SESSION 7: FUNGAL GENETICS AND GENOMICS

Changes in the expression of a gene involved in cell wall integrity are related to mycotoxin production in filamentous fungi in food

Lucía da Cruz-Cabral[1], Josué Delgado[2], Sihem Fodi[3], María J. Andrade[2], Mar Rodríguez[2], Andrea Patriarca[1] and Alicia Rodríguez[2,4]*

[1]*Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Orgánica, Laboratorio de Microbiología de Alimentos, Buenos Aires, Argentina;* [2]*Food Hygiene and Safety, Meat and Meat Products Research Institute. Faculty of Veterinary Science, University of Extremadura, Avda. de las Ciencias, s/n. 10003-Cáceres, Spain;* [3]*Agronomic Mediterranean Institute of Bari (CIHEAM IAM Bari), Valenzano, Italy;* [4]*Food Science and Nutrition, School of Agricultural Engineering, University of Extremadura, Avda. Adolfo Suárez s/n, 06071 Badajoz, Spain*

*Presenter: aliciarj@unex.es

Spoilage fungi and mycotoxigenic fungi are able to colonise a wide range of ecological niches including soil, plants and foods. External conditions and antifungal compounds may trigger different signal cascades within the fungal cell that culminate in changes at the transcriptional level, leading to the production of compatible solutes that enable the enzyme systems to function under the environmental conditions. It has been suggested that these compounds may act as stressors and provoke an increase of mycotoxin production. Fungi have different intracellular pathways that help them coping with challenging external conditions. Among them, the cell wall integrity pathway (CWI) is activated in response to cell wall stresses due to different food-related environments. So far, little is known about the relationship between the activation of the CWI pathway and mycotoxin production by important economic filamentous fungi commonly found in various cereals and fruits. The objectives of this work were to: a) evaluate the effect of external conditions (temperature, water activity) and antifungal compounds (fungicides, biocontrol agents) on growth, mycotoxin production and changes in the expression of the *Rho1* gene, one of the main regulators of CWI pathway by some *Alternaria* species and *Aspergillus ochraceus* in model systems based on wheat, tomato and raisins; b) analyse the relationship between *Rho1* gene expression and both the growth and mycotoxin production by the target filamentous fungi. Results showed that temperatures, water activity and the nature of the fungicide have different influence on both growth and mycotoxin (ochratoxin A, alternariol, alternariol monomethyl ether and tenuazonic acid) production. In addition, it has been shown that changes in the CWI pathway and the accumulation of ochratoxin A and *Alternaria* spp. mycotoxins seem to be related under specific environmental conditions. These findings will be useful in developing new strategies to efficiently control toxigenic fungus spoilage in cereals and fruits.

Regulation of mycotoxin biosynthesis by *Penicillium expansum* as an important determinant of the colonization capacity

Rolf Geisen* and Markus Schmidt-Heydt

Max Rubner-Institut, Department of Safety and Quality of Fruit and Vegetables, Karlsruhe

*Presenter: rolf.geisen@mri.bund.de

Penicillium expansum is an important post-harvest pathogen of fruits, especially apples. It is able to produce the two important mycotoxins patulin and citrinin. For both mycotoxins it was shown that their biosynthesis supports the colonization of the natural habitat. Mutants in biosynthetic genes of both toxins (*pks*CT, *pat*K) have a reduced capacity to colonize apples. Patulin is usually produced very early during growth and at much higher amounts compared to citrinin. Citrinin in contrast, can analytically be detected only lately during the infection process, generally in correlation to the production of aerial mycelium. The biosynthesis of mycotoxins is strongly dependent on the substrate and various environmental parameters. The most important parameters are temperature, water activity and pH. Changes in the pH of the substrate are sensed by the PACC signal cascade which transfers the signal to the transcriptional level thereby regulating certain responsive genes which also might include

mycotoxin biosynthetic genes. Fine-tuned regulation of mycotoxin biosynthesis under conditions of the natural habitat is important for the fungus for the colonization of the substrate. For *Penicillium expansum* it could be shown that the PACC regulated citrinin biosynthesis supports adaptation and colonization. The *pac*C gene was inactivated and it was demonstrated that the resulting *P. expansum* transformants had a reduced colonization capacity on apples. For *P. expansum* it was shown that there is an interplay between PACC regulation and the transcriptional activator of the citrinin biosynthesis gene cluster, CTNR. A higher expression of the *ctn*R gene could override PACC regulation which results in constitutive biosynthesis of citrinin. Interestingly, according to literature data, also the biosynthesis of patulin is regulated by PACC.

Genomic diversity in ochratoxigenic and non ochratoxigenic strains of *Aspergillus carbonarius*

Gemma Castellá[1], M. Rosa Bragulat[1], Laura Puig, Walter Sanseverino[2], and F. Javier Cabañes[1*]

[1] *Veterinary Mycology Group, Department of Animal Health and Anatomy. Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.* [2] *Sequentia Biotech SL, Barcelona, Spain*

[*]Presenter: Gemma.Castella@uab.cat

Ochratoxina A (OTA) is a mycotoxin with nephrotoxic effects that has been associated to kidney problems in both livestock and human populations and it can be found in a variety of common foods and beverages. This mycotoxin is produced by several species of *Penicillium* and *Aspergillus* among which *Aspergillus carbonarius* is recognized as the main OTA source on grapes and derived products. OTA production is a very consistent property of *A. carbonarius* and nearly 100% of the isolates of this species produce OTA. Little is known about the genes involved in the OTA biosynthetic pathway although recently a consensus OTA biosynthetic pathway has been proposed in *Aspergillus ochraceus*. In *A. carbonarius*, a hypothetical OTA gene cluster has been characterized. This cluster contains three genes, a nonribosomal peptide synthetase (*AcOTAnrps*) gene, a polyketide synthase (*AcOTApks*) gene and a halogenase gene (*AcOTAhal*), directly related to OTA biosynthesis. There are two other genes located in the same genomic region that could play a role in the biosynthesis pathway as part of the OTA cluster. These genes were a cytochrome p450 monooxygenase (*AcOTAp450*), and a transcription factor (*AcOTAbZIP)*. However, the OTA cluster remains not completely defined and most of the regulatory aspects underlying OTA production remain unclear. In the present study, we present the genome resequencing of four *A. carbonarius* strains, one OTA producer and three atypical non-OTA producing strains. These strains were sequenced using Illumina technology and compared with the genome reference Acv3. Besides this main objective, and due to the fact that three of these strains do not produce OTA, we performed some new specific bioinformatics analyses in genes involved in OTA biosynthesis. We focused these analyses on nonsense and missense mutation detection, and also in to identify whether large DNA sections of the reference genome Acv3 or of the newly sequenced OTA producing strain were absent in the genome of the three non-OTA producing strains. Regarding the genes potentially involved in OTA biosynthesis, the OTA-producer strain showed variants in *AcOTApks*, *AcOTAnrps*, *AcOTAbZIP* and *AcOTAp450*. In atoxigenic strains only five common missense variants in *AcOTApks* gene were found. Although some gaps of more than 1,000 bp were identified in non-ochratoxigenic strains, no large deletions in functional genes related with OTA production were found. Moreover, the expression of five genes of the putative OTA biosynthetic cluster was down regulated under OTA-inducing conditions in the non-ochratoxigenic strains. Knowledge of the regulatory mechanisms involved in OTA biosynthesis will provide a deeper understanding of these nonochratoxigenic strains.

This research was supported by the Ministerio de Economía y Competitividad of the Spanish Government (AGL2014-52516-R).

ICFM

International Commission on Food Mycology

Conference 2019

Food- and Airborne Fungi – Challenges for Food Safety and Supply

Programme and Abstracts

Freising - Germany, 3-5 June, 2019