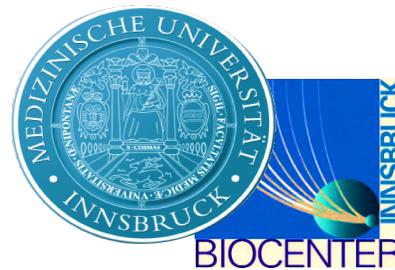




ECFG15

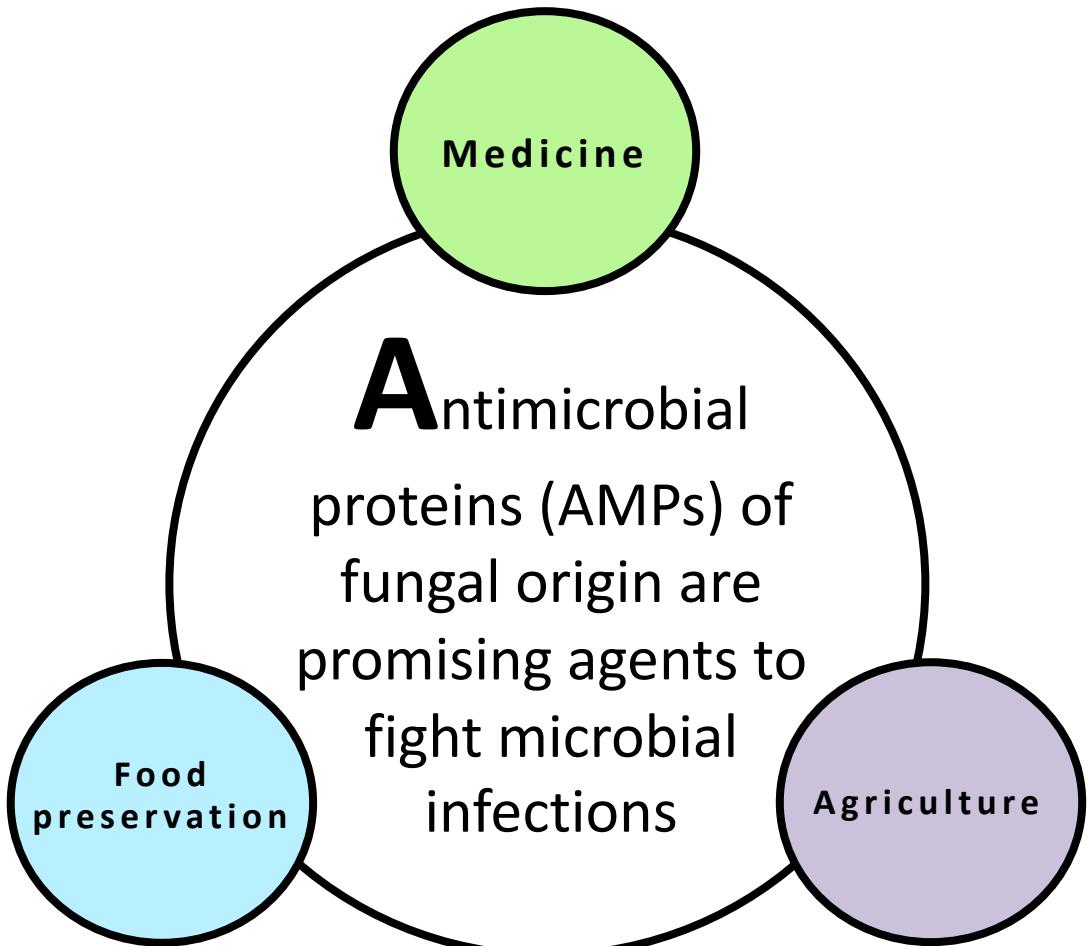
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The antifungal protein PAFB
from *Penicillium chrysogenum*
is highly expressed under
nutrient excess conditions

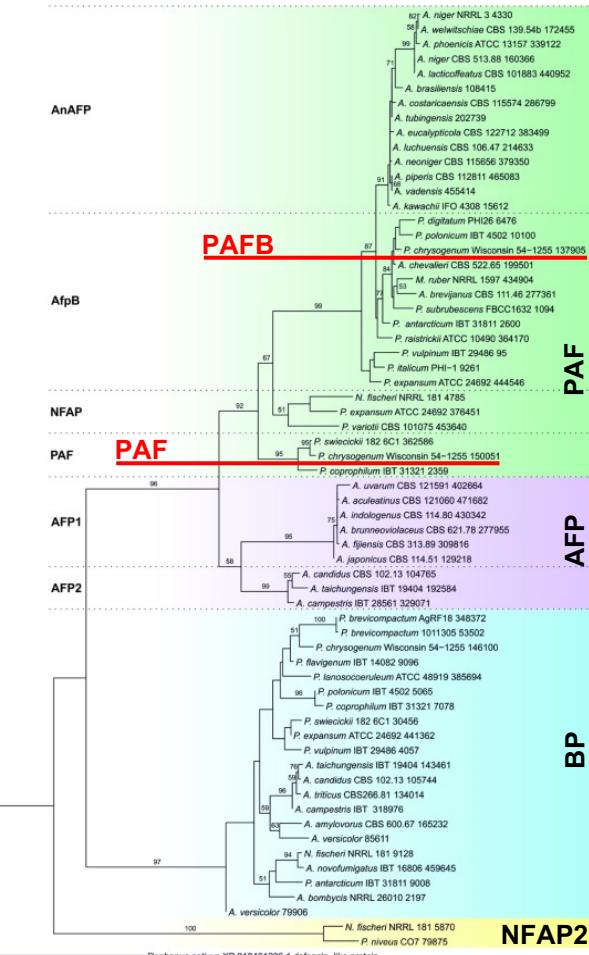
Anna Huber

Institute of Molecular Biology
Medical University of Innsbruck

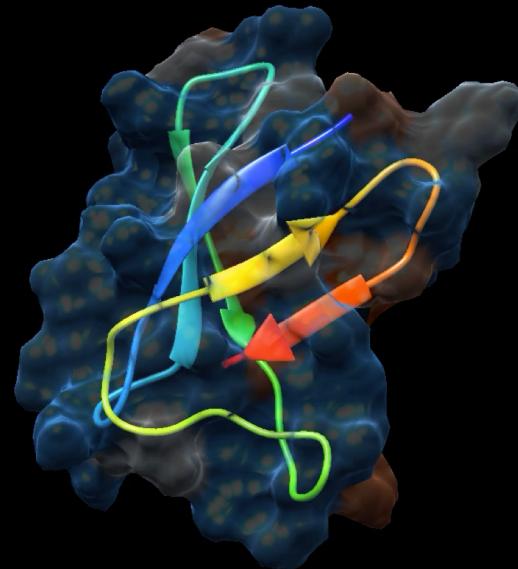


Sonderegger et al. *Front Microbiol* 2018
 Rodriguez-Martin et al. *Peptides* 2010
 Tóth et al. *AMB Express* 2016

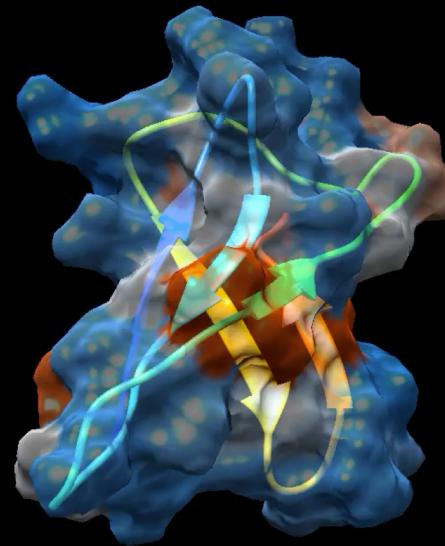
Meyer *Appl Microbiol Biotechnol* 2008
 Palicz et al. *Toxicol Appl Pharmacol* 2013
 Szappanos et al. *Naunyn-Schmiedebergs Archives of Pharmacology* 2005



PAF & PAFB – Two AMPs encoded by *P. chrysogenum*



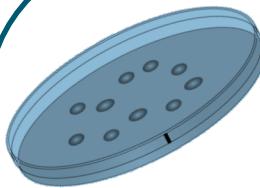
PAF



PAFB

Protein	Number of amino acids	Molecular mass (kDa)	Net charge at pH=7.0	GRAVY
PAF	55	6.25	+4.7	-1.375
PAFB	58	6.50	+5.2	-1.031

Intrinsic function of PAF in *P. chrysogenum*?

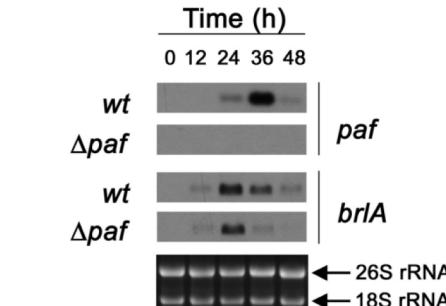
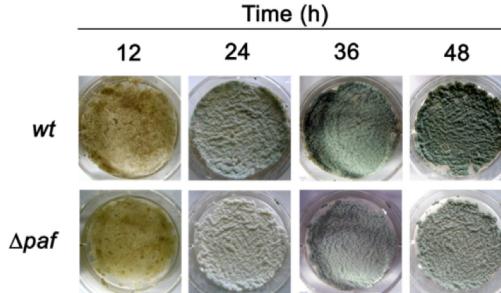


Journal of Basic Microbiology 2011, 51, 253–262

Research Paper

The *paf* gene product modulates asexual development in *Penicillium chrysogenum*

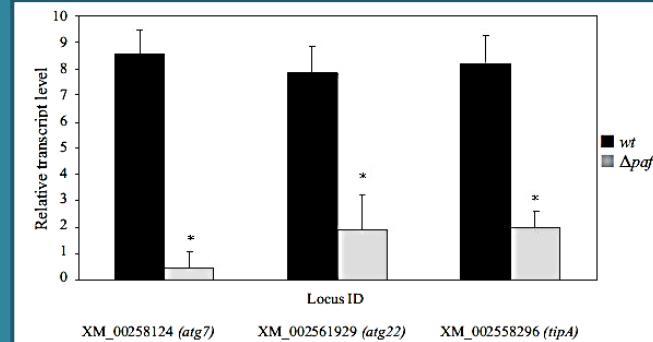
Nikoletta Hegedűs^{1,2}, Claudia Sigl², Ivo Zadra², István Pócsi² and Florentine Marx¹



Acta Microbiologica et Immunologica Hungarica, 61 (3), pp. 379–388 (2014)
DOI: 10.1556/AMscr.61.2014.3.10

PENICILLIUM ANTIFUNGAL PROTEIN (PAF) IS INVOLVED IN THE APOPTOTIC AND AUTOPHAGIC PROCESSES OF THE PRODUCER *PENICILLIUM CHRYSOGENUM*

BARBARA KOVÁCS¹, NIKOLETTA HEGEDŰS¹, MIHÁLY BÁLINT¹,
ZSUZSA SZABÓ¹, TAMÁS EMRI¹, GRÉTA KISS², MIKLÓS ANTAL²,
ISTVÁN PÓCSI¹ and ÉVA LEITER^{1*}



Knowledge about AMP gene expression is important for:

- Understanding the AMPs' function in the host
- Identifying culture conditions to improve AMP production

Regulation of AMP expression:

Aspergillus giganteus (**AFP**)

Aspergillus niger (**AnAFP**)

Penicillium chrysogenum (**PAF**)



Expression under limiting growth conditions:

- nutrient limitation
- pH stress

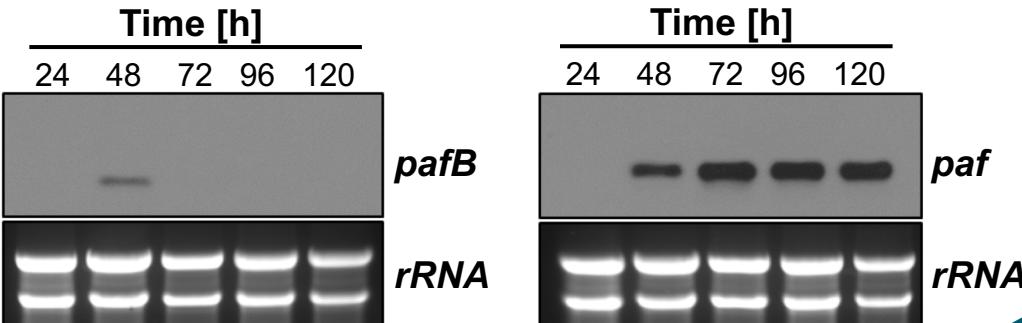
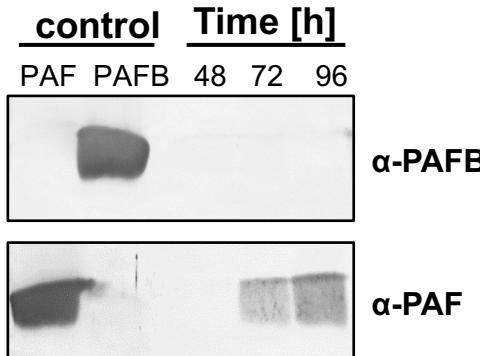


AMP function in apoptosis, autolysis or autophagy?

Which cultivation conditions favor PAFB expression in *P. chrysogenum*?

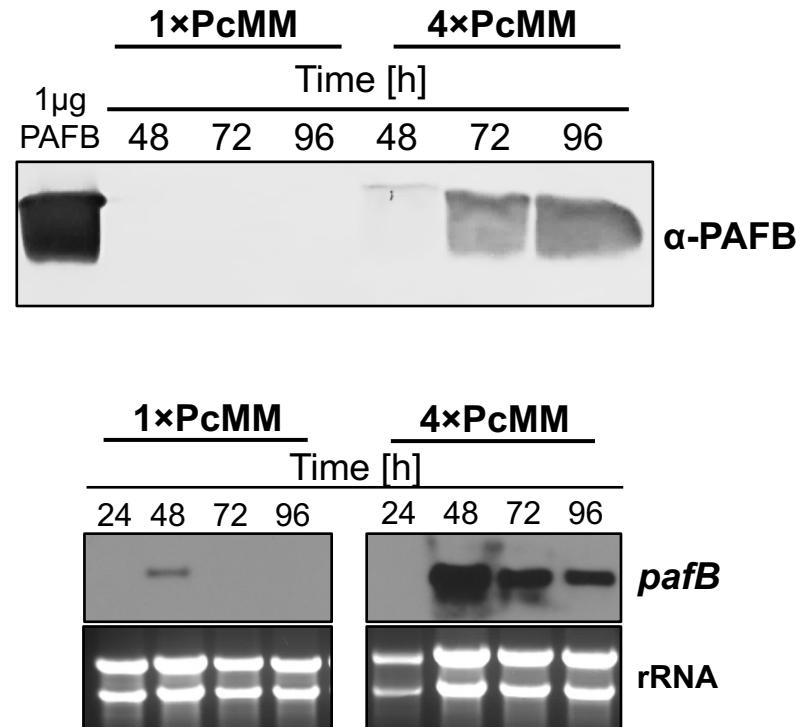
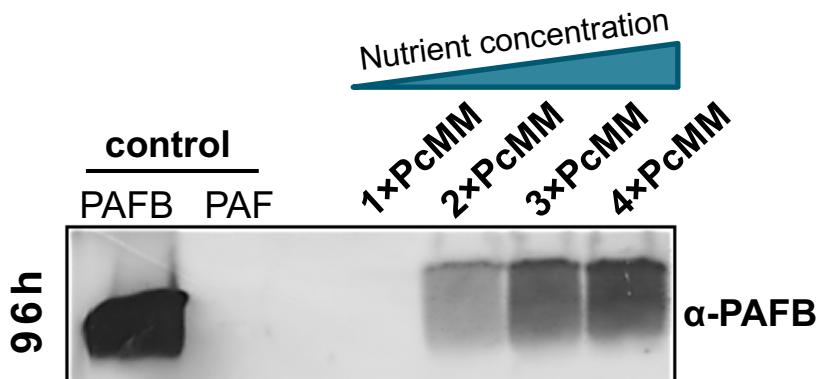


No PAFB-expression under nutrient limiting conditions...

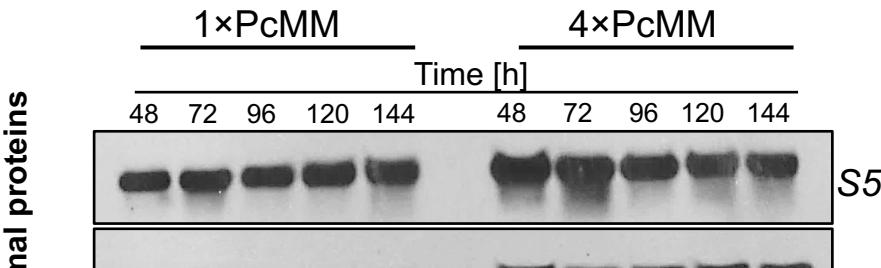
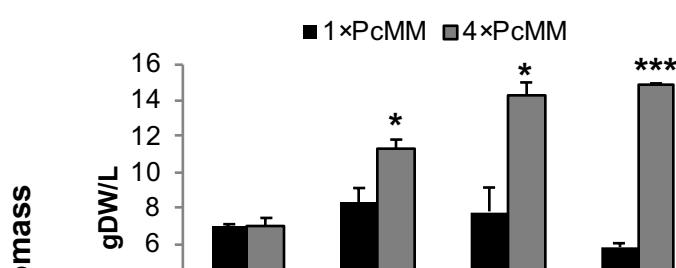


PAFB-expression is induced under nutrient excess

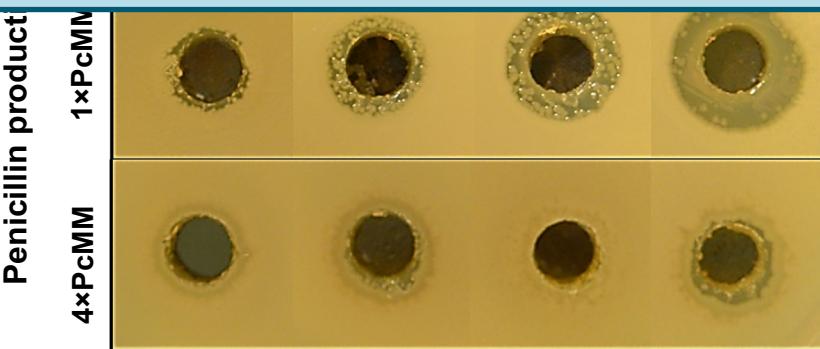
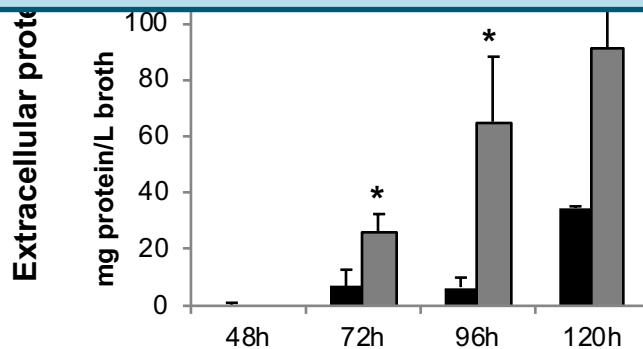
AMP expression is not exclusively induced under nutrient limitation



Pafb-expression is induced in metabolically active cultures

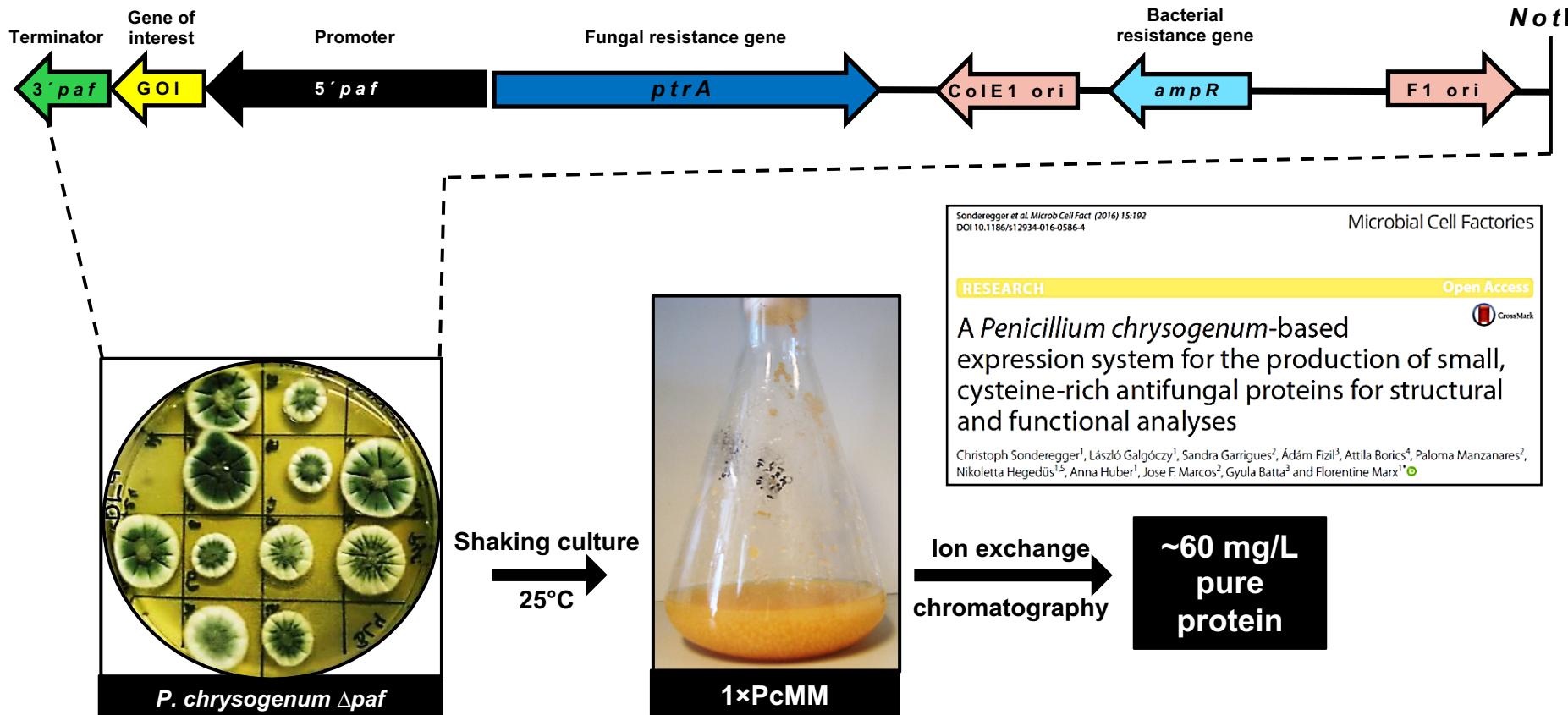


AMPs might play a role in cellular processes other than autophagy, apoptosis or autolysis



* p ≤ 0.05, *** p ≤ 0.0005

The *P. chrysogenum*-based expression system uses the strong *paf*-promoter

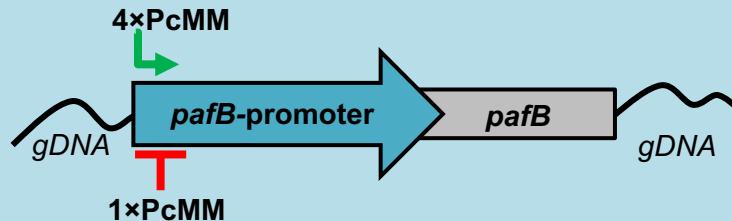


The *pafB*-promoter is strong and inducible

Efficiency of *pafB*-promoter compared to *paf*-promoter & *xyIP*-promoter?

wt

This study



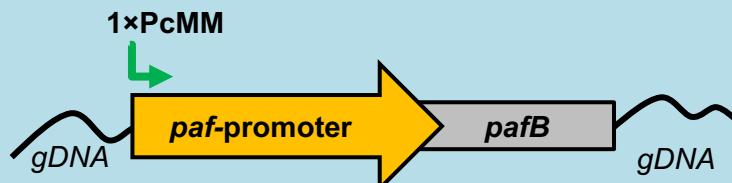
paf_prom

Sonderegger et al. *Microb Cell Fact* 2016

A *Penicillium chrysogenum*-based expression system for the production of small, cysteine-rich antifungal proteins for structural and functional analyses.

New Antimicrobial Potential and Structural Properties of PAFB: A Cationic, Cysteine-Rich Protein from *Penicillium chrysogenum* Q176.

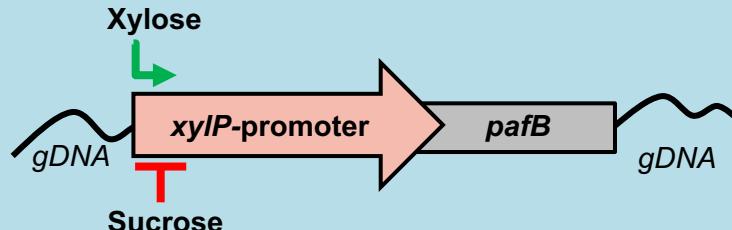
Huber et al. *Sci Rep* 2018



xyIP_prom

Zadra et al. *Appl Environ Microbiol* 2000

xyIP Promoter-Based Expression System and Its Use for Antisense Downregulation of the *Penicillium chrysogenum* Nitrogen Regulator NRE

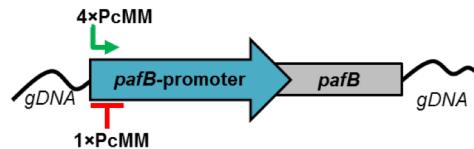


The *pafB*-promoter is strong and inducible

PAFB expression under
pafB-promoter regulation



4×PcMM
(8% Sucrose)

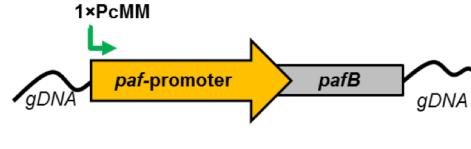


PAFB expression under
paf-promoter regulation

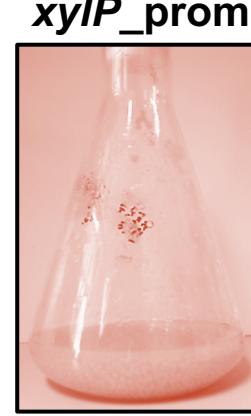


VS.

1×PcMM
(2% Sucrose)

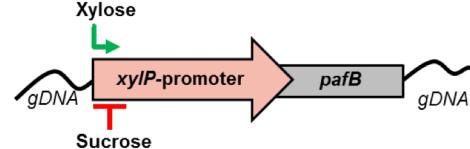


PAFB expression under
xyIP-promoter regulation



VS.

1×PcMM
(2% Xylose)



Controls:

Q176 in 1×PcMM
(2% Sucrose)

Q176 in 1×PcMM
(2% Xylose)

The *pafB*-promoter is strong and inducible

wt



4×PcMM
(8% Sucrose)

paf_prom



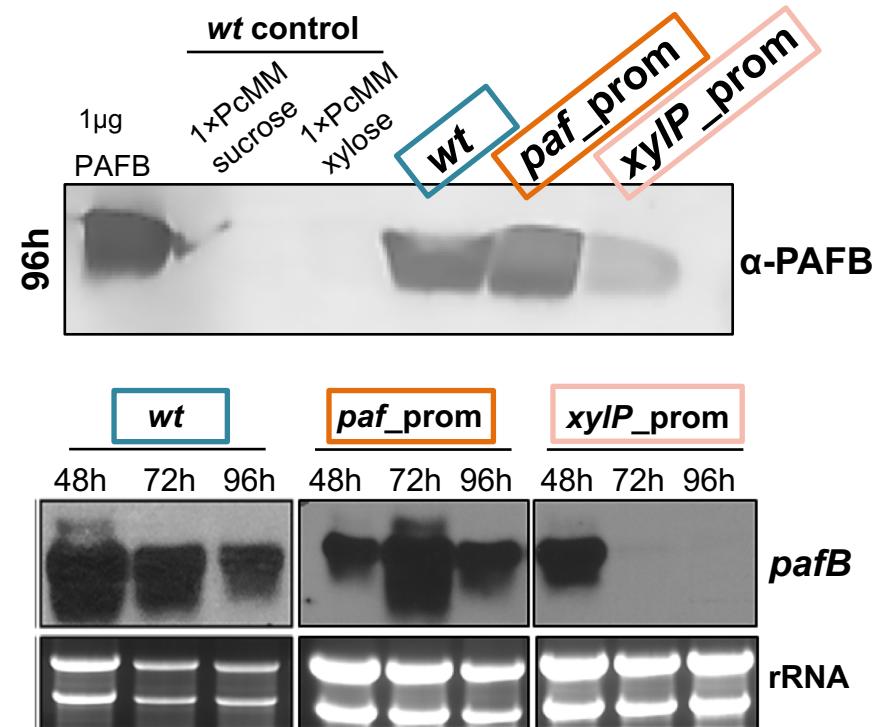
1×PcMM
(2% Sucrose)

xyIP_prom



1×PcMM
(2% Xylose)

Highest protein yields with the *pafB*- and *paf*-promoter



Summary

- The *pafB*-gene is expressed under nutrient excess conditions
→ difference to other AMPs
- Increased and prolonged mRNA-expression correlates with high PAFB-amount in culture broth
- The *pafB*-promoter is strong and inducible and opens new possibilities to be used for recombinant protein expression



Acknowledgements

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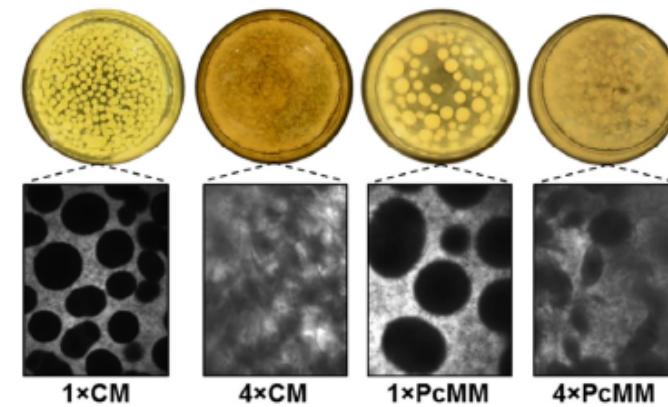
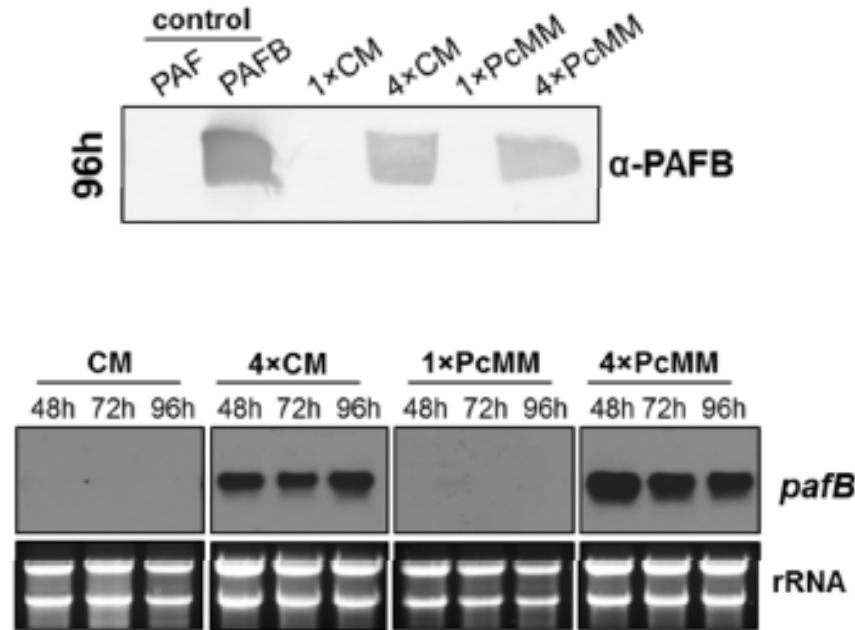
László Galgóczy

FWF
Der Wissenschaftsfonds.

EMBO Fellow

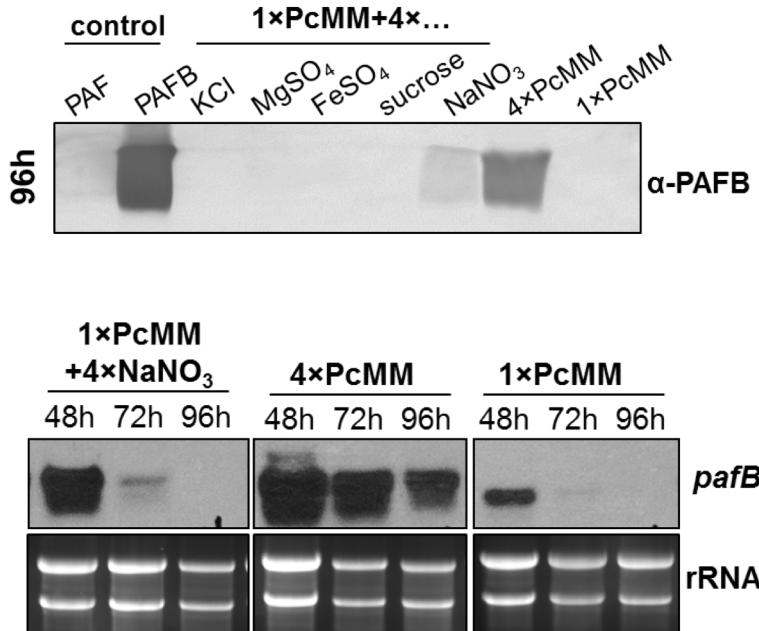
BIOCENTER
INNSBRUCK

Expression in CM



PAFB-expression is induced by nutrient excess

Nitrogen induces PAFB-expression



Impact of mycelial morphology on PAFB-expression?

