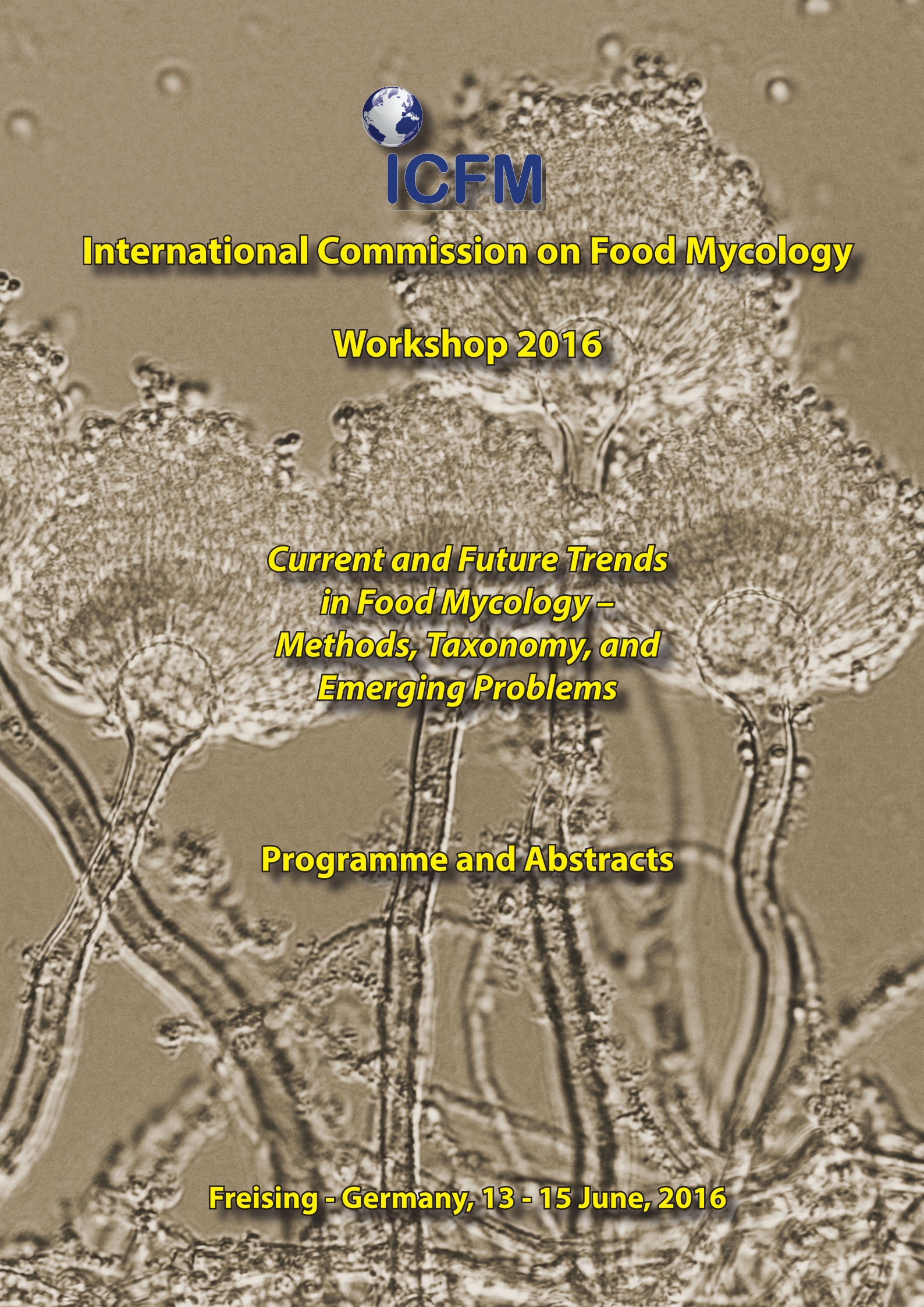

International Commission on Food Mycology

Workshop 2016

Current and Future Trends in Food Mycology – Methods, Taxonomy, and Emerging Problems

Programme and Abstracts

Freising - Germany, 13 - 15 June, 2016

International Commission on Food Mycology

Workshop 2016

Current and Future Trends in Food Mycology – Methods, Taxonomy, and Emerging Problems

Freising, Germany - 13 – 15 June, 2016

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

Venue:
Freising is a 50.000 citizen community situated 40 km north east from the city of Munich, which can be reached by train in 20 min. Munich Airport is close by with a direct bus connection.

The city is well known for its rich ecclesiastic history as well as for its importance as a centre of food science and technology as well as beer brewing. The Freising Cathedral (built 1205) it is one of the two home churches to the archbishop of Munich and Freising, one of which was Cardinal Josef Ratzinger, who was elected pope Benedikt XVI in 2005. The workshop will take place at the "Kardinal Döpfner Haus", a venue within the premises of the cathedral with lecture rooms and accommodation for participants

The eigth International Foodmycology workshop is organized by

Ludwig Niessen
Technical University of Munich, Chair for Technical Microbiology, Gregor-Mendel-Str. 4, 85354 Freising, Germany

and

Rob Samson
CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

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PROGRAMME ICFM 2016

Sunday, 12. June, 2016

16.00 Registration
18.30 Get together at "Klause" bar (drinks and finger food)

Monday, 13. June, 2016

08.00-09.00 Registration continued
09.00-09.15 Opening of the workshop and welcome addresses

Session 1: Taxonomy and new naming of food and beverage fungi (Chair: Naresh Magan).

09.15-09.40 Feng Yan Bai: The origin, domestication and taxonomy of lager beer yeast
09.40-10.05 Ulf Thrane: The rapid development in *Fusarium* taxonomy – a challenge for food mycology
10.05-10.30 Jens C. Frisvad: *Penicillium*, *Aspergillus* and *Talaromyces*: Identification and mycotoxins
10.30-10.55 Giancarlo Perrone: Two independent multilocus phylogenetic analyses support the monophyly of the genus *Aspergillus*

10.55-11.20 Coffee break

11.20-11.45 John I. Pitt: The importance of maintaining the generic name *Eurotium* for a major genus of spoilage fungi

Session 2: Food and beverage mycology, including mycotoxin contamination of food (Chair: Jens C. Frisvad)

11.50-12.15 Su-lin Leong: Proposed strategy for routine screening of pathogenic fungi in foods

12.30-13.30 Lunch at Kardinal-Döpfner-Haus

13.30-13.55 Barbara Biermaier: Dried culinary herbs – Contamination with toxigenic *Stachybotrys chartarum*
13.55-14.20 Endang S. Rahayu: The occurrence of toxigenic fungi in Indonesian dried food products
14.20-14.45 Sofia Chulze: *Fusarium* species and moniliformin occurrence in sorghum grains used as ingredient for animal feed in Argentina
14.45-15.10 Ludwig Pfenning: Production of fumonisins and moniliformin by *Fusarium* species associated with sorghum in Brazil

15.10-15.40 Coffee break

15.40-16.05 Maria L. Ramirez: Search for *Fusarium* species responsible for type A trichothecene contamination on natural grasses from a wetland ecosystem in Argentina
16.05-16.30 Marta Taniwaki: Fungi and aflatoxins in Brazilian rice: occurrence and significance in human health
16.30-16.55 Emmanuel Coton: Mycotoxin production by *Penicillium roqueforti* and genetic basis for strain-dependent mycophenolic acid production variability

17.00-18.10 Poster session (meet presenters at their poster)

18.15 Dinner at Kardinal-Döpfner-Haus

Tuesday, 14. June, 2016

Session 3 Control of food- and airborne fungi, mycotoxin producers and heat resistant molds (Chair: Jan Dijksterhuis)

08.30-08.55 Emilia Rico: Heat-resistant mold ascospores elimination in the beverage and food processing environment: Is it possible?
08.55-09:20 Elettra Berni: Heat resistant moulds: occurrence in raw materials, thermal death kinetics and effectiveness of some industrial strategies to avoid spoilage of pasteurized foods
09.20-09.45 Filipa Silva: Use of *Byssochlamys nivea* mould spores as target of pasteurization for high pressure processed fruit products
09.45-10.10 Simba Samapundo: Combined effect of pH and heat treatment intensity on the survival and outgrowth of ascospores of *Byssochlamys nivea*

10.15-10.35 Coffee break

10.35-11.00 Vasilis P. Valdramidis: Assessing the anti-fungal efficiency of filters coated with ZnO nanoparticles
11.00-11.25 Rhoda El Khoury: Use of a large-scale qPCR approach to understand the anti-aflatoxigenic effect of Eugenol
11.25-11.50 Maria J. Andrade: Influence of cured meat product constituents on *Penicillium verrucosum* growth and ochratoxin A production
11.50-12.15 Alicia Rodríguez-Jimenez: Impact of Debaryomyces hansenii on ochratoxin A production by P*enicillium verrucosum* on dry-cured sausage-based media

12.30-13.30 Lunch break at Kardinal-Döpfner-Haus

13.30-13.55 Naresh Magan: Efficacy of a fungal and bacterial antagonist for controlling growth, FUM1 gene expression and fumonisin B1 by *Fusarium verticillioides*, on maize cobs of different ripening stages
13.55-14.20 Alicia Rodríguez Sixtos Higuera: Environmental factors and ratios of pathogen:antagonist affects control of aflatoxin B1 production by *A. flavus* in vitro and on maize
14.20-14.45 Arnau Vidal: Enzyme bread improvers affect the stability of deoxynivalenol and deoxynivalenol-3-glucoside during bread making
14.45-15.10 Per Nielsen: Controlling fungal contamination and spoilage by optimizing hygienic design and air quality in the production environment

Session 4: Fungi and the environment, including industrial settings (Chair: Rob Samson).

15.15-15.35 Ángel Medina: *Aspergillus flavus* and climate change: effects of environmental changes on growth, gene expression, and aflatoxin production in vitro and in maize

15.40-16.00 Coffee break

16.00-16.20 Maria Teresa González-Jaén: Growth and toxin biosynthesis profiles of F. *verticillioides, F. proliferatum* and *F. equiseti* cultured on cereal based media at environmental conditions predicted for climate change scenarios in Spain
16.20-16.40 Davide Sardella: Evaluating the interactions within mixed biofilms of *Erwinia* spp. and fungal isolates
16.40-17.00 Brigitte Andersen: Comparison of the fungal biodiversity outdoors and indoors using cultural and molecular methods
17.00-17.20 Fabio Mascher: Airborne fungal communities in wheat grain dusts

17.30-19.00 ICFM committee meeting (closed meeting)

19.30 Downtown Freising foot walk

20.00 Dinner at “Weißbräu Huber”, traditional Bavarian restaurant, downtown Freising

Wednesday, 15. June, 2016

Session 4 continued

08.30-08.55 Jan Dijksterhuis: Fungal growth and humidity dynamics

Session 5: New methods for isolation, detection and identification of fungi and mycotoxins (Chair: Ludwig Niessen)

09.00-09.25 Jos Houbraken: Identification of foodborne yeasts and filamentous fungi using MALDI TOF MS

09.25-09.50 Sebastian Ulrich: MALDI-TOF MS as a tool for identification of *Stachybotrys* spp

09.50-10.15 Rolf Geisen: De-regulating citrinin biosynthesis of *Penicillium expansum* by heterologous expression of the ctnR gene of *Penicillium citrinum*

10.15-10.40 Gemma Castella: Effect of culture media, incubation time and temperature on poliketyde synthase gene expression and ochratoxin A biosynthesis in *Aspergillus niger*

10.40-11.00 Coffee break

11.00-11.25 Laura Puig: Comparative genomic and phylogenomic studies of 24 *Aspergillus* species with fast identification of ochratoxin A biosynthesis-related enzymes

11.25-11.50 Ayşe H. Baysal: Application of Fourier Transformed Mid Infrared (FTIR) spectroscopy as a technique for the identification of *Aspergillus* and *Penicillium* species

12.00-12.30 Final discussions, closing of the workshop

12.30 Lunch at Kardinal-Döpfner-Haus, Checkout

POSTER PRESENTATIONS

1. Albina Bakeeva *et al.*: Biocontrol activity of *Wickerhamomyces anomalus* against mould growth applied in Cameroon
2. M. Rosa Bragulat and F. Javier Cabañes: Presence of ochratoxin α in cultures of A*spergillus carbonarius*
3. F. Javier Cabañes *et al.*: Some clues in the ochratoxin A biosynthetic pathway: genome resequencing of non-ochratoxigenic strains of *Aspergillus carbonarius*
4. Guillaume Gillot *et al.*: *Penicillium roqueforti* genetic and functional diversity
5. Céline Le Lay *et al.*: Screening of bioprotective bacterial cultures against bakery product spoilage molds and identification of their antifungal compounds
6. Hugo Streekstra *et al.*: Fungal strains and the development of tolerance against natamycin
7. Isaura Caceres *et al.*: Elucidation of the molecular mechanism of action induced by the natural compound Piperine on Aflatoxin B1 production
8. Maria Helena Fungaro: *Aspergillus labruscus*: a new species of section *Nigri* isolated from grapes grown in Brazil
9. Kumaran Sivagnanam *et al.*: MALDI-TOF-MS identification and characterization of *Alternaria* species associated with cereal grains
10. Miloslava Kavkova *et al.:* Antifungal effect of whey ferment with propionate against bread moulds
11. Alessandra Marcon Gasperini *et al.:* Fungal biodiversity of Brazilian GM and non-GM maize
12. Charlotte Martin *et al.*: Comparison of infection modes of *Fusarium* in wheat, barley and oat kernels
13. Azlina Mohd Danial *et al.*: Screening of lactic acid bacteria (LABs) and a *Streptomyces* strain (AS1) for efficacy against food spoilage fungi
14. Lucía da Cruz Cabral *et al.*: A polyphasic characterization of small-spored Alternaria from Argentina
15. Massimo Ferrara *et al.*: New insight on safety and quality of salami production related to *Penicillium* species
16. Eugenia Cendoya *et al.*: Fumonisin biosynthesis and FUM gene expression by *Fusarium proliferatum* strains in a wheat-based media under different abiotic conditions
17. Eugenia Cendoya *et al.:* Impact of environmental factors and fungicides on growth of two *Fusarium proliferatum* strains isolated from Argentinian wheat grains
18. Andrew L. Robinson *et al.:* Occurrence of ochratoxin A-producing fungi in green coffee from different origins
19. Nicoletta Scaramuzza *et al.*: House mycobiota in a *Culatello* manufacturing plant: Characterization and use of biocompetitors to contrast “red moulding”
20. Beyrle, A. *et al.*: A disease-complex involving the mycotoxigenic *F. oxysporum* species is associated with the horseradish-wilt in *Armoracia rusticana*
21. Abigail Snyder *et al.:* Cytotoxicity of the novel antifungal protein, thurimycin, against mammalian cell culture
22. Elisabeth Vogt *et al.*: Influence of *Penicillium oxalicum* proteins on gushing of sparkling wine
23. Évelin Wigmann *et al.*: Effect of deep-frying and baking steps on the inactivation of *Penicillium* spp. conidia in frozen chicken nuggets processing

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SESSION 1: TAXONOMY AND NEW NAMING OF FOOD AND BEVERAGE FUNGI

The origin, domestication and taxonomy of lager beer yeast

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Lager-brewing at low temperature arose in 15th century Bavaria and has become the most popular technique for alcoholic beverage production in the world. The lager yeast *Saccharomyces pastorianus* (syn. *S. carlsbergensis*) is a domesticated microbe through the hybridization between an ale yeast *S. cerevisiae* and a cryotolerant wild yeast *S. eubayanus*. The latter firstly discovered from Patagonia, Argentina exhibits 99.5% genome sequence identity with the non-ale subgenome of *S. pastorianus*. Consequently, a Patagonian hypothesis for the origin of lager yeast has been proposed. We have shown that *S. eubayanus* commonly occurs in the Tibetan Plateau and adjacent high altitude regions in west China and exhibits surprisingly high genetic and phenotypic diversity. Three distinct lineages with over 6% inter-lineage sequence divergence were identified from the *S. eubayanus* strains from China based on multiple gene sequence analyses. A Tibetan population of *S. eubayanus* exhibits the closest known match (99.8% genome sequence identity) with the non-ale subgenome of *S. pastorianus*. Phenotypically, the Tibetan *S. eubayanus* strains are more cryophilic than the Patagonian strains. Our results suggest that *S. eubayanus* is native to Far East Asia and that the Tibetan *S. eubayanus* population is the progenitor of lager yeast. Two groups of lager yeasts have been recognized, namely group I/Saaz and group II/Frohberg, which differ in low temperature adaptation, maltotriose utilization and flavor production. The group I/Saaz strains are usually triploid (3n-1) and the group II/Frohberg strains (allo)tetraploid. The former is suggested to be referred to as *Saccharomyces carlsbergensis* and the latter as *Saccharomyces pastorianus*. We found that the lager yeasts used in China mostly belonged to *S. pastorianus* (group II/Frohberg) and their genome sizes were 3n (17%), 3-4n (28%) and 4n (50%) as determined by flow cytometry. The formation and evolution of the different groups of lager yeasts remain to be investigated further.

The rapid development in *Fusarium* taxonomy – a challenge for food mycology

Ulf Thrane

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The rapid developments in molecular biology and gene sequence facilities have had major impact on the taxonomy of all organisms, including *Fusarium* and other fungi. During the recent years many *Fusarium* species that used to be well established have been re-evaluated by molecular and phylogenetic methods that has resulted in numerous new phylogenetic based species. The new, as well as the classic species are grouped, named species complexes that help to keep a link to the former taxonomy. Having said so, it should be stressed that quite often it is impossible to transform phenotypic traits – e.g. mycotoxin profiles – from species identified in the classic *Fusarium* taxonomic regime to species in the new phylogenetic based taxonomy. This is a real challenge for applied mycologists and others that focus on the ecological function of the *Fusarium* species. The positive aspect is that more and more genes coding for biosynthetic pathways of mycotoxins and other metabolites are sequenced and add to the increasing information on the genetics behind metabolite productions. However, a whole-hearted integration of fungal phenetics and genomics, as well as critical evaluation of the exceptional amount of metabolite data being generated is crucial to ensure a valid phenotypic characterization of *Fusarium* species within the most recent taxonomy. Updated information on the *Fusarium* taxonomy, species-specific profiles of mycotoxins and metabolites will be presented and discussed with focus on the relevance for food mycology and improved knowledge on *Fusarium* and its potent mycotoxins.

Penicillium, *Aspergillus* and *Talaromyces*: Identification and mycotoxins

Jens C. Frisvad[1*], Jos Houbraken[2] & Robert A. Samson[2]

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Penicillium, *Aspergillus* and *Talaromyces* are fully polythetic genera that are monophyletic. They represent some of the most important genera of fungi in nature, and are used extensively in biotechnology. They can give problems for food mycology and indoor (air) environments because of potential allergenicity, deterioration and mycotoxin production. Correct identification to species level is of paramount importance, because a species name is predictive of a large number of important functions, such as production of extrolites including small molecules (specialized metabolites) and exoenzymes. An omnispective or polyphasic approach to classification, cladification and identification is strongly recommended. Concerning nomenclature it is recommended to use the binomial system, with an addition of the sexual morph when it is deemed necessary, e.g. *Aspergillus thermomutatus* (neosartorya morph). In scientific papers it is recommended to mention the former species name after the current name when this binomial is mentioned for the first time in the main body of the text, for example *Aspergillus thermomutatus* (formerly *Neosartorya pseudofischeri*). This is important for information retrieval at least as long as comprehensive taxonomic databases are not easily accessible. For *Talaromyces*, which now represents the former *Talaromyces* and *Penicillium* subgenus *Biverticillium*, the situation is different. When relevant, one could use the expression *Talaromyces pinophilus* (strictly anamorphic), but then still write *Talaromyces pinophilus* (formerly *Penicillium pinophilum*), the first time this name is mentioned in the text. Regarding identification, this should be based on a polyphasic approach, as described by Samson et al. [1] for *Aspergillus*, Yilmaz et al. [2] for *Talaromyces* and Visagie et al. [3] for *Penicillium*. Unfortunately, especially in the natural products literature, a high number of isolates of the important genera are misidentified, especially when only ITS sequences are used as the identification tool. Concerning mycotoxins, this misidentification problem is also of great concern, but here misidentification of the mycotoxin is unfortunately also common. An update on the producers of the most important mycotoxins produced by species in the three genera will be presented.

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Two independent multilocus phylogenetic analyses support the monophyly of the genus *Aspergillus*

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The genus *Aspergillus* is among the most abundant and widely distributed organisms on earth, and comprises approximately 350 accepted species. Economically it is one of the most important fungal genera for biotechnological and industrial use (enzymes, organic acids, active metabolites), but members of the genus are also frequently reported as foodborne contaminants (food spoilage and mycotoxin contamination), or as causative agents of human mycoses (pulmonary aspergillosis, otomycosis, keratitis). Recently, the ICN adopted the single name nomenclature which has forced mycologists to choose one name for fungi (i.e *Aspergillus, Fusarium*, *Penicillium,* etc.). In this respect, the phylogenetic approach was mainly used to settle the disputes on the right choice with decisions not affecting the majority of the concerned fungal group. This is not the case for the genus *Aspergillus*, because it is characterized

by a well-defined asexual fruiting structure, but is very broad in concept, as it is associated with eleven sections with a sexual state. Two proposals for the single name nomenclature in *Aspergillus* are presented: one attributes the name "*Aspergillus*" to clades comprising seven different teleomorphic names, by supporting the monophyly of this genus. The other proposes that *Aspergillus* is a non-monophyletic genus, by preserving the *Aspergillus* name only to species belonging to subgenus *Circumdati* and maintaining the sexual names in the other clades. The aim of our study was to test the monophyly of *Aspergilli* by a multilocus phylogenetic approach which was applied by two independent analyses. One was run on the publicly available coding regions of six genes (*RPB1*, *RPB2*, *Tsr1*, *Cct8*, *BenA*, *CaM*), on 96 species of *Penicillium*, *Aspergillus* and related taxa. Bayesian and Ultrafast Maximum Likelihood (IQ-Tree) and RaxML analyses gave the same conclusion with highly supporting the monophyly of *Aspergillus*. The other analyses were also made by using publicly available data by using the coding sequences of nine loci (18S rRNA, 5,8S rRNA, 28S rRNA (D1-D2), *RPB1*, *RPB2*, *CaM*, *BenA*, *Tsr1*, *Cct8*) of 150 different species. Both Bayesian (MrBayes) and Maximum Likelihood (RAxML) trees obtained by this second round of independent analyses strongly supported the monophyly of the genus *Aspergillus*. The stability test also confirmed the robustness of the results obtained. In conclusion both conducted statistical analyses reject the hypothesis that Aspergilli are non-monophyletic, and gives robust arguments that the genus is monophyletic and clearly separated from the monophyletic genus *Penicillium*. We therefore conclude that there is no phylogenetic evidence to split *Aspergillus* and the name *Aspergillus* can be used for all the species belonging to *Aspergillus* i.e. the clade comprising the subgenera *Aspergillus, Circumdati, Fumigati, Nidulantes,* section *Cremei* and certain species which were formerly part of the genera *Phialosimplex* and *Polypaecilum*.

The importance of maintaining the generic name *Eurotium* for a major genus of spoilage fungi

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For the past 40 years, *Eurotium* has been the name applied to a very important genus of fungi, that causes spoilage to all kinds of biological materials with a water activity just above safe levels, including textiles, paper and leather goods, paintings, museum artefacts and microscope or camera lenses. *Eurotium* is of particular importance to food mycologists, as species from this genus consistently cause large economic losses to a very wide variety of dried, concentrated and processed foods. As classically circumscribed, *Eurotium* species are very distinctive, as on suitable media the characteristic ascomycete stage is almost always formed in culture. Variations in the gross and microscopic morphology of the sexual stage are used to differentiate species. All *Eurotium* species produce a characteristic *Aspergillus* asexual state, and the transition to one name, one fungus has led to the question as to whether *Eurotium* or *Aspergillus* should be the single generic name by which the species are known in the future. This paper will put forward morphological, physiological, ecological, taxonomic and phylogenetic reasons why the name *Eurotium* should be retained for these fungi.

SESSION 2: FOOD AND BEVERAGE MYCOLOGY, INCLUDING MYCOTOXIN CONTAMINATION OF FOOD

Proposed strategy for routine screening of pathogenic fungi in foods

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Many of the questions addressed within the discipline of food mycology fall into one of three categories: food safety, namely, mycotoxins; food spoilage, where the food is not inherently unsafe but rather unfit for reasons based on quality or acceptability; and, food production, where beneficial attributes of the fungi are harnessed. Within the food safety category could be added allergies to mould spores, and the presence of opportunistic pathogens. The possibility for foodborne moulds to act as opportunistic pathogens in immunocompromised individuals has previously received little attention from food microbiologists. However, at least four cases of mycoses have been linked to various moulds present in foods, namely, a naturopathic herbal remedy, homebrew beer, spoiled yogurt, and a probiotic preparation; the last case was a fatal mycosis in a premature neonate. Three of these mycoses were gastrointestinal, in keeping with the hypothesised foodborne route of infection, and the fourth was a rhino-cerebral infection, also plausible for foodborne infection. This is in contrast to the majority of systemic mycoses where infection occurs via the respiratory route or via breach of the skin or mucosa. The proportion of immunocompromised individuals in the population is likely to grow, a result of the interplay between increasing life-spans, rise in incidence of diabetes, and improved capacity for diagnoses and interventions for cancer and organ transplants. With this increase in susceptible individuals, the number of cases of foodborne mycoses will probably also go up. Currently, yeast and mould counts are applied as 'indicators of hygiene' in many quality systems in the food industry, yet there is no screening methodology available specifically for the presence of potential opportunistic pathogens, which could, for example, inform decisions about the need to issue a product-recall. We propose some ideas which could form the basis for such a methodology, first using growth at 35°C as a selection criterion. Easy, morphology-based interpretation criteria may then be applied, as gastro-intestinal infection is often caused by mucormycetes, with their readily recognisable colony morphology; a list of other relevant fungi will also be presented. A decision-support system in the case of a positive sample will be proposed.

Dried culinary herbs – Contamination with toxigenic *Stachybotrys chartarum*

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Stachybotrys (*S.*) *chartarum* is a black mould, which grows mainly on wilting plant material such as hay and straw but also on cellulose containing building materials. The species is composed of the two chemotypes A and S, the latter of which is able to produce the highly toxic macrocyclic trichothecenes. These secondary metabolites cause severe illnesses in horses and other animals and are suspected of being responsible for the sick building syndrome in humans and for severe pulmonary haemorrhages in infants in Cleveland, Ohio. The role of *S. chartarum* and its toxins in food is hardly studied. Spices in general are known to serve as vectors for microorganisms into food products. Culinary herbs, especially in a dried form, represent a good habitat for cellulolytic moulds, due to their high cellulose content combined with relatively high humidity during the drying process. In our study, 80 samples of dried culinary herbs (marjoram, oregano, thyme and savory) were examined for their overall mycobiota with emphasis on the isolation of *S. chartarum* chemotype S. A total of 50 *Stachybotrys* spp. were isolated and further characterized by MTT- cell culture test, ELISA and LC-MS/MS. Fifteen isolates were additionally identified by sequencing the trichodiene synthase 5 gene segment (*tri5*), as well as a segment of betatubulin (*tub2*) and chitinsynthase (*chs1*) genes. Five isolates proved to be highly cytotoxic in the cell culture assay. The production of macrocyclic trichothecenes was confirmed by ELISA and LC-MS/MS. All these five toxic isolates were confirmed as being *S. chartarum* chemotype S by DNA sequencing. The ten nontoxic isolates were found to belong to the closely

related species *S. chlorohalonata*. These results show that culinary herbs must be regarded as a vector of toxigenic *S. chartarum* into food products.

The occurrence of toxigenic fungi in Indonesian dried food products

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The aim of this study was to determine toxigenic fungi in salted fish and dried chilli sold in the market and to analyze the level of aflatoxin B_1 contamination. The samples were cultivated directly in DRBC and DG-18 media and then mold enumeration was done based on colony forming units. Identification of mold was carried out based on micro- and macro-morphological characteristics using standard methods. Analysis of aflatoxin B_1 was done using ELISA test. The results of mold identification showed molds that salted fish are contaminated with *Aspergillus tamarii*, *Aspergillus flavus* which are aflatoxin producing molds, *Aspergillus sydowii*, *Aspergillus niger*, *Aspergillus versicolor*, *Penicillium citrinum*, and *Penicillium chrysogenum*. *Rhizopus* was also found in the samples. All samples were positively contaminated with aflatoxin B_1 with the range between 4.38 ppb to 75.81 ppb. In further research, genes responsible for production of aflatoxin B1 and ochratoxin A of these mold strains isolated from dried foods are being analyzed.

Fusarium species and moniliformin occurrence in sorghum grains used as ingredient for animal feed in Argentina

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Sorghum (*Sorghum bicolour* L) is the fourth most important summer crop in Argentina after soybean, corn and sunflower. In our country, its importance lies in the use of grains and fodder as a supplement for animal feed. Argentina ranks second as sorghum exporter in the world. *Fusarium* species usually associate with sorghum belong to *Fusarium fijikuroi* species complex and can produce mycotoxins that are harmful to both humans and animals. The aims of this study were to determine *Fusarium* species and the moniliformin (MON) contamination in 48 sorghum grain samples collected from two fields located in Córdoba, Argentina. The *Fusarium* species were isolated on Nash Snyder medium. A total of 688 *Fusarium* strains were randomly taken and transferred to SNA agar. After single-spore culturing, 201 isolates were identified to species level by morphological characteristics on carnation leaf agar (CLA) and potato dextrose agar (PDA). Translation elongation factor- 1 alpha (EF-1α) gene was amplified in selected strains showing morphological characteristics of *F. verticillioides*. Moniliformin was detected by UV HPLC coupled to SPE column clean up. All samples showed *Fusarium* contamination with infection levels ranging from 82.5 to 99%. Among the *Fusarium fijikuroi* species complex identified, *F. verticillioides* was the most frequently recovered (46.4%) followed by *F. proliferatum* (13.9%) and *F. subglutinans* (8.2%). Based on the EF-1α gene sequences the strains were *F. thapsinum* and *F. andiyazi*. Species within the *Fusarium graminearum* species complex also were isolated in a high frequency (26.3%). Other *Fusarium* species identified were: *F. semitectum*, *F. oxysporum* and *F. sporotrichioides.* Natural occurrence of MON was observed. The toxin levels detected ranged from 363.2 to 914.2 ng/g (mean value: 605.06 ng/g).

Production of fumonisins and moniliformin by *Fusarium* species associated with sorghum in Brazil

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Species of the *Fusarium fujikuroi species complex* are known to colonize grasses, including *Sorghum*, where they may produce mycotoxins. The objective of this study was to evaluate the *Fusarium* species that occur in association with *Sorghum* in Brazil and to determine its potential to produce fumonisins FB_1 and FB_2, and moniliformin. Within a collection of 79 isolates obtained from diverse regions in Brazil, *F. andiyazi, F. proliferatum, F. thapsinum* and *F. verticillioides* were identified based on phylogenetic analysis of the TEF region and morphological markers. Within a subsample of 23 isolates inoculated in ground maize, production of fumonisins and moniliformin was determined by HPLC. Among *F. proliferatum* and *F. thapsinum* the production of high levels of FB_1 and moniliformin was observed, while production of FB_2 occurred only eventually. Isolates of *F. verticillioides* produced high levels of FB_1 and FB_2 and differentiated from *F. proliferatum* and *F. thapsinum* by producing low levels of moniliformin. Among 10 isolates of *F. andiyazi* evaluated, a high variation of the quantity of toxins was observed. Half of the isolates produced fumonisins and moniliformin in low to high levels, while the other half did not produce toxins at all. Phylogeny and preliminary results from laboratory crosses also indicate that a different population or species may be present in *Sorghum*. This study confirmed the presence of *F. proliferatum, F. thapsinum* and *F. verticillioides* as common fumonisin producers on *Sorghum*. *Fusarium andiyazi* is reported for the first time on *Sorghum* in Brazil. The potential of this species to produce moniliformin needs to be confirmed. The production of moniliformin by isolates of *F. proliferatum F. thapsinum* and *F. verticillioides* in Brazil is reported for the first time. At this moment, no legislation is available in Brazil for acceptable levels of fumonisins and moniliformin in *Sorghum*. Nevertheless, the national agency Anvisa establishs limits of up to 1,5 μg/g for fumonisins in maize subproducts. Our findings clearly indicate that incidence of *Fusarium* species and mycotoxins in *Sorghum* should be carefully monitored to guarantee quality and safety of *Sorghum* produced in the country.

Search for *Fusarium* species responsible for type A trichothecene contamination on natural grasses from a wetland ecosystem in Argentina

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Fusarium is considered a ubiquitous fungal genus commonly isolated from the majority of bioclimatic regions and ecosystems. Also, important *Fusarium* species can cause an array of plant diseases, produce mycotoxins and adversely affect animal and human health. In a previous work we have evaluated the biodiversity of *Fusarium* species and also the natural occurrence of their mycotoxins in native grasses collected from a wetland ecosystem located in the Chaco, Argentina. This region is one of the three highest biodiversity biomes of Argentina and covers part of the Parana and Paraguay rivers floodplain complex in the Eastern border of Chaco Province. The landscape is complex open water, aquatic vegetation, grasslands and gallery forests. This temperate grassland is used for grazing cattle. A total of 70 asymptomatic grasses belonging to 12 different genera all included in the *Poaceae* family were collected. The mycological analysis of the grasses revealed that all the samples were contaminated with *Fusarium* species in levels ranging from 60 to 100% regardless the grass genera analysed. The most common species found by comparison of the elongation factor 1-α sequences against those in the NCBI database and the Fusarium ID database was *F. armeniacum* (99 - 99,24 sequences similarity). But the result of the BLAST analyses was not mirrored in the phylogenies because all the isolates formed part of a group that did not include *F. armeniacum* type strains and any other type strain. The differences with *F. armeniacum* were supported by a main morphological difference, all our isolates produced microconiadia. Also all our isolates were able to produce type A trichothecenes mainly T-2, HT-2 and neosolaniol. To clarify the identity of these isolates a more profound morphological examination and a multilocus phylogenetic analysis are in progress.

Fungi and aflatoxins in Brazilian rice: occurrence and significance in human health

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Rice is one of the most consumed cereals in Brazil and around the world. It is an excellent substrate for mycotoxin production and when toxigenic fungi have conditions to grow and produce toxins, they will produce in high amounts. A total of 121 rice samples and their fractions (husk and rice bran) were analyzed during the following stages: production, processing and marketing. They were obtained from two producing States, Rio Grande do Sul and Maranhão whose markets, along with those in São Paulo State, were included. Samples were analyzed for the fungal percentage infection, the potential of aflatoxigenic species to produce aflatoxins and the presence of aflatoxins. The mycobiota showed a difference at each stage of the processing; rice samples with higher water activity (field) had a predominance of *Fusarium incarnatum*, *F. equiseti, F. oxysporum, F. nelsonii, Phoma spp., Alternaria spp., Curvularia sp., Nigrospora oryzae* and other dematiaceous fungi, while samples with lower water activity, *Aspergillus* section *Flavi, Aspergillus candidus, Aspergillus (Eurotium) chevalieri* and *A. rubrum* had a higher predominance. A total of 85 isolates of *Aspergillus* section *Flavi* were found, of which 7 and 1 isolates were positive for aflatoxins B and B + G, respectively. Out of 121 rice samples, 13 showed detectable level of aflatoxins, 11 were lower than 5µg/kg, one sample had 23.4 µg/kg and another 71 µg/kg. These two samples were classified as red rice and although this type of rice is not commonly consumed in Brazil, this data is of concern for those who include this type of rice in their diet. Other mycotoxins such as citreoviridin and *Fusarium* toxins will also be investigated.

Mycotoxin production by Penicillium roqueforti and genetic basis for strain-dependent mycophenolic acid production variability

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Penicillium roqueforti is encountered as a contaminant in food (bread, dairy products) and feed (silage) but is also used as a ripening culture for blue-veined cheeses, in which it largely contributes to their organoleptic properties. P. roqueforti is known to produce secondary metabolites including mycotoxins such as PR toxin, roquefortine C (ROQC) and mycophenolic acid (MPA) that may have an impact on human health. Recent studies showed that mycotoxin production in cheese could be variable. The observed variations could be associated to cheese processing and/or intrinsic mycotoxin-producing potential of a given strain. Based on an unprecedented worldwide P. roqueforti collection (120 blue cheeses and 21 other substrates), 55 isolates, selected based on representative genetic diversity as well as product and geographical origins, were screened for mycotoxin production after 7-day cultures on Yeast Extract Sucrose (YES) agar by Q-TOF LC-MS. Among the targeted metabolites, no penitrem A or patulin were detected while andrastin A (ANDA), ROQC, (iso)-fumigaclavine ((ISO)-FUMI A), PR toxin, MPA and the eremefortins A and B were detected and quantified in different strains. Interestingly, ROQ C, AND A, and (ISO)-FUMI A were the 3 most widely produced metabolites while nearly 50% of strains did not produce quantifiable amounts of MPA. To further understand the genetic basis for MPA biosynthesis in P. roqueforti, a 23.5-kb putative MPA biosynthesis cluster was localized in the P. roqueforti FM164 genome sequence via an in silico analysis. Composed of seven genes (mpaA, mpaB, mpaC, mpaDE, mpaF, mpaG, mpaH), the cluster is highly similar to the one described in P. brevicompactum. In order to confirm the involvement of this gene cluster in MPA biosynthesis, RNA interference targeting mpaC was performed in a high MPA-producing P. roqueforti strain. Transformants exhibited contrasted mpaC expression and MPA production. In parallel, as mycotoxin quantification suggested strain-dependent MPA-production, the entire 23.5-kb cluster was sequenced for 3 P. roqueforti strains with contrasted productions and a 174 bp deletion in mpaC was observed in low MPA-producers. PCRs targeting the identified deleted region were carried out on 55 isolates and showed an excellent correlation with MPA quantification indicating the clear involvement of mpaC gene in MPA biosynthesis and explaining the observed MPA-production variation.

SESSION 3: CONTROL OF FOOD- AND AIRBORNE FUNGI, MYCOTOXIN PRODUCERS AND HEAT RESISTANT MOULDS

Heat-resistant mold ascopores elimination in the beverage and food processing environment: Is it possible?

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Heat-processed foods and beverages can be spoiled by heat-resistant molds (HRM). These molds produce ascospores that not only can survive the heat treatment given to these products, but also can be activated and grow during storage. These ascospores can be found in the ingredients, packaging and processing environment. They have been isolated from sweeteners, juice concentrates, juice purees, pectin, protein powders, vitamin powders, coconut water, tea leaves, corn flour and other ingredients. Even though contamination of the ingredients can give high rates of spoilage of these products, packaging and processing environment contamination can also contribute to their spoilage. This study was conducted to: (1) determine the occurrence of HRM ascospores in the beverage and food processing environment and (2) determine which sanitizer is most effective against these ascospores. Over 2,500 samples were collected from different sites in more than twenty food and beverage processing facilities. Samples collected from palletizers and pallets had the highest incidence of HRM ascospores. They were followed by samples taken from slip sheets between empty bottles, wooden pallets, palletizers, cap boxes, airveyors, bottle conveyors, flour dumping stations and others. These ascospores were also isolated in lower numbers from many of the processing areas tested such as rinsers, fillers, cappers, cooler, forklifts, and pallet jacks among others. The HRM that was isolated in higher frequency was *Byssochlamys spectabilis (Paecilomyces variotii)*, the most common cause of HRM beverage spoilage in the U.S. Of the sanitizers studied, only solutions of chlorine dioxide at 200 ppm inactivated the ascospores of the HRM studied. In conclusion, ascospores of the HRM are present in the food and beverage processing environment and current sanitation practices will not effectively eliminate them.

Heat resistant moulds: occurrence in raw materials, thermal death kinetics and effectiveness of some industrial strategies to avoid spoilage of pasteurized foods

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Heat resistant mould spores (HRMS) are well-known spoilage microorganisms of pasteurized fruit-based products. Unfortunately, there is not much information on their incidence in raw materials processed by food industries. This study was undertaken to determine their occurrence and geographical distribution in some of such matrices and to assess the efficacy of different industrial strategies to avoid acid products spoilage by some of them.
On screened raw materials, aspergilli with either *Eurotium*-type or *Neosartorya*-type ascoma proved overwhelming, totaling 93.5% of the heat-resistant mycobiota and being the only mycetes simultaneously detected in discrete concentrations on almost all matrices found positive for HRMS. *Talaromyces* spp., *Penicillium* spp. and *Monascus* proved to occur at low percentages (up to 2.1%), though they were the most frequently occurring genera in lemon cells (*Talaromyces and Monascus*) or blueberries (*Penicillium* spp.), while strains belonging to *Arthrinium, Byssochlamys*, *Hyphodermella, Rasamsonia, and Thermoascus* were only sporadically found.

Since aspergilli with *Neosartorya*-type ascoma proved to prevail in raw materials and they frequently spoil low-a_w acid products, e.g. fruit jams, the effectiveness of some technological aids such as gaseous ozone (4.4 ppm for 24, 48 or 72 hours) or aqueous peracetic acid (75 ppm for 15 minutes) has been tested on fresh berries inoculated with *Neosartorya* ascospores at a pre-pasteurization stage. Unfortunately, both of them proved ineffective, since no logarithmic reductions have been observed on treated samples at any condition tested, compared to controls.
Moreover, the degree Brix was also evaluated as a limiting factor for *Neosartorya*-type aspergilli germination and growth on strawberry- or blueberry-based media added with up to 60% sucrose and inoculated with *Neosartorya* ascospores (100 CFU/plate). Although Brix degree limit proved to be strain-dependent and matrix-independent, the upper value resulting in a lack of germination for all *Neosartorya*-type ascospores tested was higher than 56°

Brix. Based on these results, trials were carried out in a pilot plant using strawberries naturally contaminated with *Neosartorya*-type ascospores in order to reproduce what usually occurs in the industrial practice when contaminated raw material is processed. Results from industrial trials could represent a starting point for all jam producers who can apply them under the same technological conditions (initial contamination level, product formulation, pasteurization parameters), if they don't want spoilage to occur in their products.

Use of *Byssochlamys nivea* mould spores as target of pasteurization for high pressure processed fruit products

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High pressure processing (HPP), also named high hydrostatic pressure (HHP) is a modern method of food pasteurization commercially used in many countries. It relies on the application of very high pressures (up to 600 MPa) to the food/beverage to inactivate microorganisms. Since no heat or mild heat is applied, most of the original food sensory, nutrient and functional properties are retained after processing, and fresh-like fruit products with longer shelf-life are produced. In this study, a review of the resistance to HPP and HPTP (high pressure thermal process) of key bacteria, moulds and yeasts often contaminating fruit products was carried out. *B. nivea* is a mould that spoils acid fruits and can produce mycotoxins, and as most fungi is able to grow at temperatures between 11 and 43°C, water activity between 0.892 and 0.992, over a wide range of pH (3–8), under reduced oxygen conditions inside food packs and in carbonated beverages. Several studies have demonstrated that HPTP at 600 MPa at 70°C for 15 min resulted only in 1.5 to 3.2 log reductions of *Byssochlamys nivea* spores in fruit products. In addition, HPP treated commercial fruit products are normally cold stored, and therefore moulds are more problematic than bacteria. In view of the high resistance to HPTP and the acidity of fruit products, we propose *B. nivea* spores to be used as reference microorganism in the design of new HPP and HPTP processes with fruit products.

Combined effect of pH and heat treatment intensity on the survival and outgrowth of ascospores of *Byssochlamys nivea*

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The importance of heat resistant fungi is increasing as a result of the increasing use of mild(er) heat treatments to protect the nutritional, sensorial and textural properties of food products. The objective of this study was to evaluate the combined effects of heat treatment intensity (10 minutes at 70, 80 or 90°C) and pH (4, 5 and 6) on the outgrowth (activation) of ascospores of *Byssochlamys nivea*. The heat treatments applied had a large pH dependent effect on the outgrowth of *B. nivea*. Outgrowth of *B. nivea* was not observed at all pH values from ascospores which had been given a heat treatment of 10 minutes at 90°C during incubation for three weeks at 22°C. Heat treatments of either 10 minutes at 70 or 80°C resulted in a significant ($p < 0.05$) increase in the estimated radial colony growth rates compared to those of the native ascospores. Growth was generally faster when the ascospores were treated at 70°C compared to when they were treated at 80°C. Larger differences were observed between the radial outgrowth rates of colonies arising from native ascospores and ascospores heated for 10 minutes at either 70 or 80°C in media adjusted to pH 5 and 6 than those found at pH 4. This indicated that the heat activation of ascospores of *B. nivea* is pH dependent. An interesting observation was that despite the colony radial growth rates being significantly ($p < 0.05$) faster from ascospores heated at 70 or 80°C for 10 minutes, their corresponding lag phases were longer than those observed for native ascospores.

Assessing the anti-fungal efficiency of filters coated with ZnO nanoparticles

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Air handling systems should be able to remove contaminating micro-organisms from the air by filtration, but also to reduce or prevent growth of microorganisms. The efficiency of these systems is important for protected environments, such as medical and food processing/storage facilities. The aim of this study was to assess the antifungal efficiency of meltblown and non-woven filters (HS-Luftfilterbau GmbH), which were coated with 0.012 M and 0.12 M of ZnO nanoparticle solutions. Assessment was performed for coating periods varying between 0 to 50 min. Conidial suspensions of 10^5 spores/mL *Penicillium expansum* and *Rhizopus stolonifer*, previously isolated from pome fruits, were used to inoculate Sabouraud Dextrose agar at 50°C and the filters were then soaked with ten-fold diluted suspensions. The setups were incubated at 25°C and the tests were compared with controls to see whether fungal growth was inhibited. Experiments were performed in triplicate and were reported as binary responses (+,–). Results showed that *Penicillium expansum* was most inhibited on the 0.012 M ZnO coated filters and could be even inhibited when filters were coated for only 0.5 min in the case of non-woven filters. *Rhizopus stolonifer* showed less sensitivity when compared with *Penicillium expansum*. The longer the coating time, the more effective was the inhibition for all the tested fungi. The 0.12 M concentration of ZnO inhibited all the studied fungi even for the shortest coating time of 0.5 min. This work contributes to optimizing the filter coating procedure for ZnO to effectively inhibit the growth of fungi.

Use of a large-scale qPCR approach to understand the anti-aflatoxigenic effect of Eugenol

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Produced by several species of Aspergillus, Aflatoxin B1 (AFB1) is considered as one of the most dangerous mycotoxins being a carcinogenic for humans and animals. Therefore, it is essential to avoid its presence in food and for that, several methods have been developed. Utilization of fungicides is nowadays one of the most common methods; nevertheless, their use is not environmental or economically sound. Thus, the use of natural compounds capable of inhibiting fungal toxin production is investigated as an alternative strategy. Indeed, several natural extracts such as plant extracts, essential oils and spices have shown interesting anti-aflatoxinogenic effects. Among them, eugenol was identified as an inhibitor of AFB1 production in some Aspergillii. However, its mechanism of action is yet to be elucidated. Production of AFB1 is associated with the expression of a 70 kB cluster and not less than 21 enzymatic reactions are necessary for its production. Additionally, several external factors interfere with the synthesis process of this toxin and were linked to its regulation. The latter can be classified in different families such as: Velvet complex, RAS family, genes involved in oxidative stress response, transcription and environmental factors, G-protein receptors, etc. Based on the former empirical data, a molecular tool composed of 60 genes, including the entire gene cluster involved in the production of AFB1 was developed in order to understand the mechanism of action of eugenol. We demonstrated that 0.5 mM of Eugenol in MEA culture medium totally inhibits the production of AFB1 by Aspergillus flavus without a significant fungal growth modulation. We have also confirmed that the presence of eugenol induces a transcriptional inhibition of the cluster and modulates the expression of certain global regulation factors such as VeA, MtfA and MsnA among others.

Influence of cured meat product constituents on *Penicillium verrucosum* growth and ochratoxin a production

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Penicillium verrucosum has been reported as one of the species responsible for ochratoxin A (OTA) contamination in meat derivatives such as dry-fermented sausages and dry-cured ham. The influence of the environmental conditions through the ripening of such products on growth and OTA production by this species has been studied. However, not much is known about the effect of different ingredients used during their manufacturing on the colonisation and mycotoxin production of *P. verrucosum*. The aim of this study was to evaluate the effect of several concentrations (10, 20, 40 and 60 g/L) of NaCl, KCl and sucrose added to the conducive YES agar on the growth and OTA production of two *P. verrucosum* strains isolated from dry-cured meat products. Both mould strains had been previously confirmed as OTA-producers. Media were inoculated with spores of each strain and incubated at 25ºC for 7 days. Growth was evaluated every two days and OTA was quantified at the end of the incubation period. In general, the nature and the concentration of the components affected the growth rates of *P. verrucosum* strains. The lowest growth rate was detected in the presence of sucrose. Regarding the production of OTA, differences depending on the component were observed between both tested strains. In general, the presence of NaCl favoured the production of OTA. These results suggest that the ingredients added to cured meat derivatives and their concentrations could have influence on the OTA presence in such products. However further investigations are necessary in order to develop strategies based on the ingredient addition to cured meat products for reducing OTA contamination.

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Impact of *Debaryomyces hansenii* on ochratoxin A production by *Penicillium verrucosum* on dry-cured sausage-based media

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Dry-cured sausages are prone to be contaminated with ochratoxin A (OTA) because of colonisation of their surface during ripening by ochratoxigenic *Penicillia*, such as *Penicillium verrucosum*. Consequently, to protect consumer health from the hazard of exposure to OTA in meat products, control of *P. verrucosum* contamination is needed. Biopreservation is an emergent alternative to efficiently control undesirable moulds in foods. Therefore *Debaryomyces hansenii*, the most commonly encountered yeast species in cured meat products, could be used in sausages for reducing OTA accumulation due to *P. verrucosum*. The objective of this study was to examine the efficacy of *D. hansenii* against *P. verrucosum* when grown together using different yeast cells:spores ratios (100:0, 25:75, 50:50, 75:25 and 0:100) for the control of OTA production in dry-cured sausage-based matrices. To simulate the range of conditions used in dry-cured sausages ripening, the water activity (a_w) of the non-modified media (0.97 a_w) was amended to achieve 0.94 and 0.90 a_w by adding appropriate amounts of NaCl, the pH was adjusted to 4.9-5.2 and the treatments were incubated at 10 and 15 ºC over 21 days. As indicators of the antagonistic efficacy, the relative expression of the *otapks*PN and *otanps*PN genes and the production of OTA were used. Sampling was made every 1 week. This study showed that *D. hansenii* could significantly reduce the relative expression of both key genes at all the conditions studied. However, the results for OTA production were not consistent with the effects on gene expression. In most cases, *D. hansenii* stimulated or did not inhibit OTA production on dry-cured sausage-based media when the ratios of antagonist:pathogen were 50:50 and 75:25 depending on a_w and temperature conditions. These results suggest that the efficacy of such biocontrol agent to minimise OTA incidence is affected by the environmental conditions occurring through dry-cured sausages processing. Complimentary studies focused on the mechanism of action of *D. hansenii* against *P. verrucosum* under different environmental and nutritional regimes are needed to evaluate the yeast potential to control OTA accumulation.

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Efficacy of a fungal and bacterial antagonist for controlling growth, *fum1* gene expression and fumonisin B_1 by *Fusarium verticillioides*, on maize cobs of different ripening stages

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Previous studies identified two biocontrol agents (*Clonostachys rosea* 016; gram negative bacterium) with potential for control of fumonisin B1 production in vitro and in stored maize under different water availabilities (Samsudin & Magan, 2016). These have now been complemented by studies of the efficacy of these two antagonists on maize cobs of different ripening stages: R3, Milk (0.985 aw); R4, Dough (0.976 aw); R5, Dent (0.958 aw). The cobs were inoculated with 50:50 mixtures of the pathogen:antagonist inoculum ratio and stored in environmental chambers to maintain these conditions for 10 days at 25 and 30oC. The growth rate of *F. verticillioides*, the relative expression of the FUM1 gene and fumonisin B1 (FB1) production were quantified. Water activity (aw) temperature had significant impacts on growth, FUM1 gene expression and FB1 production by the strain of F.verticillioides on maize cobs of different maturities. The C. rosea 016 antagonist significantly reduced FB1 contamination on maize cobs by >70% at 25°C, and almost 60% at 30°C regardless of maize ripening stage. For the bacterial antagonist FB1 levels on maize cobs were significantly decreased in some treatments only. These results suggest that efficacy of antagonists to control mycotoxin production in ripening maize cobs needs to take account of the ecophysiology of the pathogen and the antagonist to ensure that effective control can be achieved.

Reference:

Samsudin, N.I.P. & Magan, N. (2016). Efficacy of potential biocontrol agent thresholds for control of *Fusarium verticillioides* and fumonisin B_1 production under different environmental conditions on maize-based medium. World Mycotoxin Journal 9, 205-213.

Environmental factors and ratios of pathogen:antagonist affects control of aflatoxin B_1 production by *A.flavus* in vitro and on maize

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Aspergillus flavus is a ubiquitous fungus that can infect maize, it can colonise both pre and post-harvest. The main concern of *A.flavus* infection is contamination with aflatoxin B_1 (AFB_1), the most potent carcinogenic natural compound produced by fungi. Thus, worldwide there are strict maximum legislative limits on food and feed to minimise consumer exposure. There are different strategies to try and minimise the contamination level. Among these strategies is the use of antagonistic biocontrol agents (BCAs) to outcompete toxigenic *A. flavus* strains and minimise AFB_1 contamination. The objective of this study was to understand the ecophysiology of *A.flavus* and interactions with potential BCAs under different environmental conditions to evaluate potential for control of AFB_1 production on maize. Potential BCAs isolated from Mexican maize cultivars and some established strains were screened for their ability to antagonise toxigenic *A.flavus* strains under different environmental conditions. Co-inoculation of the candidate BCAs and toxigenic strains showed that four were able to dominate or antagonise *A.flavus* under all environmental conditions tested. The effect of the potential BCAs on AFB_1 production was evaluated using different mixed spore suspensions of antagonist: pathogen (100%, 75:25, 50:50, 25:75 and 100% respectively) on maize-based media and on stored maize grain under different interacting water activity x temperature conditions. The results showed that some BCAs strains were better than others, inhibiting >80% the aflatoxin production (influenced by water availability and temperature). The best BCAS were a non-toxigenic strain

of *A.flavus* and a *Clonostachys rosea* strain. On maize grain, one BCA was able to inhibit AFB_1 production by >50%, while the other one stimulated AFB_1 production. Complimentary studies on the mechanism of action suggested that both antagonists had little effect on the sporulation of the toxigenic strain of *A.flavus* on senescent maize leaves and thus no effect on the inoculum potential in maize crop debris, regardless of water availability regimes. Studies are in progress to examine control efficacy on maize cobs at different ripening stages.

Enzyme bread improvers affect the stability of deoxynivalenol and deoxynivalenol-3-glucoside during bread making.

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Deoxynivalenol (DON) is one of the most common contaminants in cereals. In addition, wheat and wheat-containing products are considered to be the major source of human intake of DON. Wheat grains contaminated with DON may also contain deoxynivalenol-3-glucoside (DON-3-glucoside). Due to the high presence of DON and DON-3-glucoside in raw wheat, studying the stability of DON and DON-3-glucoside during the bread making process is critical. Contradictory reports exist regarding the fate of DON and DON-3-glucoside during this process due to the use of different parameters during processing (baking temperature, baking time, fermentation temperature, use of enzymes …). Given that hydrolytic enzymes may affect the release of DON and DON-3-glucoside during bread making, the effect of different enzymes (xylanase, α-amylase, cellulase, protease, lipase and glucose oxidase that are commonly used in bread making) on DON and DON-3-glucoside levels during bran bread production was assessed. The level of DON in breads without added enzymes was reduced (17-21%). Similarly, the addition of cellulase, protease, lipase and glucose-oxidase did not modify this decreasing trend. The effect of xylanase and α-amylase on DON content depended on the fermentation temperature (30 or 45 °C). The presence of these enzymes reduced the DON content in the final bread by 10-14% at 45ºC. In contrast, at 30ºC, these enzymes increased the DON content by 13-23%. DON-3-glucoside levels decreased at the end of fermentation, with a final reduction in the final bread of 19-48% when no enzymes were used. However, the presence of xylanase, α-amylase, cellulase and lipase resulted in bread with greater quantities of DON-3-glucoside when fermentation occurred at 30ºC. The results showed that wheat bran and flour may contain hidden DON and DON-3-glucoside that may be enzymatically released during the bread making process.

Controlling fungal contamination and spoilage by optimizing hygienic design and air quality in the production environment

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In the production of high quality natural food products optimization of processing time and process conditions is of extreme importance to ensure optimal quality. Heat sensitive raw materials also ad strict limits on the applied heat during processing and the increased demand to clean label requires limited or no preservatives added. In these productions the hygienic design of process equipment, hygienic layout of production facilities, zoning, sufficient air quality and well trained and skilled operators are of outmost importance. The objective of this presentation is to illustrate how these factors in combination with fungal identification, ecophysiological data and our pragmatic easy to use growth boundary model, MicroTool, can be used in combination with process data and product characteristics to resolve the root cause for spoilage problems and devising robust solutions. This will be illustrated with cases from production of natural food colors of plant origin and other food products. Case 1: The spoilage during cold storage of a natural color product was analyzed using this setup. The product was pasteurized before filling. The microbiota of empty container was dominated by *Aspergillus flavus* and *A. fumigatus*, the lids were dominated with *Penicillium citrinum* and *A. versicolor* whereas the spoiled product was contaminated with *P. brevicompactum* and P. chrysogenum. Analysis of air quality showed *Cladosporium sp*. and *P. brevicompactum, P. chrysogenum* and P. palitans. The containers were delivered with an inner and an outer wrap. The inner wrap was dominted by P. rugulosum and the outer contained *Cladosporium* sp., *P. palitants* and *P. brevicompactum*. Wooden pallets in the room were

dominated by *Cladosporium* sp., *P. palitants and P. brevicompactum*. Based on the analysis it was concluded that the containers were contaminated during filling from airborne fungi from the outer wrapping and/or wooden pallets. A change in procedure where these were removed from the filling room and the flow of people in the room was restricted resulted in significant improvement in air quality and an elimination of the spoilage problem. Case 2: A product that on release was found to have no detectable yeast and molds (<10 cfu/g) was found to have a too high level of *Zygosaccharomyces bailii* (103 cfu) after 2 months storage at 4 – 8°C. The product was pasteurized prior to filling (30s at 96°C) and all process equipment have been cleaned and chemically sterilized. The problem was only seen at one of the filling lines. A detailed review of the filling line revealed a difficult to clean valve wherefrom the same yeast could be isolated. The line was subsequently renovated completely. Predictive growth models for this and other food spoilage yeast is currently being developed in collaboration with DTU Food. The intention is to further optimize the MicroTool as a toolbox for process optimization.

SESSION 4: INFLUENCE OF ENVIRONMENTAL FACTORS ON THE PHYSIOLOGY OF FOODBORNE FUNGI

Aspergillus flavus and climate change: effects of environmental changes on growth, gene expression, and aflatoxin production *in vitro* and in maize

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There is a significant interest in the impact that climate change factors may have on mycotoxigenic fungi. We have, for the first time, examined on conducive media and maize grain the impact that three way interactions between water availability, temperature and elevated CO_2 have on: (i) growth, (ii) the relative expression of all genes in the aflatoxin gene cluster using both RT-qPCR and RNAseq, and (iii) the phenotypic aflatoxin B_1 production by *Aspergillus flavus*. On conducive media, interactions between water stress (water activity, a_w; 0.97, 0.95, 0.92), temperature (34, 37ºC) and CO_2 exposure (350, 650, 1000 ppm) were considered and the growth, AFB_1 production and expression of biosynthetic genes (*aflD*, *aflR*) studied. For maize grains, interactions between water stress (water activity, a_w; 0.99, 0.91), temperature (30, 37ºC) and CO_2 exposure (350, 650, 1000 ppm) were included. Fungal growth, AFB_1 production and expression of the all genes in the aflatoxin gene cluster by RNAseq were studied. The results showed that for growth there was relatively little effect. In contrast, the three-way interacting conditions (elevated CO_2, water and temperature stress) had a profound effect on aflatoxin B_1 production both in media and maize grains. Under slightly elevated CO_2 conditions there was a stimulation of aflatoxin B_1 production.
With regard to gene expression in conducive media results show that at 37ºC, there was a significant increase in expression of both *aflD* and *aflR* at 0.95 and 0.92 a_w and 650 and 1000 ppm CO_2. There was an associated increase in AFB_1 in these treatments. In contrast at 34ºC there were no significant differences for interacting treatments. In stored maize grain differential expression of several genes in the aflatoxin gene cluster where found in relation with these interacting factors. Aflatoxin B_1 production increased under elevated CO_2 conditions at both temperatures and a_w tested. This is the first study to examine these three-way interacting climatic factors on growth and mycotoxin production by a strain of *A.flavus*. This provides data which is necessary to help predict the real impacts of climate change on mycotoxigenic fungi.

Growth and toxin biosynthesis profiles of *F. verticillioides*, *F. proliferatum* and *F. equiseti* cultured on cereal based media at environmental conditions predicted for climate change scenarios in Spain

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The genus *Fusarium* commonly colonizes a number of crops which may become contaminated by a diverse array of toxins produced by some of these species. The mycotoxin profiles and regulation of their biosynthesis depend on the species or population considered. Additionally, environmental factors influencing fungal growth and toxin biosynthesis, which determine the final exposure of humans to dietary mycotoxins, form a complex interconnected system that starts with toxigenic fungal species interacting with crop plant and the rest of the biota in a certain geographic location. All these components are affected by environmental conditions, mainly temperature and water availability and, eventually by climate change events. Experimental *in vitro* studies were similarly conducted for Spanish strains of *F. verticillioides*, *F. proliferatum* and *F. equiseti* cultured on cereal based media at a wide set of temperatures and water potential values to gain information on their ecophysiological and toxin biosynthesis profiles. These were compared and discussed considering the current and predicted scenarios for Spain to foreseen changes on the occurrence and distribution trends of the mycotoxigenic species considered on the main cereal host species (wheat, barley and maize). Additionally, preliminary data obtained from *F. equiseti* strains isolated from

North Africa (Tunisia) were also included as well as information about a survey recently conducted on the actual distribution of these species in Spain.

Evaluating the interactions within mixed biofilms of *Erwinia* spp. and fungal isolates

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Mixed bacterial-fungal biofilms may result in antagonism or enhancement of fungal growth, bacterial utilization of fungi as nutrient sources, as well as the formation of more complex synergistic associations involving horizontal gene transfer mechanisms. *Erwinia* spp. biofilm was investigated when co-cultured with the fungi: *Penicillium expansum*, *Aspergillus* spp., *Alternaria alternata* and *Botrytis cinerea*. Growth studies were carried out on two batches of plates containing Czapek Yeast Agar (CYA) and Pear Pulp Agar (PPA). *Erwinia* spp. was inoculated onto the plates in concomitance with each of the fungi and incubated for 7 days at 25°C. The mycelium diameters were measured along two axes perpendicular to each other, twice a day. The bacterial-fungi interactions in mixed biofilms were also determined using the fluorescence dye acridine orange. Extracellular DNA from the biofilm matrix was extracted and amplified using primers for the 16S and *rpoB* genes. The most significant differences in the growth rate were observed in *A. alternata* and *B. cinerea*, whose growth was limited when co-cultured with *Erwinia* spp. and the competitive effect was more pronounced on the PPA rather than on the CYA media. Fluorescence microscopy permitted to assess not only when the fungal growth was compromised (in the case of *B. cinerea*), but also how the biofilm structure was affected by the presence of the fungus (in the case of *A. alternata*). Finally, differences in the electrophoretic pattern of the *rpoB* amplicon were found in *Erwinia* spp. grown with *Botrytis cinerea* that could be an evidence of genetic rearrangement.

Comparison of the fungal biodiversity outdoors and indoors using cultural methods and metagenomics analysis

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Airborne cross contamination is an important parameter in food production facilities as well as other indoor environments. One purpose of the study was to compare the outdoor and the indoor mycobiota during wintertime in order to evaluate the sources on indoor fungal contamination. Another purpose was to compare culture methods with metagenomics analyses. Samples were collected in February 2015 at nine different locations in Greater Copenhagen. Sample sets consisting of two sterile BBL swabs were taken outside on the upper frame of the front door and inside on the upper doorframe in the hallway. One swab was used for molecular analysis (ITS, Illumina, UNITE database and GenBank), while the other was used for culturing (streak culturing on V8 and DG18, 7 days at 23 °C, morphological ID). Sample sets were also taken directly of fungal growth if water damage was detected indoors. Quantitative comparison between culture and molecular swabs was not meaningful since the culture method only detected viable fungi that can grow on the chosen media, whereas the molecular method detected basidiomycetes as well as ascomycetes of both live and dead fungal material. However, the two methods can supplement each other in detecting sources of contamination. Preliminary results indicate that the source of *Aspergillus versicolor*, for example, was water damaged interior, since zero reads was found in the outdoor samples (mean out-OUTs: 0) but 0-139 reads were seen in the indoor samples (mean in-OTUs: 48). The corresponding culture results also showed zero CFU in the outdoor samples and two indoor samples with *A. versicolor*. One sample site had minor water damage and both molecular and culture methods showed high numbers of *A. versicolor* (growth-OUT: 4108).

Airborne fungal communities in wheat grain dusts

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Chronic exposure to airborne fungi has been associated with different respiratory symptoms and pathologies in occupational populations, such as grain workers. However, the homogeneity in the fungal species composition of these bioaerosols on a large geographical scale and the different drivers that shape these fungal communities remain unclear. In this study, the diversity of fungi in grain dust and in the aerosols released during harvesting was determined across 96 sites at a geographical scale of 560 km^2 along an elevation gradient of 500 m by tag-encoded 454 pyrosequencing of the internal transcribed spacer (ITS) sequences. Associations between the structure of fungal communities in the grain dust and different abiotic (farming system, soil characteristics, and geographic and climatic parameters) and biotic (wheat cultivar and previous crop culture) factors were explored. These analyses revealed a strong relationship between the airborne and grain dust fungal communities and showed the presence of allergenic and mycotoxigenic species in most samples, which highlights the potential contribution of these fungal species to work-related respiratory symptoms of grain workers. The farming system was the major driver of the alpha and beta phylogenetic diversity values of fungal communities. In addition, elevation and soil $CaCO_3$ concentrations shaped the alpha diversity, whereas wheat cultivar, cropping history, and the number of freezing days per year shaped the taxonomic beta diversity of these communities.

Fungal growth and humidity dynamics

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Indoor fungal growth is estimated to be occurring in about one-quarter of social dwellings in the European Union. This results in cosmetic problems on building materials, but also may be related to asthmatic problems of the inhabitants. The presence of water is regarded to be the most important factor in the occurrence of indoor fungal growth. The terms water activity (a_w) and relative humidity (RH) are used to water availability. In addition, the moisture content of the substrates were the fungi grow is an important parameter. Different moisture contents at the same water activity were compared in a closed system for the fungus *Penicillium rubens*. Gypsum soaked in a solution with a certain water activity was compared to gypsum equilibrated in air with the same relative humidity (a_w.100%). Growth of this fungus was faster in soaked gypsum having the higher moisture content. The response of this fungus towards changing relative humidities was studied. Studies that address the role of humidity dynamics during growth of fungi are very limited compared to those that monitor the ability of fungi to grow at a steady state water activity. However changes in humidity are very common inside human dwellings. Among the indoor fungi, *Cladosporium* species are predominant, and we present evidence that species belonging to the species complex *C. sphaerospermum* are overrepresented with respect to growth on indoor surfaces. It is of interest that these species also are the most xerophilic ones within the genus. The ability of different developmental stages of the fungi *Aspergillus niger*, *Cladosporium halotolerans*, and *P. rubens* to survive changes in a_w dynamics was studied. *C. halotolerans* was found to be more resistant to a_w dynamics than *A. niger* and *P. rubens*, despite its limited growth compared to these fungi.

SESSION 5: NEW METHODS FOR ISOLATION, DETECTION AND IDENTIFICATION OF FUNGI AND MYCOTOXINS

Identification of foodborne yeasts and filamentous fungi using MALDI-TOF MS

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Since conventional identification techniques are often time consuming and expensive, the need for the development of a fast, cost-effective and accurate identification technique has risen. In the late 1980's Matrix assisted laser desorption ionization was introduced as a soft ionization technique for large biomolecules which, since recently, has been successfully introduced into the clinical laboratory for routine bacterial identifications. However, this technique is less commonly used in food mycology and we investigated the possibility of this technique for rapid identification of foodborne yeasts and filamentous fungi. Recently, various articles were published of the use of MALDI TOF MS for identification of clinical yeasts species. In order to reliably identify foodborne yeasts, a database with reference spectra was generated. This database was subsequently validated with relevant yeasts that were isolated from various food samples in the last decade. There remain some issues when MALDI TOF MS is applied to filamentous fungi. The standard liquid cultivation method employed is labor intensive and time consuming. The aim of this study was to develop a cultivation method which can replace the standard liquid cultivation technique. A database containing MSP spectra obtained from strains belonging to *Paecilomyces* and *Aspergillus* sect. *Fumigati* was generated and validated.

MALDI-TOF MS as a tool for identification of *Stachybotrys* spp.

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Currently applied methods for the identification of mycota like phenotypic description are time-consuming and limited as regards the reliable identification at species level or very cost-intensive like molecular techniques. In recent years, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has proven to be a fast and easy method for the identification of microorganisms after minimal sample pretreatment. However, for filamentous fungi the routine methods of sample preparation are often not suited for the generation of high quality mass spectra for their reliable identification. We report on an optimized protocol for the extraction of ribosomal proteins from filamentous fungi like *Stachybotrys* (S.) spp. for subsequent measurement by MALDI-TOF MS. Certain *Stachybotrys* spp. are able to produce highly cytotoxic secondary metabolites such as the macrocyclic trichothecenes. They can grow on wilting plants like hay and straw but also on wallpapers and culinary herbs thus exhibiting a risk for likewise human and animal health. The optimized protein extraction protocol enabled the generation of MALDI-TOF MS reference mass spectra for 11 *Stachybotrys* species which were successfully applied for the fast and reliable identification of a set of isolates (n=45) from different habitats (hay/straw, indoor materials, culinary herbs; n=15 each). For verification of the MALDI-TOF MS results we applied molecular techniques. Eighteen and 27 isolates were consistently identified as *S. chlorohalonata* and *S. chartarum*, respectively, by MALDI-TOF MS and by sequencing.

De-regulating citrinin biosynthesis of *Penicillium expansum* by heterologous expression of the *ctn*R gene of *Penicillium citrinum*

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Both, *P. expansum* and *P. citrinum* are able to produce the mycotoxin citrinin. *P. expansum* is a postharvest pathogen of apples and is in addition able to produce patulin. Recently it was demonstrated that patulin is a pathogenicity factor which supports the colonization of apples. The biological importance of the biosynthesis of citrinin in *P. expansum* is not that clear. The biosynthesis of citrinin in *P. expansum* is strongly regulated. In a kinetics experiment it could be shown that patulin is produced in the early, whereas citrinin mainly in the later growth phase of *P. expansum*. Moreover the pH conditions also have a strong influence on the regulation. Under acidic conditions the production of patulin is preferred, whereas under neutral to more alkaline conditions citrinin is produced in *P. expasum*. In contrast the citrinin biosynthesis in *P. citrinum*, which is a ubiquitously occurring fungal species, is much less affected by environmental parameters. Changes in pH have almost no effects on citrinin biosynthesis. First results show, that the pacC signaling pathway, which reacts upon changes in pH, is differently involved in the regulation of citrinin biosynthesis in both organisms. In order to analyse the differential regulation the citrinin regulatory transcription factor *ctn*R of *P. citrinum* was expressed in *P. expansum*. Interestingly the citrinin biosynthesis of the resulting transformant was less dependent on changes in pH and the overall profile of citrinin biosynthesis resembles more that of *P. citrinum* than that of *P. expansum*. These results suggests that *ctn*R of *P. expansum* is more tightly regulated by the pacC signaling cascade via *ctn*R compared to that of *P. citrinum*.

Effect of culture media, incubation time and temperature on poliketyde synthase gene expression and ochratoxin a biosynthesis in *Aspergillus niger*

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Aspergillus niger is a fungus commonly found in foods and feedstuffs and used for biotechnological purposes. It has been reported that some strains are able to produced ochratoxin A (OTA), a potentially carcinogenic mycotoxin. Among the ochratoxigenic fungi, it is well known that a polyketide synthase gene (*pks*) is required in the initial steps of the pathway. In *A. niger*, the genome sequencing of strain CBS 513.88 revealed the presence of a *pks* gene An15g07920 that has a strong similarity to the *pks* gene of *A. ochraceus* involved in OTA biosynthesis. In our laboratory, we showed that a fragment of this gene was specific for ochratoxigenic strains of *A. niger*, being present only in the strains of *A. niger* that were able to produce OTA. Interestingly, genome sequencing of *A. niger* strain ATCC 1015, which does not produce OTA, showed that this strain has a 21-kb deletion in this predicted biosynthetic cluster, including this *pks* gene. However, no functional genetic studies have been performed to confirm its role in OTA biosynthesis in *A. niger*. The aim of this study is to demonstrate that *pks* gene expression appears to correlate with OTA production in the fungus indicating a possible role for the product of this gene in OTA biosynthesis in *A. niger*. We also evaluated the influence of culture media, incubation time and temperature on *pks* gene expression and OTA production in *A. niger*. The *pks* gene is up-regulated in medium supporting OTA production and a high induction of the *pks* gene can be determined already about 12 hours before an increase in OTA concentration is detected. This work describes for the first time the correlation between *pks* gene expression and OTA production in *A. niger*, and represents an important first step in increasing our understanding of the genetic mechanism of OTA biosynthesis.

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Comparative genomic and phylogenomic studies of 24 *Aspergillus* species with fast identification of ochratoxin A biosynthesis-related enzymes

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Fungal species of the genus *Aspergillus* are highly relevant due to their ability to cause spoilage of food products. Some of these species are able to produce mycotoxins, among which ochratoxin A (OTA) is particularly relevant to human health, due to its activity as a potent nephrotoxin, they were classified as a class 2B carcinogen. The biosynthesis of OTA is still not fully understood at the moment, although it is known that some PKS and NRPS enzymes take an important part in this process. In this study we analysed the genomes of 24 *Aspergillus* species with comparative genomics and phylogenomics approaches, with special focus to the ochratoxigenic species and the OTA-related PKS and NRPS enzymes. For this purpose, syntenic blocks were identified among the *Aspergillus* genomes. On the other hand we identified groups of orthologous proteins among the genomes, which were phylogenetically analysed. With our methodology, we confirmed the identity of known OTA-PKS and NRPS enzymes. We also identified putative OTA-related enzymes in different *Aspergillus* species, based on their phylogenetic relationship and conserved synteny. This is the first work that studied with a comparative genomics and phylogenomics approach the genomes of 24 *Aspergillus* species. This study provides a methodologic framework for the characterization of relevant compounds in the fungal genomes.

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Application of Fourier Transformed Mid Infrared (FTIR) spectroscopy as a technique for the identification of *Aspergillus* and *Penicillium* species

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Fourier transformed infrared (FTIR) spectroscopy is considered to be a rapid, nondestructive, reliable, sensitive, and a cost-effective technique, which could be used for characterizing the chemical composition (or identifying functional groups) of various microorganisms. FTIR technique has been successfully applied for identification of various spoilage and pathogenic bacteria as well as yeasts. Since bio-molecules, such as lipids, carbohydrates, and nucleic acids, have their own unique 'vibrational' fingerprints and characteristic functional groups, which correspond to specific infrared light frequencies, the FTIR spectrum obtained for any compound gives the information on the unique 'fingerprint'. The objective of this study was to identify the ability of employing FTIR for identifying and differentiating different species of *Aspergillus* and *Penicillum* genera. The *Penicillium* and *Aspergillus* isolates were grown on Malt Extract Agar (MEA) and Czapek Yeast Agar (CYA) at 25°C for 10-15 days. All the fungi were identified by using standard taxonomic references and molecular method and FTIR were employed as a rapid method for detection of these species. It was highlighted that FTIR was able to differentiate genera and species of the filamentous fungi showing its potential as a rapid, reliable, low-cost alternative method.

POSTER ABSTRACTS

1. Biocontrol activity of *Wickerhamomyces anomalus* against mould growth applied in Cameroon

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Sorghum is second most important cereal used for food and for brewing traditional African beers; the grain of 21st century in Africa. This study reviews moulds, yeasts and lactic acid bacteria (LAB) associated with sorghum stored in Cameroon. To assess the efficacy of the yeast *Wickerhamomyces anomalus* (syn. *Hansenula anomala*, formerly *Pichia anomala*) biopreservation system for nutritional improvement and applying it as biocontrol agent, the natural microbiota present in sorghum was characterized. Key microbiota of red sorghum "Jigari" from Extreme North of Cameroon, was studied at harvest and after two, five and eight months of sun drying and airtight storage with the biocontrol agent (results will be presented). The initial hygiene status of the grain was poor; Enterobacteriaceae contamination was 106 cfu/g. Mould infection of the sorghum was 105 cfu/g and potentially toxigenic *Fusarium oxysporum, F. chlamydosporum* species complex, *Penicillium citrinum* were present at harvest. *Phoma herbarum, Cladosporium oxysporum, Eurotium amstelodami, Alternaria longissima* were also present on sorghum kernels, but aflatoxigenic moulds were absent. LAB (107 cfu/g) and yeasts (106 cfu/g) were also high. In order to inhibit moulds and improve the grain hygiene during storage the biocontrol yeast *W. anomalus* was added to airtight stored moist sorghum. This biocontrol system has previously been demonstrated in moist crimped wheat and barley in Sweden. When grain is harvested and stored moist in airtight conditions, LAB naturally initiate fermentation. The decreased pH due to lactic acid production, together with the anaerobic environment, generates a stable storage system in which moulds and other microbes are inhibited. Inoculating grain with the biocontrol agent confers additional storage stability. *W. anomalus* inhibits moulds and minimizes the risk for mycotoxins via products of glucose metabolism, mainly the volatile, ethyl acetate. It also inhibits Enterobacteriaceae by an unspecified mechanism and yields a hygienic product with reduced levels of potentially pathogenic bacteria. Furthermore, W. anomalus produces phytase that degrades phytic acid (phytate), which in turn releases soluble phosphorus as well as the trace metals chelated by phytate (calcium, zinc, iron). Airtight storage of grains combined with biocontrol is a promising, cheap and energy-efficient technique for minimizing mould growth and the risks for mycotoxin production during storage.

2. Presence of ochratoxin α in cultures of *Aspergillus carbonarius*

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Aspergillus carbonarius is the main responsible source of ochratoxin A (OTA) in food commodities such as wine, grapes or dried vine fruits from main viticultural regions worldwide. Besides, OTA production is a very consistent property of this species and for this reason non-OTA producing isolates of A. carbonarius are very rarely found in natural environments. However, little is known about the presence of ochratoxin α (OTα) in both toxigenic and atoxigenic isolates of A. carbonarius. Metabolic studies of OTA in the mammalian system have shown that OTA is hydrolysed to the much less toxic products, OTα and phenylalanine. On the other hand, OTα has also been reported to occur in cultures of OTA producing species. OTα has been proposed to have a strong role in the biosynthesis of OTA using labeled precursors to growing cultures of an OTA producing strain of A. ochraceus. However, more recently it has been supported the hypothesis that OTα is not involved in OTA biosynthesis. In this meeting the presence of OTα in cultures of wild strains of A. carbonarius will be presented. Six strains from our fungal collection were first three point inoculated on Czapek Yeast extract Agar (CYA) and incubated at 15, 25 and 30ºC. After 7 and 15 days of incubation at each temperature assayed and from each strain, three agar plugs were removed from different points of the colony and extracted with 0.5 ml of methanol. Two replicates for each strain and incubation condition assayed were used. Using a HPLC technique, first results have shown that OTα

was present in cultures of ochratoxigenic strains of A. carbonarius but it was not detected in cultures of non-OTA producing strains of this species. However, these preliminary results must be confirmed by mass spectrometry.

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3. Some clues in the ochratoxin A biosynthetic pathway: genome resequencing of non-ochratoxigenic strains of *Aspergillus carbonarius*.

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Ochratoxin A (OTA) is a potent nephrotoxin which is found mainly in cereals and their products, but it also occurs in a variety of common foods and beverages such chocolate, dried fruits, coffee or wine. This mycotoxin is produced by several species of *Penicillium* and *Aspergillus* among which *Aspergillus carbonarius* is the main responsible source of this mycotoxin in wine or dried vine fruits from main viticultural regions worldwide. This species is also found in cocoa and coffee beans contaminated by OTA. In comparison to the well characterized aflatoxin biosynthetic gene cluster of *Aspergillus flavus* which is largely responsible for the aflatoxin contamination of maize crops, little is known about the genes involved in OTA biosynthesis. Different polyketide synthase (PKS) encoding genes involved in OTA biosynthesis have been identified in various OTA producing species. To date, only a PKS gene involved in the initial steps of the OTA biosynthetic pathway and a nonribosomal peptide synthetase (NRPS) gene have been related to this biosynthetic gene cluster in *A. carbonarius*. Recently, we used the Ion Torrent technology to resequence the genome of an atoxigenic wild strain. Due to the fact that our strain did not produce OTA, we focused some of the bioinformatics analyses in genes involved in OTA biosynthesis. We detected that while no differences were observed in the sequences of the functional NPRS gene, several SNP were found in the PKS gene which may be responsible for atoxigenicity in the non OTA-producing strain of *A. carbonarius*. We also detected that in the atoxigenic strain there was a high accumulation of nonsense and missense mutations in other not characterized PKS and NRPS encoding genes. The high mutation rate of these genes could explain the lack of production of OTA by the atoxigenic strain. Now, we have just resequenced three non-OTA producing strains of *A. carbonarius* using Illumina technology and we plan to perform also RNA sequencing on these strains in order to contribute to elucidate its OTA biosynthetic gene cluster.

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4. *Penicillium roqueforti* genetic and functional diversity

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Penicillium roqueforti is used as a ripening culture for blue-veined cheeses and is greatly responsible for their organoleptic quality and typicity. These features are largely due to different manufacturing methods but also to the specific *P. roqueforti* strains used. Indeed, *P. roqueforti* inoculated strains, via their proteolytic and lipolytic activities, have an effect both on texture and flavor of blue-veined cheeses but may also be involved in producing other secondary metabolites such as mycotoxins. This study investigated an unprecedented large worldwide *P. roqueforti* collection from 120 blue-veined cheeses and 21 other substrates in order to test: (i) whether *P. roqueforti* phenotypic diversity corresponds to a complex of species, (ii) what is the *P. roqueforti* intraspecific diversity and population structure, and (iii) whether the assessed differentiated populations correspond to distinct functional traits (proteolytic activities, aroma production and mycotoxin production). A Genealogical Concordance Phylogenetic Species Recognition analysis confirmed the existence of a single species. Overall, 28 haplotypes were identified

among 164 isolates and three highly differentiated populations were revealed linking isolates to blue-cheese types. Then, 55 representative strains were screened for different metabolic properties including proteolytic activity, aroma compounds (using HS-Trap GC-MS) and mycotoxin production (via LC-MS/Q-TOF). Mini model cheeses were used for aroma production and proteolysis analysis while Yeast Extract Sucrose (YES) agar medium was used for mycotoxin production. This study highlighted key differences in functional traits. Noteworthy, when *P. roqueforti* strains isolated from Protected Designation of Origin (PDO) or Protected Geographical Indication (PGI) blue-veined cheeses were only considered, a clear relationship was demonstrated between genetic diversity, population structure and the assessed functional properties.

5. Screening of bioprotective bacterial cultures against bakery product spoilage molds and identification of their antifungal compounds

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In bakery products, the use of bioprotective cultures represents an interesting alternative to chemical preservatives. The aim of this study was to evaluate the *in vitro* and *in situ* antifungal activity of lactic acid bacteria (LAB) and propionibacteria against bakery product spoilage fungi. Firstly, an *in vitro* screening showed that the most active isolates against selected fungal targets belonged to the *Leuconostoc* spp., *Lactobacillus reuteri* and *Lactobacillus buchneri* groups among the 270 tested LAB, as well as to the *Propionibacterium freudenreichi* and *Propionibacterium acidipropionici* species for the 50 tested propionibacteria. Secondly, *in situ* tests showed that cultures of isolates belonging to the *Leuconostoc citreum*, *Lactobacillus spicheri*, and *Lactobacillus reuteri* species could delay one or several fungal targets after bakery product surface-spraying. Moreover, different strain cultures led to delayed fungal growths after incorporation in milk bread rolls. Finally, an analysis of culture supernatants using LC, LC-QToF and GC revealed that several organic acids and ethanol acting in synergy were probably involved in this antifungal activity. This study provides further evidence of the potential of bioprotective cultures to delay fungal spoilage in bakery products.

6. Fungal strains and the development of tolerance against natamycin

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A selection of 20 fungal strains was subjected to increasing concentrations of the food preservative natamycin for prolonged time in order to evaluate the development of antimicrobial tolerance against this polyene, a procedure designated as “training”. The range of Minimum Inhibitory Concentrations (M.I.C.) before (1.8-19.2 μM) and after (1.8-19.8 μM) training did not change significantly, whereas the average M.I.C. increased from 6.1 to 8.6 μM. Out of the 20 strains tested, 4 strains showed a more than 2-fold increase of tolerance after training. One strain (of *Aspergillus ochraceus*) also showed increased tolerance to amphotericin B and nystatin. However, two *Fusarium* strains showed similar or even decreased tolerance for these other polyene antifungals.

7. Elucidation of the molecular mechanism of action induced by the natural compound piperine on aflatoxin B_1 production.

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Aflatoxin B_1 (AFB_1) is a worldwide public health issue usually considered as a frequent contaminant in tropical regions but also identified as emerging hazard in Europe. This carcinogenic mycotoxin is produced by *Aspergillii* belonging to the *Flavi* section but mainly *Aspergillus flavus* that is a frequent contaminant of many agricultural commodities, such as maize, peanuts, cereals, spices and dry fruits.

Once synthesized by fungi, AFB_1 is very stable and may resist to almost all food processes; it is estimated that between 500 and 750 million people are exposed to this toxin. Therefore, toxin limitation in food and feedstuffs is a major concern. Many strategies have been developed to reduce AFB_1 contamination, acting either by preventing the fungal development or by blocking the toxin production. Among these strategies, the use of natural compounds has been demonstrated as an effective anti-aflatoxinogenic method. For instance, piperine, an alkaloid present in *Piper longum L* and *Piper nigrum,* has been proved as an effective AFB_1 inhibitor but its mechanism of action has not been elucidated.

The aim of this work was to investigate the mechanism of this inhibition. For that, we studied the impact of piperine on *Aspergillus flavus* cultures on the expression of genes belonging to AFB_1 cluster as well as other external regulatory factors involved related with toxin biosynthesis. Analyses were done using a q-PCR molecular tool previously developed in our laboratory. We demonstrated that piperine inhibits AFB_1 through a transcriptomic effect on the AflR/AflS complex, which lead to a down regulation of all other genes of the cluster. We also observed the implication of different external regulatory genes related with AFB_1 production, among which catalases and superoxide dismutases genes are over-expressed in the presence of piperine. It suggests that piperine acts on AFB_1 synthesis by a modification of oxidative status of the medium.

8. *Aspergillus labruscus*: a new species of section *Nigri* isolated from grapes grown in Brazil

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Fungi belonging to *Aspergillus* section *Nigri*, dubbed "the black aspergilli", have been show to occur frequently throughout the world on grapes for wine production, such as *Vitis vinifera.* However, black aspergilli populations on grapes for the production of concentrated grape juice, especially *Vitis labrusca*, have yet to be described. Among 89 grape samples, 5 (0.29 %) revealed the presence of a novel fungal species, *Aspergillus labruscus.* This new species belonging to *Aspergillus* subgenus *Circumdati* section *Nigri* was described using morphological characters, extrolite profiles and molecular data. *A. labruscus* is uniseriate, has yellow mycelium, poor sporulation on CYA at 25 °C, abundant yellow sclerotia and rough conidia. The *CaM*-based phylogram placed our isolates on a branch clearly separated from all other species of *Aspergillus* section *Nigri*. The novel isolates were found to belong to a clade including *Aspergillus homomorphus* and *Aspergillus saccharolyticus*. Interestingly, the ITS amplicon length, obtained with the ITS1-ITS4 primer-pair, from *A. labruscus* was smaller than that of all other *Aspergillus* section *Nigri*. They exhibit a 38-39 bp deletion in the ITS1 region when compared to *A. homomorphus* and *A. saccharolyticus.* Regarding to ß-tubulin partial gene sequence, *A. labruscus* is more similar to *A. homomorphus* ex-type strain CBS 101889, but with only 85 % of sequence identity. Metabolite analysis indicated that *A. labruscus* does not produce ochratoxins and fumonisins. Neoxaline and secalonic acid D were consistently produced by isolates in this new species. The strain type of *A. labruscus* sp. nov. is CCT 7800 (T) = ITAL 22.223 (T) = IBT 33586 (T) The GenBank accession numbers are: ITS Barcode (KU708544); alternative markers= *BenA* (KT986014) and *CaM* (KT986008).

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9. MALDI-TOF MS IDENTIFICATION AND CHARACTERIZATION OF ALTERNARIA SPECIES ASSOCIATED WITH CEREAL GRAINS

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A variety of fungal microorganisms such as *Alternaria* are known to infect plants, causing foliar and seed diseases that give rise to extensive loss of yield or crop quality. In addition to causing damage to cereal grains and other plants, they have shown adverse effects toward human health due to their propensity to form mycotoxins and airborne allergens. These increasing threats justify the need to develop a powerful, rapid screening method to better characterize fungal microorganisms based on their protein and metabolite profile. This study aimed at developing a more comprehensive MALDI-TOF-MS spectral library for *Alternaria* species associated with Canadian grains. Mycelia of 5-day old cultures were used for extraction of proteins directly spotted onto target plates. An alpha-cyano-4-hydroxycinnamic acid (CHCA) matrix solution was applied for mass spectrometry analysis, which was performed on an Ultraflex MALDI-TOF/TOF system. Spectra were collected in the positive ion mode as an average of 240 laser shots within a mass range of 2000 Da to 20,000 Da. A total of 79 *Alternaria* cultures were isolated from Canadian grains and analyzed using a MADI-TOF-MS system. Acquired spectra were loaded into the MALDI Biotyper 3.1 software and preprocessed using standard algorithms for baseline subtraction, smoothing, and normalization. The generated main spectra were subjected to cluster analysis using correlation as the distance index. A comprehensive spectral library of *Alternaria* isolates was created based on their main spectra constructed. Furthermore, all the main spectra of *Alternaria* were categorized into four clusters using a clustering approach that was based on the similarity scores implemented in the software. These findings were compared with results from phylogenetic analyses of our *Alternaria* isolates in order to achieve an in depth molecular level understanding of fungal populations. We conclude, MALDI-TOF-MS technology can be a valuable tool for fungal taxonomy and the rapid identification of *Alternaria* isolates.

10. ANTIFUNGAL EFFECT OF WHEY FERMENT WITH PROPIONATE AGAINST BREAD MOULDS

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The increasing demands for biopreservants with antifungal effect in bakery products is leading to the utilisation of propionic acid from natural resources such as whey fermented with *Propionobacterium freudenreichii*. The aim of our study was to evaluate the antifungal effect of ferment whey containing 4% of propionate against food borne fungi "*in vitro*". The five moulds were isolated from sliced bread and identified by traditional and molecular methods (*Rhizopus oryzae, Eurotium repens, Alternaria alternata, Aspergillus niger, Penicillium commune*). The germination of conidia and development of fungal colonies was evaluated within 14 days based on developmental index scale. The fungi were cultured on 2% agar (A), Czapek-Dox agar (CZA) and 5% malt agar (MA) enriched with whey ferment containing the propionate (4%). Final amount of propionate in media ranged from 0.4, 0.6, 0.7 to 0.8 % .The cultivation of moulds on pure media and A, CZA and MA enriched with equal amounts of chemical sodium propionate were managed simultaneously at 25°C. The culture medium including modification with chemical and natural propionate and experimental time showed to be significant parameters that influenced development of mould species in time ($F_{(2,265)}$ = 125.15, p≤0.05). In general, the germination was postponed and the number of active conidia was significantly decreased. *Rhizopus oryzae* and *Alternaria alternata* were the most sensitive to minimal amount of propionate in medium. The development of *R. oryzae* and *A. alternata* was totally supressed by addition of 0.7 and 0.8% of ferment propionate and 0.6% of chemical propionate in CZA and MA. The addition of propionate (0.7-0.8%) postponed the start of sporulation of *Eurotium repens* from 7th to 10th day. The propionate in media inhibited germination of *Aspergillus niger* during five days and the percentage of active conidia was about 30 - 40%. The start of sporulation occurred on day 10. The colony growth including sporulation of *Penicillium*

commune on media enriched with propionate was the most intensive in comparison with other fungal species. *P. commune* and *A. niger* showed significant resistance to higher levels of propionate in all media.

11. Fungal biodiversity of Brazilian GM and non-GM maize

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Maize is at the centre of global food security as one of the most important cereal crops in diets worldwide. In Brazil, maize represents an important economic and social product in both family farming and agribusiness, and genetically modified (GM) adoption currently is around 82%. Maize is prone to infection by *Aspergillus flavus* and contamination with aflatoxins during ripening and poor post-harvest storage. However, there is little information on the correlation between GM crops, fungal contamination and levels of aflatoxins. Thus, the objective of the present work was to study the fungal diversity in 7 GM and non-GM maize cultivars from Brazil. Both serial dilution and direct plating on DG18, MEA and NA were used to examine the diversity and overall populations of fungi and total bacteria in these GM and non-GM cultivars. Overall, the population of microorganisms (log_{10}CFU g^{-1} dry sample) of all the cultivars for fungi and bacteria was <8.60 and <6.8, respectively. Both surface sterilised and unsterilized direct plating of maize kernels was made for all the cultivars examined. The fungal occurrence in each sample was determined and *Aspergillus* species isolated for further identification. *Fusarium* sp., *Penicillium* sp. and *Eurotium* sp. were predominant, and *Aspergillus flavus* was identified in 6 of 14 samples. The a_w of the samples was between 0.58 and 0.90 and the moisture content variated between 11% and 22%. Ecological studies have been carried out with *A. flavus* strain on GM- and non-GM based maize media to examine the impact on growth and aflatoxin B_1 production. This work will be complemented with interaction studies between *A. flavus* and other fungi on GM- and non-GM cultivars to identify potential biocontrol agents and the impact of climate change parameters on aflatoxin contamination of these two types of maize cultivars.

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12. Comparison of infection modes of *Fusarium* in wheat, barley and oat kernels

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Fusarium head blight is one of the most devastating diseases in all cereal species, impacting yield and food safety due to the accumulation of harmful mycotoxins. The disease is due to a number of fungal species belonging to the genera *Fusarium* and *Microdochium*. Each *Fusarium* species produces a specific array of mycotoxins. *Fusarium graminearum* is common in all cereals but is the predominant on barley and wheat and is known to produce deoxynivalenol. *F. poae* and *F. langsethiae* are frequently found on oats, producing nivalenol and T-2/HT-2 toxins, respectively. In barley and wheat, *F. graminearum* penetrates into the flower at anthesis and spreads throughout the spike, infecting and contaminating the developing kernels with mycotoxins. While the infection pathway and the resistance reactions in wheat and barley are rather well understood, the infection mode in oats has been poorly studied. In fact, after infection, hardly any symptoms are visible on the panicle or on the grains. Only the presence of mycotoxins witnesses the presence of the pathogen. The present investigation compares the impact of the kernel infection by *F. graminearum* in oats, wheat and barley on the content of mycotoxins and health promoting compounds (HPCs), such as anthocyanins and ß-glucans. Preliminary results from field studies with artificial infection showed that in barley and wheat, levels of HPCs were not altered or diminished with infection. However, in oats, ß-glucan contents increased after infection. In addition, these trends varied with the resistance potential of individual varieties and suggest an interaction with resistance mechanisms at the kernel level.

13. Screening of lactic acid bacteria (labs) and a *Streptomyces* strain (as1) for efficacy against food spoilage fungi

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Lactic acid bacteria (LAB) have been used for food preservation for many years and are considered safe as bio-preservatives because of their production of lactic acid, acetic acid and bacteriocins. Actinomycete strains particularly strains of *Streptomyces* are known to produce metabolites with antimicrobial properties. The objective of this study was to screen 66 lactic acid bacteria (LAB) isolated from Malaysian fermented foods and a *Streptomyces* strain *(*AS1) isolated from peanuts for efficacy against *A. flavus*, *F. verticilloides*, *P. nordicum* and *P. verrucosum*. In agar well diffusion assays five LABs showed antifungal activity against *F. verticilloides.* Physiological modification by environmental stress ($a_{w;}$ 0.955, 0.985, 0.995; pH 4.5, 5.5, 6.0) on the strains during growth was conducted for trying to enhance metabolite production. Antifungal activity of LABs was influenced by a_w, with better activity when water was freely available (0.995 a_w). There was no influence of the prevailing pH. Using the agar overlay method four LAB strains showed efficacy against *F. verticillioides.* However, these four strains (*L. plantarum* strain MCC 2156, *L. plantarum* strain HT-W104-B1, *P. acidilactici* 1498 and *P. pentosaceus* 1426) had weak activity against the other spoilage fungi screened. The *Streptomyces* AS1 strain had a broad spectrum of antifungal activity and reduced growth of all the fungi examined with inhibition between 40-90% in dual culture assays. *P. verrucosum* was particularly sensitive to metabolites produced by the AS1 strain. Studies are in progress to examine the relative concentrations of the metabolites to inhibit germination, growth and mycotoxin production (aflatoxin B1, ochratoxin A and fumonisin B1).

14. A polyphasic characterization of small-spored *Alternaria* from Argentina

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Accurate identification of fungal contaminants in food is critical in the development of prevention strategies at pre- and post-harvest stages. Even though *Alternaria* is frequently isolated from Argentinean crops, little is known on the variability and differentiation of its populations in this country. The objective of this study was the characterization through a polyphasic approach of 45 small-spored *Alternaria* spp. obtained from edible parts from tomato fruits, pepper fruits, wheat and blueberries, involving morphological, metabolomic and molecular analyses. The isolates were grown on Potato Carrot Agar at 25ºC under an alternating light cycle (8 h light, 16 h darkness) for 7 days. Morphological classification was achieved according to Simmons (2007). Metabolite profiling was carried out in DRYES (14 days, 25ºC) by micro-extraction with ethyl acetate-1% formic acid. Detection was performed by HPLC-MS (ESI-TOF). Amplification and sequencing of a portion of the endopolygalacturonase gene (*endoPG*) and subsequent Bayesian analysis using a Kimura 80 model were carried out. Morphologically, isolates were classified as *A. tenuissima* sp.-grp. (29/45), *A. arborescens* sp.-grp. (11/45), and *A. alternata* sp.-grp. (2/45). The 3 remaining strains exhibited intermediate characteristics among the three mentioned groups and were referred to as *Alternaria* sp. By secondary metabolites analysis, no compound representative of morphological groups could be detected; all isolates showed overlapping profiles. Bayesian analysis from *endoPG* gene yielded two clades, both containing isolates belonging to the three species-groups and the four substrates with short branch length, indicating low number of substitutions per site. Even though morphological differences were observed, and most of the isolates could be assigned to a species-group based on them, the whole set of analyses suggested that they were strongly related. No correlation between morphospecies and phylogenetic or metabolomic studies could be established. These results are in agreement with recent *Alternaria* organization systems, which incorporate these 3 species-groups in a single section within the genus (*Alternaria* sect. *Alternaria*).

15. New insight on safety and quality of salami production related to *Penicillium* species

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Fungi have an important role in the production of dry-cured meat products, especially during the seasoning period, when the salami surface, both industrially and handmade, is quickly colonized by a composite mycobiota. This mycobiota could have beneficial or undesirable effects on the products depending on its peculiar composition. Various genera of fungi could colonize salami (i.e. *Aspergillus*¸ *Cladosporium*, *Eurotium*, *Penicillium*), but *Penicillium* species are predominant, being *P. nalgiovense*, *P. olsonii*, *P. brevicompactum*, *P. chrysogenum* and a new recently described species *P. salamii,* the main occurring. As part of the Ministerial project "SAFE-MEAT", aiming to increase food safety and quality of pork-based products, new interesting results to prevent and control ochratoxin A (OTA) risk, and improvements of the quality of salami production have been achieved. In comparison with *P. nalgiovense, P. salamii* has been proved to be a fast growing mould on dry-cured sausages casing, well adapted to the seasoning process, with higher lipolytic and proteolytic enzymatic activities that could contribute to confer typical sensory characteristics to meat products. Thus, *P. salamii* resulted a promising candidate for new fungal starter formulations for meat industry.

However, salami could be also colonized by *P. nordicum*, an important and consistent producer of the potent nephrotoxin OTA, widely reported as undesirable contaminant of dry-cured meat products. To this purpose, a high sensitive and easy to use LAMP assay, has been developed for *P. nordicum* detection on salami surface co-inoculated with *P. nalgiovense* and *P. nordicum* at different rates. Moreover, monitoring gene expression of a key gene of OTA biosynthesis in *P. nordicum* and toxin accumulation in meat during the seasoning process revealed that expression profile was consistent with OTA accumulation. Gene expression was observed since the 4th day after inoculation and progressively increased up to the 10th day when OTA reached the maximum level. Indeed, contamination of dry-cured meat products by *P. nordicum* could represent a serious concern for salami production and therefore molecular tools, such as LAMP and gene expression assay, should be considered for new HACCP plans in order to prevent and control OTA risk in dry-cured meat production.

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16 Fumonisin biosynthesis and *fum* gene expression by *Fusarium proliferatum* strains in a wheat-based media under different abiotic conditions

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Fumonisins are toxic fungal metabolites produced mainly by *Fusarium* species. Fumonisin B1 is the most significant in terms of occurrence and toxicity; it can cause severe disorders in animal's health and has been epidemiologically associated with oesophageal cancer and neural tube defects in some human populations. Several Fusarium species are able to produce fumonisins, but the two most important ones are F. verticillioides and F. proliferatum, which are common fungi associated with maize, but can also be isolated from other substrates such as wheat. Wheat is the most important cereal consumed by the Argentine population. Fungal growth and mycotoxin production result from the complex interaction of several factors and, therefore, an understanding of each factor involved is essential to predict and prevent mycotoxin development. The aim of this study was to analyze the effects of abiotic

factors, temperature and water activity (aW), on growth, fumonisins biosynthesis and FUM8 and FUM19 gene expression in 3 *F. proliferatum* strains isolated from durum wheat in Argentina, using a wheat-based medium. The effect of both aw and temperature on growth was examined in vitro, the effect on fumonisin production was examined by HPLC-MS-MS, and the effect on FUM gene expression was quantified by specific real-time reverse transcriptase-PCR assays. Although all isolates showed similar profiles of growth, the fumonisin production profiles were slightly different. Regarding FUM gene expression, both FUM8 and FUM19 showed similar behavior in all tested conditions. Both genes where repressed when there was not fumonisin production, with the exception of the control condition at 15 °C, where there was no fumonisin biosynthesis but gene expression was not repressed, suggesting that genes were expressed in the same way than in the control condition at 25 °C for all tested strains. This study provides useful base line data on conditions representing a high and a low risk for contamination of wheat by fumonisins.

17. Impact of environmental factors and fungicides on growth of two *Fusarium proliferatum* strains isolated from Argentinian wheat grains

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Wheat is an important grain cereal mostly used for human consumption. During non-FHB epidemic years the predominant *Fusarium* species is *F. proliferatum.* This species is able to produce fumonisins, which are mycotoxins that can cause various adverse health effects in animals and humans. There is also evidence of fumonisin contamination in wheat grains and wheat-based products. The use of fungicides is a complementary control measure when weather conditions are conducive to FHB infection from anthesis to harvest. In this study, the impact of four commercial fungicides used for controlling FHB (epoxiconazole + metconazole, tebuconazole, pyraclostrobin + epoxiconazole, and prothioconazole) on growth of two *F. proliferatum* strains was evaluated in relation to water activity (a_W; 0.99, 0.97, 0.95) and temperature (15°C and 25°C) on a wheat-based media. Both strains showed a similar behavior in all tested conditions. All fungicides reduced growth rates when compared to the control, with the exception of tebuconazole used in the lowest concentration at 15 °C. This reduction increased as the fungicide concentration increased. The best fungicide resulted to be prothioconazole, because it was able to inhibited fungal growth at 15°C in all tested conditions, and at 25°C at the lowest a_w tested. Our results show that reduction in growth rate in the presence of fungicides is influenced by complex interactions between a_w, temperature, and fungicide concentration in both *F. proliferatum* strains. Such information could be useful for effective control of *F. proliferatum* growth and possible fumonisin production on wheat grains.

18. Occurrence of ochratoxin a-producing fungi in green coffee from different origins

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Ochratoxin A (OTA) is a nephrotoxic compound produced by *Aspergillus* and *Penicillium* species and classified as a possible human carcinogen. While OTA may contaminate a wide range of agricultural commodities, high level of OTA has been detected in green coffee beans. Eighteen green coffee samples from six countries were collected and potential ochratoxigenic species were surveyed with 18% glycerol (DG18) and dichloran rose-bengal chloramphenicol (DRBC) agar. About 65% of green coffee beans analyzed by direct plating presented fungal infection. The range in occurrence of mold differed based on the country of origin - Peru and Sumatra showed the highest incidence with 100% and 95%, respectively, while the beans from Brazil and Guatemala showed the lowest incidence with 21% and 23%, respectively. *Aspergillus* species were the dominant mold flora, which included *A. flavus*, *A. niger*, and *A. steynii* with more than 90% of incidence in green coffee beans imported from Uganda, Sumatra, and Peru. Black aspergilli isolates represented 47% from the total number of isolates from green coffee beans whereas *A. ochraceus* was not detected. An abundantly sporulating and sclerotia producing type of *A. oryzae* as well as *Eurotium herbarium* were also frequently isolated. Other less frequent contaminants included

various *Rhizopus* species, *Absidia corymbifera*, and *Syncephalastrum racemosum*. The prevalence of *A. flavus* and black aspergilli in coffee samples from every country highlight the natural occurrence of these species.

19. House-mycobioata in a Culatello manufacturing plant: Characterization and use of biocompetitors to contrast "red moulding"

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Sampling was carried out in an artisanal plant manufacturing *culatello* (a typical Italian dry-cured product) during summer and winter, in order to assess the mycobiota occurring in the air of the ripening rooms and on products, with special attention given to undesired moulds and potential mycotoxin producers. Among the house-mycobiota, fungal strains potentially producing ochratoxin A were sporadically isolated and proved to be the least prevailing species collected from culatelli, while fungal strains producing unpleasant spots on the casings were massively found in the first steps of the ageing process, even if their presence proved to diminish at the end of the ageing time. Among those strains, *Sporendonema casei* was a matter of concern for product appearance, giving its surface a characteristic flame-red colour throughout ageing. For this reason, a study concerning the use of fungal autochthonous strains as biocompetitors was carried out on natural substrate (*culatello*), in order to find out a possible solution to the so-called "red moulding" of meat products.

Aspergillus pseudoglaucus (Eurotium repens), Aspergillus tonophilus (Eurotium tonophilum) and *Penicillium brevicompactum* were selected to be used as biocompetitors, since their combination was thought to be suitable for possibly contrasting *S. casei* throughout the ageing process. In biocompetition tests, though *S. casei* was not detected in the first part of the maturing process, after 180 and 360 days, the mould reached concentrations that were significantly higher than those of *P. brevicompactum,* but significantly lower than those of aspergilli with *Eurotium*-type ascospores on all samples. Despite this, *S. casei* gave both inoculated and uninoculated samples a spotted, flame-red appearance throughout the ageing process, so it can be stated that use of our autochthonous biocompetitors is not an effective counter-action against red moulding of meat products.

20. A disease-complex involving the mycotoxigenic *Fusarium oxysporum* species is associated with the horseradish-wilt in *Armoracia rusticana*

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Introduction: Horseradish, *Armoracia rusticana,* is a perennial plant belonging to the family of *Brassicaceae*, which has been cultivated since 1.500 B.C. Nowadays, in Germany horseradish is cultivated in a volume of about 1.800 tons/anno (2010). Like other species included in this taxonomic family, horseradish is often infested by a disease called (horseradish)-wilt, which is described to be a result of an infection by filamentous fungi of the species *Verticillium* and to lesser extent *F. solani*. In the framework of comparative mycobiome analyses of horseradish plants, we found, beside the species *Verticillium* and *Fusarium*, also some bacteria and various other fungal species showing mycotoxigenic and phytopathogenic potential.

Results and Discussion: The disease "horseradish-wilt" in *Armoracia rusticana* shows a world-wide distribution and has been described since decades as a symptom caused mainly by filamentous fungi of the species Verticillium and to lesser extent F. solani. In comprehensive molecular analyses of naturally infected "Fechser"- and horseradish-plants using methods like e.g. DNA-fingerprinting and ITS-Cladogram-Analyses, we could identify various fungal species with mycotoxigenic and phytopathogenic potential inside and outside the horseradish plants. Comparing the grade of infestation of different harvest-charges of horseradish plants with corresponding weight and length using one-factorial analyses, reveals a correlation between infection and possible growth retardations. Furthermore, it was possible to identify a bacterial complex composed mainly of the three species *Stenotrophomonas* spp., *Burkholderia* spp. and *Serratia* spp. In subsequent greenhouse trials with horseradish plants which have been inoculated either with the bacteria or with fungi of the species *Verticillium dahliae* or *F. oxysporum*, it could be shown, that both, an infection with *F. oxysporum* respectively *V. dahliae* lead to substantial deregulations in root development and growth

of the horseradish plants. Interestingly, in cases were the plants were solely infected by bacteria, the horseradish showed normal growth rates and morphology. Moreover, there is a strong indication that an infection of the plants with the bacterial complex composed of *Stenotrophomonas* spp., *Burkholderia* spp., - spp seems to protect the horseradish against fungal contaminations. Hence, using competitive, non-pathogenic bacterial species could be a successful opportunity for a biological control of fungal infections in soil-borne plants which in principle could be a cause for public health concern.

21. Cytotoxicity of the novel antifungal protein, thurimycin, against mammalian cell culture

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The use of commercial antifungal agents to control food spoilage is limited by the availability of efficacious compounds that are not also toxic towards humans. Thurimycin is a novel antifungal protein that has previously been shown to inhibit *Byssochlamys fulva* proliferation in juices and beverages with an MIC of 50.0 µg/ml or less, depending on the food matrix. In this study, the cytotoxicity of thurimycin was assayed using the human intestinal epithelial cell line, Caco-2, and the human liver epithelial-like cell line, HepG2. Metabolic activity was evaluated through a colorimetric assay dependent on oxidoreductase activity in viable cells. Membrane integrity was evaluated by quantification of luminescence generated through a luciferase reaction as a consequence of adenylate kinase leakage from cell membrane permeabilization. Thurimycin, natamycin, and vancomycin were applied in concentrations ranging from 25 µg/ml to 250 µg/ml to cell monolayers in order to obtain dose-response curves. For antimicrobials which did not achieve an EC_{50} within that range, additional testing was performed at higher concentrations as allowed by compound solubility. Application of either vancomycin or thurimycin at levels up to 1,000 µg/ml decreased cellular viability <10%. In contrast, the EC_{50} for natamycin, a commercially available antifungal agent utilized in dairy products, was determined to be 55 µg/ml against HepG2. With the Caco2 cell line, 250 µg/ml natamycin decreased viability by 31%. The *in vitro* work presented here suggest thurimycin is a promising antifungal additive candidate with a sufficient therapeutic index; however, additional toxicological work is required to substantiate its safety.

22. Influence of *Penicillium oxalicum* proteins on gushing of sparkling wine

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Gushing describes the spontaneous over foaming of carbonated beverages directly after opening of the bottle despite correct handling. This phenomenon causes substantial economic losses for beverage companies. In contrast to the brewing industry, gushing in sparkling wine is a widely unexplored phenomenon. It is well established that hydrophobin proteins produced by filamentous fungi contribute to gushing in beer. We concluded a similar involvement of fungal proteins in gushing of sparkling wine. The aim of this study was to screen different grape-associated fungi in order to investigate their potential influence on the gushing of sparkling wine. A set of 74 strains of filamentous fungi from 40 fungal species was tested for their influence on the surface activity of culture supernatants. Furthermore, culture supernatants were screened for the presence of secreted low molecular weight proteins by SDS-PAGE followed by silver staining with a focus on potential hydrophobins with a molecular weight of ~10 kDa. A subset of strains showing promising characteristics in previous experiments was screened for the induction of gushing in sparkling water and in sparkling wine after addition of spumate. Addition of a spumate obtained by foam fractionation of *Penicillium oxalicum* cultures was demonstrated to induce gushing in sparkling water and sparkling wine. Not only the sample volume and the temperature, but also the pH of the beverage had massive influence on the amount of over foam and hence on the gushing phenomenon. Currently, work is ongoing to identify the gushing inducing proteins in the exoproteome of *P. oxalicum*. Our results indicate that surface active proteins from *P. oxalicum* may be a possible reason for the occurrence of gushing in sparkling wine. Accordingly, we

suggest the infection of grapes with this fungus to be one of the key factors leading to gushing in sparkling wine.

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23 Effect of deep-frying and baking steps on the inactivation of *Penicillium* spp. conidia in frozen chicken nuggets processing

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Several studies have reported the occurrence of filamentous fungi in meat products. In the specific case of frozen chicken nuggets, fungal spoilage losses are estimated in approximately 1.0-1.5 %. Previous study have found *Penicillium glabrum*, *P. polonicum*, *P. commune*, *P. solitum* and *P. crustosum* as the main species associated with spoilage (visible mycelia) of frozen chicken nuggets. This study aimed to evaluate the impact of deep-frying and baking applied during frozen chicken nuggets processing on the inactivation of *Penicillium* spp. Firstly, it was found that the conidia of *Penicillium* were not able to survive the heat shock in phosphate buffer at pH 7.2 in thermal death tubes (TDT) at 80 °C/30 minutes. Subsequently, each *Penicillium* strain was inoculated in frozen chicken nuggets, which were subjected to the following treatments: i) only deep frying (frying oil at 195-200 °C), ii) only baking 120-130 °C until the internal temperature reached 70 °C and iii) deep frying followed by baking (frying oil temperature of 195-200 °C and baking temperature of 120-130 °C, until the internal temperature of nugget reached 70 °C). The results indicated that *P. polonicum* NGT 23/12, P. *commune* NGT 16/12, *P. solitum* NGT 30/12 and *P. crustosum* NGT 51/12 were able to survive after exposition to the combined treatment (deep frying followed by baking) when inoculated in chicken nuggets. *P. polonicum* NGT 23/12 was the most resistant strain to the combined treatment (deep frying and baking), as its population was reduced by 3 log cycles CFU/g when the internal temperature reached 78 °C after 10:30 minutes of baking. The present data show that if *Penicillium* spp. is present in high numbers in raw materials, such as breading flours, it could survive to the thermal processing applied during chicken nuggets production. Furthermore, increasing target temperature for thermal processing at the cold spot of the product from 70 °C to about 78 °C may be an alternative to ensure the microbiological stability of these products during its shelf life.

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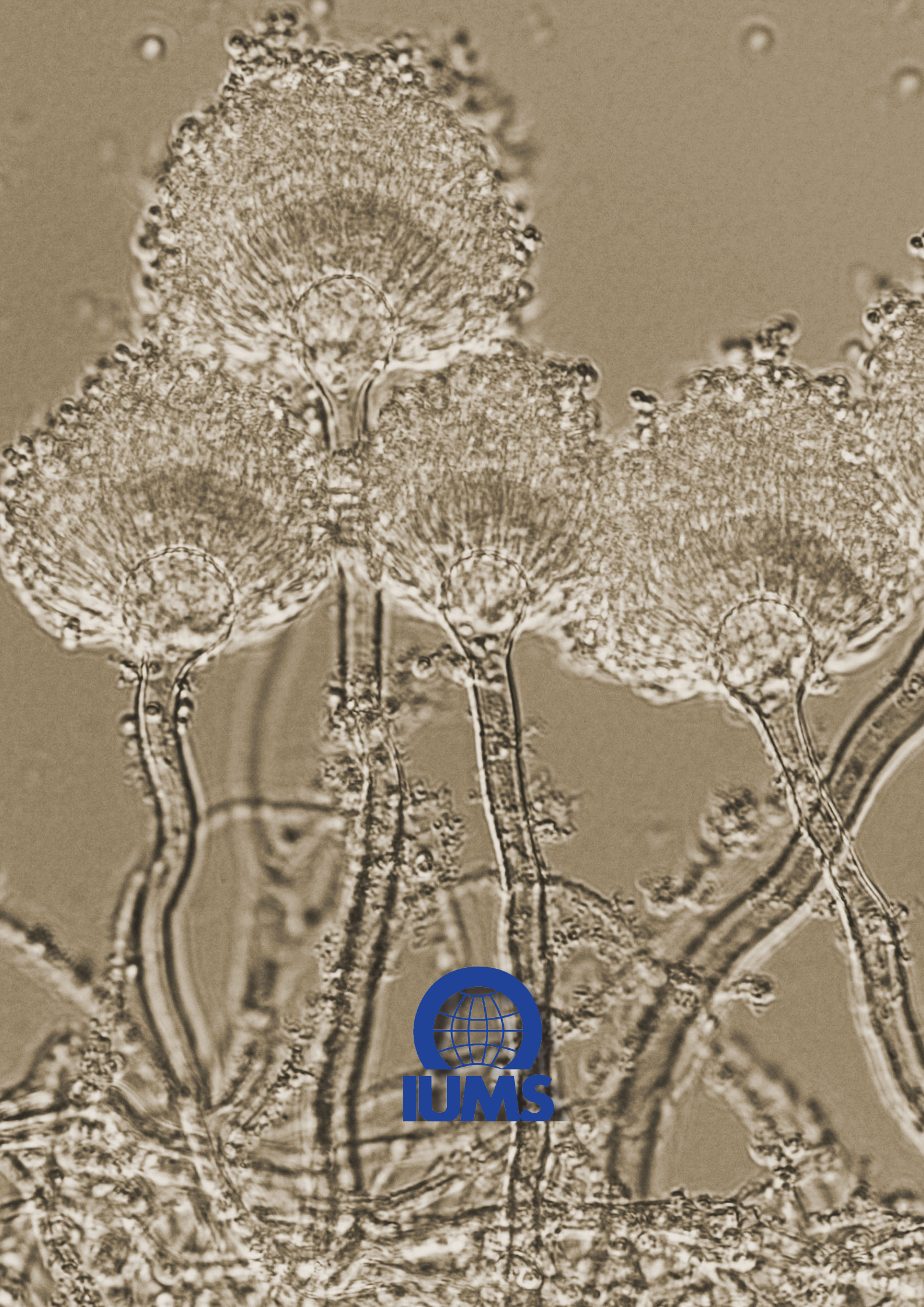
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