Alternative Modelle zur Untersuchung von Pathogenitätsfaktoren von Mucorales

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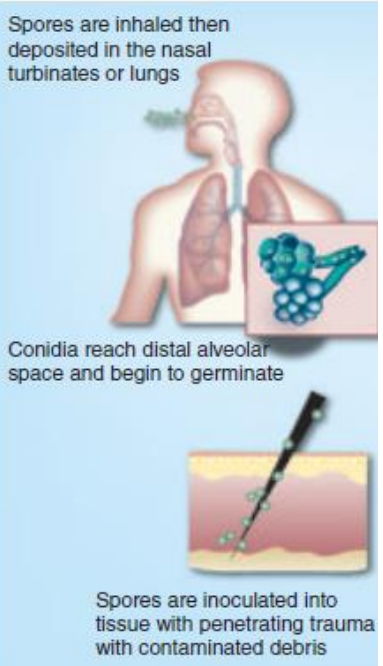
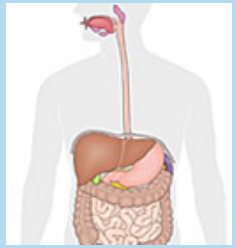
PEG Tagung Mai 2023

Key characteristics of Mucormycosis

transmission

manifestation





uptake of contaminated food

rhino-orbital/rhino-cerebral mucormycosis

pulmonary mucormycosis

cutaneous mucormycosis (primary)

gastrointestinal mucormycosis

high likelyhood of dissemination

secondary cutaneous

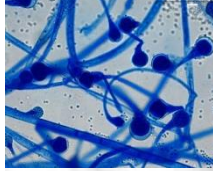
unique risk factors

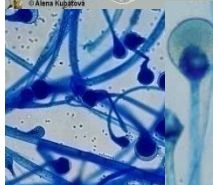
- non/poorly controlled diabetes
- +/- ketoacidosis
- iron overload
- deferoxamine therapy

clinical features

- fulminant progression of disease
- extensive tissue necrosis
- angioinvasion

variety of causative agents





adapted from Lewis & Kontoyiannis Future Microbiol 2013

Animal models….

understanding infectious diseases

therapy development

limited access to patient material & difficulty in designing clinical trials

- relatively low incidence
- variety of causative agents
- different routes of infections
- variations in underlying disease and risk factors

limitations & challenges

- variations in technical details
- lack of standardization: mouse strains, dosing, immunosuppression
- readout parameters

Alternative models

NON-  MODELS & MUCORMYCOSIS

Alternative vertebrate models

Less sentient animals/stages:
embryos or fetuses

Embryonated chicken egg model
Zebrafish model

Alternative invertebrate models

insects
nematodes

Drosophila melanogaster
Galleria mellonella
Caenorhabditis elegans

Cell culture models

immoratlized cell lines
3D-cell culture models
4D-cell culture models

aim

- **advantages**
- **limitations**
- **usage/questions addressed**

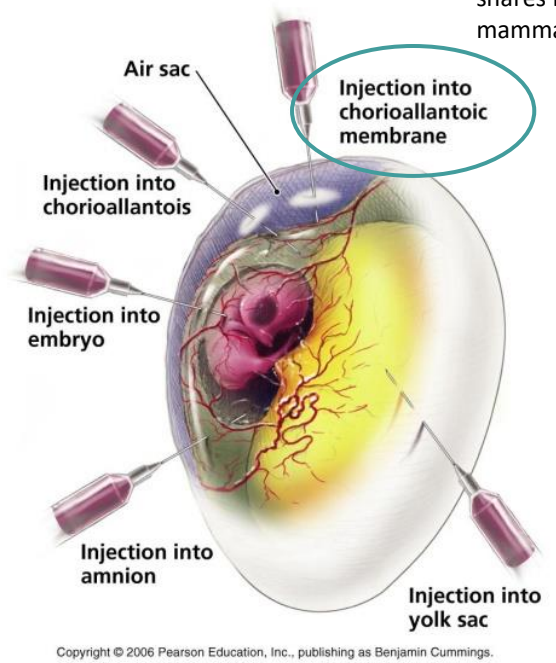
alternative model systems…..

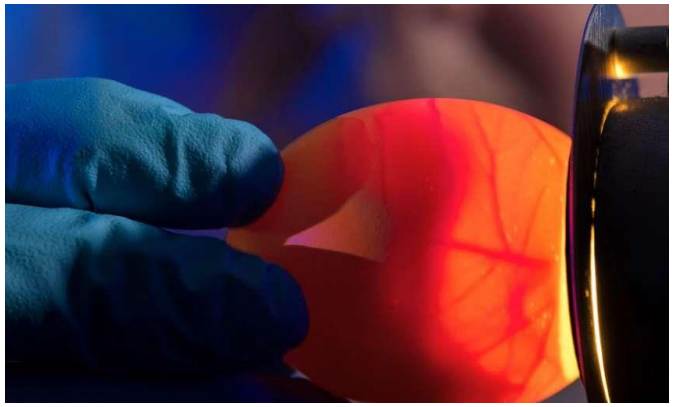
- facil model organism – to breed (or to get), to keep, to use
- no/low ethical concerns & regulations
- low price
- possibility of high throughput screening (e.g. pathogen: knock out libraries/chemical libraries for antifungal compounds)
- easy to use, no special facilities
- correlation in outcome to other (vertebrate) models

Alternative vertebrate models

Embryonated chicken eggs

shares features with mammalian lung





until day 21 not under animal protection law!

optimal temperature for this model = 37,6 °C !!

➔ dental drill, syringes, wax, lamp for candling, incubator

Jacobsen ID et al., Mol Biol. 2012

Jacobsen ID et al., Infect Immun. 2010

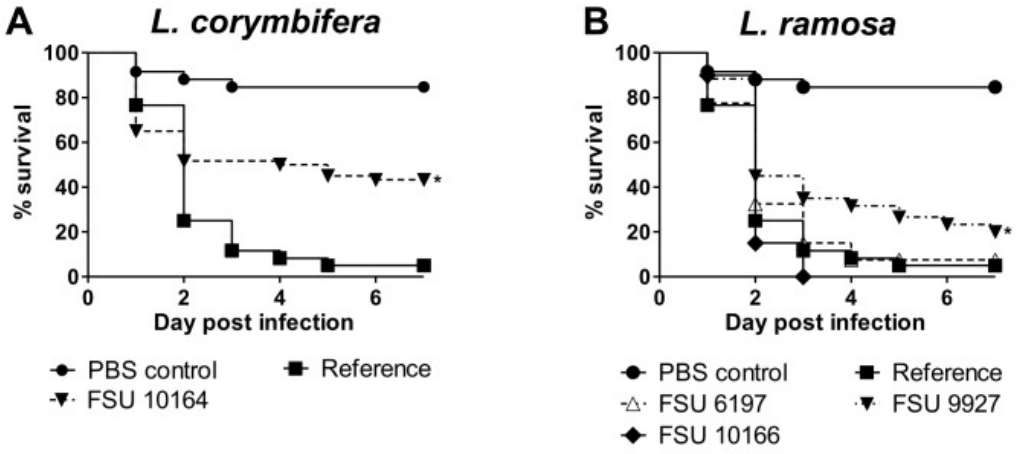
Alternative vertebrate models

Embryonated chicken eggs

- mainly used for high throughput screening & intra- and interspecies comparison of virulence potential
- „virulence profile" correlated to other models (Galleria larvae, murine model)

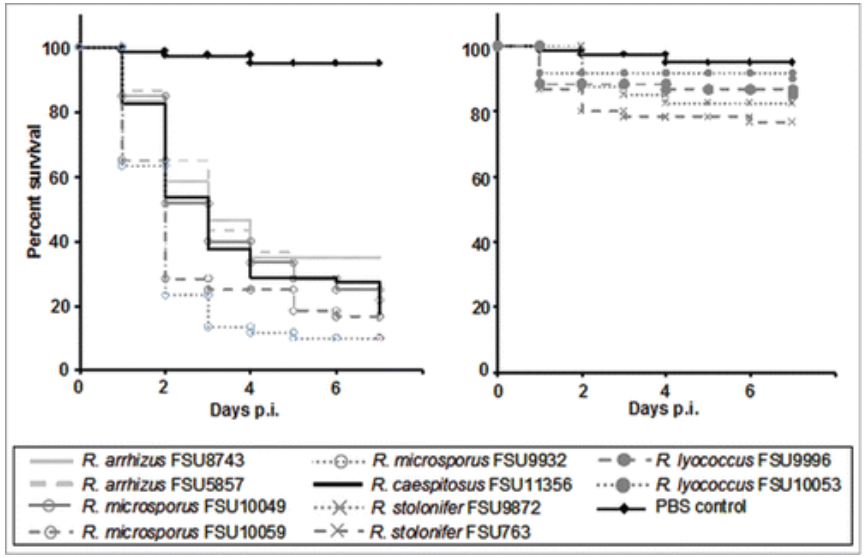
Schwartze et al. Plos One 2012

- virulence potential of different Lichtheimia species
- clinically relevant species show higher virulence potential than other (*L. hyalospora*)

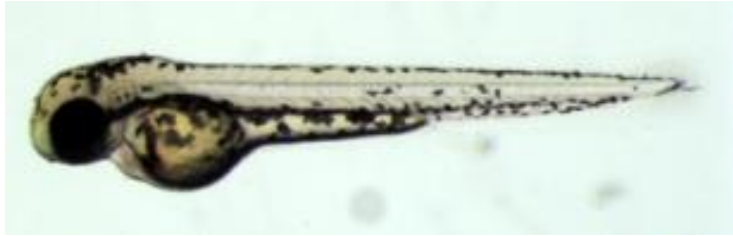


Kaerger et al. Virulence 2015

- correlation thermotolerance and virulence of *Rhizopus* sp.
- similar profile in Galleria larvae, although incubated at 30°C

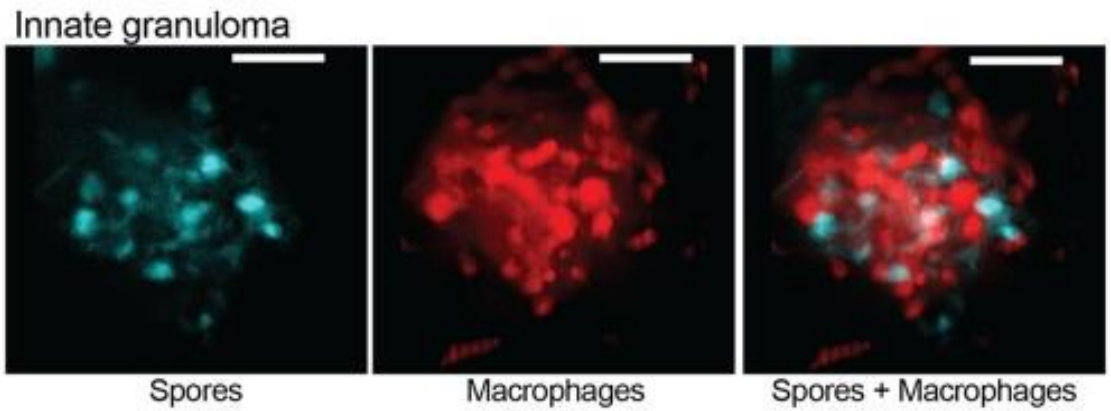


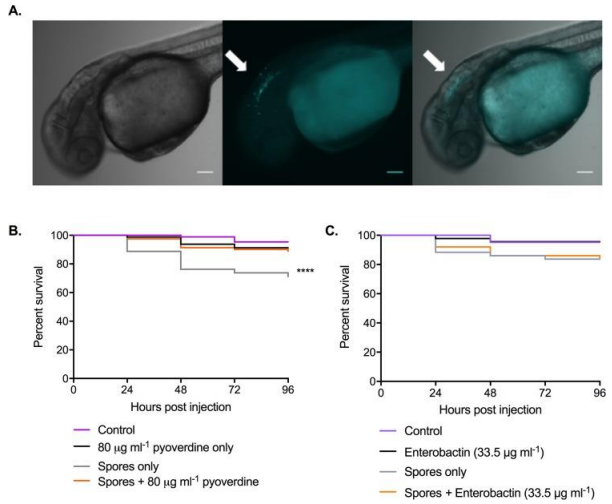
Alternative vertebrate models
zebrafish larvae



Voelz et al. Dis Model Mech. 2015
Inglesfield et al. Mbio 2018
Kousser et al. Scientific Reports 2018

- differential virulence of *M. lusitanicus* in 2 infection sites, tissue specific immune response, higher virulence in immunosuppressed larvae
- host immune response to *M. lusitanicus* depends on the rapid recruitment of phagocytes and inhibition of spore germination
- control of infection by formation of early granuloma
- pathogenicity & iron: Pseudomonas- siderophore pyoverdine protects zebrafish larvae from *R. microsporus* infection

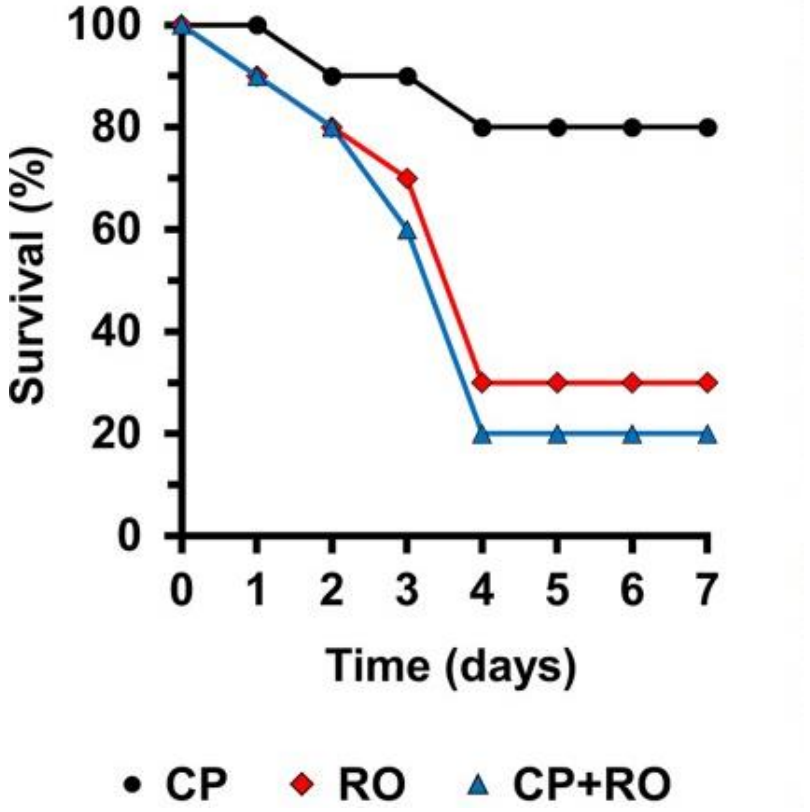




Alternative vertebrate models
zebrafish adult

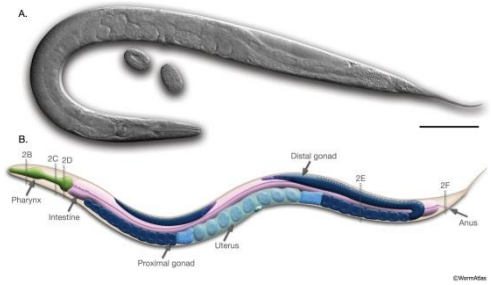
Tatara et al. AAC 2018

- no significant difference in survival of immunosuppressed (cyclophosphamide) fish vs. non-immunosuppressed infected with R.oryzae
- but – hyphal formation was seen earlier in immunosuppressed



Invertebrate model systems

Nematodes - *C. elegans*



1mm

Drosophila melanogaster

larvae or adult used

Galleria mellonella

larvae used

Invertebrate model systems

C. elegans

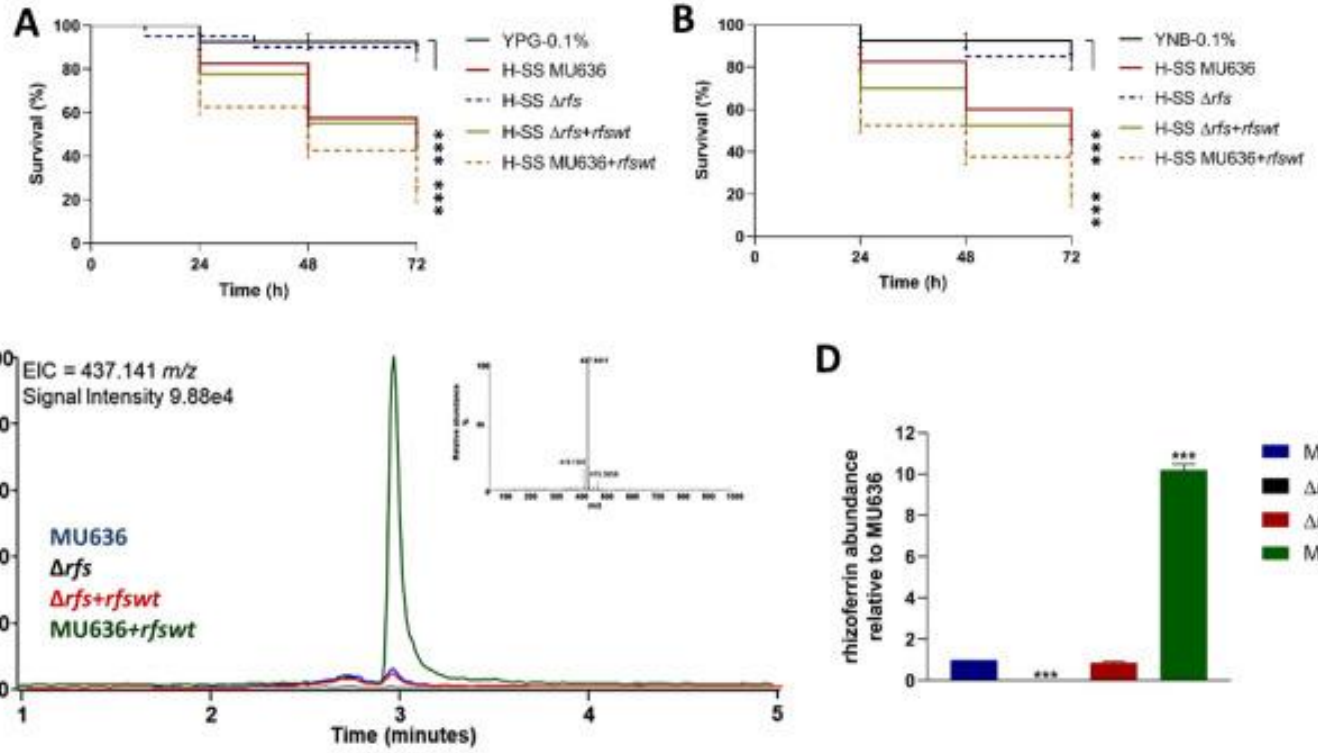
Virulence studies of kock out strains – investigations of secreted effectors

Alejandre-Castañeda et al. Scientific Reports 2022

- rhizoferrin in supernatant contributes to virulene of *M. lusitanicus:*

  Δrfs higher survival of nematodes compared to wt

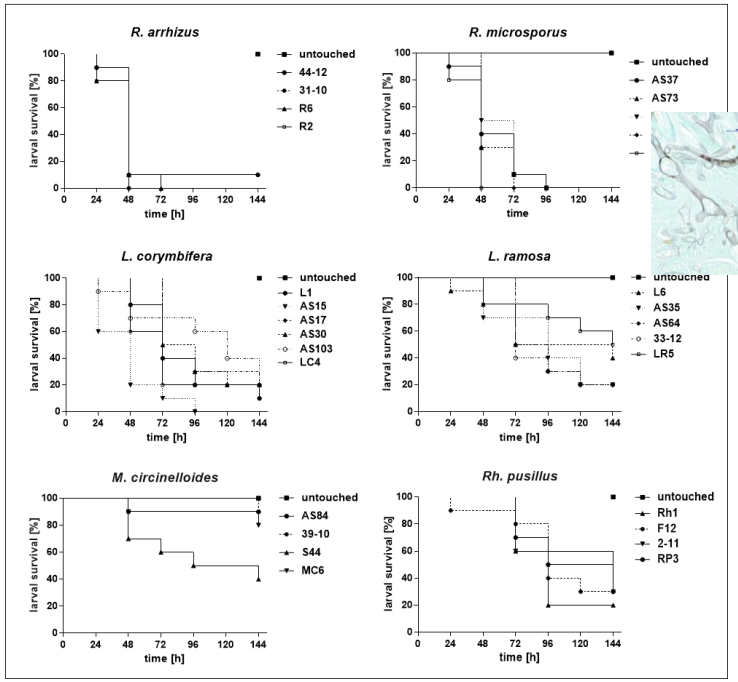
  overespression of rfs results in higher mortality

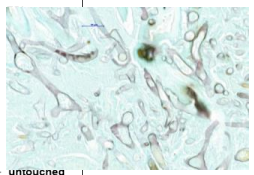


Invertebrate model systems

Galleria mellonella

Maurer et al. Med Mycol 2018

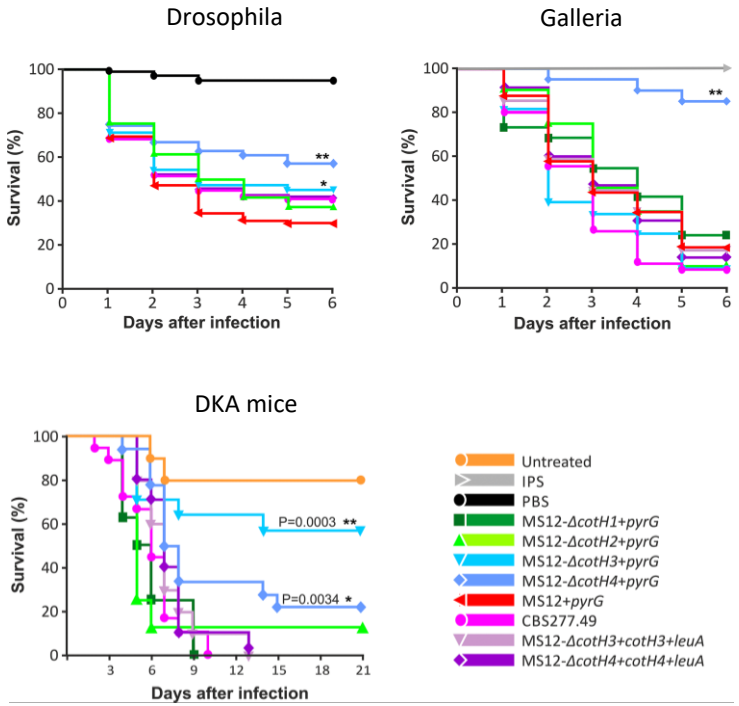




- high throughput studies: *Rhizopus* strains exhibit strongest virulence potential
- survival data correlate with species-specific features : e.g. *in vitro* growth rates and resistance to oxidative stress
- killing by active growth of fungi → histological examination, heat inactivated spores

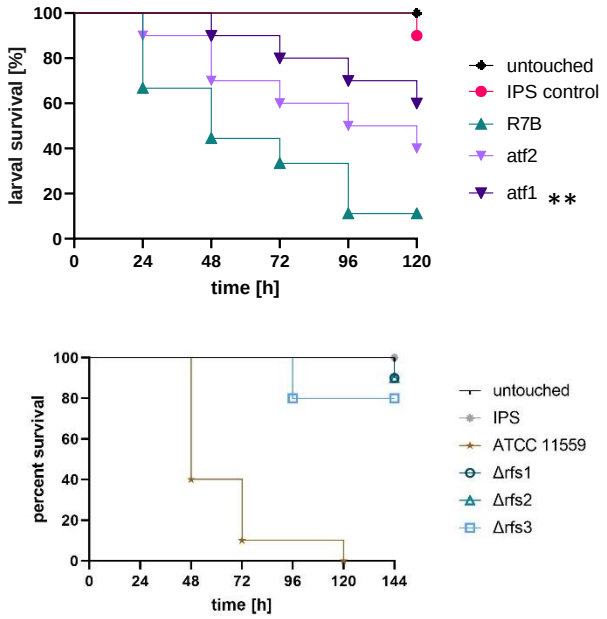
Virulence studies

Szebenyi et al. Mbio 2023



- spore coating protein (CotH3 and CotH4: protein kinases) proteins are relevant for full virulence in two invertebrate models and a diabetic ketoacidosis (DKA) mouse model

Binder et al. unpublished



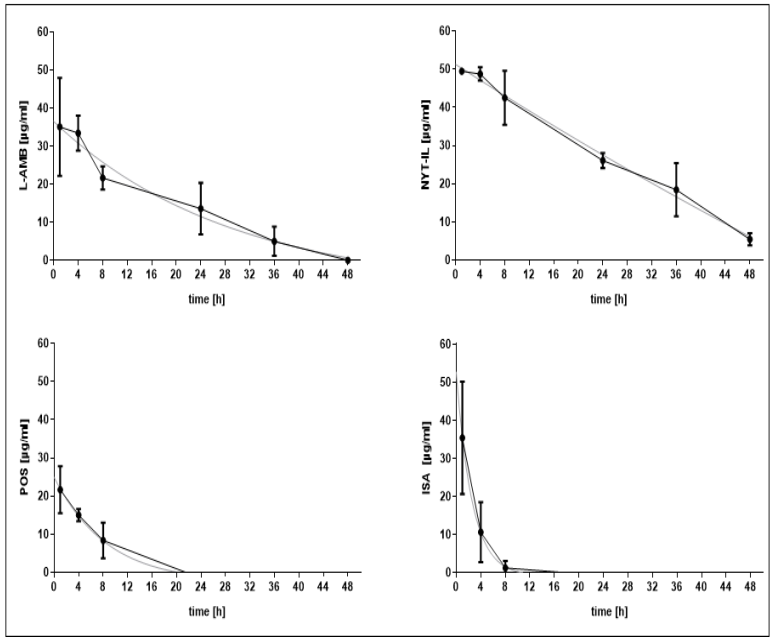
- atf1 expression (putative transcription factor) is essential for full virulence of *M. lusitanicus*
- correlates to murine data
- rfs is essential for virulence of *R. microsporus*

Pharmacokinetics and antifungal efficacy in *Galleria mellonella*

Maurer et al. Med Mycol 2018

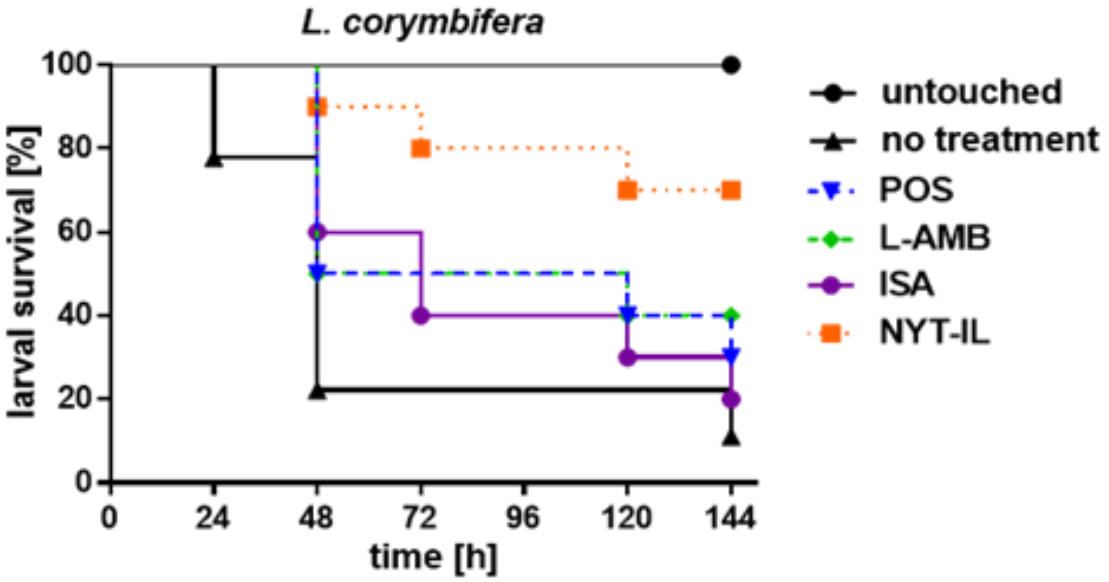
L-AMB

POS



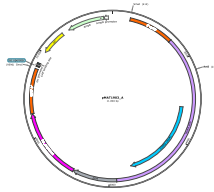
NYT-IL

ISA



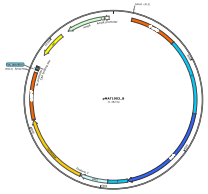
- polyenes were more stable in larval hemolymph compared to azoles
- overall poor outcome, NYT-IL had highest efficacy
- reasons: drugs only applied once + fungistatic activity

Monitoring mucormycosis and testing antifungal efficacy by the use of reporter strains



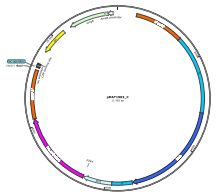
A) ***Mucor*** **- optimized** luciferase sequence under control of ***Pzrt1***

⇨ **R7B_lucA**



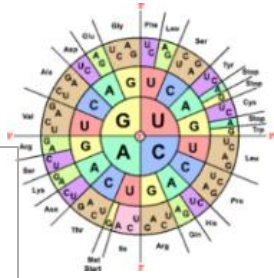
B) Mammalian luciferase under control of ***Phis3***

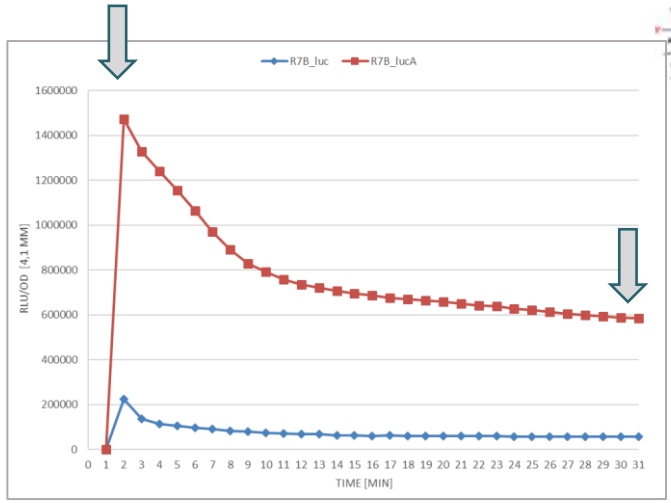
⇨ **R7B_lucB**



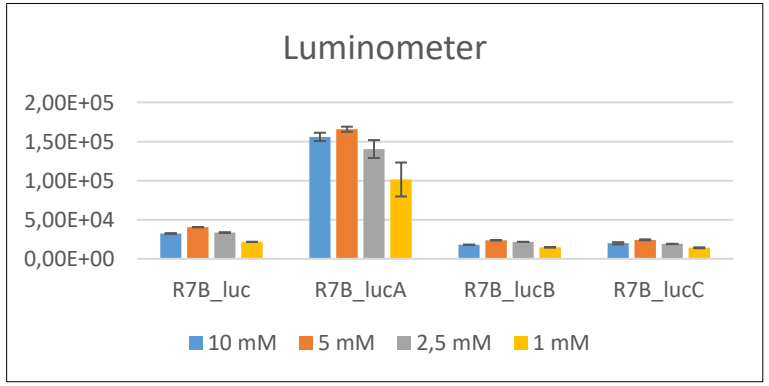
C) ***Mucor*****-optimized** sequence under control of ***Phis3***

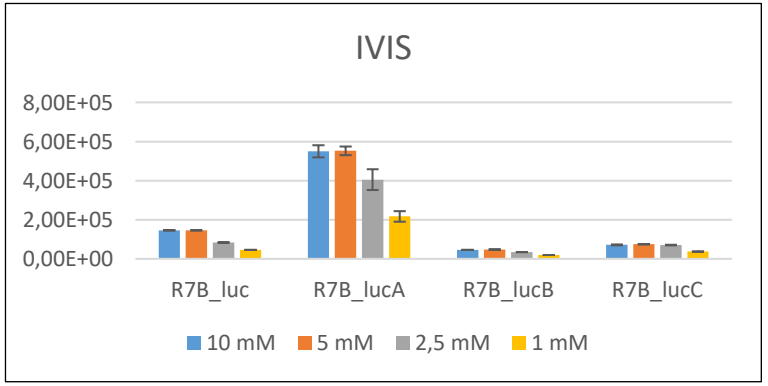
⇨ **R7B_lucC**

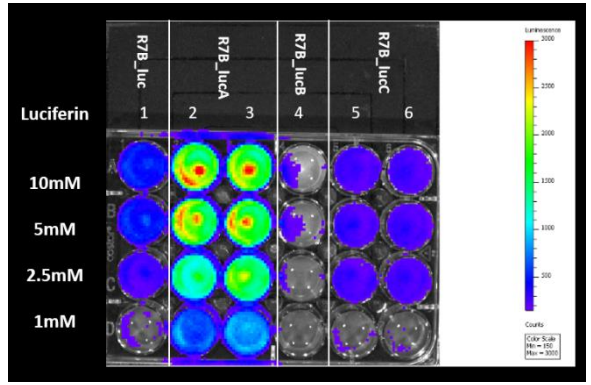




➢ expression of codon optimized luciferase resulted in significantly higher and stable light signal



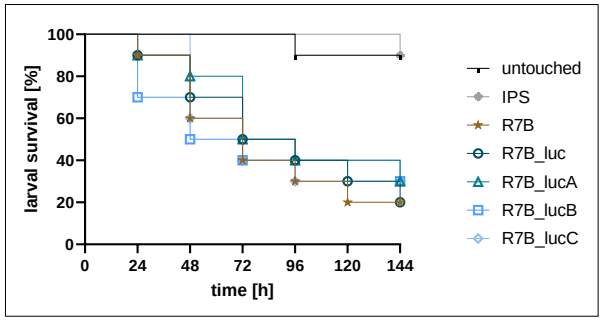


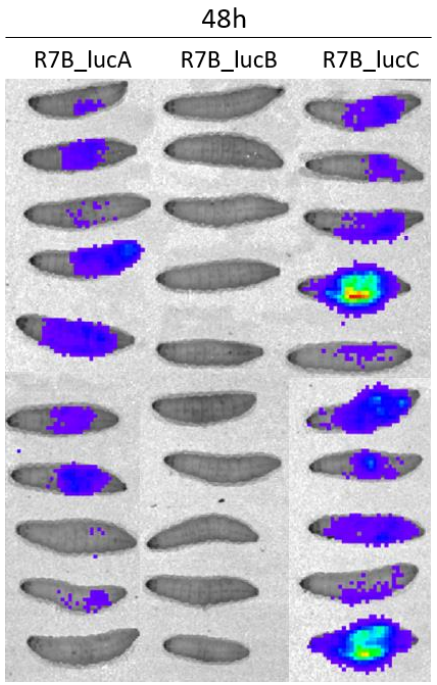


➢ strongest signals were detected in strains expressing codon optimized luciferase under control of zrt1 promoter

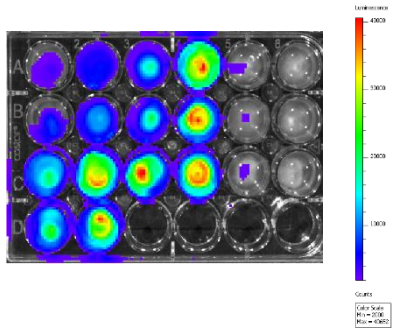
➢ luciferin concentrations could be reduced in microtiter plate assays

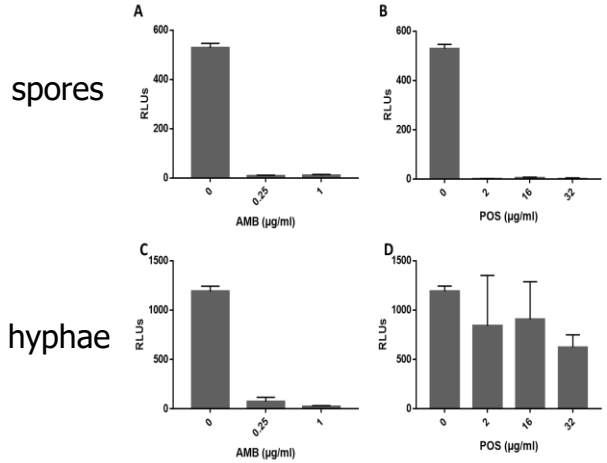
Virulence potential, *in vivo* BLI & antifungal efficacy

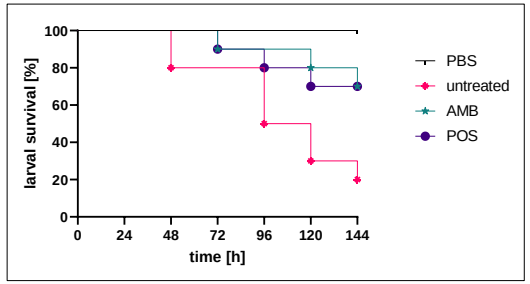


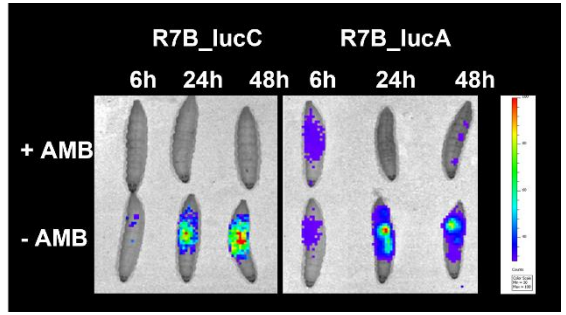


- virulence potential in Galleria is not altered by the insertion of the luciferase gene
- infection can be vivualized by IVIS system
- R7B_lucC infected larvae show strongest signals
- signal intensity rates varies between „plate assays" & larvae









- AMB (15mg/kg) and POS (10mg/kg) increase larval survival significantly
- AMB showed efficacy against R7B in vivo
- generated strains represent a useful tool to study efficacy of (novel) antifungals *in vitro* and to monitor disease in a spatial and temporal manner in animal models in the future

Summary: Benefits & drawbacks of alternative model systems in mucormycosis

	Chicken eggs	*zebrafish*	*C. elegans*	*Drosophila melanogaster*	*Galleria mellonella*
high throughput screenings	yes	yes (larvae)	yes	yes	yes
Temperature 37°C	37,6 °C	only in adult, not well tolerated	no	no	yes
related to disease in human patients	limited	limited	no	limited	limited
induction of risk factors	no	yes	no	(yes)	(yes)
Genetically tractable	no	yes	no	yes	no
application of antifungal drugs	n.d.	n.d.	n.d.	yes	yes
focus on innate immunity	yes	larvae	yes	yes	yes
adaptive immunity	yes, dep on age, slowly developing	adults	no	no	no
specialised facilities and staff	no	yes	no	yes	no
defined inoculum size	yes	yes	no (no injection)	no	yes

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