many programs are dedicated to diminishing these losses. The role of fungi in this, causing disease of agricultural crops or spoilage of processed food is not known, but indications that a fifth to a quarter of all food losses are proposed. This means that research on the precise ways fungi infect crops and spoil food may lead to novel methods to prevent losses and make food available without an expansion of the areas of food production. This has been a theme during the history of mankind after the era of hunter-gathering when food was produced in larger amounts as immediately needed and needed to be stored for prolonged times. In this contribution, examples will be given, how specific food spoilage can be and how diverse possible routings to prevent spoilage can be. A number of specific cases as the use of the polyene antifungal natamycin and the weak organic acid propionic acid are discussed.

The combined impact of organic acids and modified atmosphere on fungi resistant to modified atmosphere packaging

Frank Segers, Dieneke van Houwelingen-de Jong, Tessa van Houwelingen-Hoogenhuijzen, Florence Postollec

Corbion Innovation Center, Gorinchem, The Netherlands

Modified atmosphere packaging (MAP) is commonly used in Europe to prevent fungal spoilage in baked products. However, some species can tolerate this environment and still cause spoilage. The most common spoilers in MAP bakery products are "Chalky moulds," which resemble chalk on bread. These include *Saccharomycopsis fibulgera* and *Hyphopichia burtonii*. The study examines the effect of MAP on these resistant moulds, combined with the antimicrobials acetic acid and propionic acid. This is done in vitro using live-cell imaging, agar plate techniques, and challenge tests.

Spoiled bakery products under MAP conditions are used to isolate spoilage-causing strains. These strains are identified and screened for antimicrobial tolerance in broth using an oCelloScopeTM. Simultaneously, the strains are inoculated on small agar plates with adjusted Malt extract agar (MEA) containing additional antimicrobials and placed in black trays. A challenge test is also performed on specially produced bread containing both organic acids. The atmosphere of these packages is adjusted to 80% CO2 and 20% N2, as commonly used in the industry. Growth on oCelloScopeTM is analyzed for growth rates, while growth on agar plates and during the challenge test is visually observed and documented.

The combination of oCelloScopeTM and agar plates under MAP shows a wide diversity in resistance to preservatives among chalky moulds. Chalky moulds are not only resistant to modified atmosphere but also to commonly used preservatives. Saccharomycopsis fibulgera is generally more tolerant to preservatives compared to *H. burtonii*, but there is significant diversity in the growth abilities of these strains, which can strongly impact shelf-life. Increasing knowledge on MAP-resistant fungi using live-cell imaging, agar plates, and challenge tests can significantly contribute to developing targeted preservative solutions to tackle spoilage issues in MAP bakery products.

Impact of UV and/or biocides on the inactivation of *Aspergillus brasiliensis* ATCC 16404

Mélanie Cadoret[1]*, Son Ho Van[1], Philippe Dantigny[1], Emmanuel Coton[1]

[1]Univ Brest, INRAE, Laboratoire Universitaire de Biodiversité et Écologie Microbienne, F-29280 Plouzané, France

Presenter: Mélanie Cadoret

Fungal contamination is a major concern in the food production chain, particularly in the dairy industry. Traditional cleaning and disinfection techniques, though effective, can produce undesirable by-products (. chloramines) and may not align with increasing consumers demands for natural, non-toxic and environmentally

friendly solutions. This study explores the efficacy of "green" biocides (ethanol, peracetic acid, acetic acid) and UV irradiation – individually and in combination – against *Aspergillus brasiliensis* ATCC 16404, a reference strain used in disinfection evaluation norms. Unlike many previous studies that were solely based on hydrated conidia, we also studied dry conidia – mechanically harvested without contact with liquids – to better reflect the physiological conditions encountered in agri-food environments. Both hydrated and dry conidia (mimic airborne conidia) were exposed to various biocides or UV radiations for different duration and intensities. Initial spore counts were determined using a Malassez cell and post-treatment viability was evaluated by thallus enumeration. Inactivation was quantified as $\log_{10}(N/N_0)$ and were modelled using the Weibull equation. Overall, dry spores exhibited higher sensitivity to alcohol-based biocides, whereas they show similar resistance when exposed to a peracetic acid-based biocide compared to hydrated spores. Combined biocide/UV treatments (either at the same time or sequentially) are currently under investigation for potential synergic effects. Finally, this research aims to assess the applicability of combined disinfection technologies in the dairy industry by evaluating their efficacy on various actual fungal dairy contaminants in different physiological states (hydrated and dry conidia).

Genetic basis and evolution of resistance to a polyene preservative natamycin

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Presenter: A. Grum-Grzhimaylo

The polyene antifungal natamycin is used for the protection of cheese and sausages against fungal growth, posing risk for resistance evolution. Resistance to polyenes is considered rare compared to other antifungal compounds. Here, we discover high-level resistance conferred by a natamycin-degrading enzyme, which we call natamycinase, in the cheese-spoiling fungus *Penicillium discolor*. The gene is present within a large transposable element carrying many other genes of unknown function. In resistant genomes, we found multiple identical gene copies coding for natamycinase, also likely spread by transposons. Further genomic analyses and phylogenetic reconstructions of resistant and sensitive strains demonstrated that the acquisition of resistance likely happened once and clonally spread across the globe. The clonal mode of propagation and the inability to form anastomoses between resistant and closely related sensitive *P. discolor* strains suggests a low risk of horizontal spread of natamycin resistance within species. Homologs of the natamycinase gene are present in unrelated fungal species (mainly aspergilli), also conferring resistance to natamycin. As some of those fungi can cause human infections, natamycin would not be the preferred antifungal for treatment.

Practical use of weak acid preservatives in meat-analogues and other products

Petter Melin

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Within a greater research centre entitled FINEST-Food Innovation for Sustainable System Transition, we have been exploring business-related as well as technological challenges to invent and commercialise new and sustainable food products. Examples of specific tasks are better use of the great resource of wild Swedish berries, and legume-based alternatives to meat products.

Berries harbouring substances will well-known antioxidant properties, and of particular interest is the lingonberry *(Vaccinium vitis-idaea)*, which harbours substantial amounts of benzoic acid. We have shown that lingonberry juice can be used as a complement in blends where intrinsic benzoic acid enables safe products, and the sour taste of the berries can be reduced using malolactic fermentation (Bergentall et al. 2024). In contrast, the remaining press-cake could not successfully be used to produce microbially safe products despite the high content of benzoic acid. Simply, amounts of press-cake required results in foods unattractive both in structure and taste, although the added red colour was positive. In another product, we explored the possibility to preserve meat-analogues after the food-packaging has been broken, which is relevant for any product not consumed directly, e.g. sandwich spread, sausage and cheese analogues. Addition of sorbate was the most efficient preservative as growth of the tolerant fungi *Penicillium roqueforti, Pichia fermentans* as well as the bacterium *Listeria monocytogenes* were completely inhibited (Melin, 2024).

From both projects, we could conclude that the weak acid preservatives were active also at rather high pHs where only a fraction of the molecules are in their active uncharged form. Our working hypothesis is that trapped, and membrane-integrated preservatives no longer contribute to the acid equilibrium. This hypothesis is not in contrast with proposed mode of actions, including the weak acid theory, but nevertheless, it has practical implications when developing food preservation strategies.

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Natural antimicrobials for enhanced food bio-preservation

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Presenter: Roya Choupannejad

Food spoilage fungi are a major food safety issue due to mycotoxin production and are responsible for substantial annual revenue losses in the food and beverage industries. Conventional preservatives such as sorbic acid, calcium propionate, and benzoic acid are commonly employed to prevent spoilage, however some such as sorbic acid pose health and environmental drawbacks. Moreover, these preservatives are not efficient against all food spoilage fungi, limiting their usage. As a result, there is a growing demand for natural antimicrobial compounds that could be used as food bio-preservatives. Especially, fungi and bacteria are recognized as good resources for such natural antimicrobial compounds. This study aims to evaluate bacterial and fungal strains for the production of compounds with antifungal activity against two main food spoilage fungi, *Aspergillus niger* and *Penicillium paneum*. To this end, thirty-two thermophilic bacterial and fungal strains sourced from the NCBB and CBS biobanks, were fermented and screened for antifungal activity using Bioscreen C Pro assay. The 32 culture filtrates (CFs) were heat-treated and tested, resulting in 10 CFs showing significant inhibition of both *A. niger* and *P. paneum*. Secondary metabolites from these 10 CFs were then extracted with different organic solvents, and antifungal assay of these organic extracts showed that the antifungal activity produced by strain F07 is present in the ethyl acetate fraction, suggesting it is likely a secondary metabolite. Bioactivity-guided purification of the active compound is being performed using preparative HPLC, and the pure active compound will be characterized by UV-HPLC-MS, HRMS and NMR. Overall, thermophilic bacterial and fungal strains have been found to produce antifungals that can effectively control the growth of food spoilage fungi. These findings suggest a promising avenue for developing natural antimicrobial solutions that withstand processing, thereby extending food shelf life.

Natural preservation of bakery products

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Microbial spoilage of bakery products by moulds, yeast and bacteria is a serious problem resulting in a significant food waste. Fungal development leads to bakery products sensory defects varying from visual deterioration to noticeable odour, flavour, or texture changes. Fungal growth can also have negative health impacts due to mycotoxin production by some moulds. To tackle this economic and safety issue, the bakery industry has been working to identify treatments which allow bread safety and extended shelf-life. Physical methods and chemical preservatives have long been used. However, public authorities encourage the food industry to limit the use of chemical preservatives and develop natural methods for food preservation. This is accompanied by a strong societal demand for 'clean label' food products, as consumers are looking for more natural, less processed, and safer products. Whereas natural preservatives have been rather successful in replacing chemical preservatives in various types of foods and drinks, finding effective natural preservatives for bakery products, especially bread, has remained a challenge. This is related to complex matrix of dough/bread, shear force of mixing, intense heat-treatment during baking, and potential negative sensory impact of the preservatives. During ICFM workshop, I will discuss challenges and opportunities of using natural compounds for preservation of bakery products.

SESSION 2: MYCOTOXIN CONTAMINATION AND EXPOSURE RISK IN FOOD

Deciphering ochratoxin A biosynthesis and degradation in *Aspergillus niger*: functional insights from halogenase and ochratoxinase mutants

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Presenter: Ana-Rosa Ballester

Ochratoxin A (OTA) is a potent mycotoxin with significant implications for food safety, human health, and economic stability. Aspergillus niger, a filamentous fungus widely used in industrial applications, is also one of the main OTA-producing species within the Aspergillus section Nigri. Despite previous research, the complete biosynthetic and regulatory pathways of OTA remain unclear, limiting the development of effective mitigation strategies. In this study, we investigated two key enzymatic steps influencing OTA levels: halogenation in OTA biosynthesis and enzymatic degradation by ochratoxinases.

First, we examined the role of a flavin-dependent halogenase in A. niger, hypothesized to catalyze the conversion of ochratoxin B (OTB) to OTA by introducing a chlorine atom at the C-5 position. Using gene knockout strategies, we generated Δhal mutants and assessed their impact on OTA production through chromatographic analysis. Our results confirmed that the absence of the halogenase gene completely halted OTA synthesis, leading to a marked accumulation of OTB, thereby demonstrating that halogenation represents the final step in OTA biosynthesis in A. niger.

In parallel, we investigated the role of two putative ochratoxin-degrading enzymes, ochratoxinase 1 (OTase1) and ochratoxinase 2 (OTase2), using CRISPR-Cas9-mediated gene editing. Single and double knockout mutants were generated to assess their impact on OTA degradation and overall mycotoxin levels. Surprisingly, high-performance liquid chromatography (HPLC) analysis of three independent transformants revealed that disruption of these genes resulted in a significant reduction of both OTA and OTB production, contrary to our initial hypothesis that OTA levels would increase due to impaired degradation. This unexpected finding suggests that ochratoxinases may play a more complex role in the regulatory network of OTA synthesis and degradation than previously thought.

Our study provides novel insights into the molecular mechanisms governing OTA biosynthesis and degradation in A. niger. The confirmation of halogenation as the final biosynthetic step reinforces its potential as a target for mycotoxin control strategies, while the unexpected regulatory role of ochratoxinases highlights the need for further research into the interplay between biosynthesis and degradation pathways. A deeper understanding of these mechanisms will contribute to developing innovative approaches for mitigating OTA contamination in food and agricultural products.

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Mycotoxins on Indonesian agricultural commodities: Challenges and mitigation approaches

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Presenter: Andika Sidar

The presence of mycotoxin is a global concern due to the serious health risks posed to livestock and humans. Additionally, they present significant challenges to the food industry, affecting food safety, quality, and economic stability. In Indonesia, the occurrence of mycotoxin contamination remains a major issue, primarily due to the country's high temperatures, humidity levels, and inadequate food safety practices, all of which promote the growth of mycotoxin-producing fungi. Several cases have been reported in important agricultural commodities from Indonesia, including nutmeg, coffee beans, and cocoa beans. Aflatoxin contamination in nutmeg has been detected at all levels of the market chain, from farmers and collectors to exporters, indicating that aflatoxin

presence in nutmeg is common and severe. Moreover, significant detention of Indonesia nutmeg exports including those entering the European market due to aflatoxin levels exceeding permissible limits, requires urgent attention. Mycotoxin contamination in cocoa and coffee beans, especially with ochratoxin-A also poses major challenges in Indonesia. Consequently, mitigation strategies against mycotoxin are needed to ensure the safe consumption of these products. To prevent mycotoxin contamination in cocoa beans, indigenous lactic acid bacteria (LAB) with antifungal potential were selected during cocoa bean fermentation. The use of *Lactobacillus plantarum* HL-15, either individually or in combination with *Candida famata* HY-37 and *Acetobacter* spp. HA-37, successfully inhibited the growth of ochratoxin A producing fungi. As further attempt, those isolated will be screened for laccase activity. Laccase is a multi-copper oxidase enzyme containing four copper ions that catalyzes the oxidation of a wide range of phenolic compounds. The oxidative potential of laccase is considered crucial for breaking down the aromatic moieties of mycotoxin. A previous study demonstrated the unique structure of small laccase (SLAC) from *Streptomyces* and its ability to degrade lignocellulosic biomass, which also contains aromatic phenolic compounds. The potential of laccase and SLAC derived from BAL and *Streptomyces* isolates, along with creating rational design of laccase enzyme for mycotoxin detoxification will be investigated. Given that enzymes offer an ecological-friendly solution for mycotoxin removal, this approach offer a promising option for biotechnological approaches to mycotoxin reduction.

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Towards climate change resilient biocontrol to avoid OTA contamination in Robusta coffee production

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Presenter: Angel Medina

Ivory cost produce 66 million kilograms of Robusta coffee every year. This production is impacted by the contamination of Ochratoxin A (OTA) mainly produced by Aspergillus westerdijkiae and Aspergillus carbonarius. With an EU maximum limit of 3 µg/kg for roasted coffee and above 5 µg/kg for soluble coffee there is a need to develop innovative and resilient solutions to prevent OTA accumulation within the Robusta coffee supply chain.

Following a review on the current mitigation strategies along the coffee supply chain. This study first identified both high OTA producers in Ivory Coast coffee cherries and candidate biocontrol agents. The potential of indigenous yeasts as biocontrol agents was further explored and confirmed in in vitro conditions.

For the first time, the most promising candidates were also investigated for their capacity to prevent OTA production considering predicting climate change conditions. This novel approach led to the selection of one biocontrol candidate (Meyerozyma caribbica Y4) with the best resilience with reduction of both growth (50%) and OTA production (70%) under all the scenarios tested.

This research is pivotal in the pursuit of climate-resilient strategies for mycotoxin management, contributing to both food safety and agricultural sustainability.

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How to evaluate mycotoxin exposure due to moldy foods at the consumer level. A case study on *Alternaria* mycotoxins in tomatoes

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Presenter: Monika Coton

Food losses are a major worldwide challenge [1] undermining the sustainability of food systems. These losses are partly due to mould spoilage at the consumer level, especially in industrialized countries [2]. One possible way to reduce food waste due to mouldy foods would be to avoid a too conservative approach that leads to discarding entire or large portions of a product when fungal growth is observed. However, there is also a food safety risk as moulds can produce mycotoxins that migrate into foods without being detected by consumers [3].

To better understand consumer habits and evaluate the mycotoxin danger, a citizen science strategy was used to collect and identify moulds from mouldy foods in French households, determine consumer habits and evaluate to what extent consumer may be exposed to mycotoxins.

Based on feedback from ~350 consumers and ~500 collected mouldy food samples, results showed that most samples belonged to fruits (37%), vegetables (17%), cheeses (14%), other dairy products (6%), jams (12%), bakery products (10%) and meat products (3%). Consumer habits showed that meat and dairy products are most often thrown away while mouldy fruits, vegetables and jams are typically consumed after scraping or removing the moldy sections even though more frequently observed on these products.

Nearly 70% of molds belonged to Penicillium although Fusarium, Cladosporium, Aspergillus, Mucor, Botrytis and Alternaria were common contaminants. Among them, over 50% were potential mycotoxin-producers. Based on the obtained data, multiple food-fungi associations have been selected for further study.

As a case study, mycotoxin migration in Alternaria alternata contaminated tomatoes was evaluated using our worst-case strategy and 3D sampling procedure [3-4] in conditions mimicking consumer storage. Fungal growth and mycotoxin content (HR-LC-QTOF) were monitored in increasing width wise and depth wise portions. Simultaneous production of up to 5 mycotoxins was observed with highest mycotoxin contents accumulating near the mouldy lesions. Only small lesion sizes were correlated to minimal mycotoxin migration while larger lesions were linked to much further migration.

In vitro mycotoxin toxicity of the detected mycotoxins in tomatoes was evaluated on 2D and 3D intestinal and hepatic cell models; these models are used to mimic the human digestive tract and detoxification organ mycotoxins encounter after food consumption. So far, results showed dose-dependent exposure effects.

The next step will be to link consumer habits to mycotoxin data to better evaluate the mycotoxin risk at the consumer level and provide simple consumer recommendations to limit food waste while ensuring consumer safety.

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Probabilistic assessment of deoxynivalenol (DON) exposure from pita bread consumption: a Greek population study

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Presenter: Myrsini Kakagianni

Flatbreads are a widely consumed food globally, but they may contain deoxynivalenol (DON), a common mycotoxin produced by Fusarium fungi. Due to its frequent presence in cereal-based foods and potential health risks, this study evaluated DON exposure in the Greek population through pita bread consumption using a probabilistic risk assessment approach. Data from 710 individuals of all age groups were obtained from the Hellenic National Nutrition and Health Survey (HNNHS), representing all regions of Greece. Based on EFSA reports, the mean DON contamination level for unleavened bread, crispbread, and rusk was 43.5 µg/day (median concentration). Food consumption and occurrence data were classified using EFSA's FoodEx2 system. Chronic dietary exposure was calculated by combining daily pita bread intake with mean DON levels, adjusted for body weight. The mean and 95th percentile exposures were derived using the ImproRisk model (V0.5.4). Results showed that DON exposure from pita bread did not exceed the tolerable daily intake (TDI: 1 µg/kg bw/day) for the Greek population. Toddlers, children, and adolescents exhibited the highest exposure levels, yet their Hazard Quotient (HQ) remained below 1, indicating no significant health concern. These findings suggest that DON contamination in Greek pita bread may pose minimal risk to both average and high consumers.

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Toxigenic fungi from Mozambican maize, peanuts and rice: what is the associated risk?

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Presenter: Paula Rodrigues

Toxigenic fungi and mycotoxins are persistent contaminants in food, causing great economic losses, and when ingested, can result in acute or chronic poisoning. In Mozambique, these contaminations are frequent in basic foods, due to lack of knowledge, climatic conditions and improper storage, among other factors. This work aimed to evaluate the risk of contamination by toxigenic fungi of three important food crops from southern Mozambique: maize, rice and peanuts. To this end, contaminating fungi from samples of maize (14 samples), rice (13 samples) and peanut (12 samples) were isolated, identified and characterized for their mycotoxigenic ability. For each sample, 25 seeds were sown in Petri dishes containing DRBC medium. After an incubation of 5-7 days at 25 °C, fungi were isolated and grouped into morphotypes. Representative isolates were molecularly identified by Sanger sequencing of the ITS region, and CL, EF and BT genes. Isolates were characterized for mycotoxin production by LC-MS/MS. All peanut, rice and maize samples showed fungal contamination, with an average incidence per sample of 84 %, 58.5 % and 87 % for peanut, rice and maize, respectively. From the 347 isolated fungi, 24 genera were identified. The rice samples showed higher fungal diversity (18 genera) than the peanut samples (10 genera) and the maize samples (8 genera). The genus Aspergillus was dominant in all products (53 % of the total isolates), followed by Fusarium (10 %) and Penicillium (6 %). Aspergillus flavus (aflatoxin-producing species) was

detected in 91.7 % of the peanut samples, while in rice 46.2 % of the samples were contaminated and in maize 78.5 % of the samples were contaminated. Aspergillus niger (OTA-producing species) had lower expression in all crops. Penicillium citrinum (citrinin-producing species) was the most predominant species of the Penicillium genus in the samples (85 % of all Penicillium isolates), while in the Fusarium genus the predominant species was Fusarium verticillioides (fumonisin-producing species) (54.3 % of all Fusarium isolates), with a higher incidence in maize samples. Correlations will be established between toxigenic fungi incidence and mycotoxin contamination of samples. The high incidence of A. flavus in the samples agrees with the high level of aflatoxin contamination of these crops (Matusse et al. 2024), and strengthens the need for control strategies.

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Expanding the mycotoxin horizon: Analytical approaches for fungal metabolites in lupins and forage grasses

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Presenter: Sylvia Kalli

The evolving landscape of food and feed production—driven by climate change, sustainability goals, and regulatory constraints—has led to increased interest in alternative crops such as lupins and safety of forage grasses. The novel food sources, while promising, are not immune to fungal contamination and associated mycotoxins, many of which remain under-characterized and unregulated. Phomopsins, produced by Diaporthe toxica, are a notable concern in lupins, that are rapidly gaining popularity in the global shift toward more climate-friendly protein sources. However, several congeners are lacking analytical standards and regulatory limits in Europe. Similarly, forage grasses can contain tremorgenic indole-diterpenes, ergot alkaloids, originating from fungal endophytes or environmental contaminants. These compounds present significant analytical challenges due to matrix complexity, low natural concentrations, heterogeneous distribution, and structural diversity.

Recent developments using targeted and high-resolution mass spectrometry have enabled the detection and semi-quantitative analysis of a wider range of mycotoxins in these matrices. Artificial contamination techniques and in vitro fungal incubations have supported the identification of novel phomopsin congeners, while multi-analyte LC-MS/MS methods have been validated for tremorgenic, ergot, alkaloids in grass-based feeds.

Initial occurrence surveys indicate sporadic but measurable contamination, underscoring the need for continued methodology improvements and monitoring. These findings highlight the relevance of expanding the research and analytical scope beyond regulated mycotoxins, particularly as non-traditional crops gain importance in the food and feed sectors and while the climate change brings yet unpredictable challenges for the future of food and feed safety.

An increasing risk driven by climate change: aflatoxins and the urgent need for biocontrol

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Presenter: Sofía Noemí Chulze

Maize (*Zea mays* L.) is an important crop in Argentina and worldwide, used for food, forage, and bioenergy. Pathogenic microorganisms cause 20–40% losses, many of them linked to mycotoxins such as aflatoxins (AFs). Climate change (CC), with increasing temperatures, CO_2 levels, and alternating droughts and intense rainfalls, promotes their synthesis. Biological control agents (BCAs) offer a promising strategy to mitigate contamination. Studies have demonstrated that native atoxigenic *Aspergillus flavus* strains (AFCHG2 and ARG5-30) effectively reduce AFs at field stage and during six months of storage (Alaniz Zanon et al., 2022). This study aimed to evaluate BCA effectiveness under CC conditions. Twenty grams of maize were inoculated with spore suspensions of toxigenic and non-toxigenic strains, individually or combined in Petri dishes. These treatments were incubated for 7, 14, and 21 days under current climate conditions (30°C, *a*w 0.98, 400 ppm CO_2) and simulate CC (35°C, *a*w 0.94, 1000 ppm CO_2). Kernels were dried, ground, and AFs were analyzed using the QuEChERS method (Bursić et al., 2013) and determined by HPLC/FD. Results showed the BCAs reduce AFs contamination under both conditions, emphasizing the importance of this study in enhancing mycotoxin control and ensuring food safety in an scenario of climate change.

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SESSION 3: FOOD SPOILAGE REDUCTION – BIOCONTROL AND PROCESSING.

Evaluation of the antagonistic activity of indigenous *Trichoderma* species against *Colletotrichum gloeosporioides*, the fungal pathogen causing mango anthracnose in Senegal

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Presenter: Maodo Malick Cissé

Mango is a major crop that represent 63% of fruit production in Senegal. The mango business suffers however from major phytosanitary constraints such as mango anthracnose caused by *Colletotrichum gloeosporioides*. Fungicides are usually sprayed for the control of this fungal pathogen with their downside of negative impacts on human health and on environment. Fungi of the genus *Trichoderma* are reported to have great potential for biological control on pathogens. Their ability to control *C. gloeosporioides* was tested through confrontation with three isolates of *Trichoderma asperellum*(S3M4ZIG, F3GP3 and F3GP3-5), one isolate of *Trichoderma viride* (F2KV3) and a fifth isolate of *Trichoderma* sp. (S5M5ZN). These isolates, obtained from mango tree organs in Senegal,were used in direct confrontation against *C. gloeosporioides* in vitro and further on in vivo tests on fruits. The incidence and severity of anthracnose were evaluated after 20 days of incubation.In dual culture in the petri dishes, the isolate S5M5ZN inhibited totally the mycelial growth of *C. gloeosporioides*,followed by the strains F3GP3 (T.asperellum), F3GP3-5 (*T. asperellum*), F2KV3 (T. viride) and S3M4ZIG (T.asperellum), that caused respective reduction of mycelial growth of the pathogen by 89.41 ± 18.33%, 89.01 ±19.01%, 77.25 ±19, 77%, 77.25 ± 12.95%.For the in vivo tests, the mangoes soaked with distilled water (negative control) and those inoculated with only *C. gloeosporioides*(positive control) had a disease severity of respectively 20.36±4.39% and 50.69±6.58%. The *Trichoderma* isolate S3M4ZIG, showed the best efficacy on mango with the lowest disease severity of 5.55±00.00%.

Reference :

Cissé MM, Mbaye N, Diédhiou PM, Lahoucine H, Bencharki B. 2022. Evaluation of the antagonistic activity of indigenous Trichoderma species against Colletotrichum gloeosporioides, the fungal pathogen causing mango anthracnose in Senegal. Int. J. Adv. Res. 10 (08): 650-660

Heat-resistant moulds (HRM) spoilage of thermal-processed beverages: has anything changed in the last 35 years?

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Nowadays, consumers desire less processed, non-preserved products. Controlling their spoilage can be challenging. One way to control mould spoilage in these types of beverages is by pasteurization. However, even pasteurization can be ineffective preventing spoilage of thermal-processed beverages by both heat-resistant moulds (HRM). Heat-resistant moulds (HRM) have spores called ascospores that can survive both the pasteurization treatment and hot filling. These ascospores can be found in the ingredients, packaging, and the processing environment. In 1990, a beverage company that manufactured sport drinks switched from glass bottles to PET bottles. Since the PET bottles could not be filled at more than 82ºC (180º), they decided that the beverage should be pasteurized also at this temperature. The beverage had a 60% spoilage rate caused by an HRM, Paecilomyces variotii. Ascospores of this species were found in the sugar tanks that were not even cleaned once a year. The solution was to pasteurize at a higher temperature of 95ºC (203ºF) for 26-30 seconds and cool down the product to 82ºC (180º) prior to filling the PET bottles. However, even using this pasteurization temperature and time, sport drinks as well as juices and other beverages are still spoiled by HRM on a regular basis. So, has anything changed in the last 35 years? This presentation will review all the changes that have been done to prevent this type of spoilage if any.

Antifungal properties of lactic acid bacteria isolated from Maltese sheep milk and cheese

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Presenter: Muhammad Ahmed Ihsan / Vasilis P. Valdramidis

Lactic acid bacteria (LAB) exhibit potent antifungal activity through production of organic acids and bacteriocins, providing natural solutions to fungal spoilage and mycotoxin risks in dairy. This study aimed to evaluate the antifungal activity of LAB isolates from Maltese raw sheep milk and cheese against *Penicillium expansum* and Candida albicans, and to develop a predictive model for fungal growth inhibition to support dairy product safety. A total of 129 LAB isolates from traditional Maltese sheep cheese (Ġbejna) were screened, and 27 with strong antifungal activity using overlay method and were identified via 16S rRNA sequencing. Four strains (*Lactplantibacillus plantarum* S2 and S8, *Levilactobacillus brevis* 5.17, Limosilactoabcillus fermentum 5.10) were selected to develop a polynomial logistic regression model predicting fungal growth/no-growth in a miniature cheese with serial two-fold dilutions of bacterial inocula between 4.15-8.97 log CFU/ml and fungal inoculum levels between 3.4-5.6 log CFU/ml for incubation periods between 0-15 days.

Most LAB isolates demonstrated antifungal activity against *P. expansum,* except one strain of L. paracasei. Strains such as *L. plantarum* S2 and S8, *L. brevis* 5.17, L. *casei, C. halodurans* I2.8, and L. fermentum 5.10 exhibited inhibition zones ranging from 11.16 ± 1.30 to 24.23 ± 6.63 mm against *A. terreus, A. flavus, A. niger, S. cerevisiae,* and *Rhizopus*. Logistic models yielded high R^2 values (>0.88) for *L. plantarum* against both fungi, requiring minimum of 5.96 log CFU/mL to inhibit *P. expansum* until day 10 and 4.5 log for C. albicans until day 12 and then higher bacterial size is required to inhibit 5.6 log inoculum of *Penicillium* and *Candida* spp. In contrast, L. brevis 5.17 required 7.16 log CFU/mL to suppress *P. expansum* until day 10 and displayed a similar response to *C. albicans* (R^2 = 0.89). L. fermentum 5.10 showed moderate efficacy, requiring 6.56 log CFU/mL to inhibit *P. expansum,* although lower concentrations were effective against *C. albicans*. This work identifies autochthonous LAB from Maltese sheep dairy products with bioprotective properties against spoilage fungi and modelling of LAB-fungal interactions offers novel insights for enhancing the safety and shelf life of such products.

Discovering the effect of two antagonistic yeasts on metabolites involved in aflatoxin biosynthesis of *Aspergillus flavus* in a dried fig-based medium

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Presenter: Alicia Rodríguez

Aflatoxins (AFs) are highly toxic and carcinogenic mycotoxins, mainly produced by *Aspergillus flavus*. Dried figs (*Ficus carica* L.), due to their composition and drying and storage conditions, are particularly susceptible to this contamination. New strategies are needed to counteract the colonisation of this species in dried figs. Yeasts have emerged as an effective and sustainable strategy to replace not always desirable traditional fungicides. This work aimed to study the influence of two antagonistic yeasts, *Hanseniaspora uvarum* L793 and Metschnikowia pulcherrima L672, on the metabolites involved in the aflatoxin (AF) biosynthetic pathway of A. flavus L144 on dried fig-based medium (DFBM). For this, 6 batches were evaluated on DFBM and incubated for 8 days: a) BL (blank without inoculation); b) AF (A. flavus M114); c) 672C (M. pulcherrima L672); d) 672AF (co-culture of A. flavus M114 and *M. pulcherrima* L672); e) 793C (H. uvarum L793); and f) 793AF (co-culture of A. flavus M114 and H. uvarum L793). Samples were freeze-dried for extraction of the metabolites by a biphasic solid-liquid extraction, where two solvent mixtures were used: MTBE:methanol (3:1, v/v) and water:methanol (3:1, v/v). Only the polar fraction was analysed by LC-TWIMS-HRMS. Results showed the identification of the six metabolites in positive ionization mode in the batch AF, including AFB1, AFB2, AFM1, AFM2, sterigmatocystin, and O-methyl

sterigmatocystin. On the other hand, AFB1 and O-methyl sterigmatocystin were found in batch 672AF. None of these metabolites was found in batch L793AF. At the same time, non-targeted analyses were performed using the Progenesis software. So far, preliminary results show clear metabolomic differences between the control and the corresponding yeast co-cultures. Next steps will include the tentative identification of the chemical classes that vary between the sample groups. In addition, the effect of these yeasts on transcriptomics and metabolites involved in other parallel routes can be further investigated. These results highlight the necessity to understand the mode of action of biocontrol strategies to ensure their efficacy in controlling and preventing the development of toxigenic mould species.

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Comparative heat activation and inactivation of *Talaromyces trachyspermus* ascospores inside and outside ascocarps

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Heat-resistant moulds (HRM) are microorganisms known for their ability to endure high temperatures encountered during heat treatment [1,2]. HRM exhibit both an asexual phase, characterized by the production of non-heat-resistant spores called conidia, and a sexual phase, which results in the production of heat-resistant ascospores [3]. These ascospores are formed inside asci, many of which are enclosed in a large fruiting body called an ascocarp [4]. In their natural state, ascospores remain dormant and require activation by an external trigger, such as heat or high pressure, to germinate [5]. The spoilage caused by HRM is a major challenge for the fruit juice industry, resulting in considerable economic losses, and a key issue in this sector is the presence of Talaromyces trachyspermus.

The aim of this study was to investigate the influence of ascocarp structure on the thermal activation and inactivation of *T. trachyspermus* ascospores. Specifically, we compared the heat resistance of ascospores when they are still enclosed within ascocarps to heat resistance after being released.

The fruiting bodies of *T. trachyspermus* were produced on malt extract agar under different incubation periods of 2 months and 6 months at 25 °C. Thermal treatments of 80 °C and 85 °C for up to 15 minutes were carried out to determine the temperature required to activate or inhibit heat-resistant ascospores.

The results showed that mechanical disruption of ascocarps prior to heat treatment led to significantly higher activation of ascospores compared to when the ascocarps were only broken after thermal exposure. This suggests that the ascocarp structure may act as a protective barrier, reducing the effectiveness of heat activation when ascospores are still enclosed during treatment.

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The antifungal activity of lactobacilli against spoilage fungi in milk, bakery and vegetable matrices

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Presenter: Miloslava Kavková

The strains of three species of lactobacilli, *Lactiplantibacillus plantarum, L. pentosus,* and *L. sakei,* isolated from various environments, were characterised for their functional and technological properties. The primary focus was to select strains for protective purposes in milk and bakery products, as well as for application in protective biofilms for stored vegetables and legumes, considering current trends aimed at green-labelling food without the use of chemical preservatives. The characteristics included the detection of functional genes and their products, probiotic properties, exopolysaccharide production, the production of short-chain organic acids in various forms, and the inhibitory effect on mycelial growth and conidial germination of five species of *Fusarium* spp., ten species of *Aspergillus* spp., and *Cladosporium* spp. The inhibitory effect on fungal contaminants was tested both *in vitro* and in milk and sourdough matrices. The biofilms containing verified strains of lactobacilli were applied as a protective coating layer on vegetables and legumes to prevent infection by the fungal pathogens *Cladosporium cladosporioides, Botrytis cinerea,* and *Sclerotinia sclerotiorum*. The cell-free supernatants from lactobacilli suppressed or postponed the mycelial growth of all the tested fungi. The strains of lactobacilli that produced bacteriocins Class 2a, chitinases and chitin-binding protein (LACO CBP), and produced high amounts of organic acids, completely inhibited conidial germination and mycelial growth in artificial media, as well as in milk and flour matrices. The effective concentration of living cells was up to 10^6. mL^{-1}, or when 20-30% of the concentrated extracellular filtrate (≤30 kDa) from lactobacilli was added to the medium or food matrix. The factorial analysis revealed that the production of antifungal proteins and the forms of lactic and acetic acid can contribute to the antifungal activity of particular strains of lactobacilli. The production of organic acids with an inhibitory effect on fungal growth significantly varied due to factors such as microbial strains and matrices. The sensitivity of fungal species, particularly those of *Aspergillus* spp. and *Fusarium* spp., to extracellular products of lactobacilli is a significant factor in selecting the targeted protective microbial strain and its use. Strains of *Latilactobacillus sakei* isolated from legumes performed the protective effect of vegetables against spoilage fungi when applied in biofilms based on methylcellulose or sodium caseinate.

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SESSION 4: FUNGI FOR ALTERNATIVE PROTEINS AND FOOD FERMENTATION.

MEDDLING WITH MYCOPROTEIN - NOVEL STRAIN DEVELOPMENT OF *FUSARIUM VENENATUM*

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Presenter: Alex J. Pate

Fusarium venenatum is used by Quorn to produce mycoprotein, the main ingredient in its meat-alternative products. The current production strain (A3/5) has been in use for several decades and generates a product high in protein and fibre, and low in fat. There are nevertheless environmental and economic incentives to improve or replace A3/5 with a superior strain for mycoprotein production. However, there are barriers to altering the production process, such as the risk of mycotoxin production from using different sugar feedstocks, and the risk of increased accumulation of texture-distorting mutants if using different production isolates of *F. venenatum* (Whittaker et al, 2020). To address these issues, a global collection of F. venenatum strains has been established, and is being screened to first determine if a better candidate strain already exists. All strains were whole genome sequenced and then production of trichothecene mycotoxins on glucose or sucrose sugar feedstocks was compared using a Kluveromyces marxianus bioassay and HPLC analysis. In parallel, bioinformatic analysis of whole genome data was employed to investigate the conservation of genes responsible for mycotoxin production. Interestingly, there was evidence for a novel species closely related to but distinct from F. venenatum, with possible use in mycoprotein production. Conidiation rate, protein content, and hyphal branch length have also been evaluated between isolates as traits of interest due to their effects on the texture and quality of the industrial product. Secondly, attempts have been made to identify a sexual cycle as a possible method for generating new strains by outbreeding of compatible MAT1-1 and MAT1-2 isolates. Finally, complementary auxotrophs of A3/5 will be used to develop heterokaryons as potentially more stable production strains. As well as being of industrial benefit, these study areas present opportunities to learn more about the general biology of Fusarium species, and possible reasons for asexuality in ascomycete fungi.

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MYCOLOGICAL FERMENTATION OF PLANT-BASED SUBSTRATES FOR BLUE CHEESE ANALOGUE PRODUCTION

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Presenter: Kollia Eleni

The growing demand for sustainable and ethical alternatives to traditional dairy products has accelerated innovation in the development of plant-based cheese analogues. Among these, the formation of plant-based alternatives to mold-ripened dairy products, has proven to be an important challenge for the food industry (Fabiszewska et al., 2024). Especially the blue cheese analogues present a unique challenge due to their complex organoleptic characteristics, traditionally derived from microbial activity during ripening. In this study, Penicillium roqueforti isolated from traditional Roquefort cheese together with lactic acid bacteria, were employed to ferment cashew and tofu-based substrates for the development of blue cheese analogues.

FTIR analysis demonstrated that the plant-based cheese analogues exhibited spectral similarities to dairy Roquefort, particularly in regions associated with fatty acids and proteins, indicating successful lipolytic and proteolytic activity by *Penicillium roqueforti*, producing key compounds linked to blue cheese aroma/flavor. The titratable free acidity and pH values of plant-based blue cheese analogues were found to be similar to those of dairy Roquefort (26.87% acidity, pH 5.76), although some variation was observed. The cashew-based variants exhibited acidity values ranging from 19.19% (w/w) to 20.47% (w/w), with pH values between 5.06 and 5.25,

while tofu-based variant showed an acidity of 20.5% (w/w) and pH value of 6.36. In terms of fat content, dairy Roquefort exhibited a fat level of 38.81% (w/w), whereas the plant-based alternatives contained lower amounts. The cashew-based cheese ranged from 34.11% to 34.78% (w/w) fat, while the tofu-based cheese had a notably lower fat content of 14.45% (w/w). Moreover, image analysis further revealed that the blue vein percentages were 0.81%–1.10% for the cashew-based cheese analogues and 0.23% for the tofu-based cheese, reflecting the extent of the growth and sporulation of *Penicillium roqueforti* in cheese cavities.

These findings highlight the potential of plant-based substrates in replicating the complex characteristics of mold-ripened blue cheese, while also emphasizing the influence of substrate composition on fermentation dynamics.

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Fabiszewska, A., Wierzchowska, K., Dębkowska, I., Ślicznіak, W., Ziółkowska, M., Jasińska, K., Kobus, J., Nowak, D., & Zieniuk, B. (2024). Plant-Based Alternatives to Mold-Ripened Cheeses as an Innovation among Dairy Analogues. Foods, **13** (14), 2305.

Unravelling the enzymatic dynamics of mould-ripened Camembert and Brie Cheese

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Presenter: Asaph Kuria

The production of outer mould-ripened cheeses such as Camembert and Brie by the fungus Penicillium camemberti dates to at least the early 20th century. However, despite its economic significance, P. camemberti has not been widely studied and its mechanism of action with regards to cheese production is still unclear. Although it is widely accepted that enzymatic activity is the key factor for the ripening of cheese, the specific enzymes involved in this process are unknown. This study aims to bridge this knowledge gap by identifying and characterizing at a molecular genetic level the extracellular enzymes that are key for flavor, taste, and appearance of these cheeses. It is anticipated that this would help identify potential areas for strain improvement. To achieve this goal, parallel approaches have been used to identify candidate lipolytic and proteolytic genes of P. camemberti involved in cheese maturation. Firstly, bioinformatic analysis based on the genome of the FM013 strain identified 20 and 49 candidate extracellular lipolytic and proteolytic enzymes, respectively. Secondly, a combination of gene expression techniques, initially involving end-point RT-PCR, and a proteomics approach were used to further identify key enzymes. For lipolytic enzymes, five genes appeared to be primarily responsible for lipolysis and work is ongoing to further characterize these genes. A similar approach has been adopted for proteolytic enzymes, with qRT-PCR work ongoing to select candidate genes from a list of 18 genes already identified using end-point RT-PCR and proteomics techniques. Finally, profiling of metabolites from mutants, generated via CRISPR-Cas9 mediated genetic modification protocol, against wild-type strains will help identify genes involved in the ripening of Camembert and Brie cheeses.

Metabolite profile variability in *Penicillium roqueforti* populations: a footprint of ecological niche specialisation and domestication

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Presenter: Emmanuel Coton

Fungi are known to produce many chemically diversified metabolites, yet their ecological roles are not always fully understood. The blue cheese fungus Penicillium roqueforti thrives in different ecological niches and is known to produce a wide range of metabolites, including mycotoxins. To date, five P. roqueforti populations were identified using comparative genomics. Three, named Roquefort, non-Roquefort and Termignon, are associated with blue cheeses, with two exhibiting domestication footprints. The other two are associated with silage and lumber/spoiled food. In this study, we looked for differences in targeted and untargeted metabolite

production profiles between populations using HR-LC-Q-TOF. The non-cheese populations produced several fatty acids and different terpenoids, lacking in cheese strains potentially due to the maintenance of metabolite diversity, which is likely important in these ecological niches. The Termignon cheese population displayed intermediate metabolite profiles between cheese and non-cheese populations, as previously shown for other traits. The non-Roquefort cheese population with the strongest domestication syndrome, produced the lowest quantities of measured metabolites, including mycophenolic acid and PR toxin due to mutations in key biosynthetic genes. In the Roquefort population, we detected no PR toxin nor eremofortin intermediates, but found no indel or frameshift mutation, suggesting downregulation. More recently, we have focused on studying the patulin biosynthetic gene cluster (BGC) and identified an unexpected number of genetic organizations. However, regardless of organization, no P. roqueforti strains produced patulin in the tested conditions. Variability in secondary metabolite BCGs provides great grounds to understand how this species has adapted to a wide range of ecological niches including through domestication.

SESSION 5: ECOLOGICAL INSIGHTS INTO FUNGAL COMMUNITIES AND MYCOTOXIN FORMATION IN FOOD.

Aspergillus Section *Nigri* and ochratoxin A accumulation in raisins: A comparative study of drying systems

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Presenter: María L. Ramirez

Ochratoxin A (OTA) is a nephrotoxic and carcinogenic mycotoxin (IARC Group 2B) commonly found in grapes and by-products. In Argentina, raisin production is concentrated in the Cuyo region, where intense solar radiation and low rainfall allow for sun-drying of raisins using three main methods: 1) gravel drying yard, 2) on elevated mesh trays and 3) dry on-vine (DOV). Understanding the relationship between drying techniques and mycotoxin levels is crucial for ensuring food safety and maintaining the quality of raisins produced in this region. The objectives of the present study were to evaluate the natural occurrence of OTA-producing fungi and to identify critical stages of OTA accumulation across the different drying methods used. Samples of Flame Seedless grapes were collected from five vineyards in San Juan at four stages: during dehydration (M1–M3) and storage (M4). Moisture, water activity (aW), Aspergillus section Nigri incidence, and OTA levels were measured. Results showed high fungal presence throughout drying, with A. section Nigri dominating. A. carbonarius, the primary OTA producer, was especially prevalent in DOV samples during all drying period. OTA was detected in 35% of all samples (16.4–1382.9 ppb), exceeding the legal limit (10 ppb). The highest OTA levels were found during the storage stage independently of the drying method used, where 100% of samples were contaminated. Slow dehydration, particularly in the DOV method, correlated with higher OTA levels, likely due to gradual reductions in aW and favourable conditions for fungal growth. Furthermore, this study highlights storage as a critical control point for OTA contamination. To mitigate the risk of OTA accumulation, it is essential to implement stringent monitoring and control measures during both the drying and storage phases. Future research should focus on optimizing drying techniques and exploring alternative storage solutions.4.10 Andrea Patriarca, Cranfield University, UK

Ecophysiology of Alternaria strains from tomato producing AAL toxins

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Presenter: Andrea Patriarca

The production of host-specific toxins, such as AAL (A. alternata f. sp. lycopersici) toxins, has been suggested as a taxonomic criterion for distinguishing different pathotypes of A. alternata. However, AAL-producing strains are rare in nature. Although the tomato pathotype, which causes tomato stem canker, has been isolated from fruits, its full toxigenic capacity has not yet been evaluated.

In this study, the role of temperature in the differential growth and mycotoxin production of AAL-producing Alternaria species isolated from tomato fruit was investigated. Two wild strains, A. arborescens and A. tomaticola, along with a reference strain of A. arborescens (EGS 39128) were studied. These strains were grown on tomato pulp agar at four different temperatures: 12, 25, 30, and 35 °C. The radial growth rate was measured and the following Alternaria toxins were quantified: AOH, AME, TeA, ALT, TEN, ATX-I, ATX-II, AAL-TA, and AAL-TB.

The results showed that the optimal temperature for growth for all three strains was between 25 and 30 °C, although they were all able to grow across the entire temperature range. The maximum toxin production occurred at 30 °C, but the relationship between temperature, strain, and toxin production was complex and dependent on

both factors. AAL-TA was produced by all three strains at every temperature evaluated, while only the wild type A. tomaticola and the reference strain were able to produce AAL-TB. These findings highlight the potential for contamination of tomato products with both AAL toxins and non-host-specific Alternaria toxins.

Beyond the flavor: assessing the risks and rewards of Brazilian artisanal cheese

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Presenter: Marta Taniwaki

Cheese provides a favorable environment for fungal growth. While some fungal species contribute significantly to cheese flavor development, others can produce harmful mycotoxins. Artisanal cheeses are particularly vulnerable because they are predominantly made with raw milk and involve more basic and manual processes, which give them variations in sensory profile and chemical composition typically linked to the local terroir. This study examined the fungal biodiversity in 130 Brazilian artisanal cheeses with natural moldy rinds at various maturation stages, along with the prevalence of toxigenic fungi and mycotoxins. We isolated 741 molds, with the most common genera being *Aspergillus* (22%), *Penicillium* (19%), and *Geotrichum* (19%), followed by *Cladosporium* (13%) and a smaller group including *Trichoderma, Phoma, Fusarium,* and dematiaceous fungi. Molecular analysis identified the most frequent *Penicillium* species as *P. biforme, P. copticola, P. brevicompactum,* and *P. terrigeneum.* Among *Aspergillus* species, we detected ochratoxin-producing (*Aspergillus westerdijkiae, Aspergillus ostianus,* and *Aspergillus steynii*) and sterigmatocystin-producing (A. amoenus, A. hongkongensis, and A. tennesseensis) species. Ochratoxin A (OTA) was found in 22% of the samples, and sterigmatocystin (STC) in 6%. These findings highlight the critical need for consistent surveillance and stringent quality assurance practices in artisanal cheese production to minimize consumer exposure to mycotoxins. Given the significant cultural and economic importance of Brazilian artisanal cheese production, dedicated efforts are essential to ensure its microbiological safety. This requires a thorough understanding of fungal biodiversity and the potential for mycotoxin contamination.

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Competitiveness study among black aspergilli strains

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Presenter: Julia Marques

Many studies have shown that A. carbonarius is the main responsible source of ochratoxin A (OTA) in wine and dried vine. OTA is produced during the infection of grapes in vineyards, mostly from the veraison to harvest. These black aspergilli live as saprophytes in vineyard soil, and it has been described that air movement deposits spores from the soil onto the grape berry surface, thus the risk of contamination with OTA in wines might be related to the presence of toxigenic strains in the soil. To reduce OTA in grapes, non-ochratoxigenic strains of black aspergilli could be used in vineyards to compete with naturally toxigenic A. carbonarius strains present in the field. There are few studies on the in vitro competition between A. carbonarius and non-ochratoxigenic black aspergilli and most of them were done by co-culturing strains to be tested on solid media. These methods are time-consuming and are not suitable when many isolates must be screened. In the present study, we developed a method to study competitiveness among black aspergilli strains using microtiter plates. An ochratoxigenic strain of A. carbonarius and five non-ochratoxigenic strains of A. uvarum were co-cultured in microtiter plates at 50:50 ratio. Their competitiveness was evaluated by qPCR and OTA production was assessed by HPLC. Results showed that variation in competitive ability among strains of A. uvarum was strain dependent. One strain was able to outcompete to A. carbonarius, reducing both the percentage of A. carbonarius population and OTA production. The rest of the strains showed a low or no competitiveness compared to A. carbonarius strain, and the reduction of OTA level was low and, in some cases, an increase on OTA was observed. Thus, this method allows for proper

screening of the competitiveness of non-ochratoxigenic strains and can be applied in biocontrol research for easily quantifying fungal population and OTA reduction.

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Exploring the mycobiota and mycotoxin contamination in traditional Iranian foods

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Presenter: Mahshid Saedi

Fungi can grow in foods and commodities worldwide, leading to the production of secondary metabolites (SM) in these substrates. Mycotoxins are toxic SM, and contamination in agricultural and food products poses a serious risk to human health, livestock and the environment. In the current study, we aim to investigate the associated mycobiota of traditional foods, nuts and dried fruits originating from the Kurdistan Province in Iran, and analyse the presence of important mycotoxins in these substrates and selected isolated strains. Sampling was conducted in nine cities during the summer and autumn of 2022, and the mycological analysis resulted in a large fungal strain collection. Morphological examination of the strains showed that the majority of isolated fungi belonged to *Alternaria, Aspergillus, Penicillium* and *Rhizopus*. A large selection of strains was identified to species level using a molecular-based identification approach, which involved sequencing various barcode genes (e.g., BenA, CaM, GPDH, ITS and RPB2). Mycotoxin analysis of a representative selection of substrates, and determination of metabolite profiles from the isolated fungi, were performed using LC-MS/MS. This study investigates the mycobiota and mycotoxins in diverse substrates from Iran, and discusses the obtained data in relation to their prevalence and potential health concerns.

Ecological niche shapes fungal communities from vine to wine and impacts FMA detection in wine

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Presenter: Marie Belair

In viticulture, grape berry quality is highly influenced by the pedoclimatic conditions in vineyards but also by the microbial communities colonizing the berry surface during ripening and persisting up to wine making. To date, grape microbiota from French eastern region vineyards, especially mycobiota, has not been fully explored and could impact wine quality. More specifically, fungal interactions and their metabolic activities potentially contributing to off-odor defects like fresh mushroom aroma (FMA) in wines have yet to be fully understood.

In this context, we first focused on deciphering microbiota diversity and dynamics at five key stages from vine to wine using a dual cultural and metabarcoding strategy. To do so, 30 vineyards were sampled at fruit set, veraison and harvest stages during three successive years (2021-2023) as well as in the resulting musts and still wines. Species interactions (molds, yeasts and bacteria) were also determined using co-occurrence networks. This data was also compared to sensorial analyses of still wines as well as with climatic, soil and growing practices; the goal was to determine to what extent fungal composition and microbial interactions led to the FMA defect in wines. We clearly observed distinct shifts in mycobiota composition from vine to wine with berries mainly dominated by yeasts (i.e. *Aureobasidium pullulans, Vishniacozyma* spp.) followed by a progressive increase in mold diversity. *Cladosporium* spp. were more abundant in unmature berries before other molds colonized grapes, especially *Penicillium* spp. (15 different species) and *Botrytis cinerea*. We specifically observed Penicillium counts at significantly higher levels in musts leading to the FMA defect in corresponding wines. Co-occurrence networks also pinpointed positive interactions between fungal species (e.g. *Vishniacozyma, Cladosporium, Penicillium* and *Botrytis*) and negative interactions between Penicillium and bacterial species (e.g.

Acetobacter and Gluconobacter).

We are now focusing on screening individual or mixed cultures of isolates for their capacity to produce FMA off-odors in conditions mimicking grapes and musts. So far, *Penicillium* species tend to be more associated with FMA, while *Cladosporium* species are associated with fruity aroma. These microbial interaction studies will provide in-depth knowledge about fungal and bacterial species associated with FMA defect in wines.

Mycotoxin production by Penicillium species during refrigerated storage of plant-based analogues of cheese, fraiche and pâté.

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Presenter: Su-lin L. Leong

Vegetarian substitutes for dairy and meat products have a different composition and nutritional profile than the traditional products. If the products become mouldy at consumer level, this could mean differences in which mycotoxins are formed, as well as in what concentrations and how they spread. To test this hypothesis, strains of Penicillium used in previous publications were single point-inoculated on the surface of dairy light crème fraiche vs oat-based fraiche, Gouda cheese vs Coconut-oil based cheese-analogue, and pork liver pâté vs plant-based pâté and stored at 8°C for 14 days (fraiche and pâté) or 21 days (cheese/analogues). At the end of incubation, samples were taken with a cork-borer and analysed for mycotoxins and secondary metabolites by a LC-MS multi-analyte method at the mould spot, and in two layers under the mould spot.

Overall, differences in metabolite production between matrices were small. The production patterns varied for different metabolites with some being higher in plant-based products than in traditional products and vice versa. For example, P. crustosum produced slightly more penitrem A in animal-based than in plant-based substrates but made more roquefortine C in plant pâté than in liver pâté. Andrastine A produced by P. expansum and P. roqueforti tended to be higher in plant-based than in animal-based substrates. Patulin was the mycotoxin produced in overall highest levels, with greater production in plant-fraiche and plant pâté than in the original animal-based products .

Another significant mycotoxin, Ochratoxin A, was produced by P. verrucosum but only in animal cheese and pâté in the time frames tested and not in their plant-based equivalents. For the plant-based cheese, we notably had chosen a formulation with zero protein and the mould had barely grown after 21 d at 8°C.

We chose 8°C as the incubation temperature as this is the maximum storage temperature recommended by the manufacturer. However, it should be pointed out that our extended storage times are longer than the manufacturers' recommendations that pâté and plant-based cheese analogues should be consumed within 5-7 days of opening.

In future experiments, we will perform inoculation on a larger variety of products with varying nutritional profiles and incubate the samples for longer periods/different temperatures. Hopefully, this will further elucidate metabolite production patterns and underlying causes.

SESSION 6: GUIDELINES AND NEW INSIGHTS IN THE IDENTIFICATION OF MYCOTOXIGENIC FUNGI

Morphological and molecular characterisation of *Alternaria alternata* from tomato Lycopersicon esculentum fruit

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Tomato, *Lycopersicon* esculentum (L.) abundantly grown in tropical regions of the world, is the main source of the nutrient. Alternaria is the main destructive agent responsible for black rot disease in tomato fruit. This study aimed to isolate, identify, and characterization of *Alternaria alternata* from tomato fruit. The Black-spotted tomatoes were collected from local markets of Tandojam, Kotri, Jamshoro, and Hyderabad, were brought to Mycology & Plant pathology laboratory at the institute of plant sciences at the University of Sindh, Jamshoro. The infested tomatoes were surface sterilized with sodium hypochlorite and then inoculated in Petri dishes by following the standard agar plate method and DNA was extracted by CTAB method, PCR and ITS region sequencing were outsourced and sequences were analyzed on MEGA 5. Six fungal species namely; Alternaria alternata, *Colletotrichum* spp., *Fusarium oxysporum*, *Drechslera* spp., *Bipolarus oryzae* and *Curvularia lunata* were also isolated and identified. Amongst all species, *A. alternata* was frequently observed. The morphological characters were examined after six days of culturing; the measurement of *A. alternata* colony was taken. The highest to lowest growth was recorded at 9.2cm and 4.8 cm and the width was counted at 9.0 cm and 4.9 cm with circular growth patterns. From the lower side of Petri dishes, isolates seemed brown, dark brown, and brown to black. The isolates were observed in dark green, olive green, gray-green, and lettuce green around with circular white colour margins and on the surface region of the Petri dishes found with variation in color patterns. The surface texture contains white granulated crystals that vary in shape. The microscopic characters showed cross-walled (septate) hyphae. The hyaline conidiophores are frequently observed individually but in small groups in simple and straight forms. Similarly, the conidia were found with oval, obclavate, and obpyriform shapes. The golden to dark brown conidia contains longitudinally septa which measured 2 to 7 transverse walls with oblique and longitudinal sections. The highest and lowest; length/width values of *A. alternata* conidia diameter were also measured through an ocular micrometer. The mean value of low length from A. alternata isolates was found from 15±1µm to 11.25±1.7µm however; mean value of low width from isolates was found from 7.9±0.8µm to 6.5±0.6µm. The mean values of high length of conidia measured from 32.5±3.7 to 29.4±µm. Moreover, the mean value of high width was recorded from 13.75±0.8µm to 11.88±1.2µm. One isolate was selected from Tandojam, Kotri, and Hyderabad for sequencing of ITS region for appropriate identification at the molecular level, the results revealed that the isolate of Tandojam, Kotri, and Hyderabad contains 754 bps, 570 bps, and 1277 bps of nucleotides, respectively. Sequences of isolates were blasted on NCBI, which confirmed that all three isolates belonging to *A. alternata* as they shared 98-99% similarity with already reported isolates of the same species, the phylogenetic analysis resulted from that isolate of Tandojam was similar to the isolate of *A. alternata* KY949585.1, the isolate of Kotri resembled with an isolate of *A. alternata* KX073995.1 and the isolate of Hyderabad sheared maximum characters with MH553296.1. The generated results of current studies will help to prevent the misidentification of *Alternaria* species by utilizing the results; as well these documented results also help to develop strategic measures to control this threatening pathogen of fruit rot of tomato disease

Chemistry and morphology are excellent for separating *Aspergillus oryzae* and *Aspergillus flavus*, but difficult to achieve using genome sequencing

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Chemistry and morphology are excellent for separating *Aspergillus oryzae* and *Aspergillus flavus*, but separation difficult to achieve using genome sequencing. Thom and Church (1927) described the *Aspergillus flavus-oryzae* group and mentioned, as representatives for *A. oryzae* several isolates from koji and other fermented foods (mostly from Takamine in Japan) but first mentioned a strain from an undetermined source (NRRL 447)

as representative of A. oryzae and later suggested this strain as the basis for the neotype of that species. The problem with NRRL 447 is that it produces sclerotia and is probably not domesticated. The raison d'etre for Aspergillus oryzae as a taxonomic name is that it should be domesticated, else it could just be called *A. flavus*. *A. oryzae* and A. *flavus* have very similar genomes, so phenotypic methods must be used to distinguished the two. *A. oryzae* can be distinguished (from *A. flavus*) by larger more smooth conidia, longer conidiophore stipes, more floccose colonies, a more brownish yellow green conidium colour en masse, non-production of aflatoxins, non-production of flavimin, non-production of aspergillic acid, non-production of sclerotia (and sclerotial specialized metabolites (SMs)), so on balance the first genome sequenced strain of *A. oryzae* RIB 40 is rather an A. flavus. However many other SMs have been reported from both taxa, and results will be presented on other SMs reported, whether they are produced by both species, including aflatrems, aflavarins, aflavinines, aspergillomarasmin, asperopterins, aspirochlorins, asperfuran, CSY-pyrones, flavucides, kojic acids, kojistatins, maltoryzin, miyakamides, oryzaeins, oryzins, parasiticolides, penicillin, sporogen AO1, ustilaginoidins, ustiloxin, and most importantly the mycotoxins aflatoxins (and sterigmatocystins), cyclopiazonic acid and 3-nitropropionic acid.

An update on *Aspergillus*, *Penicillium* and *Talaromyces* taxonomy

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Presenter: Jos Houbraken

Fungal spoilage and mycotoxin contamination present significant challenges to global food safety, security, and public health. Accurate identification of species causing spoilage and mycotoxin contamination is essential for effective communication and understanding of their unique properties, including resistance to antifungals, mycotoxin production, and enzyme profiles. Specific species are linked to certain food and feedstuffs due to biotic and abiotic factors, forming distinct associated mycobiota. Although the mycobiota of various foods is well-studied, new associations and species are occasionally observed, highlighting the need for taxonomic and ecological studies of food and feed.

Aspergillus and Penicillium encompass well-known food spoilage species and mycotoxin producers. Recent insights into these genera have led to the introduction of numerous new species and name changes. While species delimitation in Penicillium appears clear-cut without intergrading strains, recent studies in Aspergillus have shown increased variability, resulting in more robust species boundaries. Some Aspergillus and Penicillium species used in food fermentation processes are closely related to mycotoxin producers. For example, Aspergillus oryzae and A. sojae are typical industrial molds, and their wild counterparts (A. flavus, A. parasiticus) can produce aflatoxins. The presence of these fungi in natural fermentations can thus be concerning.

This abstract emphasizes the importance of continued studies on the biodiversity and (chemo)taxonomy of food-associated fungi to enhance our understanding of mycotoxigenic fungi and mitigate food safety risks.

Barcoding *Aspergillus*, *Penicillium* and *Talaromyces* strains from the CBS biobank

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Presenter: Ya Bin Zhou

The genera Aspergillus, Penicillium, and Talaromyces within the order Eurotiales are among the most economically and scientifically significant fungi, playing crucial roles in ecosystems, industry, biotechnology, and medicine (Houbraken et al., 2020). Accurate species identification within these genera is essential for various applications, yet traditional morphological methods often lack resolution due to phenotypic plasticity. DNA barcoding has emerged as a powerful tool for fungal taxonomy. This study applied DNA barcoding to a comprehensive set of Aspergillus, Penicillium, and Talaromyces strains from the CBS biobank. Genomic DNA was extracted from cultures, and targeted loci were amplified and sequenced. Phylogenetic analyses using Maximum Likelihood and Bayesian inference approaches were conducted to refine species boundaries. Morphological analyses were performed on selected new species strains to complement molecular data. A total of 1,989 Aspergillus, 2,891

Penicillium, and 497 Talaromyces strains were examined, leading to the accurate identification of known species and the discovery of numerous cryptic taxa. In total, 113 strains belonging to 64 new species are identified and described in this study. The expanded DNA barcode dataset enhances our understanding of fungal diversity and evolution, providing a valuable resource for mycological research. The study highlights the continued need for comprehensive sequencing efforts to refine fungal taxonomy and support various biotechnological and medical applications.

Reference:

Houbraken J, Kocsubé S, Visagie CM, Yilmaz N, Wang XC, Meijer M, Kraak B, Hubka V, Bensch K, Samson RA, Frisvad JC (2020). Classification of Aspergillus, Penicillium, Talaromyces and related genera (Eurotiales): An overview of families, genera, subgenera, sections, series and species. Studies in Mycology 95: 5-169.

Penicillium section *Brevicompacta*: new insights in taxonomy

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***Presenter: Ioanna Pyrri, ipyrri@biol.uoa.gr**

Penicillium section *Brevicompacta* includes species with compact conidiophores of multiramulate mainly penicilli with long stipes. Species are slowly growing and able to develop both at very low water activities and temperatures and exhibit small phenotypic differentiation. Currently the section includes 11 species distributed in four series, *Brevicompacta, Buchwaldiorum, Olsoniorum* and *Tularensia* (Houbraken et al. 2020). Most species have a worldwide distribution, from the tropics to the arctics and, on diverse habitats. They are common in soil and on food commodities. *Penicillium brevicompactum* is ubiquitous, frequently isolated from indoor environments, food, feed, plants, soil, etc. Species in this section produce extrolites like mycophenolic acid and raistrick phenols from representatives in series *Brevicompacta*, or asperphenamate from almost all species of the section (Frisvad et al. 2013). The present study aims to produce an overview of section *Brevicompacta* and update its taxonomy with the implementation of a polyphasic approach. Detailed study of the morphological features, the phylogenies of four genetic markers, partial ß-tubulin, calmodulin, RNA polymerase II subunit 2 and ITS as well as the extrolites produced have resulted in the delimitation of four new species.

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SESSION 7: METHODOLOGY DEVELOPMENT

Whole genome sequencing of *Penicillium* spoilage mould from food producers

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Presenter: Laura García-Calvo

Mould spoilage has significant economic consequences for the producers of cheese, dry cured meat and bakery products and leads to food waste. Nofima and the Norwegian Veterinary Institute have in previous projects worked with Norwegian industries to identify spoilage moulds and contamination sources. However, in order to understand why the mould issue increases periodically, further knowledge of the spreading of problem strains and efficient reduction technologies are needed.

The recently initiated MouldReduce project will perform sampling in production facilities, and spoilage moulds from facilities, products, and previous studies will be differentiated at the strain level using whole genome sequencing (WGS). The resulting data will be used for phylogenetic analysis to give insights into how the strains spread. The mould isolates collected during previous projects and in the context of MouldReduce will constitute a unique strain collection that can be used to gain a deeper understanding of contamination routes. Moreover, knowledge on genotypic and phenotypic characteristics of these moulds will give valuable insights for development of better control strategies. The dominant strains responsible for product contamination will be characterised. Suitable technologies for prevention and reduction of mould spores will be tested in a pilot plant and in production facilities.

Knowledge generated in MouldReduce will be used to develop guidelines for food producers for prevention and control of moulds, and to reduce food waste. Initial results from WGS of P. commune show that the same strains may persist in production environments for extended periods of time.

Development of a droplet digital PCR assay for population study of ochratoxigenic and non- ochratoxigenic *Aspergillus carbonarius* strains

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Presenter: Kaitlyn Parra

Aspergillus carbonarius is the main responsible source of ochratoxin A (OTA) in wine or dried vine fruits from main viticultural regions worldwide. The percentages of ochratoxigenic isolates cited in this species are usually high and achieve 100% in some studies. Hence, atoxigenic isolates of *A. carbonarius* are very rarely found in natural environments. In previous studies, we found three atypical and unique non-OTA-producing strains of *A. carbonarius* showing a mutation in the AcOTApks gene which was sufficient to prevent OTA production. Moreover, one of these strains was able to reduce OTA production when co-inoculated with an ochratoxigenic strain of A. carbonarius. In the present study, we developed a ddPCR assay that simultaneously enables the specific detection and quantification of the non-ochratoxigenic as well as ochratoxigenic A. carbonarius strains. Primers and probes were designed using the sequence of the acyltransferase domain of AcOTApks gene. In vitro interactions were conducted between an ochratoxigenic strain of *A. carbonarius* and these non-ochratoxigenic strains to evaluate their competitiveness and potential for controlling OTA production. Different mixed spore suspensions of OTA-producing strain and non-OTA-producing strain ratios were inoculated in Czapek Yeast broth. After incubation, genomic equivalent ratios were determined by ddPCR and OTA production was measured by HPLC. Variation in competitive ability among different strains influenced the population reduction of the ochratoxigenic strain by the non-ochratoxigenic strains. In general, non-ochratoxigenic strains had higher competitiveness compared to the ochratoxigenic strain, as indicated by higher final genomic equivalent ratios of these strains compared to the spore ratios used for inoculation. Furthermore, the three non-ochratoxigenic strains were able to decrease OTA production when were co-inoculated with *A. carbonarius*, although the percentage

of OTA reduction depended on the strain assayed. This method allows for rapid and accurate determination of population sizes of both ochratoxigenic and non-ochratoxigenic strains and can be applied in biocontrol research.

This research was supported by the Ministerio de Economía y Competitividad of the Spanish Government (PID2020-116152RB-I00).

Challenges in sample preparation of *Alternaria, Cladosporium* and *Fusarium* species for MALDI TOF analyses

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Presenter: Manuela Zadravec

This study evaluated the impact of various sample preparation methods on the identification accuracy of three filamentous fungal species—*Alternaria alternata, Cladosporium cladosporioides,* and *Fusarium verticillioides*—using MALDI-TOF MS. Three isolates per species were cultivated on solid (DG 18 agar) and liquid media (Sabouraud broth and buffered peptone water). Solid media samples were subjected to direct formic acid method and additional treatments, including mechanical disruption using ceramic or metal beads, heat treatment at 65 °C, and at -80 °C. Liquid media samples underwent standard protein extraction protocols. MALDI-TOF MS analysis was conducted on two spots per isolate, and spectra were matched against the Bruker Biotyper and an in-house database. To enhance identification outcomes, a modified protocol incorporating increased laser intensity was also assessed. Using the manufacturer's standard scanning method, no species-level identifications were achieved, and only one *Fusarium* isolate was identified at the genus level following processing with ceramic beads. In contrast, the modified high-intensity laser protocol significantly improved results. For *Alternaria*, the highest identification rate (100%) was achieved using solid media with metal beads, followed by 67% using the direct formic acid method, 50% with buffered peptone water, 33% with ceramic beads, and 17% with Sabouraud broth. For *Fusarium*, the direct formic acid method yielded 50% success, with reduced performance from Sabouraud broth (33%) and peptone water (17%). *Cladosporium* could not be identified to the species level, though genus-level identification was most successful with peptone water (50%). Freezing, cooking, or their combination yielded no successful identifications. These findings emphasize the importance of optimized sample preparation in fungal MALDI-TOF MS diagnostics.

Reference:

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Hyperspectral imaging for early fungal detection and prediction of mycotoxins in apples

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Mouldy core (MC) is a common post-harvest fungal disease in apples caused primarily by Alternaria tenuissima. This pathogen produces various mycotoxins, posing health risks, especially in processed apple products. Detecting MC is challenging due to the lack of visible external symptoms. The objective of this study was to detect MC using non-invasive reflectance hyperspectral imaging and assess its link to mycotoxin accumulation.

A total of 111 organic Golden Delicious apples were used, with 60 artificially infected in the core with an Alternaria tenuissima spore suspension and 51 serving as controls. The apples were incubated at 21 °C and hyperspectral images using the Specim FX10 and FX17 hyperspectral cameras were taken at different time points within 35 days. Spectral data were processed with perClass Mira, and mean spectra were extracted. At 20 days post-infection (dpi), half of the apples were cut open for MC inspection, and six Alternaria mycotoxins were

quantified. The remaining apples were incubated until 35 dpi for further imaging, MC assessment, and mycotoxin analysis.

At 20 dpi, 12% of infected apples showed visible MC symptoms, increasing to 54% by 35 dpi. However, hyperspectral imaging detected spectral differences as early as 5 dpi. By 20 dpi, mycotoxins—predominantly alternariol (AOH) and alternariol monomethyl ether (AME)—were already present, suggesting a role in fungal colonization. Differences in mean spectra of the groups, were dominantly observed at 500 – 700 nm range, where the ripening of the fruit can be followed due to chlorophyll breakdown. This difference increased over time, with the average mean reflectance spectra of the artificially infected apples being lower than the controls. Therefore, the infected apples were ripening slower, which could be caused by MC development and possibly mycotoxin production. These findings demonstrate the potential of hyperspectral imaging for early, non-invasive detection of MC and associated mycotoxin contamination, offering a promising tool for improving apple quality control and food safety.

ABSTRACT POSTERS

Study of *Alternaria alternata* on tomato agar by VOCs, mycotoxin and metabolomic analysis

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Presenter: Alberto Martín

Alternaria alternata is a phytopathogenic mould that primarily affects tomato crops, producing the disease known as black mould. It is a highly ubiquitous species capable of producing mycotoxins, including alternariol (AOH), altenuene (ALT), alternariol methyl ether (AME), altertoxin-I (ATX-I), and tenuazonic acid (TA). The presence of these hazardous compounds in tomatoes results in economic losses and a risk to consumer health, making it necessary to search for a control method. This study examined the volatile organic compounds (VOCs) and mycotoxins that *A. alternata* produced during its growth. For this, a tomato agar culture medium was prepared, simulating the food matrix. The mould was inoculated at a concentration of 10 CFU/mL and incubated at 25ºC. Sampling was carried out at days 0, 3, 4, 5, and 7. The VOCs were analysed using a prior extraction with HS-SPME before quantification and identification through a gas chromatograph coupled to a mass detector (GC/MS). On the other hand, the determination of mycotoxins was performed using triple quadrupole (LC-QqQ) . In addition, a metabolomic analysis was performed using LC-TWIMS-HRMS using the Acquity I-Class UPLC coupled to a Vion IMS QTOF. Results showed that a total of 36 VOCs were obtained, with differences found between the non-inoculated tomato agar and the batches inoculated with *A. alternata*. Regarding the mycotoxins, the largest quantities of AOH, AME, ALT and ATX-I were found on the 7th day of sampling. Finally, the metabolomic analysis shows a variability of the compounds throughout the different incubation times, showing a similar trend to those obtained using LC-QqQ. These findings may contribute to the scientific foundation for the early identification of *A. alternata* in tomatoes, hence preventing the development of mycotoxins.

Acknowledgements

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Isolation of filamentous fungi from beans, maize and peanuts from Cuanza Sul, Angola

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Presenter: Bruna Sepúlveda

Mycotoxins are toxic secondary metabolites produced by certain filamentous fungi, posing serious health risks to humans and animals through contaminated food products. This study aimed to isolate and identify fungi and detect mycotoxins from beans, maize, and peanuts in Cuanza Sul, Angola. Ten samples of each commodity were collected in 2023, and twenty-five kernels from each sample were surface-disinfected and plated on Dichloran Rose-Bengal Chloramphenicol (DRBC) medium. Fungal colonies were isolated after 5 to 7 days at 25 ºC. DNA

extraction was carried out to proceed with molecular identification through sequencing of the ITS region.

Preliminary results revealed diverse fungal communities across all food commodities, including presumptive species from the *Aspergillus* and *Penicillium* genera—both commonly associated with mycotoxin production (Greeff-Laubscher et. al., 2019). Fungal contamination was observed in 75.2% of maize kernels (188/250), 64.4% of peanut kernels (161/250), and 36.4% of bean kernels (91/250), based on the subsamples where counts were completed. A total of 97 fungal isolates were obtained from maize, 162 from peanuts, and 81 from beans. These isolates are currently being preserved and prepared for molecular identification and future assessment of their toxigenic potential.

These findings highlight a high incidence of fungal contamination in staple foods from Cuanza do Sul, contributing to a better understanding of food safety challenges in Angola and other tropical regions.

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Ability of selected plant essential oils to inhibit cyclopiazonic acid production by *Penicillium commune* strains

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Presenter: Dana Tančinová

Essential oils are natural substances with potential antimicrobial properties, and some also have the ability to suppress mycotoxin production. Penicillium commune is frequently involved in cheese spoilage. The aim of this research was to test the ability of selected essential oils to affect the production of cyclopianozonic acid (CPA) by P. commune strains. The tested strains (6) of P. commune were isolated from mouldy cheeses. The effect of selected plant essential oils on the growth of individual P. commune strains under in vitro conditions was tested by gaseous diffusion. Czapek yeast agar (CYA) was used for analysis in three 90 mm diameter two-section Petri dishes. The test strains were single-point inoculated into each Petri dish section. A 60 mm filter paper was placed in the center of the Petri dish lid. Then, 50 µl (625 µl.l-1 air) of a specific plant essential oil onto was pipetted onto the filter paper. For the control samples, 50 µl of distilled water was applied to the filter paper instead of essential oil. All analyses were performed in triplicate. The Petri dishes were carefully sealed with parafilm and incubated for 14 days in the dark, in a thermostat at 25 ± 1 °C. After 14 days of cultivation, three 1 x 1 cm squares were cut out of each Petri dish using a lancet, including the grown colony and the corresponding culture medium (six replicates). The excised sections were placed in 1.5 ml microtubes. Subsequently, 500 µl of extraction reagent (chloroform:methanol, 2:1 ratio) was added, and the tubes were mixed in Vortex for two minutes. After mixing, 30 - 50 µl of the liquid phase was removed and applied to a chromatography plate along with the CPA standard. The plate was placed in a developing system consisting of toluene, ethyl acetate and formic acid in a 5:3:1 ratio. After the development was completed, CPA was visualized on the chromatographic plate in daylight after applying Ehrlich reagent and heating the chromatographic plate to 130 ºC for about 8 minutes, appearing as a purple spot with a tail. According to the inhibitory effect on CPA production by P. commune strains, the tested essential oils were ranked as follows: Eucalyptus globulus (100 %) = Melaleuca quinquenervia (100 %) = Ocimum basilicum (100 %) = Salvia officinalis (100 %) Mentha citrata (77.78 %) Rosmarinus officinalis (66.67 %) Cajeput aetheroleum (38.89 %) Pimpinella anisum (16.67 %) Laurus nobilis (13.89 %) Foeniculum vulgare (8.33 %).

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Influence of reduced water activity on *Monascus ruber* heat- and sorbate-resistance

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Presenter: Elettra Berni

The spoilage of pasteurized vegetable products mainly occurs by yeasts and filamentous fungi due either to a re-contamination of the finished product or to the presence of fungal ascospores in raw materials, surviving to pasteurization treatments. If ascospore-forming fungi like those with Neosartorya morphs have been for a long time associated with spoilage of various heat-treated acid products, those with Monascus morphs have gained an increasing interest just recently, due to the increasing number of spoilage cases in heated foods different from olives (e.g. vegetable pates and purees). The objective of the present study was to assess the sorbate-resistance and the heat-resistance of three strains of the ascospore-forming fungus Monascus ruber. Tests were carried out in a control medium and, in the case of heat-resistance, also in a spreadable vegetable pesto, in order to assess the influence of reduced water activity (aw) on the above-mentioned parameter.

Sorbate-resistance tests (pH 3.5-4.5): M. ruber strains succeeded to grow in broths added with 100 ppm of sorbic acid, whereas higher concentrations resulted in an inhibiting (200-300 ppm) or inactivating (400+ppm) effect regardless of the pH considered. When 100 ppm of sorbic acid were tested, as the pH decreased the time needed for growth increased, ranging from 7 (pH 4.5) to 18-35 (pH 3.5) days.

Inactivation tests: M. ruber ascospores in a tomato-based pesto showed significantly higher D_T-values (D_{75}=38.1'; D_{80}=4.9'; D_{85}=0.5') than those obtained in two different control solutions (e.g. D_{75}=16.0'; D_{80}=2.3'; D_{85}=0.2'), heat-resistance in pesto being from 2 to 3 times higher. The calculated z-values did not prove to significantly vary, ranging from 4.9 to 5.2°C regardless of the matrix considered.

Predictive modeling for bread spoilage prevention: simplifying complex data

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Spoilage due to fungal growth is a major concern in the food industry, especially in bakeries. To prevent bread from spoiling, several measures can be taken. These include increasing plant hygiene, using modified atmosphere packaging (MAP), UV radiation, alcohol spray, and adding preservatives like natural mold inhibitors.

Major bread spoilage fungi are characterized by their ability to grow in the presence of antimicrobials and environmental factors. This helps enhance predictive modeling in bakery products. Over 30 strains isolated from bakery products and environments were tested for their resistance to antimicrobials (acetic acid and propionic acid) at pH 5.2. Growth was quantified using diameter measurements on agar and the oCelloscope™.

The combination of growth rates for each preservative and environmental factor predicts the number of days before mold growth is visible on bread. This makes complex knowledge accessible to non-specialists. The study identified four groups of fungi with different tolerances to natural mold inhibitors.

Understanding these fungi is key to better predicting and preventing mold spoilage in bread. The Corbion Natural Mold Inhibition Model (CNMIM) is a predictive modeling tool available to the bakery industry. It helps speed up time to market using robust and scientific datasets.

Effectiveness of encapsulated lemon thyme and prince herb essential oils against *Stemphylium vesicarium* and *Alternaria* spp. isolated from Portuguese "Rocha" pear prchards

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Portuguese 'Rocha' pear production is suffering substantial losses due to brown spot of pear disease (BSP), caused by the fungus Stemphylium vesicarium [1],[2]. However, recent research has highlighted a widespread presence of Alternaria species on both symptomatic and asymptomatic pear and leaves, suggesting that this genus may also be contributing to the problem. Importantly, Alternaria includes species that produce toxins and climate change may increase its prevalence [3].

Driven by increasing fungicide resistance and environmental impact, essential oils (EO) have emerged as a sustainable and eco-friendly alternative to synthetic fungicides due to their antifungal properties [4]. This study aimed to evaluate the efficacy of three EO - prince herb and lemon thyme - in inhibiting the growth of S. vesicarium and Alternaria spp. isolated from "Rocha" pear showing BSP symptoms.

To address the volatility and instability of these compounds, the oils were encapsulated with HI-CAP® 100, a modified starch-based encapsulating agent. In vitro, atomized samples of these three encapsulated EO – each at concentrations of 15% and 30% – were tested against S. vesicarium strains. To evaluate antifungal activity, S. vesicarium was grown on potato dextrose agar (PDA) media enriched with varying concentrations of the encapsulated EO formulations: 80 mg/mL, 40 mg/mL, 20 mg/mL, 10 mg/mL and 1 mg/mL. At 15%, prince herb and lemon thyme completely inhibited fungal growth at a minimum concentration of 20 mg/mL, while at 30%, they were effective at a minimum concentration of 10 mg/mL. The minimum effective concentration at 15% and 30% was subsequently tested against Alternaria spp., where it exhibited similar inhibitory effects.

Sustainable strategies that reduce reliance on synthetic products are crucial for ensuring food security while safeguarding both the environment and human health [4]. This study demonstrated that prince herb and lemon thyme essential oils encapsulated with HI-CAP® 100 effectively control the growth of S. vesicarium and Alternaria spp. from "Rocha" pear at low concentrations, highlighting their potential as a natural alternative to synthetic fungicides.

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Enzymes from wood-decaying fungi as tools for waste hydrolysis

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Presenter: Linda Mezule

The need for sustainable and improved waste management strategies has enabled the popularity of waste valorization at all stages of industrial production. A common biotechnological approach for waste valorization is the hydrolysis of biological waste with enzymes. However, this approach is most of the time limited to the availability of commercial products and narrow composition of the feed. In response to these challenges, a screening of 28 fungal isolates from boreal coniferous and nemoral summer green deciduous forests to assess their biodegradation potential for various biomass components and waste originating from wastewater treatment plants. Primary screening tests showed intensive enzyme secretion by certain isolates, particularly white rot fungi identified as Trametes pubescens and Cerrena unicolor. Furthermore, Fomitopsis pinicola showed the highest cellulose-degrading potential among the studied fungi, achieving cellulase activity over 107 U/L and 28% of saccharification. The application of enzyme produced by Irpex lacteus resulted in the release of sugars from all sewage-related substrates, demonstrating the versatility of this enzyme. The findings of this study will support the set-up of new lignocellulolytic enzyme producers to advance in-house enzyme production systems in temperate climatic zones.

Optimization of an HPLC-fluorescence method for quantification of fumonisins FB1 and FB2 in food matrices and synthetic culture media

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Presenter: Santiago Ruiz-Moyano

Fumonisins, mainly produced by species of *Fusarium* spp. and *Aspergillus* section *Nigri*, are common contaminants in maize and cereal grains, posing health risks that require reliable detection methods. This study presents and optimises high-performance liquid chromatography with fluorescence detection (HPLC-FLD) method for the quantification of fumonisin B1 (FB1) and B2 (FB2) in different food matrices and synthetic culture media.

Unlike most methods reported in the literature, which use potassium phosphate as the mobile phase, the optimised method uses formic acid, which is more suitable for liquid chromatography systems. An automated on-line precolumn derivatisation using o-phthaldialdehyde (OPA) was optimised using experimental design and response surface methodology. This approach achieved baseline separation of FB1 and FB2 derivatives in less than 20 min, with good quality parameters (limits of detection of 6.92 µg/L for FB1 and 3.01 µg/L for FB2 and intra-day relative standard deviation (RSD) of 0.84 and 0.83%, respectively).

The method was validated using synthetic media and different food matrices (figs, raisins, dates, corn, corn meal, wheat flour and rice). Various SPE cartridges were evaluated to optimise the clean-up process. Among these, MultiSepTM 211 columns showed better performance on figs and raisins, while FumoniStar immunoaffinity columns were optimal for other matrices, achieving recoveries ranging from 60-125% for FB1 and 65-105% for FB2.

The developed method provides a robust and efficient tool for both mycological studies and food safety applications.

Fungal strains of industrial food by-products fermentation and its techniques for mycelium and food production

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Presenter: Simas Borkertas

Different biomass growing conditions using fungal cultures increase the amounts of bioactive components and proteins found in the biomass. In this research the total phenolic compounds (TPC) and water soluble proteins (WSP) of mycelium will be presented together with the technique how it was grown and monitored. Only GRAS (Generally Recognized As Safe) fungal strains such as *Aspergillus oryzae* and *Fusarium venenetum* were used for this experiment due to the further use of the materials for food experiments (Singh & Gaur, 2021). TPC were examined in a samples determined by the Folin-Ciocalteu reagent in ethanol/water extract (70/30, v/v %) from different samples and WSP were measured by using methodology for protein measurement in unhopped wort by spectrophotometry. The experiment shown that TPC in solid state fermented biomass varies from 50mg GAE/100g to 300 mg GAE/100g and WSP are between 200 mg/mL and 1200 mg/mL. These results suggest that it is possible to convert food by-products into nutritious biomass which can be used for further processing (Green Chem., 2021).

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Green Chem., 2021, 23, 5150

Fungal ecology along the production line of Portuguese goat cheese

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Presenter: Teresa Vale Dias

Cheese surface contamination by moulds is frequent, with the production environment widely regarded as a major source (Kure & Skaar, 2019). The growth of filamentous fungi can compromise the product's quality and appearance and may even pose health concerns due to the potential formation of mycotoxins and other secondary metabolites. Ripening conditions tend to favour fungal development (Camardo et al., 2020), allowing resident moulds in the maturation rooms to colonize the cheese surface. This study explores the airborne and cheese fungal communities in a Portuguese factory.

Air samples were collected (in triplicates) through an impaction devise (Air test OMEGA) using two different media (DRBC and MEA) and two volumes (100L and 1000L). Samples were collected in 4 different places at three different moments. Plates were incubated at 25 ºC, and colonies were counted after 3 days. Cheese samples were collected (in triplicates) in 5 different stages of the cheesemaking process and were directly inoculated in three different culture media. Identification of fungal isolates was performed by Sanger sequencing of the ITS region.

The levels of moulds were much higher in the production room than in the curing chambers. Preliminary results showed that mycobiota of the air was mainly composed by filamentous fungi from different genera such as Penicillium spp., Cladosporium spp. and Aspergillus spp., among many others. Yeasts were also present. The composition of the cheese mycobiota evolved throughout the production process. Initially, yeasts were the most frequently isolated organisms, including species such as Yarrowia lipolytica, Candida spp., and Trichosporon spp. Filamentous fungi like Cladosporium spp. and Didymella spp. were also commonly detected in the early stages. As maturation progressed, the fungal community shifted, with Aspergillus spp. and Penicillium spp. becoming the dominant genera. The detection of shared species in air and cheese samples suggests a possible connection, but further evidence is needed to establish air as the source of contamination. Fingerprinting studies are under development to establish genetic relationships between isolates and to determine the main sources of cheese fungal contaminants.

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Ochratoxin A producers in green coffee beans

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Presenter: Zuzana Barboráková

Green coffee beans are the raw, unroasted seeds of the coffee plant (*Coffea* L.), which are the basic product for coffee production. They have a different chemical composition compared to roasted coffee and are often used in health supplements. As a result of contamination during growing, harvesting, drying, and improper storage of coffee beans, toxigenic microscopic filamentous fungi can multiply in them. In particular, the occurrence of ochratoxin A (OTA) in coffee or coffee beans has received much attention. OTA is a probable human carcinogen that is nephrotoxic and is produced mainly by micromycetes of the genera *Aspergillus* and *Penicillium*. In our study, we monitored endogenous mycocenosis of green coffee samples *Coffea arabica* (46) originating from different regions of Africa, South and Central America, Asia, and Oceania. The water activity of green coffee beans ranged from 0.407 to 0.562. After surface sterilisation with 0.4% Chloramine T solution, green coffee beans were plated at 100 beans per sample on culture media (50 beans on DRBC-Dichloran Rose Bengal Chloramphenicol agar, HiMedia M1000, India; 50 beans on DG18-Dichloran Glycerol agar, Biolife 4013942, Italy). Cultures were cultivated for 7 days at 25±1°C. Representatives of the genus *Aspergillus* had the highest relative density (RD, percentage of isolates out of the total number of isolates, 90.5%). A total of 4547 isolates of this genus were isolated. Within the genus *Aspergillus*, isolates belonging to the section *Nigri* (RD 44.7%), *Flavi* (23.7%), *Aspergillus* (20.1%), and *Circumdati* (0.7%) had the highest RD. In the context of coffee, we paid particular attention to the potential producers of OTA, i.e., the aspergilli belonging to the *Nigri* and *Circumdati* sections. We tested the ability of selected isolates to produce OTA *in vitro* by thin layer chromatography (TLC). Out of the 115 *Aspergillus* isolates of the *Nigri* section tested, 10.4% had the ability to produce OTA. All 14 tested *Aspergillus* isolates (100.0%) of the *Circumdati* section produced OTA. We used high-performance liquid chromatography with fluorescence detection (HPLC-FLD) to quantify the amount of OTA directly in green coffee samples. HPLC analysis was performed on an Agilent 1260 Infinity system. AflaOchra immunoaffinity columns (LC VICAM, Waters, USA) were used for sample preparation. The presence of OTA was detected in 78.3% of the analyzed green coffee samples, but at low concentrations. Unmeasured concentrations ranged from 0.335 to 1.829 ng.kg ^{1}.

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