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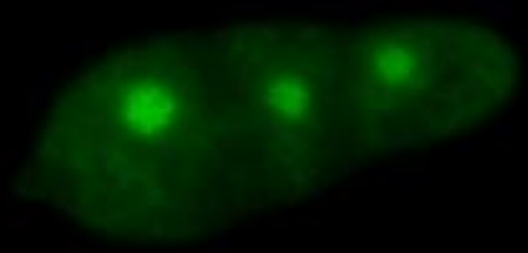
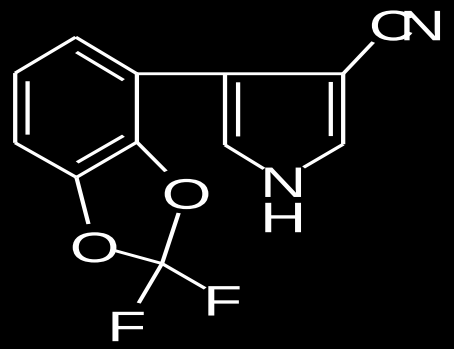
UND WIRKSTOFF-FORSCHUNG

ibwf

Signaling networks in fungal pathogens

Osmoregulation as fungicide target



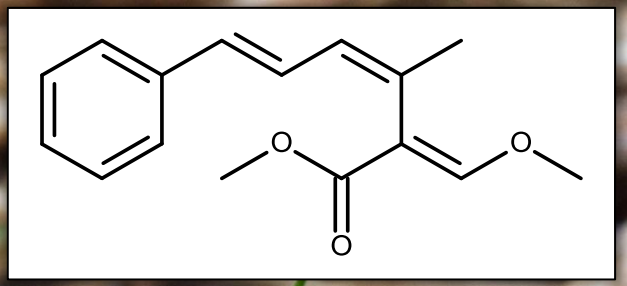


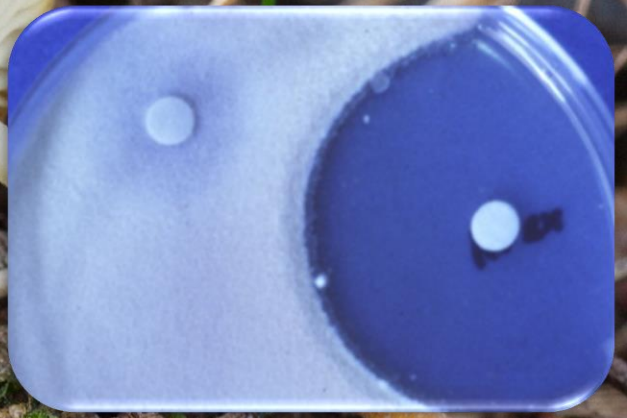
Dr. Stefan Jacob

„Modern Agriculture without chemical pesticides?“

AlPlanta - Institute for Plant Research

Neustadt, September 3rd 2018



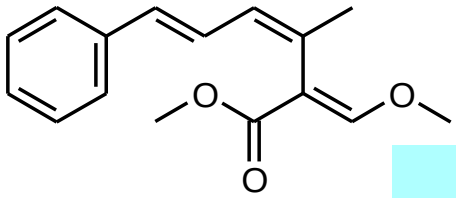


Strobilurus tenacellus

Pinecone cap

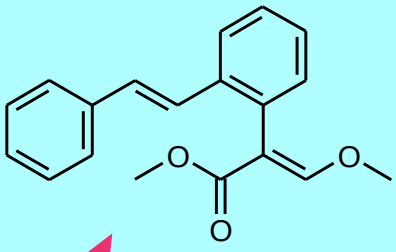
Bitterer Kiefern-Zapfenrübling

Fungal secondary metabolites as lead structures for agro-chemicals

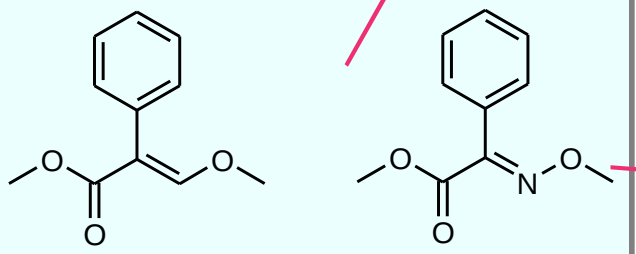


Strobilurin A

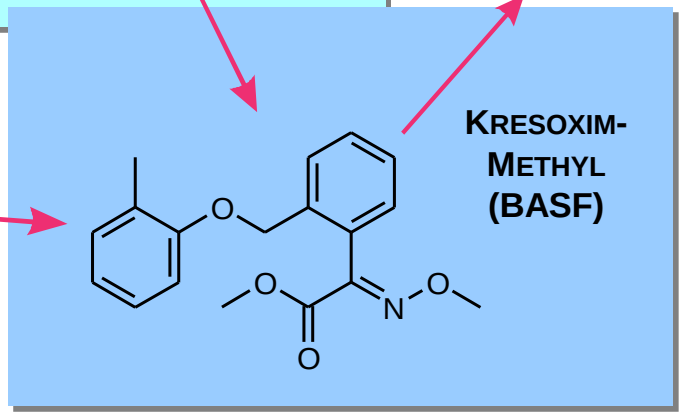
Strong antifungal activity, non-toxic for mice;
MoA: inhibition of electron transport in the respiratory chain (bc1).



B. Schwalge, Dissertation 1986



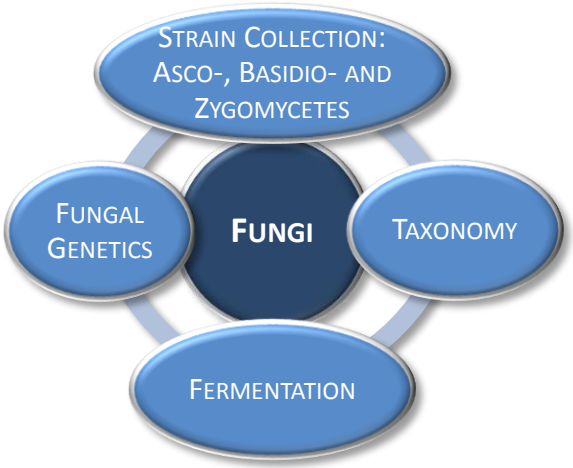
G. Schramm, Dissertation 1980

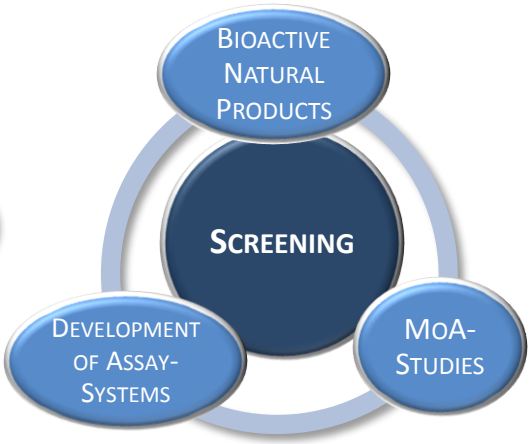


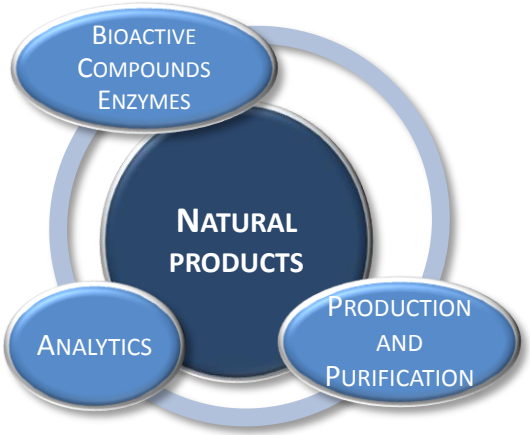
Kresoxim-Methyl (BASF)

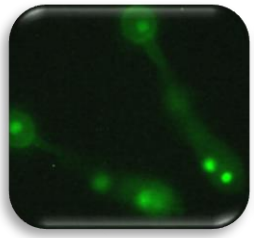
Sauter, H.; W. Steglich & T. Anke: Strobilurine: Evolution einer neuen Wirkstoffklasse. Angew. Chem. 111, 1416-1438 (1999); Int. Ed. 39, 1328-1349 (1999).

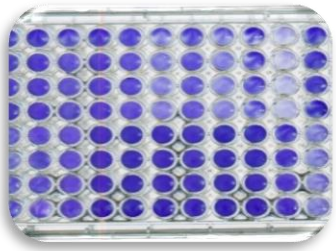
Biotechnology at the IBWF gGmbH

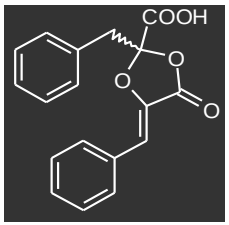








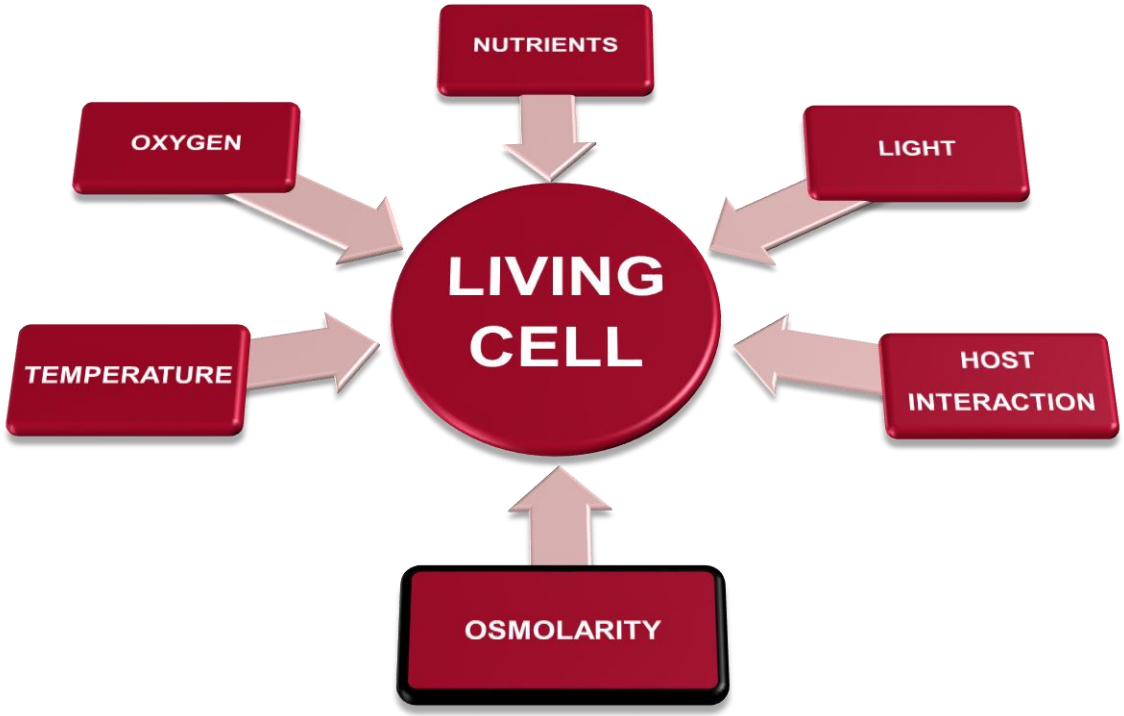




Outline

- **Part I: Introduction**
  - Signaling and Osmoregulation
  - Phytopathogenic fungus *Magnaporthe oryzae*
- **Part II: Fungal genetics**
  - "Loss of function"-mutants
  - Biological characterization of mutant strains
  - GFP-tagging for pathway visualization
- **Part III: *In vivo* Test system**
  - Target based *in vivo* test system for the identification of inhibitors
  - Validation of Inhibitor activity
- **Conclusion and discussion**

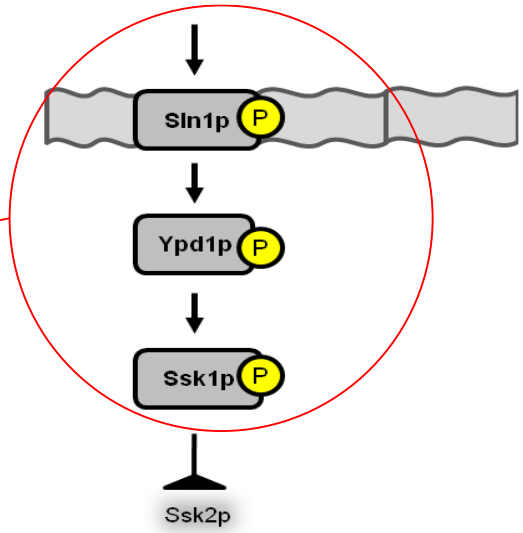
MICROORGANISMS AND THEIR ENVIRONMENT



MICROORGANISMS HAVE TO ADAPT RAPIDLY TO CHANGING ENVIRONMENTAL CONDITIONS!

HIGH OSMOLARITY GLYCEROL (HOG) PATHWAY IN YEAST

NORMAL ENVIRONMENTAL CONDITIONS



MEMBRANE

PHOSPHORELAY SYSTEM
- ACTIVE -

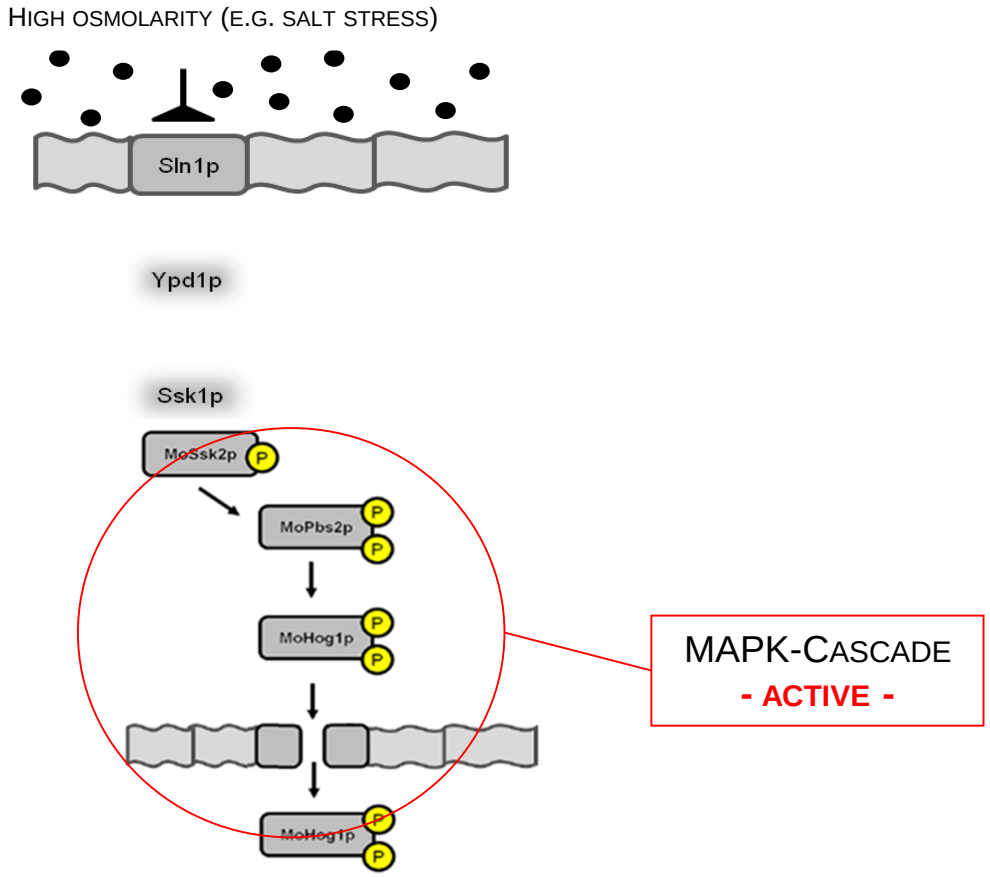
Pbs2p

Hog1p

MAPK-CASCADE
- INACTIVE -

NORMAL ENVIRONMENTAL CONDITIONS: REGULATORY SYSTEM **INACTIVE**

HIGH OSMOLARITY GLYCEROL (HOG) PATHWAY IN YEAST

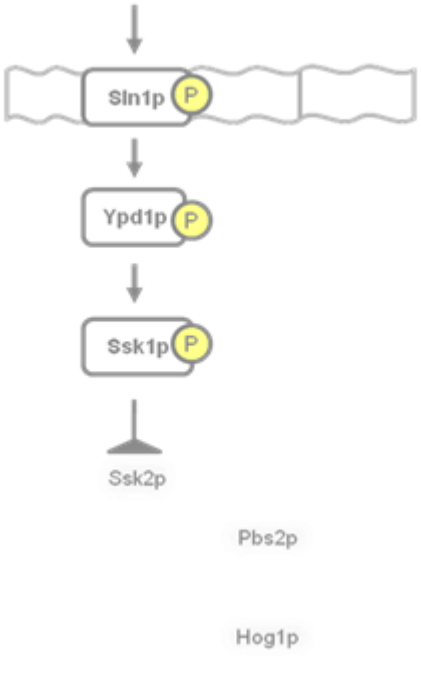


PHOSPHORELAY SYSTEM
- INACTIVE -

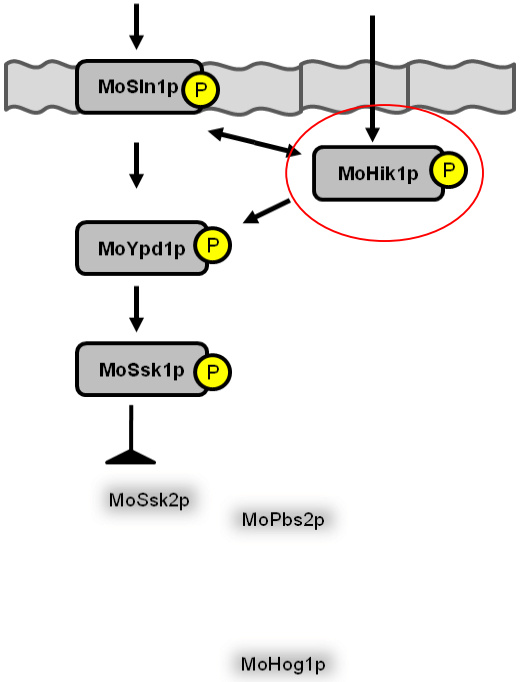
HIGH OSMOLARITY: REGULATORY SYSTEM **ACTIVE** (E.G. GLYCEROL PRODUCTION)

SIGNAL PERCEPTION IN FILAMENTOUS FUNGI IS DIFFERENT TO YEAST

SACCHAROMYCES CEREVISIAE

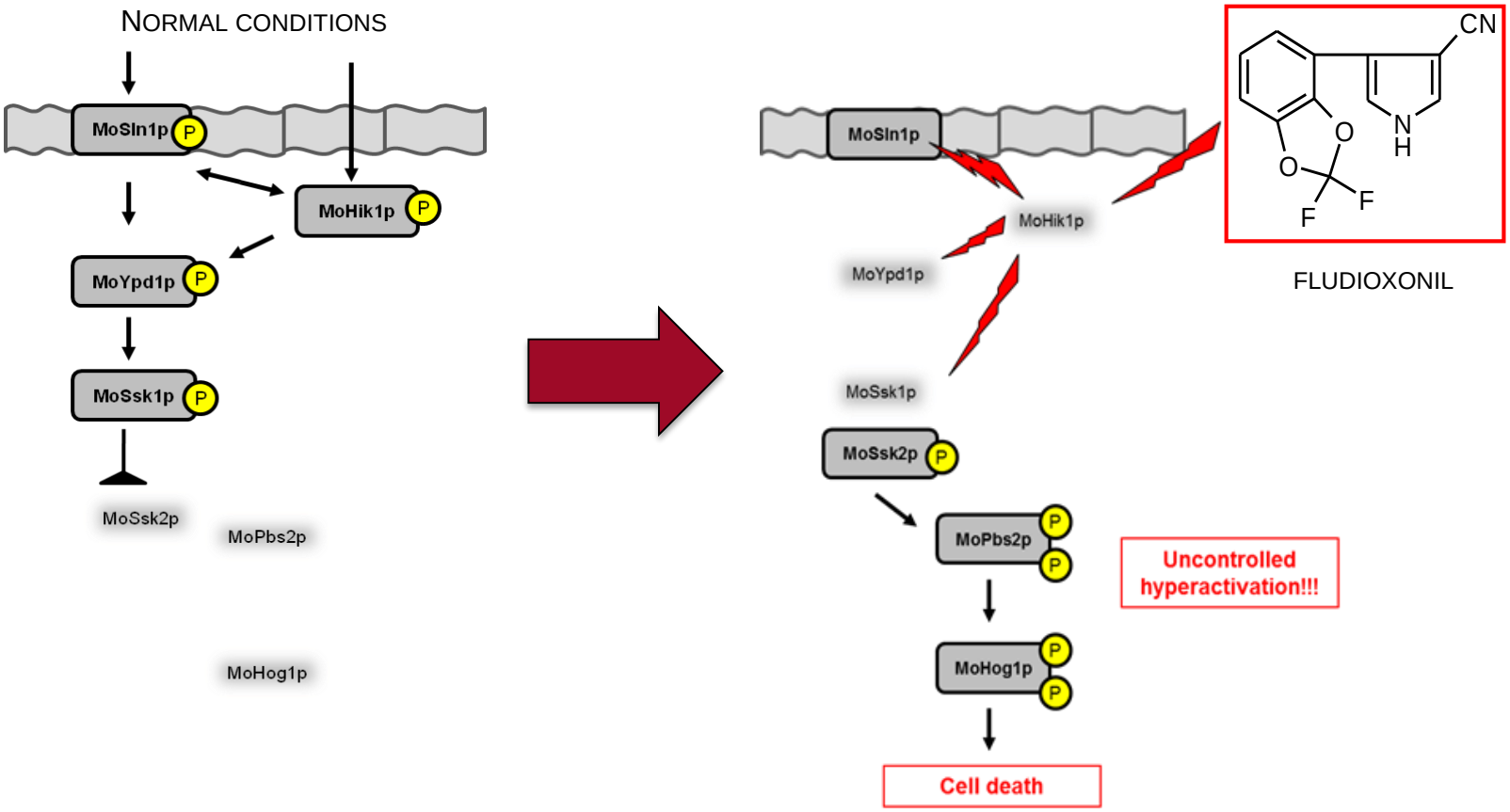


FILAMENTOUS FUNGI



HOG SIGNALLING IN FILAMENTOUS FUNGI COMPRISES MORE ELEMENTS, REFINING BUT ALSO COMPLICATING THE REGULATION SYSTEM!

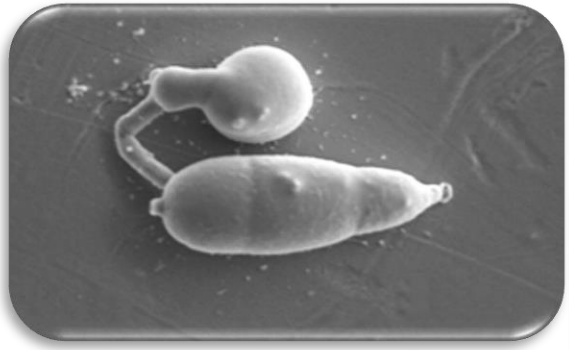
FLUDIOXONIL HYPERACTIVATES THE PATHWAY



FLUDIOXONIL APPEARS TO INACTIVATE THE PHOSPHORELAY SYSTEM BY INTERACTION WITH MOHIK1!

MAGNAPORTHE ORYZAE – RICE BLAST DISEASE

BIOSPRCTRUM ASIA, 2013



SKAMNIOTI/GURR, 2007

AGRICULTURAL RISK MANAGEMENT IN DEVELOPMENT, 2012

- HEMIBIOTROPHIC ASCOMYCETE, FACULTATIVE PATHOGEN
- GOOD TO HANDLE IN LABORATORY
- GENOME SEQUENCED SINCE 2002
- GENETIC MANIPULATION POSSIBLE (E.G. *AGROBACTERIUM TUMEFACIENS*)

PART II:

FUNGAL GENETICS

MOLECULAR TOOLBOX FOR THE MANIPULATION OF FUNGAL GENOMES

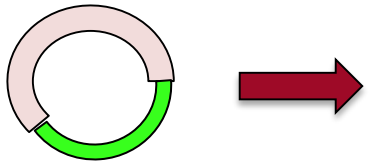
OBTAIN SEQUENCE OF TARGET GENE

ISOLATE FUNGAL GDNA, AMPLIFICATION BY MEANS OF PCR, RUN AGAROSE GELS, PURIFY DNA, …

FUNGAL DNA

CLONING & MOLECULAR MANIPULATION

PCR-PRODUCT LIGATION IN VECTOR, RESTRICTION ANALYSIS, INTERRUPT OR REPLACE CODING SEQ, SELECTION OF BACTERIAL TRANSFORMANTS, …



Resistance-gene interrupts or deletes the coding sequence of the candidate gene

GENERATION OF FUNGAL MUTANT STRAINS (LOSS-OF FUNCTION MUTANTS)

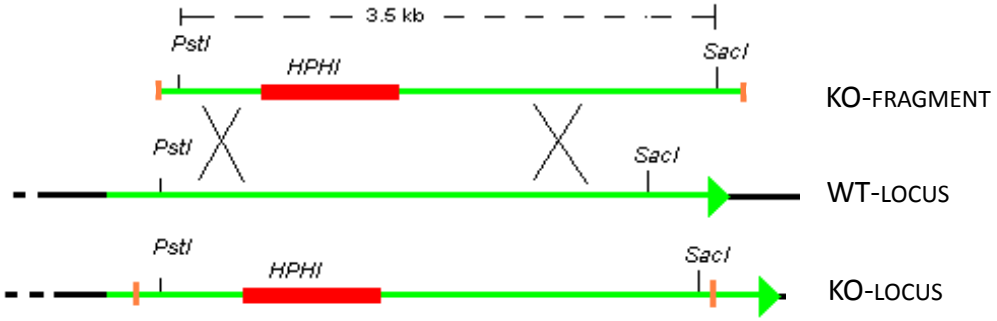
AGROBACTERIUM TUMEFACIENS MEDIATED TRANSFORMATION, SELECTION OF TRANSFORMANTS, SOUTHERN ANALYSIS, PHENOTYPIC CHARACTERIZATION, …

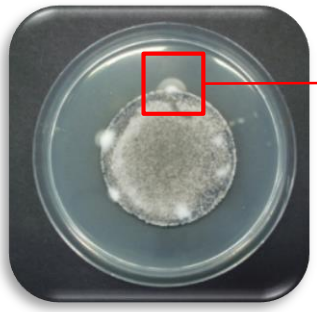
INTERNATIONAL SOCIETY FOR MICROBIOL ECOLOGY

MALCOLM STOREY, 2010, WWW.BIOIMAGES.ORG.UK

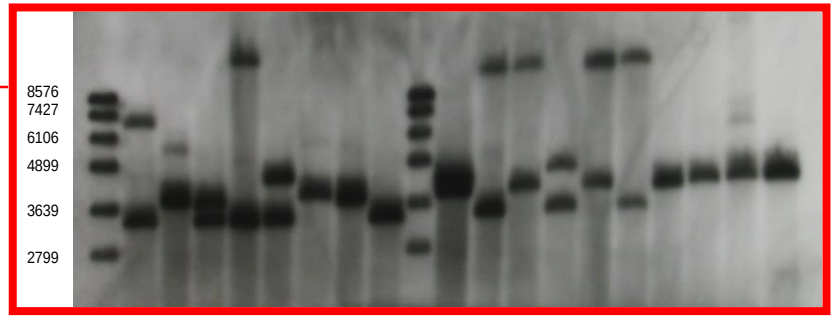
HOMOLOGOUS RECOMBINATION AND ANALYSIS OF MUTANTS

HOMOLOGOUS RECOMBINATION OF THE T-DNA INTO THE FUNGAL GENOME



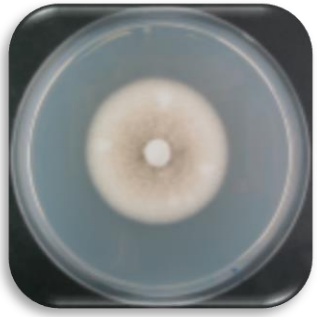


SELECTION OF TRANSFORMANTS

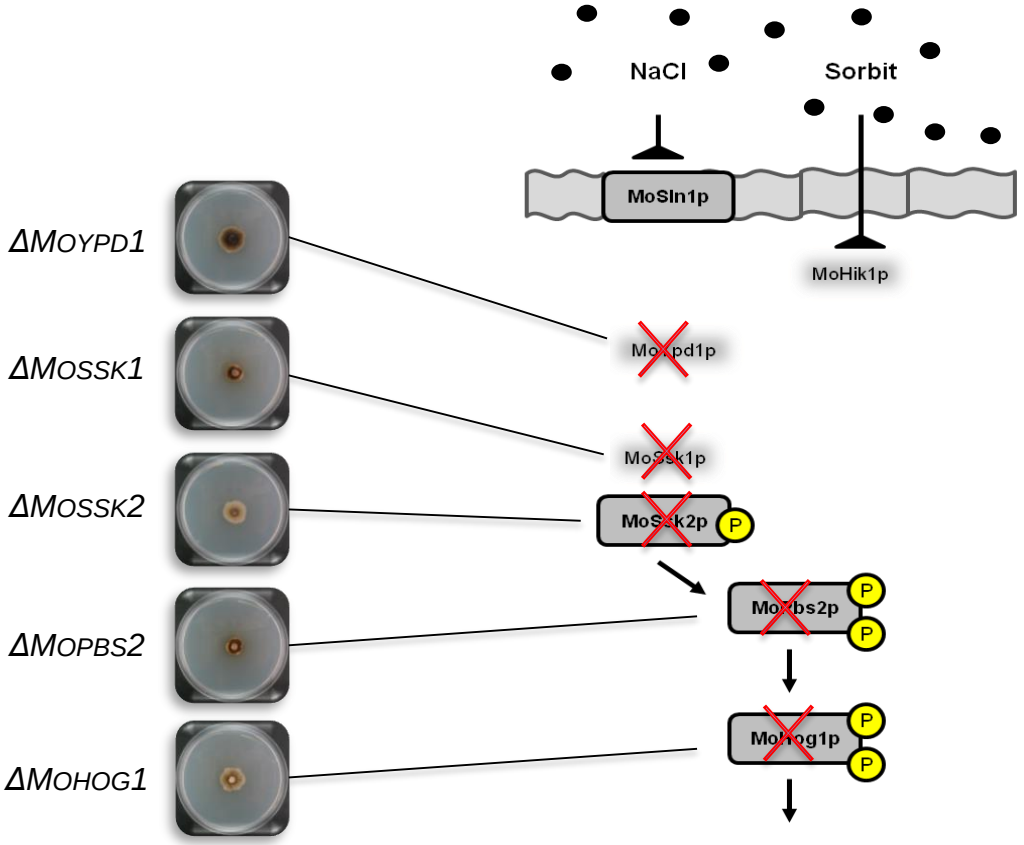


SOUTHERN ANALYSIS OF TRANSFORMANTS

„LOSS OF FUNCTION“-MUTANTS: PHENOTYPE CHARACTERIZATION

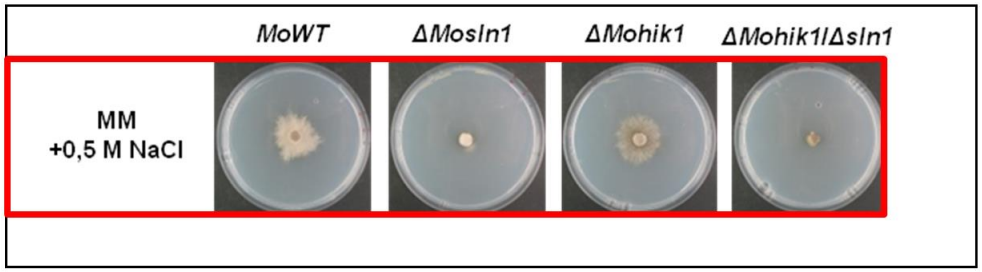


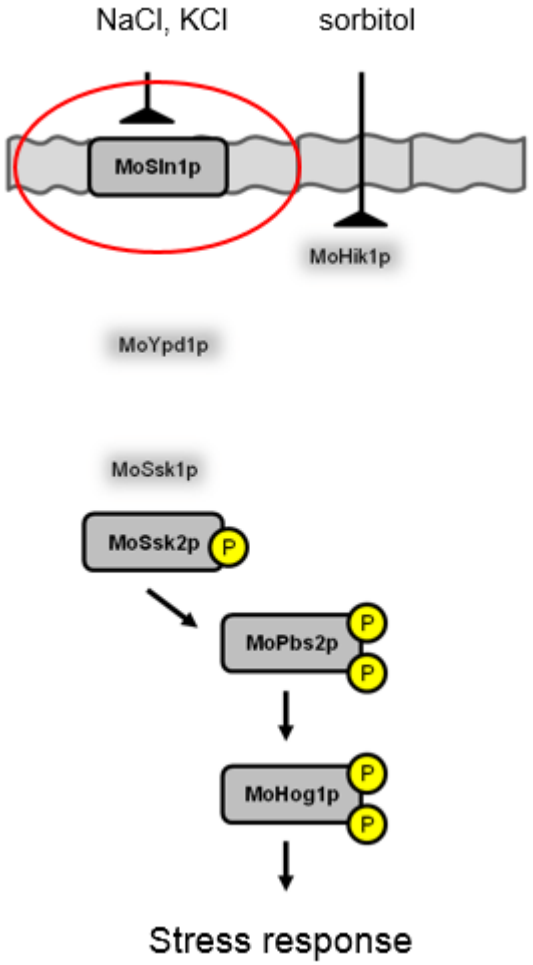
WILDTYPE STRAIN



THE MUTANTS ARE **UNABLE TO ADAPT TO** OSMOTIC STRESS (I.E.NACL, SORBITOL)

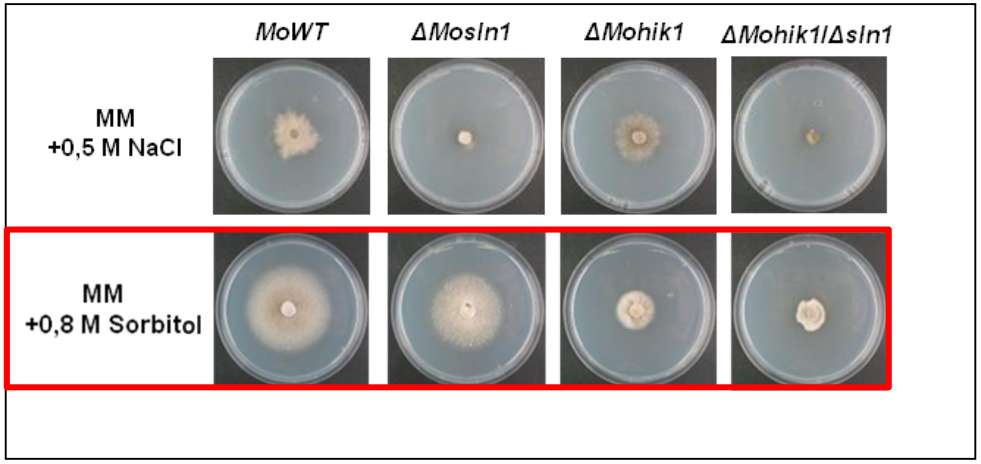
MOSLN1P: A PUTATIVE SALT SENSOR

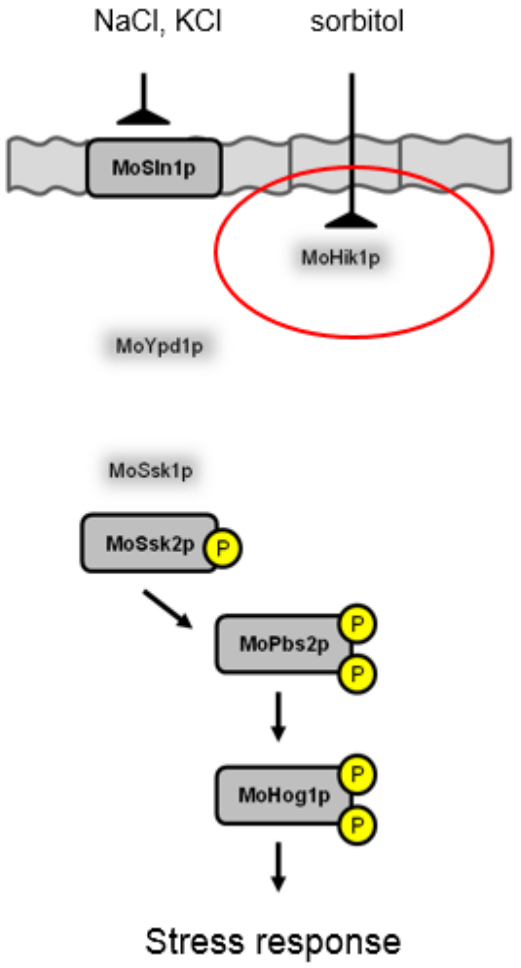




MOSLN1P APPEARS TO BE ESSENTIAL FOR **SALT** SENSING!

MOHIK1P: A PUTATIVE SUGAR SENSOR

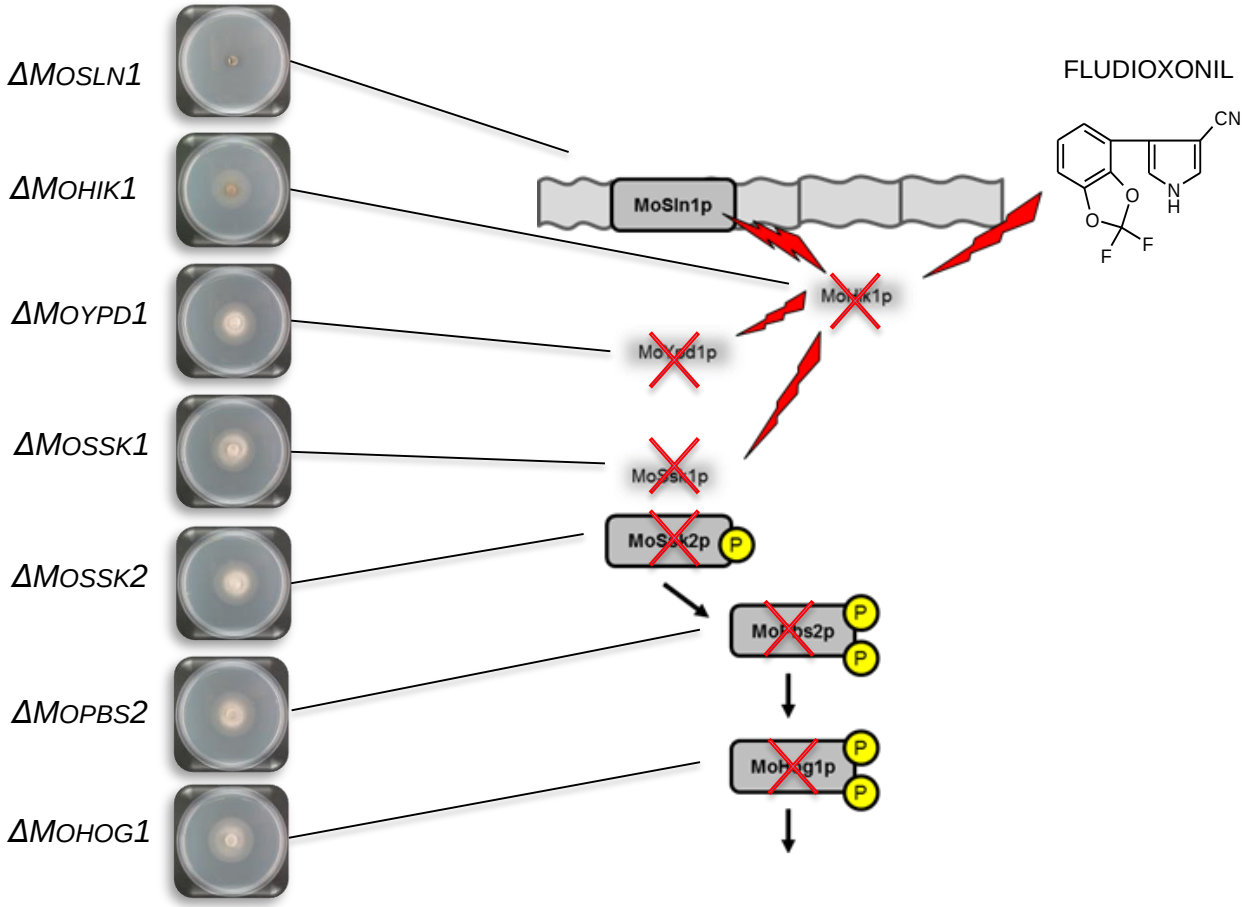




MOHIK1P APPEARS TO BE ESSENTIAL FOR **SUGAR** SENSING!

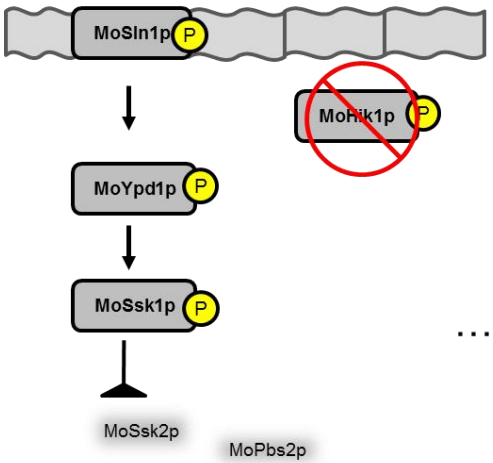
PHENOTYPE OF THE „LOSS OF FUNCTION“-MUTANTS - FUNGICIDE RESISTANCE -

WILDTYPE STRAIN



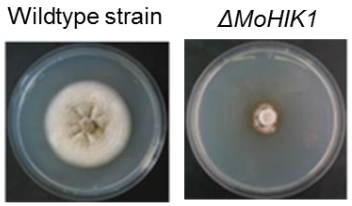
THE MUTANTS (EXCEPT *ΔMOSLN1*) ARE **RESISTANT** TO HOG EFFECTORS (I.E. FLUDIOXONIL)

GENE INACTIVATION OF *MoHIK1* IS NOT EQUAL TO FUNGICIDE ACTION

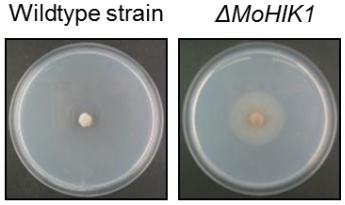


…but…

Osmosensitive



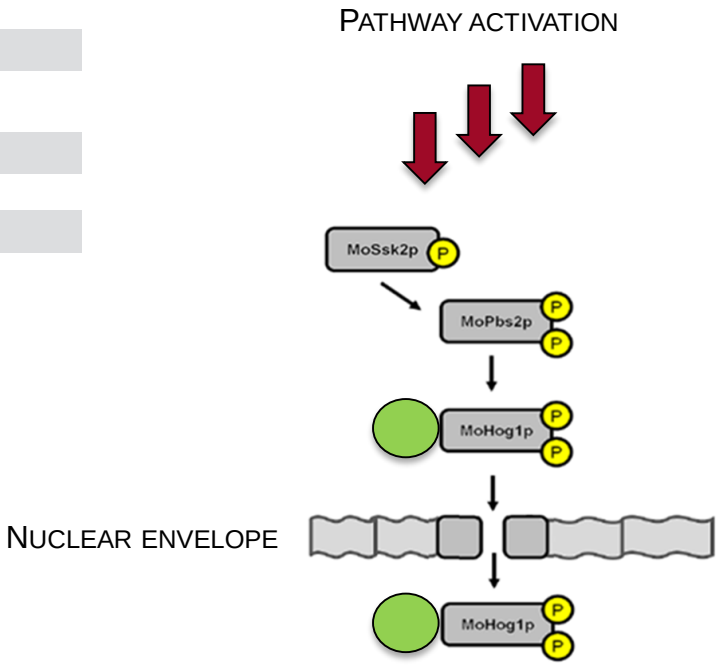
Fludioxonil resistant

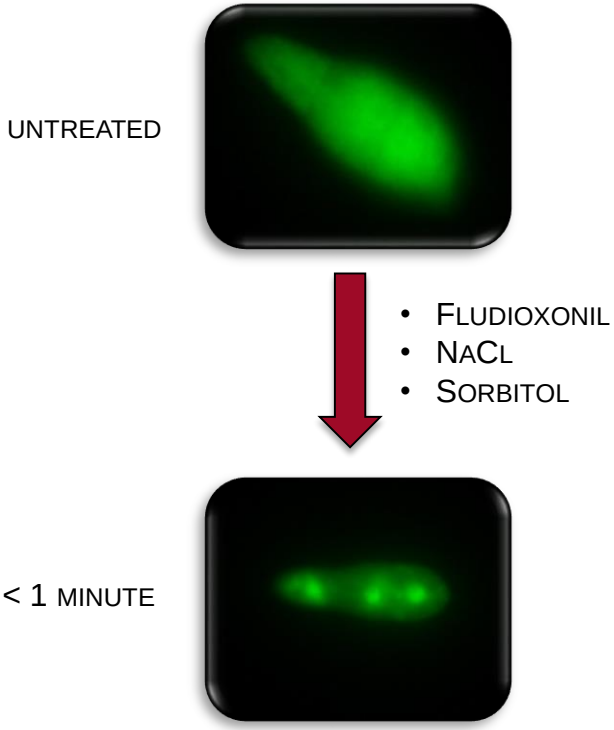


No cell death, phosphorelay is maintained through MoSln1p

MOSLN1P APPEARS TO BE ABLE TO MAINTAIN THE PHOSPHORYLATION PATTERN NEEDED FOR VITALITY!

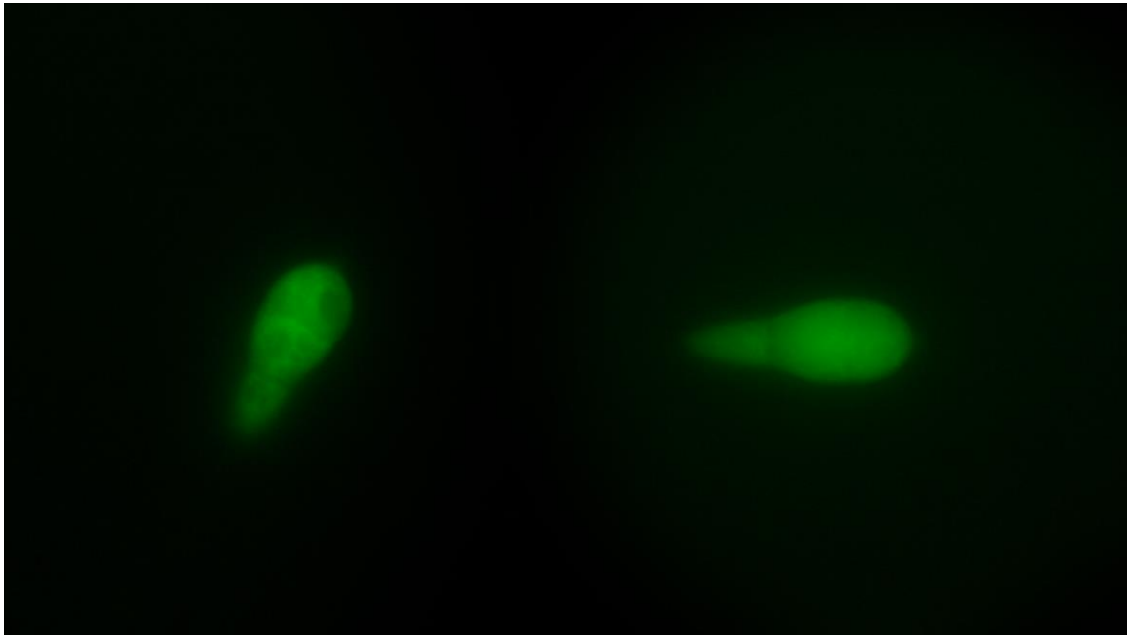
HOG1-GFP FUSION AS TOOL FOR VISUALIZING FUNGICIDE ACTIVITY

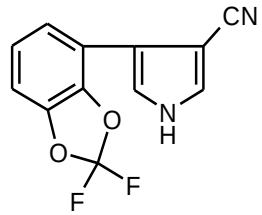




MOHOG1-GFP FUSION PROTEIN CAN BE USED AS A SUITABLE TOOL FOR ***„REAL-TIME“*** VISUALIZATION OF FUNGICIDE ACTION!

HOG1-GFP FUSION AS TOOL FOR VISUALIZING FUNGICIDE ACTIVITY





FLUDIOXONIL

ibwf

PART II:
„TAKE HOME“-MESSAGE

MUTANT STRAINS WITH INACTIVATED HOG SIGNALING ARE:

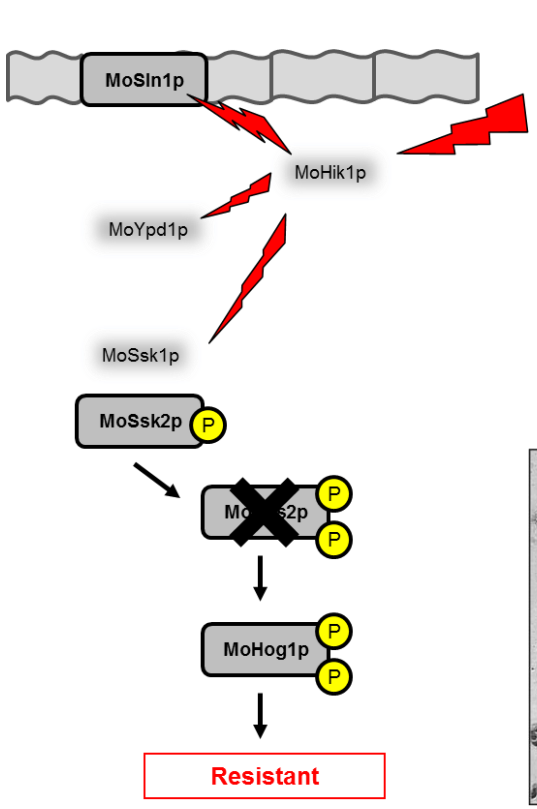
- **SENSITIVE** TO ENVIRONMENTAL STRESSES
- **RESISTANT** TO HOG-EFFECTORS (I.E. FLUDIOXONIL)

PATHWAY ACTIVITY CAN BE VISUALIZED IN „REAL-TIME“

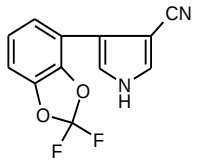
PART III:

A TARGET BASED *IN VIVO* TEST SYSTEM

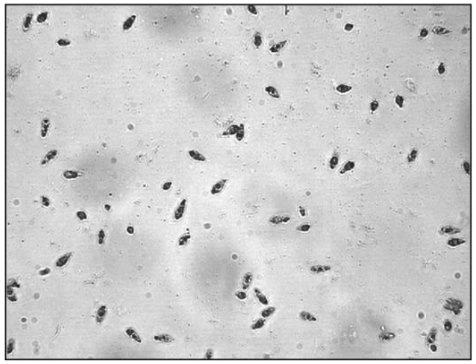
A TARGET BASED *IN VIVO* TEST SYSTEM
(1) USE OF "LOSS OF FUNCTION" MUTANTS



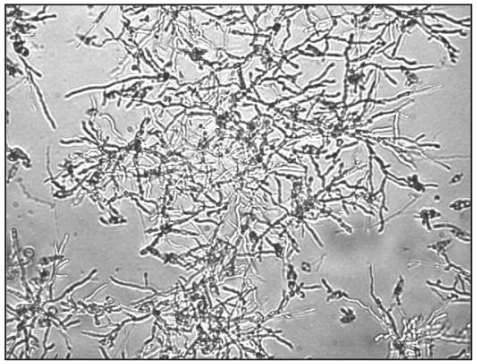
FLUDIOXONIL



WILDTYPE STRAIN

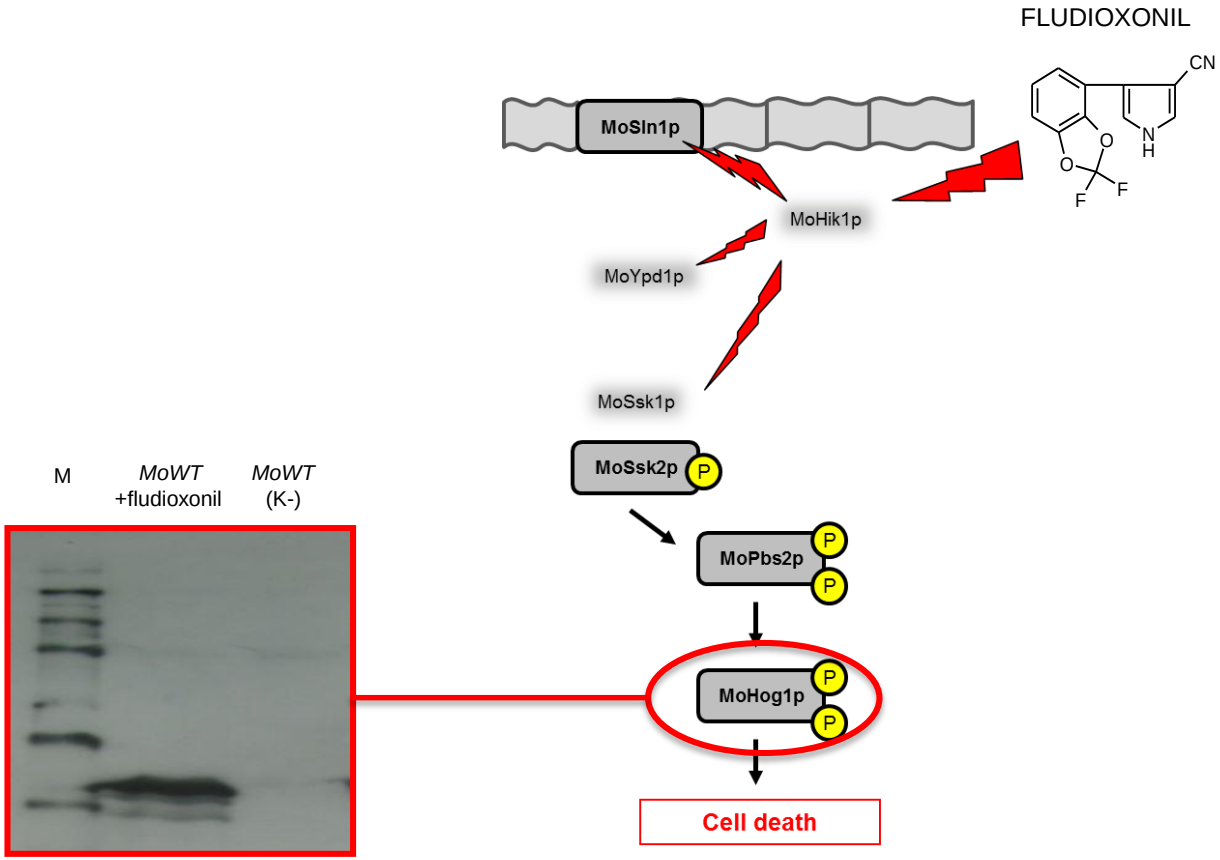


ΔMOPBS2



THE MUTANT STRAINS ARE RESISTANT! AS A CONSEQUENCE WE CAN ASSUME THE TARGET LOCATION IS LOCALIZED WITHIN THE HOG PATHWAY!

A TARGET BASED *IN VIVO* TEST SYSTEM
(2) WESTERN BLOTS TO SHOW PATHWAY ACTIVATION



WESTERN BLOTS CAN PROVIDE FURTHER EVIDENCE OF „HOG-ACTIVITY“!

A TARGET BASED *IN VIVO* TEST SYSTEM
(3) GFP-TOOL FOR „REAL-TIME“ VISUALIZATION

Pest Management Science
SCI
Research Article | Full Access

Visualizing fungicide action: an *in vivo* tool for rapid validation of fungicides with target location HOG pathway

Stefan Bohnert, Hendrik Neumann, Eckhard Thines, Stefan Jacob

THE *MOHOG::GFP*-MUTANT AS EXCELLENT TOOL FOR VISUALIZATION OF „HOG-ACTIVITY“!

Summary

- **Part II: Fungal genetics**
  - "loss of function"-mutants
  - biological characterization of mutant strains
  - GFP-tagging for pathway visualization
- **Part III: *in vivo* Test system**
  - target based *in vivo* test system for the identification of inhibitors
  - Validation of Inhibitor activity

ACKNOWLEDGEMENT

PROF. DR. ECKHARD THINES

DR. LUIS ANTELO
DR. KARSTEN ANDRESEN
DR. ALEXANDER YEMELIN

M.SC. STEFAN BOHNERT

imP Institute of Molecular Physiology

Mikrobiologie und Weinforschung

ibwf INSTITUT FÜR BIOTECHNOLOGIE UND WIRKSTOFF-FORSCHUNG

JG|U UNIVERSITÄTSmedizin. MAINZ

BASF We create chemistry

Carl Zeiss Stiftung

DFG Deutsche Forschungsgemeinschaft