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MRI
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Comparative genome analyses uncover citrinin- and ochratoxin biosynthesis gene clusters in the genus *Penicillium*

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Introduction:

Species of the genus *Penicillium* mainly belong to the saprophytes which cause substantial food loss if plants are infested in the field or during postharvest storage under suboptimal conditions. Most Penicillia are able to produce harmful substances such as mycotoxins whose ingestion lead to health issues such as mycotoxicoses in human and animal. Moreover, spores of filamentous fungi in general act as potent antigen and may trigger mycogenic allergies in exposed individuals. Genome sequencing and subsequent mining for unknown secondary metabolite gene clusters is an important prerequisite for an adequate risk assessment.

Results and Discussion:

Sequencing the genomes of different species of the genus *Penicillium* followed by genome mining analyses unraveled a highly diversity of secondary metabolite gene clusters within the analyzed species. An important outcome of investigations, focused on the genomes of Penicillia occurring on salt-rich habitats, such as *P. verrucosum, P. nordium* and *P. citrinum*, was the identification of gene clusters coding for two important mycotoxins, ochratoxin and citrinin. The citrinin gene cluster identified in *Penicillium* shows a high homology to the citrinin genes in *Monascus purpureus* (Chen et al., 2008); the identified, cryptic, ochratoxin gene cluster instead is highly homologous to the ochratoxin cluster described by Gil-Serna et al. (2014) in *Aspergillus steynii*. In *P. nordicum* an ochratoxin polyketide synthase "pksPN" and a non-ribosomal peptide synthase "npsPN" have been described more than 10 years ago (Geisen et al., 2006). Gallo et al., (2017) and other authors recently discussed that more than one polyketide synthase may be involved in the formation of molecules such as ochratoxin. If the additionally identified OTA gene cluster in *Penicillium* is actively involved in ochratoxin biosynthesis in this species, too, is now under investigation and will help to complete the view about biosynthesis of this important mycotoxin.

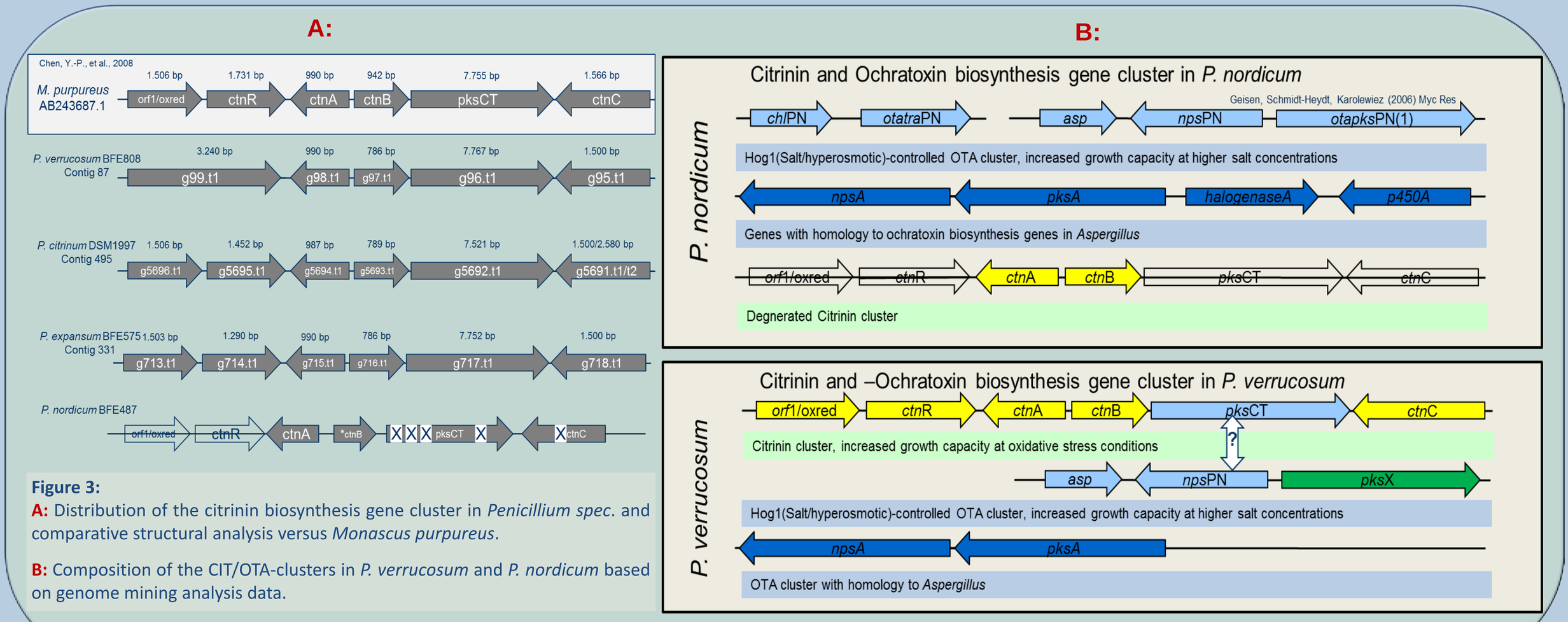

Figure 3:

A: Distribution of the citrinin biosynthesis gene cluster in *Penicillium spec*. and comparative structural analysis versus *Monascus purpureus*.

B: Composition of the CIT/OTA-clusters in *P. verrucosum* and *P. nordicum* based on genome mining analysis data.

Methods:

The genome data of the analyzed Penicillia has been recruited from online genome data bases or was sequenced by our own using a Miseq sequencer. Subsequent analyses of these genome sequences have been carried out using automatic annotation with blast2go, cluster mining by using SMURF, the MUSCLE algorithm provided by CLC Sequence Viewer Version 7.6, AntiSMASH and Augustus. HCG-microsyntheny analyses have been carried out using MegAlignpro15 from the LASERGENE genomic work suite. Sequencing was performed on a MiSeq stand-alone benchtop sequencing platform (Illumina, Inc.; San Diego, California, USA). *P. verrucosum* BFE808 and *P. nordicum* BFE487 were inoculated for 7 days at 25°C in YES broth (20 g/l yeast extract, 150 g/l saccharose). DNA was extracted using the DNA extraction Kit NucleoSpin Plant II (MACHEREY-NAGEL GmbH & Co. KG; Düren, Germany) according to manufacturer's instructions, while the mycelium was disintegrated by use of the FastPrep-24 BeadBeater (MP Biomedicals; Santa Ana, California, USA). Quality and quantity of the probes were determined using NanoDrop 1000 (VWR International GmbH; Erlangen, Germany) and Qubit ds DNA HS assay on a Qubit 3.0 (Thermo Fisher Scientific Inc.; Waltham, Massachusetts, USA). The sequencing library was created under use of the Illumina Nextera sample prep Kit and its specific workflow, which gained a sequencing library analyzed and quality checked by using Experion DNA1k Analysis (Bio-Rad Laboratories GmbH; Munich, Germany). Sequencing was carried out on a MiSeq V3 flowcell enabling 2x300 reads, where we gained raw sequencing data.

The raw data first was processed online on BaseSpace, using the provided FASTQ Toolkit v1.0 application to trim adaptor sequences. Reads were *de-novo* assembled using SeqmanNGen v.15 (DNASTAR, Inc.; Madison, Wisconsin, USA). Re-Naming of the resulting contigs was done using FaBox (Villesen, 2007). Gene prediction was facilitated using AUGUSTUS v.3.0.2 (Sommerfeld *et al*., 2009; Stanke *et al*., 2004). Prediction of secondary metabolite gene clusters was carried out using antiSMASH (Medema *et al*., 2011) and SMURF (Khaldi *et al*., 2010).

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