

Elucidating the biosynthetic pathway of the anticancer secondary metabolite calbistrin in *Penicillium decumbens*

Grijseels, Sietske; Pohl, Carsten ; Wasil, Zahida; Froslev Nielsen, Jens Christian ; Nygård, Yvonne ; Nielsen, Jens; Frisvad, Jens Christian; Nielsen, Kristian Fog; Workman, Mhairi; Larsen, Thomas Ostenfeld; Driessen, Arnold J.M.; Frandsen, Rasmus John Normand

Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

Citation (APA):
Grijseels, S., Pohl, C., Wasil, Z., Froslev Nielsen, J. C., Nygård, Y., Nielsen, J., ... Frandsen, R. J. N. (2017). Elucidating the biosynthetic pathway of the anticancer secondary metabolite calbistrin in *Penicillium decumbens*. Abstract from 29th Fungal Genetics Conference, Pacific Grove, United States.

DTU Library

Technical Information Center of Denmark

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

38T Thinking outside the box: NADPH oxidases as signaling hubs are more than just simple ROS producer. R. Marschall, P. Tudzynski Institute of Plant Biology and Biotechnology, Münster, DE.

The filamentous fungus *Botrytis cinerea* is one of the most devastating plant pathogens. Molecules, which are directly involved in the communication between host and pathogen are reactive oxygen species (ROS). They are produced as mere by-products of the respiratory chain or in highly conserved processes by e.g. NADPH oxidase complexes. In *B. cinerea* two different Nox complexes do exist, both regulating a huge variety of developmental processes. While for the NoxA complex the elucidation of the complex composition and interacting proteins (Bclqg1, BcNoxD) is already sophisticated^{1,2}, there are hardly any information about proteins that directly interact with the second catalytic subunit BcNoxB or the overall regulator BcNoxR.

Most recently, we identified a large set of proteins in a pulldown screening with the catalytic subunit NoxB and the regulator NoxR. While potential interaction partner that were previously postulated as putative Nox complex members could not be verified, other target proteins were fished in the pulldown approach and verified by Y2H experiments.

Surprisingly, in four independent experiments proteins were pulled down with BcNoxB that all belong to a predicted PKS5-NRPS7 cluster. Subsequent Y2H analyses revealed that indeed proteins of the cluster interact directly with BcNoxB, which might indicate a role for BcNoxB in the secondary metabolism. Moreover, different cluster proteins seem to build up a complex since some of them are interacting with each other.

However, not only BcNoxB seems to be more than just a simple ROS producer. The first catalytic subunit BcNoxA was shown to interact with the cytochrome C peroxidase. Thus, for the first time in fungi a direct link came apparent between mitochondria, the respiratory chain and the NoxA complex. Since BcNoxA is additionally associated with the protein disulfide isomerase BcPDI it can be speculated that BcNoxA is contributing to ROS levels, but is also working as a signaling hub, connecting essential intracellular processes such as protein folding, redox homeostasis, respiration and ROS production.

¹Marschall et al., 2016: Update on Nox function, site of action and regulation in *Botrytis cinerea*. Fungal Biology and Biotechnology 2016:3:8

²Marschall and Tudzynski, 2016: Reactive oxygen species in development and infection processes. Semin Cell Dev Biol. 2016 Sep;57:138-46.

39F Identification and characterisation of polyketide synthases in the barley pathogen *Ramularia collo-cygni*. F. Dussart^{1,2}, P.N. Hoebe¹, S.H. Spoel², N.D. Havis¹, G.R.D. McGrann¹ 1) Crop and Soil Research Department, SRUC, Edinburgh, GB; 2) School of Biological Sciences, University of Edinburgh, Edinburgh, GB.

The filamentous fungus *Ramularia collo-cygni* (Rcc) causes the late season barley disease Ramularia Leaf Spot (RLS). In the past twenty years it has become an important disease in northern Europe and other temperate regions of the world. RLS can quickly cause substantial yield losses and reduce grain quality. Limited options to control RLS have given this disease a higher profile and attracted scientific attention enhancing the understanding of the Rcc life cycle which indicated that this fungus is an endophyte with a necrotrophic phase.

Rcc produces a series of anthraquinone toxins called rubellins, thought to be involved in RLS development. Anthraquinones are known to be synthesised through the polyketide pathway. In this study, ten putative polyketide synthases (PKS) were identified in the genome of Rcc. Using *in silico* genome walking eight secondary metabolite-related gene clusters were identified near core PKS genes. Clusters with no PKS core genes were also identified. Gene expression of six core PKS genes was assessed during disease development in barley seedlings. Expression of most PKS transcripts declined during disease development. Co-regulation of secondary metabolism-related genes that clustered with core PKS genes was also observed. Together these data imply that, if produced; polyketide-derived secondary metabolites in Rcc might not be associated with disease symptom development. Instead, secondary metabolites may act as antifungal agents, as we found that Rcc inhibited the growth of several major barley fungal pathogens *in vitro*, suggesting these compounds may be important for Rcc niche exploitation.

40W Elucidating the biosynthetic pathway of the anticancer secondary metabolite calbistrin in *Penicillium decumbens*. Sietske Grijseels¹, Carsten Pohl², Zahida Wasil¹, Jens Christian Nielsen³, Yvonne Nygård², Jens Nielsen³, Jens C. Frisvad¹, Kristian Fog Nielsen¹, Mhairi Workman¹, Thomas Ostenfeld Larsen¹, Arnold Driessen², Rasmus John Normand Frandsen¹ 1) Department of Biotechnology and Biomedicine, Technical University of Denmark, DK 2800 Kgs. Lyngby, Denmark; 2) Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands; 3) Department of Biology and Biological Engineering, Chalmers University of Technology, SE412 96 Gothenburg, Sweden.

Filamentous fungi are important producers of secondary metabolites, low molecular weight molecules that often have bioactive properties.

One interesting secondary metabolite is calbistrin, a compound recently found to have bioactivity against leukemia cells. This compound consists of two polyketides linked by an ester bond; a decalin containing polyketide similar to lovastatin, and a linear 12 carbon dioic acid structure. Calbistrin is known to be produced by several uniseriate black Aspergilli, *Aspergillus versicolor*-related species, and several Penicillia. Among the Penicillia, the recently genome sequenced *P. decumbens* is interesting as it produces several putative intermediates of the calbistrin pathway, such as decumbenone A and B and versiol. In this study, the molecular and enzymatic mechanisms underlying the biosynthesis of calbistrin are elucidated using a combinatorial approach of bioinformatics, molecular biology and analytical chemistry. Comparative studies of the polyketide synthase (PKS) sequences from the three genome sequenced species *A. versicolor*, *A. aculeatus* and *P. decumbens* resulted in the identification of a putative gene cluster for production of the decalin part of calbistrin. Implementation of CRISPR/Cas9 technologies in *P. decumbens* facilitated the deletion of the putative PKS in this species. Subsequent UHPLC-MS analysis of extract metabolites revealed that calbistrin and putative intermediate compounds were absent, proving the involvement of the PKS in calbistrin production. Further characterization of the predicted gene cluster is achieved by targeted deletion of the individual biosynthetic genes in the cluster.

41T Mechanisms of capsaicin tolerance in fungal seed pathogens of wild chili peppers. C.A. Adams^{1,2}, K. Zimmerman^{2,4}, K. Fenstermacher³, D. Geiser³, A. Pringle^{1,5} 1) Plant and Microbial Biology, UC Berkeley, Berkeley, CA; 2) Department of Organismic and Evolutionary Biology, Harvard University, Cambridge MA; 3) Department of Plant Pathology and Environmental Microbiology, Penn State University, University Park, PA; 4) Gingko Bioworks; 5) Department of Botany and Bacteriology, University of Wisconsin-Madison,