



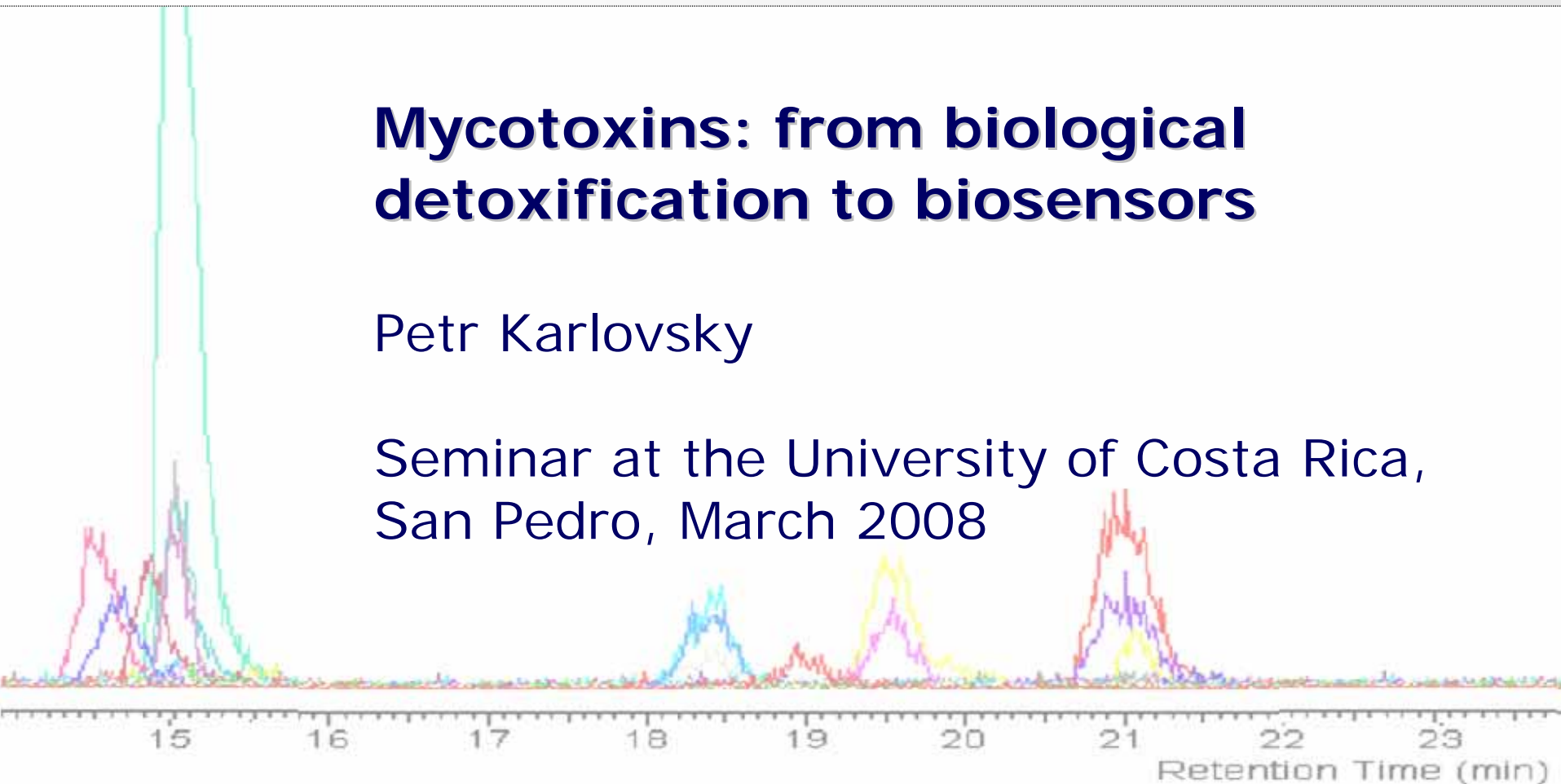
Göttingen University
Department of Crop Sciences
Molecular Phytopathology & Mycotoxin Research Unit

www.gwdg.de/~instphyt/karlovsky

Mycotoxins: from biological detoxification to biosensors

Petr Karlovsky

Seminar at the University of Costa Rica,
San Pedro, March 2008



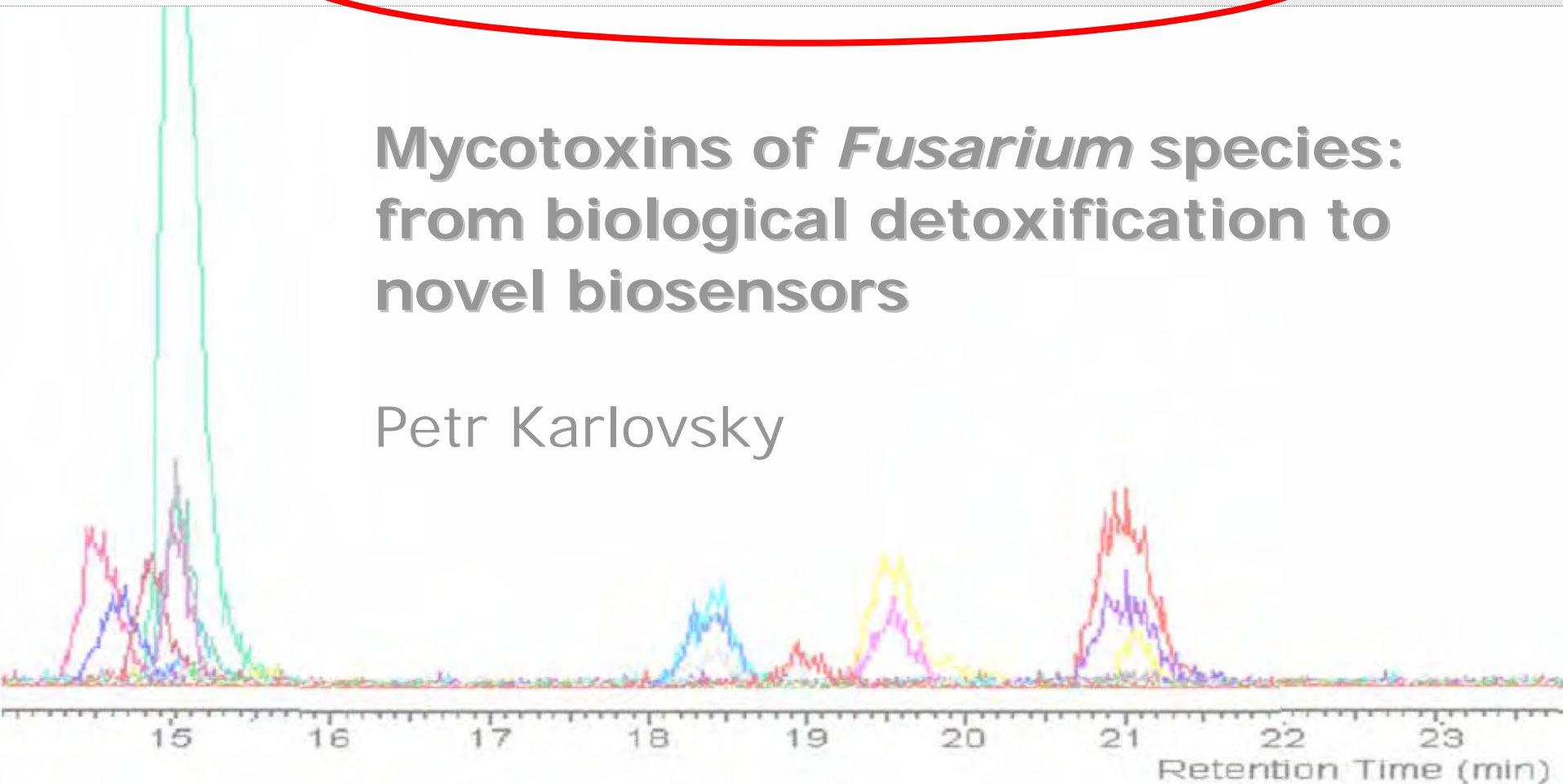


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Mycotoxins of *Fusarium* species: from biological detoxification to novel biosensors

Petr Karlovsky



Karlovsky lab: Molecular Phytopathology and Mycotoxin Research

Images are click



We study the biological role of secondary metabolites in interactions among organisms using tools of analytical chemistry and molecular genetics. Our objects are sesame/*Macrophomina*, *Brassica/Verticillium*, maize/*Fusarium*, *Gliocladium roseum/Fusarium* spp., *Piriformospora indica* and truffle fungi. Our second research focus are mycotoxins.

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Topics

- Mycotoxins: introduction
- Projects
 - ▶ Detoxification of deoxynivalenol
 - ▶ Diagnose of toxigenic *Fusarium* spp.
 - ▶ Zearalenone
- Secondary metabolite research
- Techniques



Mycotoxins

are fungal secondary metabolites toxic to animals.

Health risks

due to mycotoxin ingestion:

- Cancer (AFL, FUM)
- Heart muscle damage (MON)
- Nephropathy (OTA, DON)
- Hyperestrogenism, telarche (ZEA)
- Immunosuppression (Gliotoxin, DON)
- ...



Risk assessment of food contaminants

- Misconception: "natural" equals "healthy"
- Verbal bias: Bio-, Ecological, Natural, Organic...

Xenobiotics versus natural toxins

- Toxicity minimization vs. optimization
- Legal limits and application restrictions



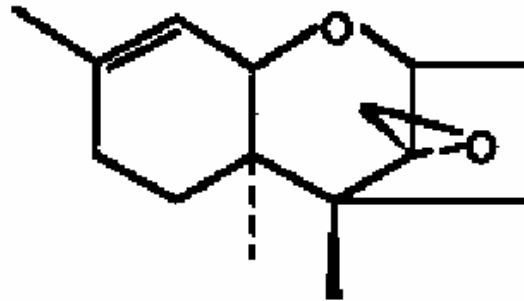
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Trichothecenes in the headlines

Transgenic mycotoxin-resistant pigs

Anticancer drugs

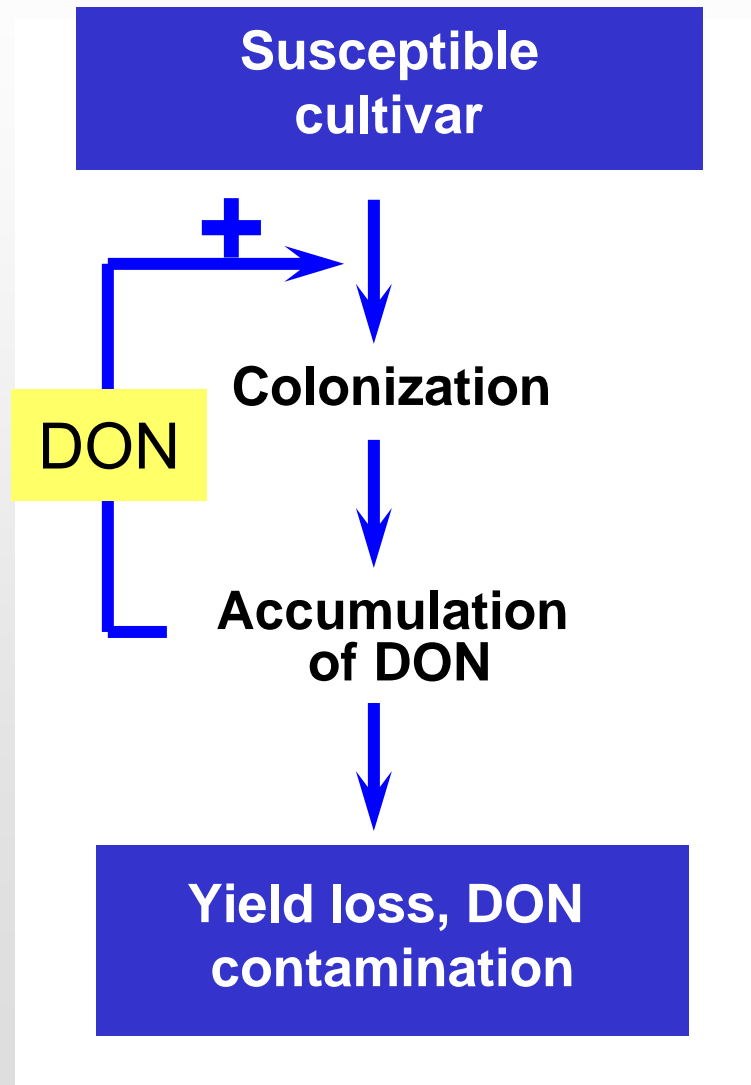


Yellow Rain and chemical warfare

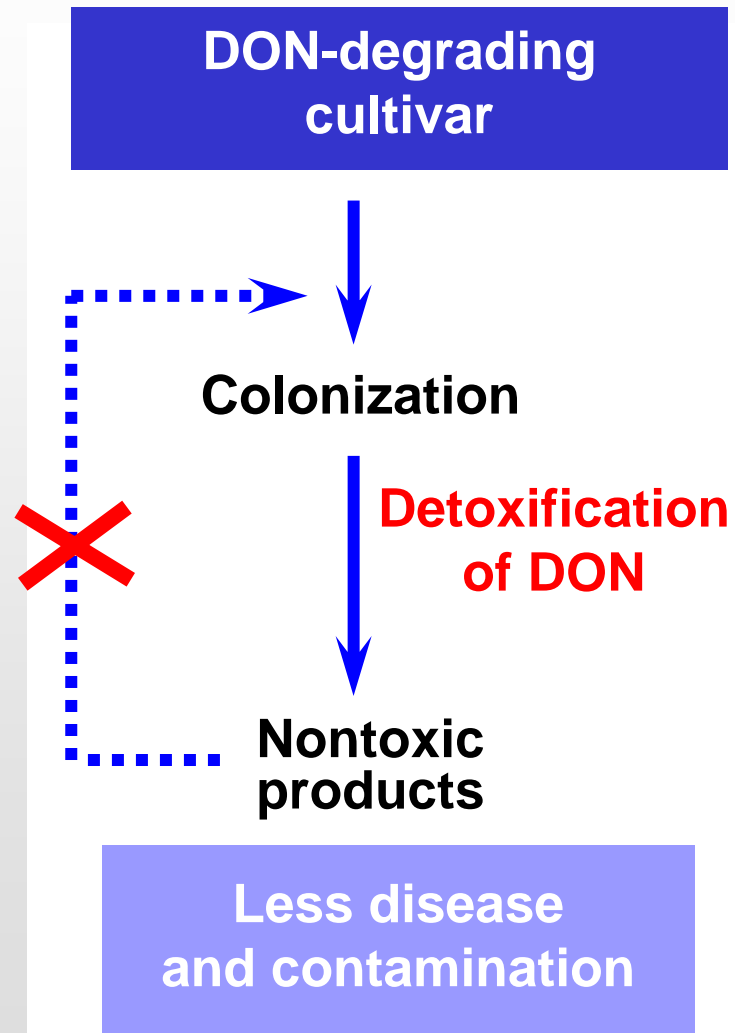
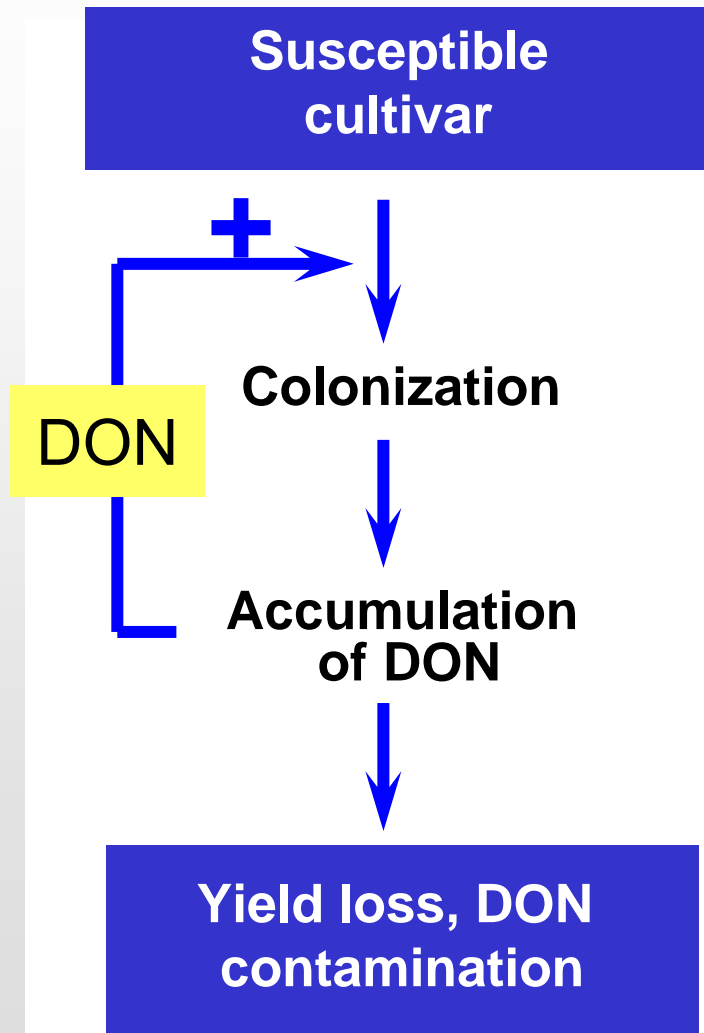
Virulence factor in the head blight

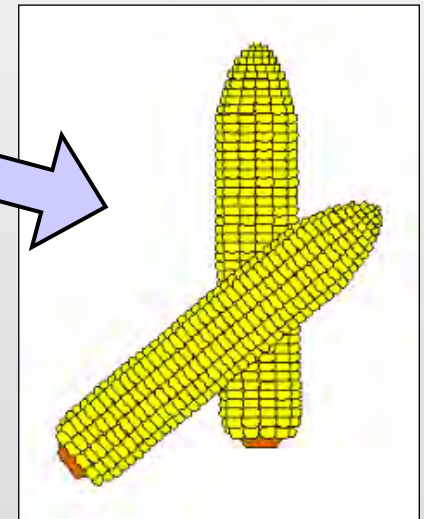
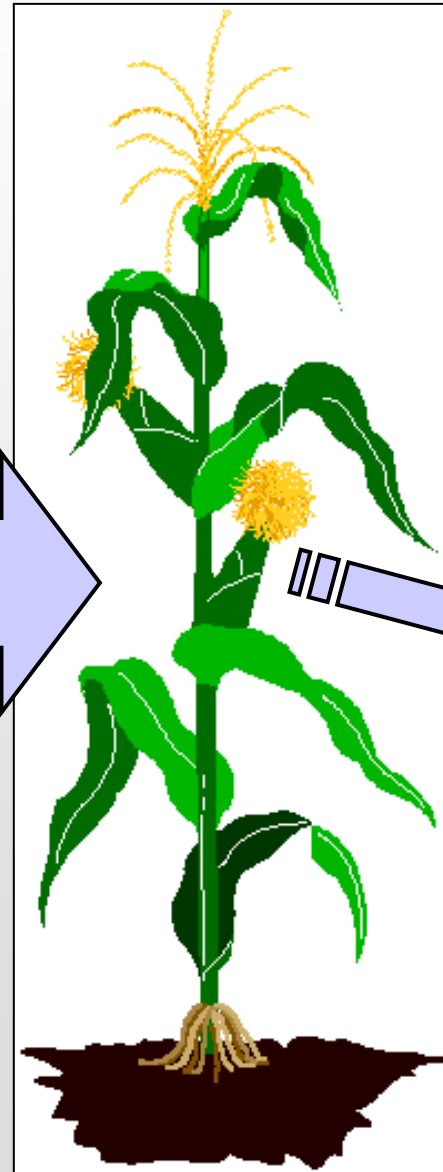
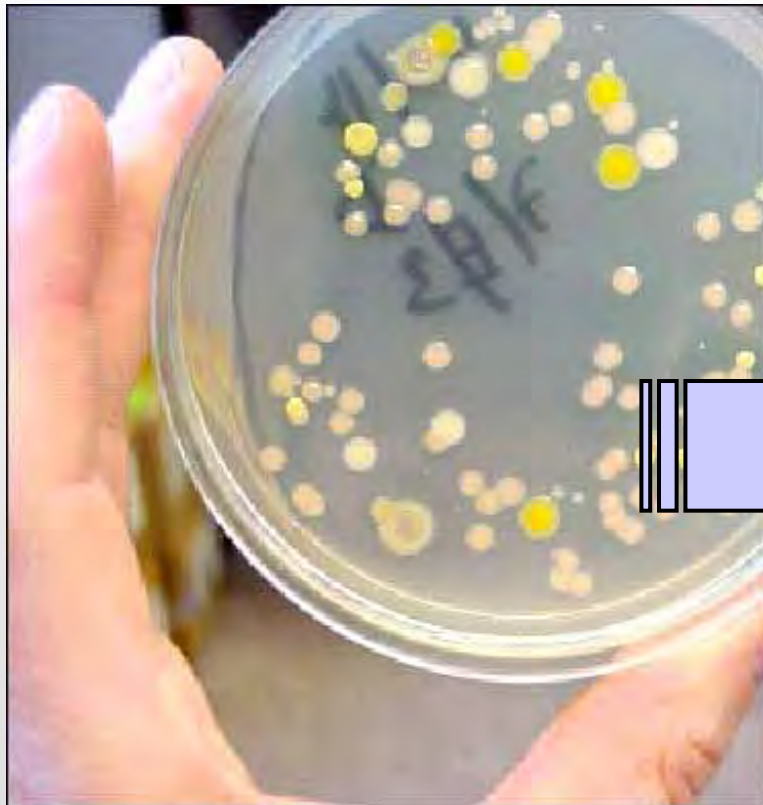
Balkan endemic nephropathy (?)

Detoxification strategy

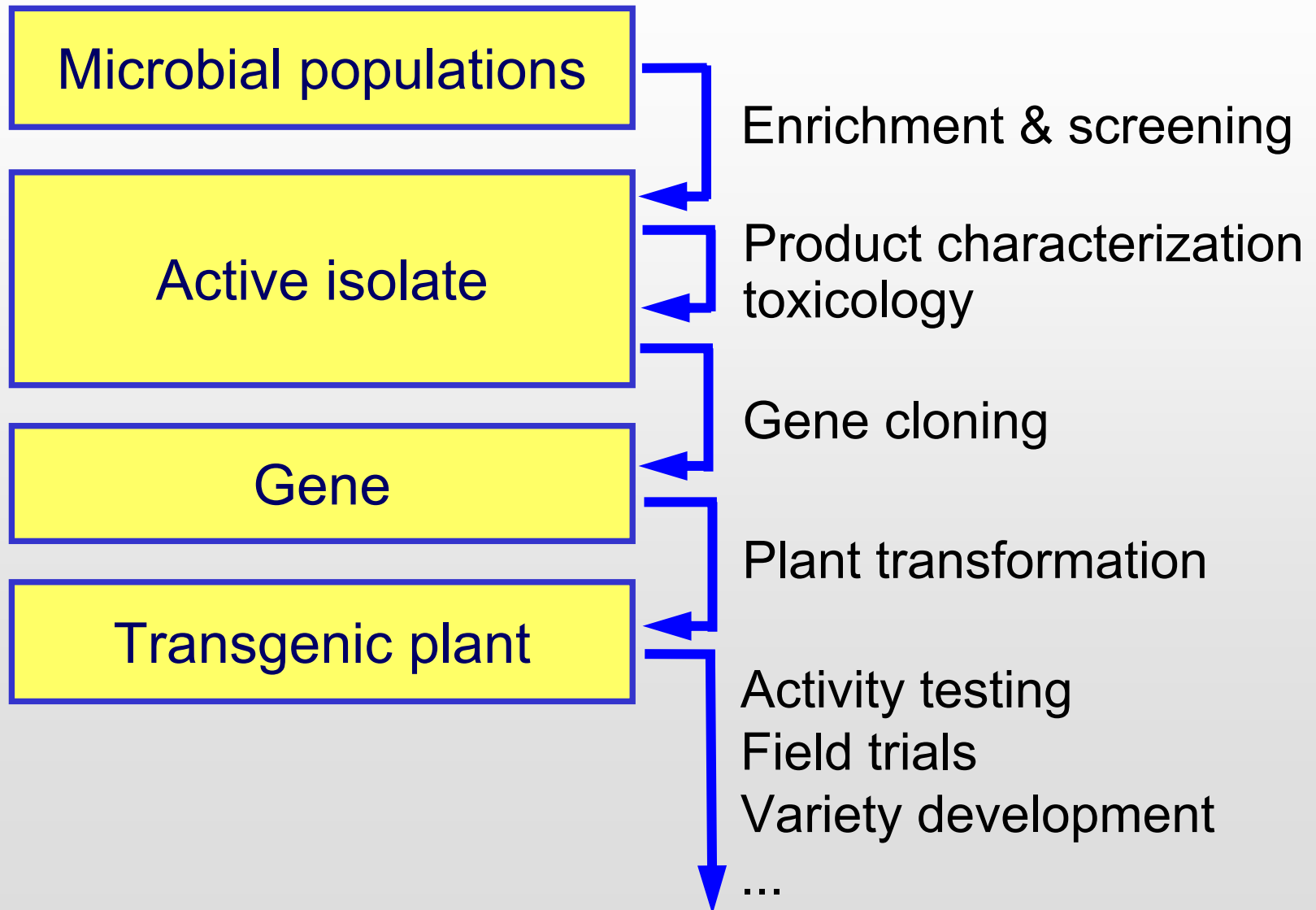


Detoxification strategy





Search for detoxification activity

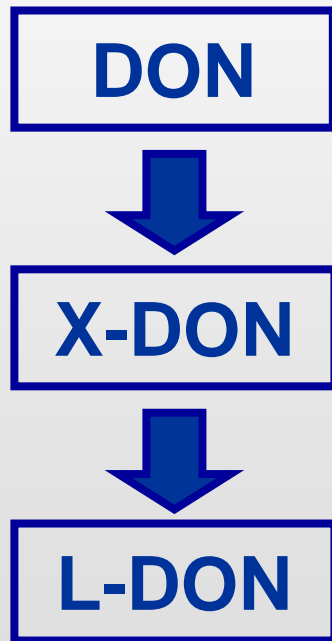




Search for DON-degrading activity

Source	Assays
Mixed cultures from grains	819
Pure cultures from grains	1434
Mixed soil cultures	200
Pure cultures from soil	975
Other sources	198
Culture DANNY => HOH107	1

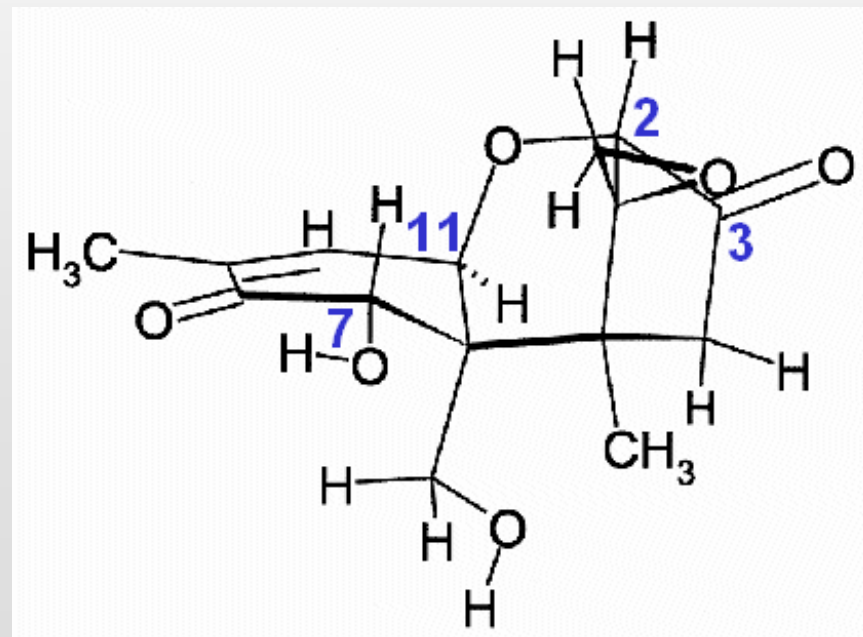
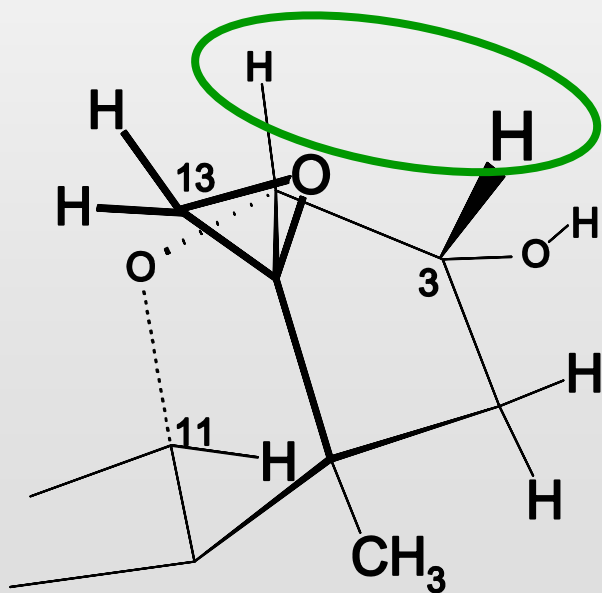
Toxicity of transformation products

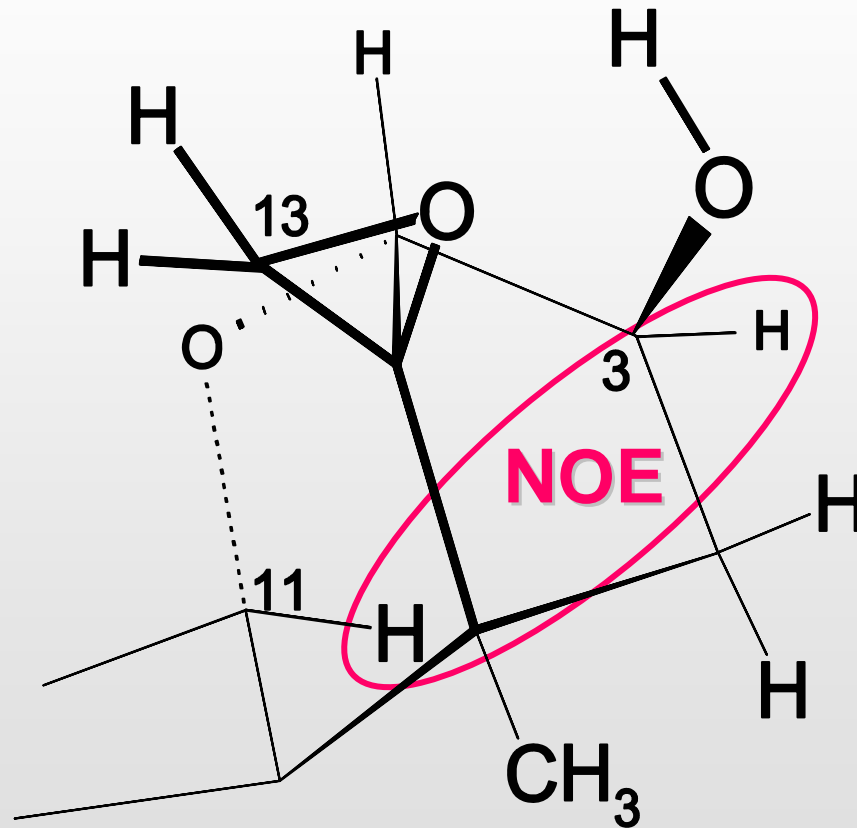


	DON [$\mu\text{g/ml}$]	X-DON [$\mu\text{g/ml}$]	L-DON [$\mu\text{g/ml}$]
<i>A. salina</i> [LC_{50}]	52	78	194
Immuno- supp. [IC_{30}]	\approx 0.1	$>$ 1.07	
MTT-assay [LC_{50}]	\approx 15	200	>> 200

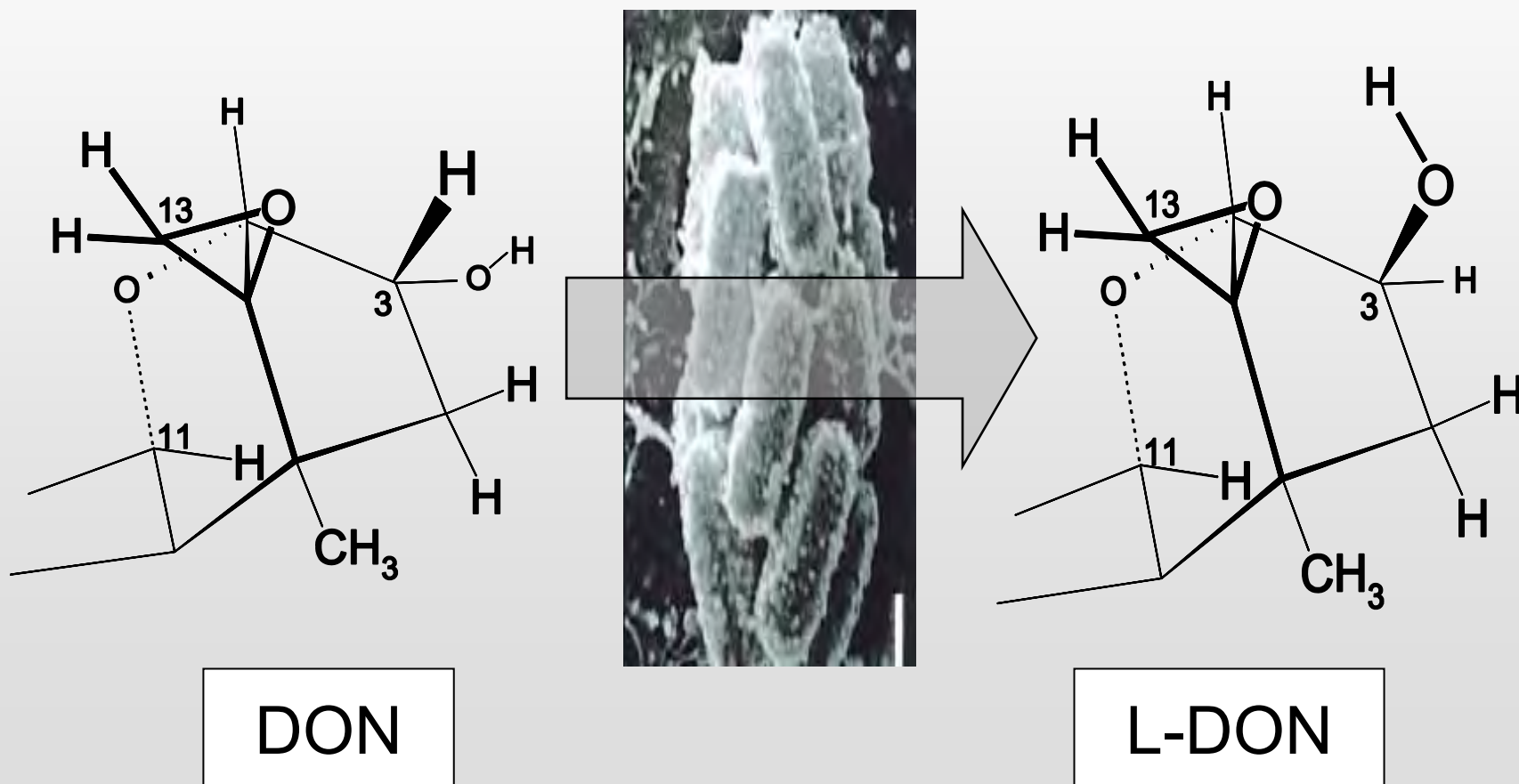
Structure elucidation of L-DON

- MW = 296
- MS fragmentation identical with DON
- ²H- and ¹³C-NMR nearly identical with DON





L-DON is 3-epi-DON



HOH107

Morphology
Physiology
Molecular data



New species of
 α -proteobacteria

"Monia trichothecina"



Photos by Anne Heller



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Real-time qPCR assays

established for the following *Fusarium* species:

- *F. graminearum*
- *F. culmorum*
- *F. verticillioides*
- *F. proliferatum*
- *F. poeae*



Fusarium Head Blight: Epidemiology and the origin of deoxynivalenol

Photo J. Weinert

Epidemiology of Fusarium head blight



Fusarium species isolated from infected ears:

F. graminearum, *F. culmorum*,
F. acuminatum, *F. avenaceum*,
F. crookwellense, *F. equiseti*,
F. poae, *F. solani*...

Which species is relevant for FHB?



Sampling in Germany

- 1200 ear samples
- Rachis isolation
- *Fusarium* assay



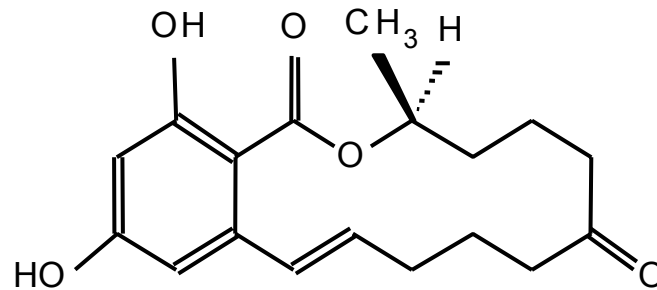
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Zearalenone in the headlines

Hog farms
in trouble

EC embargo
on US beef



Therapy of
menopausal
syndrome

Homosexuality
in rabbits



Biological function of zearalenone

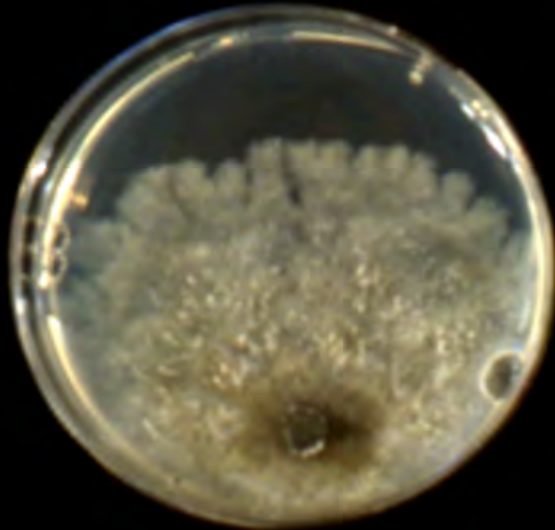
- Regulation of sexual reproduction: **No**
- Virulence factor: **No**
- Ecological metabolite?



Zearalenone as fungicide

Sordaria fimicola

Control



2 $\mu\text{g}/\text{ml}$ ZEN

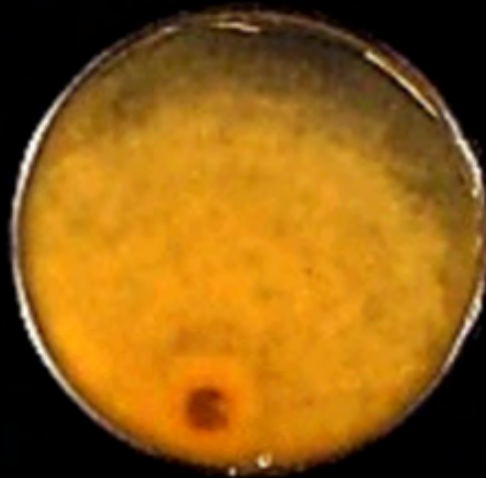


10 $\mu\text{g}/\text{ml}$ ZEN

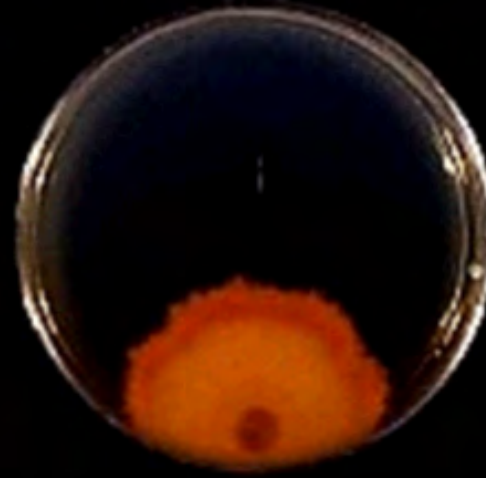


Zearalenone as fungicide

Epicoccum purpurascens



Control



10 µg/ml ZEN



Zearalenone does not affect *G. roseum*

Gliocladium roseum

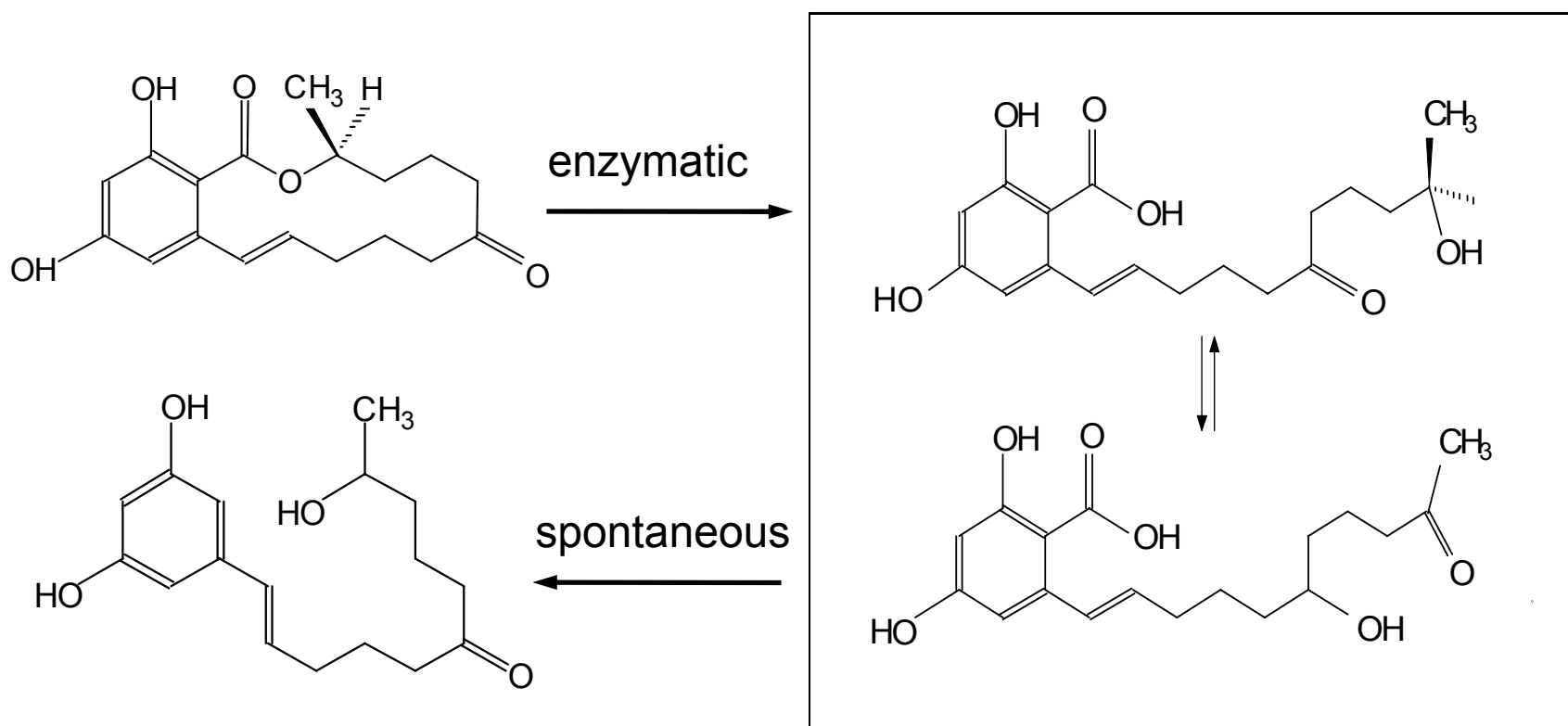


Control

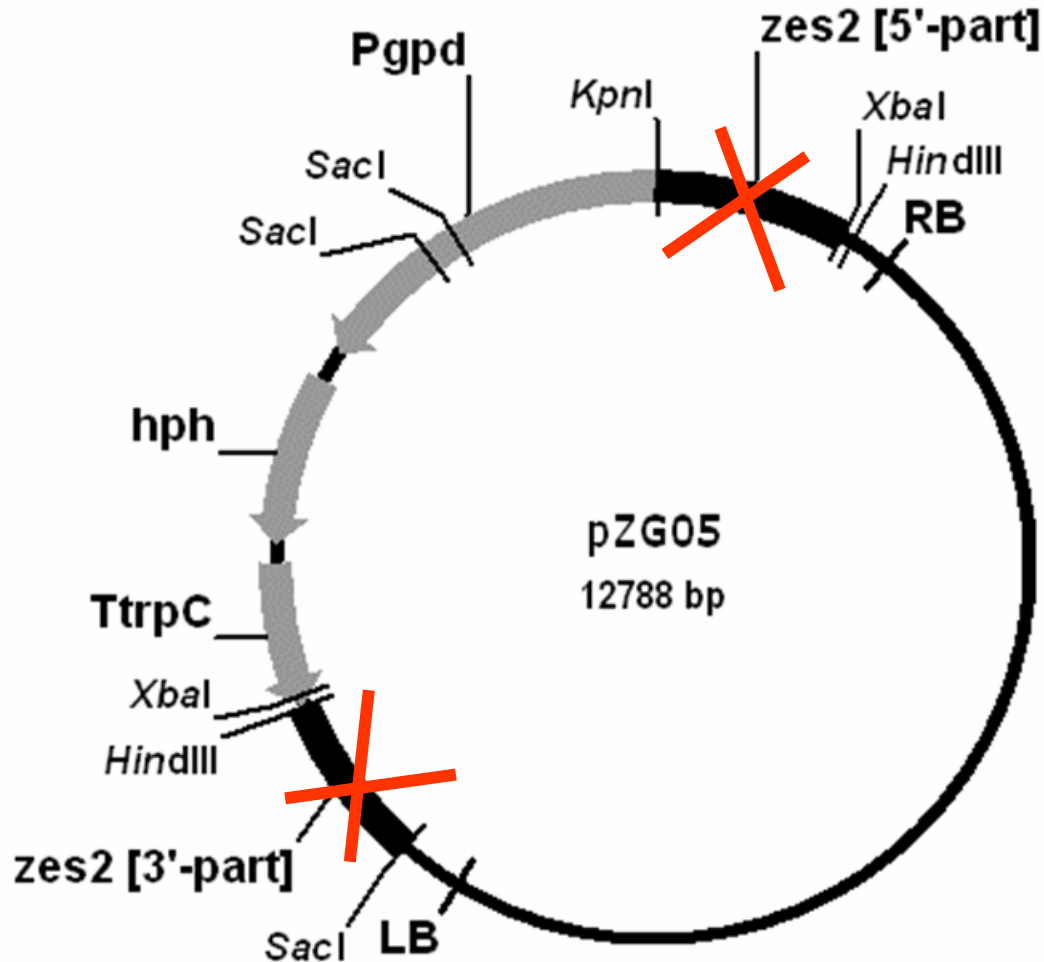


20 µg/ml ZON

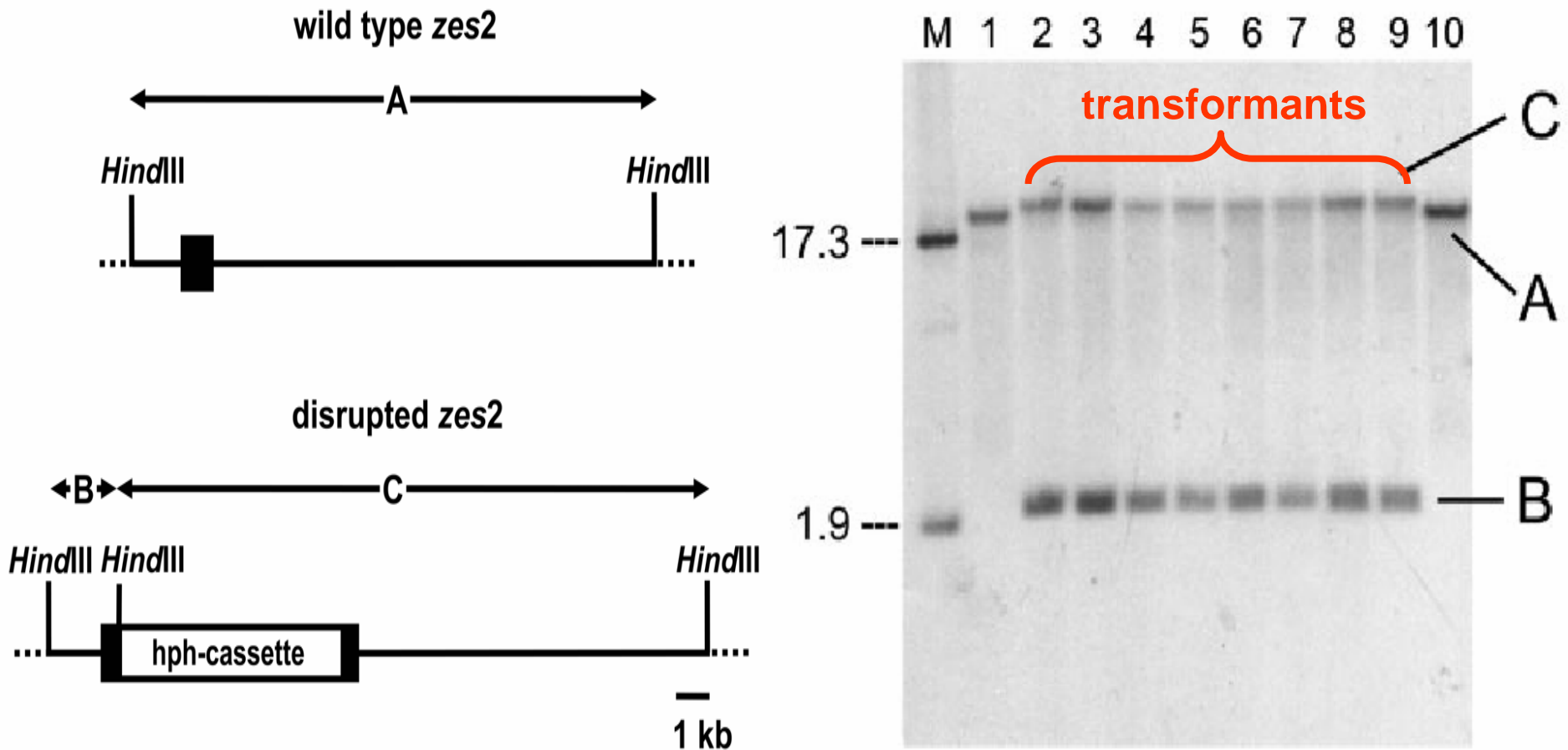
Detoxification of ZEN by *G. roseum*



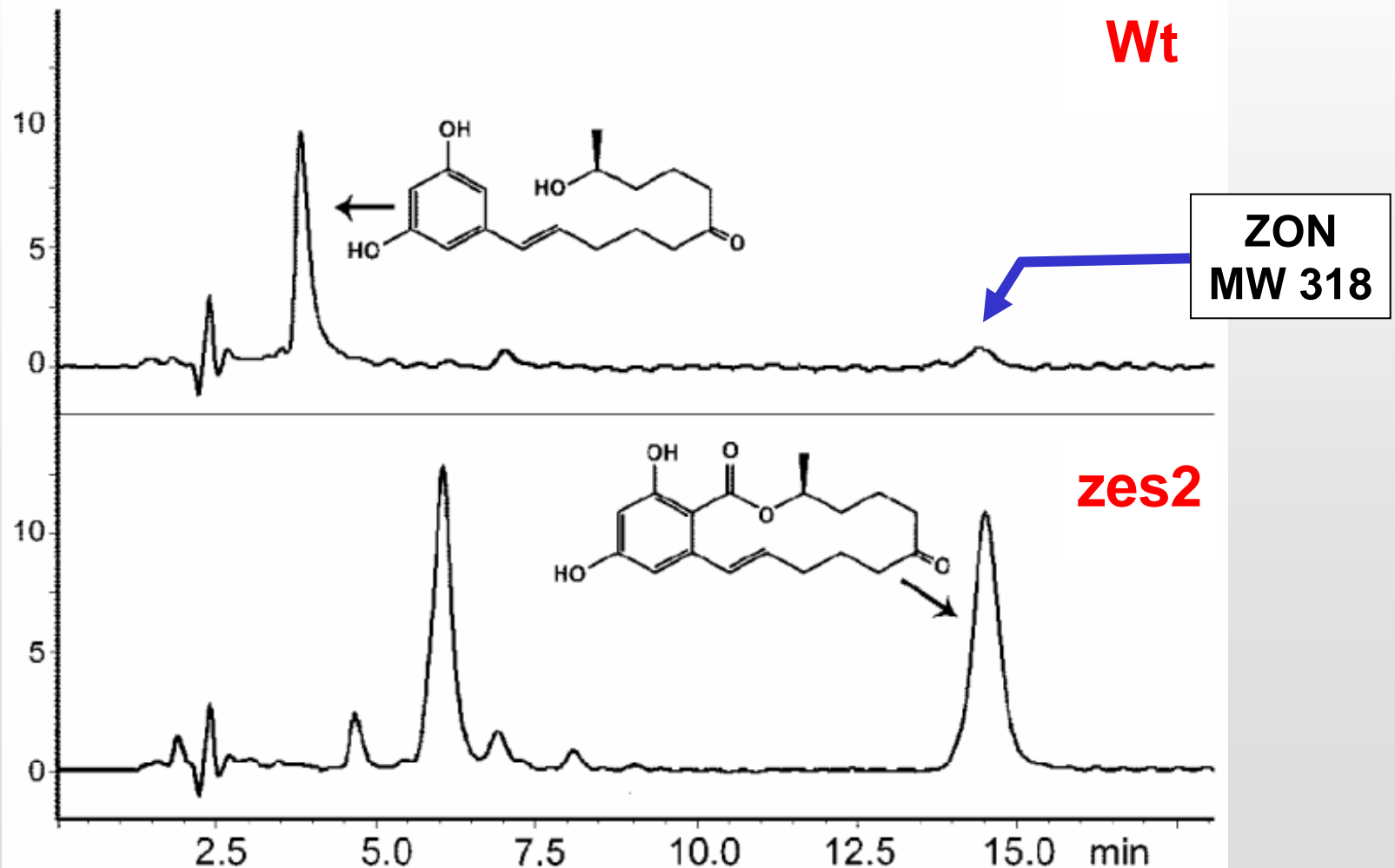
Agrobacterium tumefaciens-mediated transformation of *G. roseum*



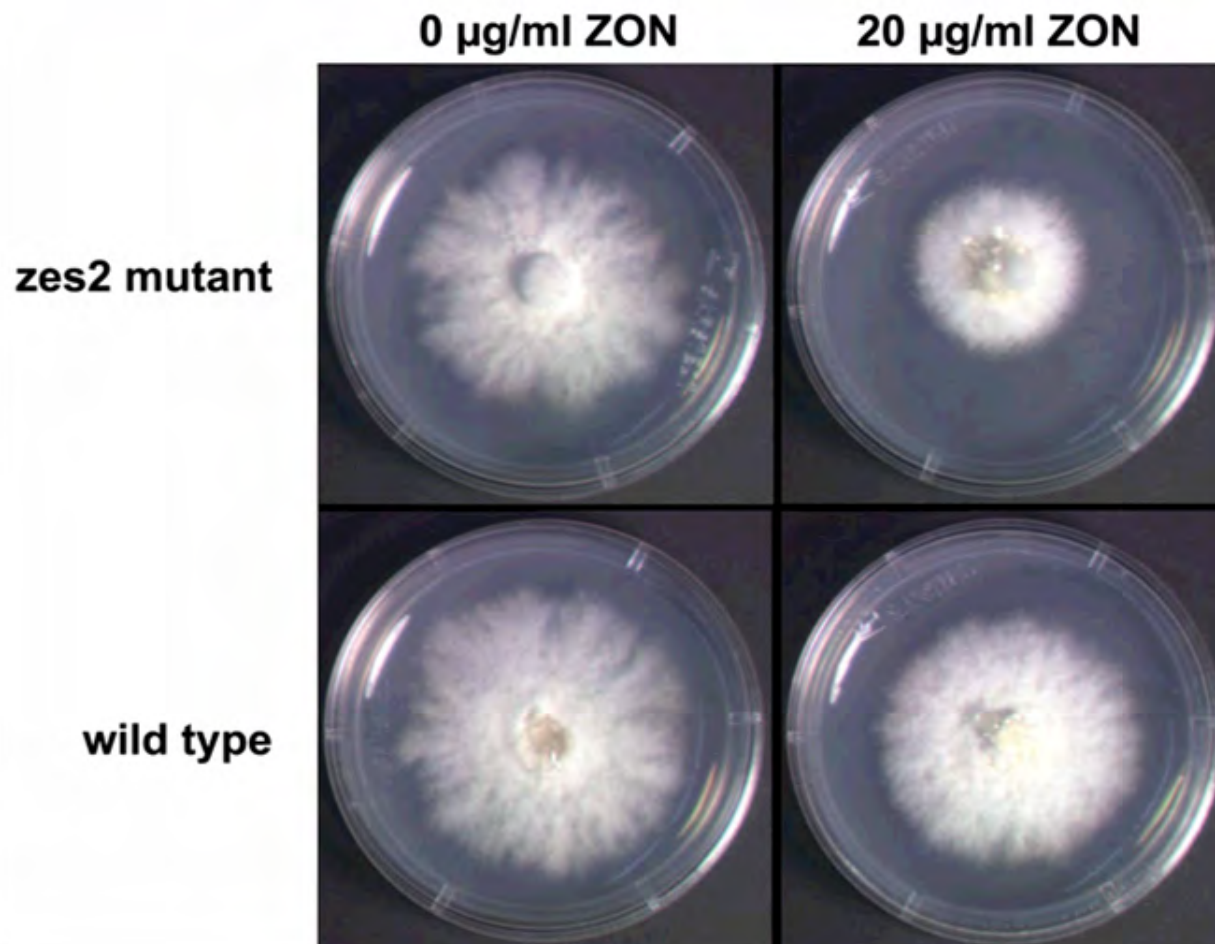
Disruption of *zes2* in *G. roseum*



Disruption of *zes2* in *G. roseum*



Disruption of *zes2* in *G. roseum*



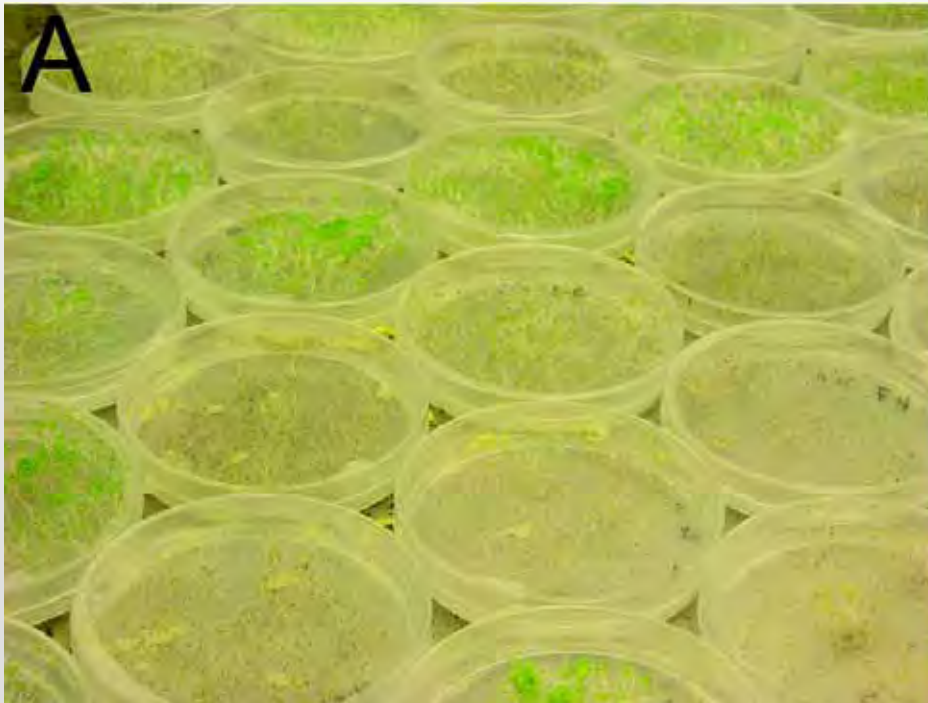


Conclusions

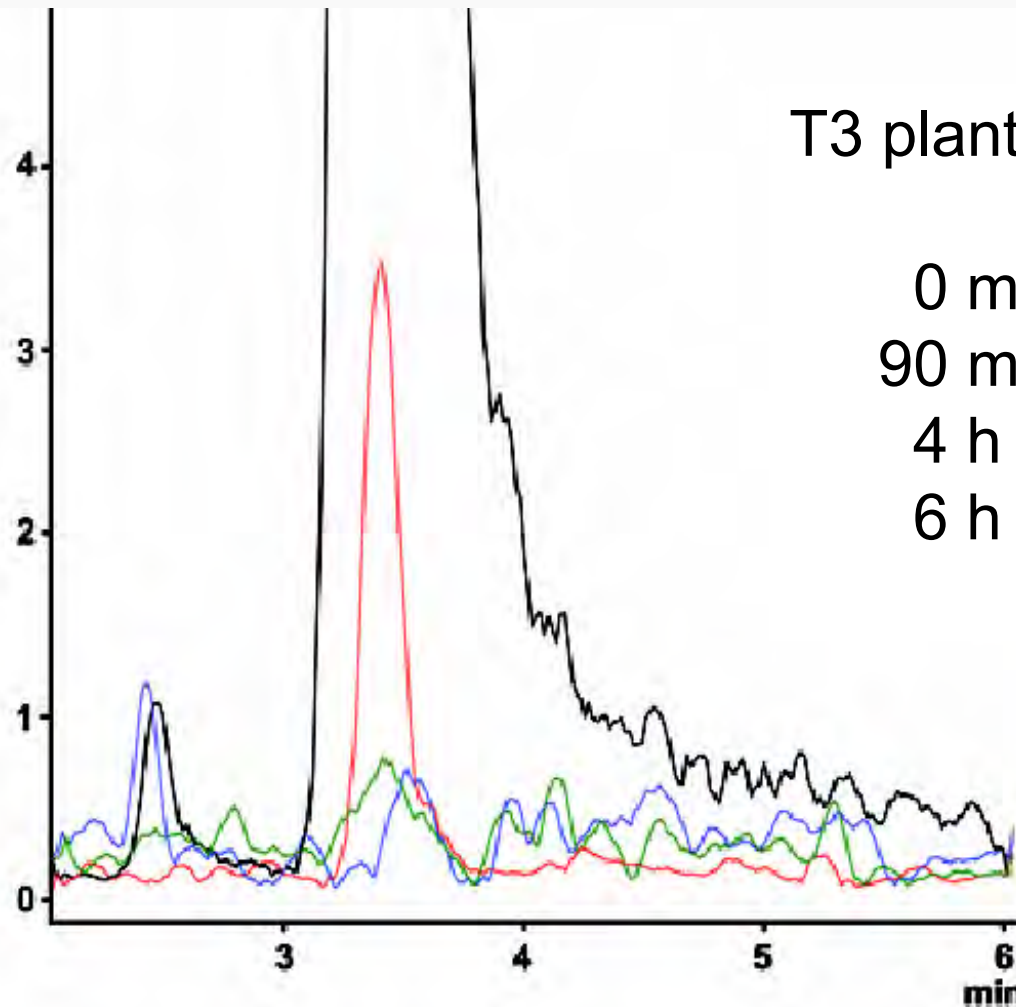
- Zearalenone is agent of interference competition
- *zes2*-lactonase protects *G. roseum* from zearalenone



Expression of ZON lactonase in *Arabidopsis*



Expression of ZON lactonase in *Arabidopsis*

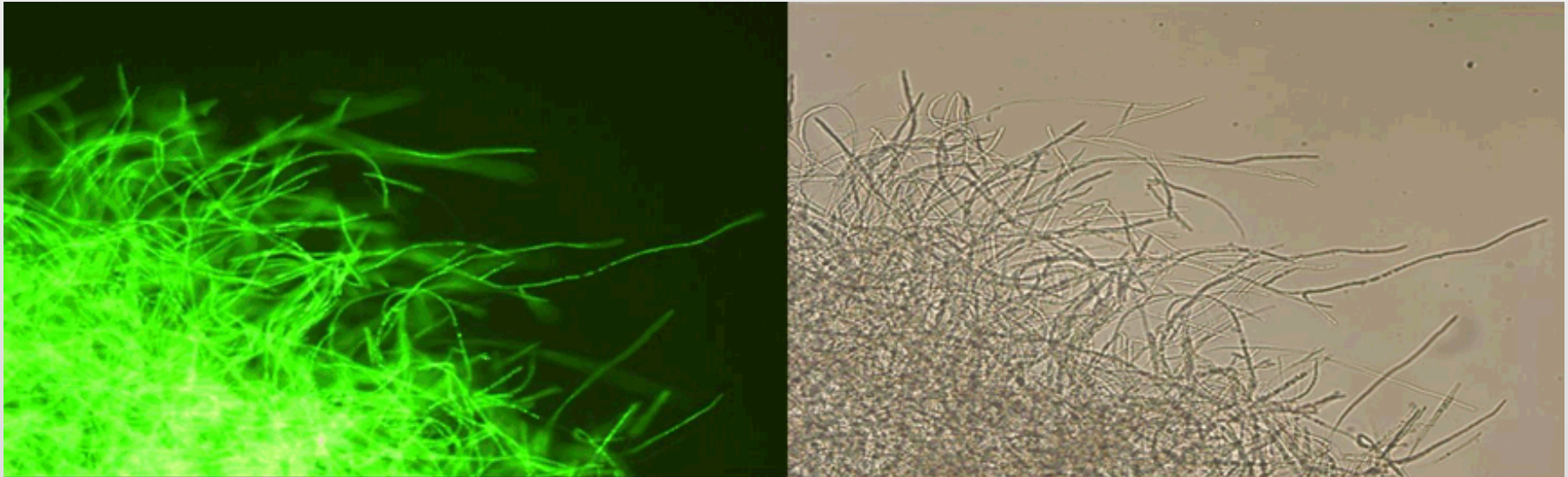
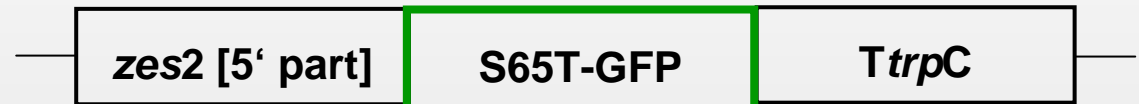


T3 plant leaf extract:

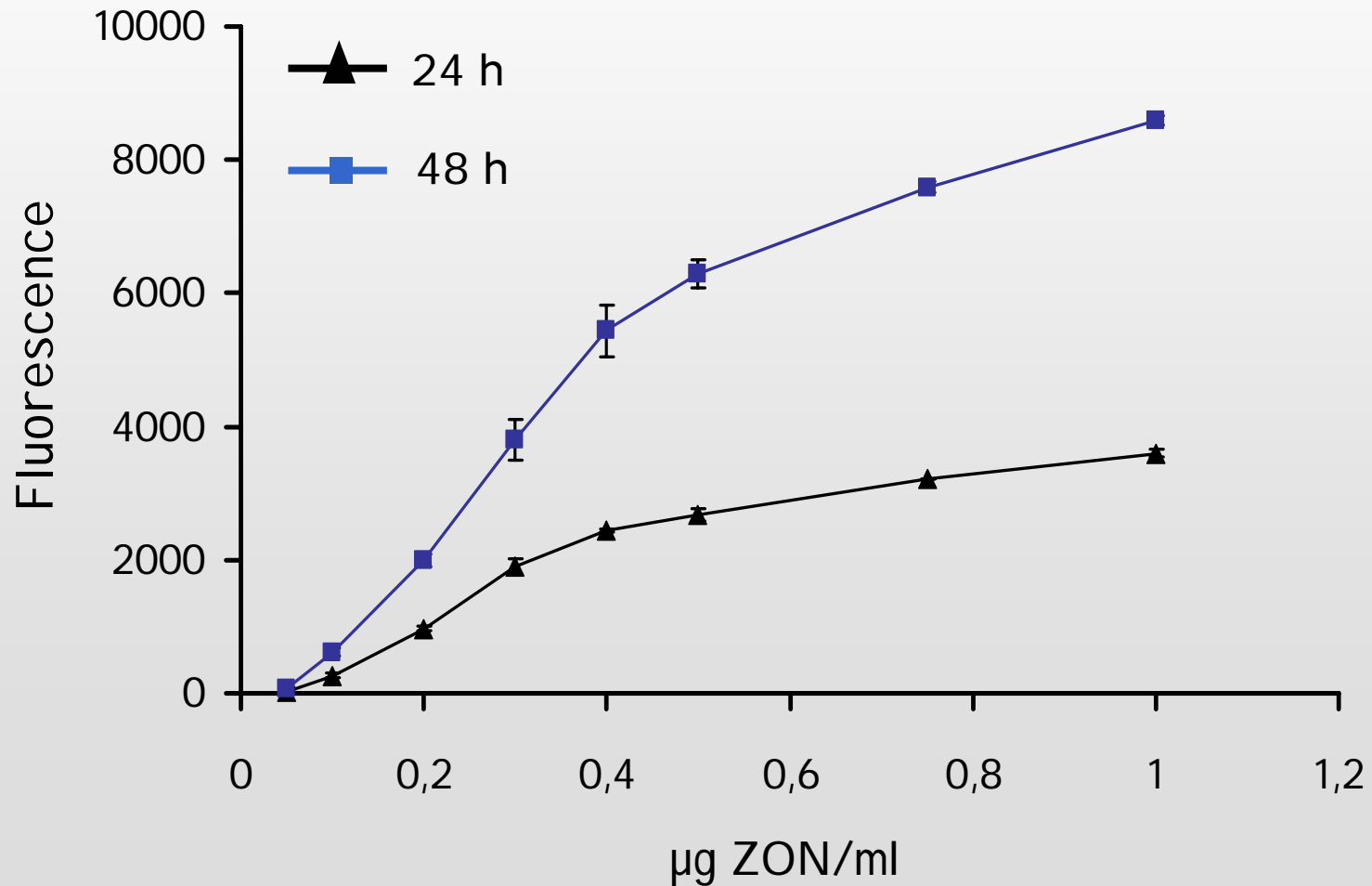
0 min —————
90 min —————
4 h —————
6 h —————

Reporter fusion of *zes2* with GFP

G. roseum zes2::GFP



Reporter fusion of *zes2* with GFP



Reporter fusion of *zes2* with luciferase

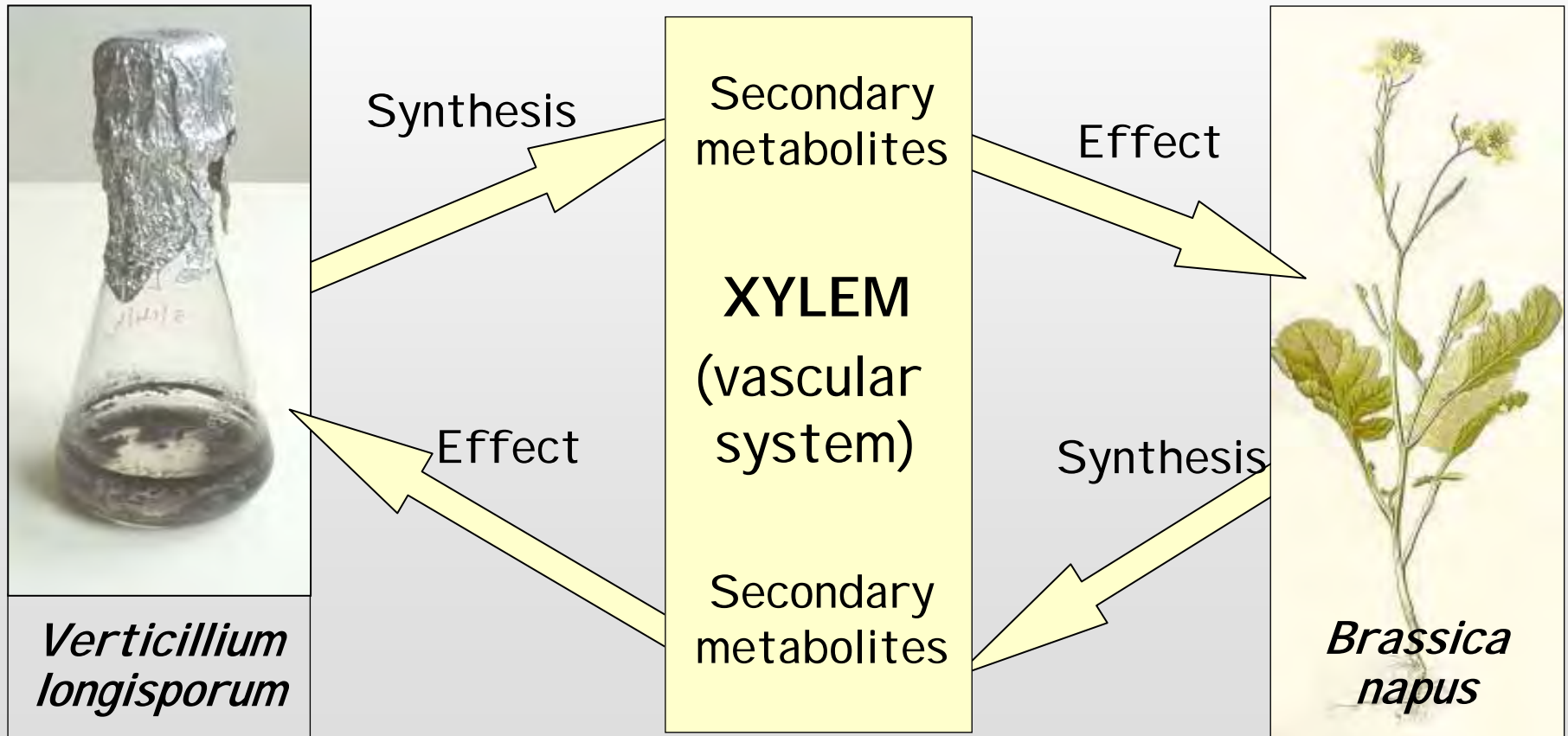




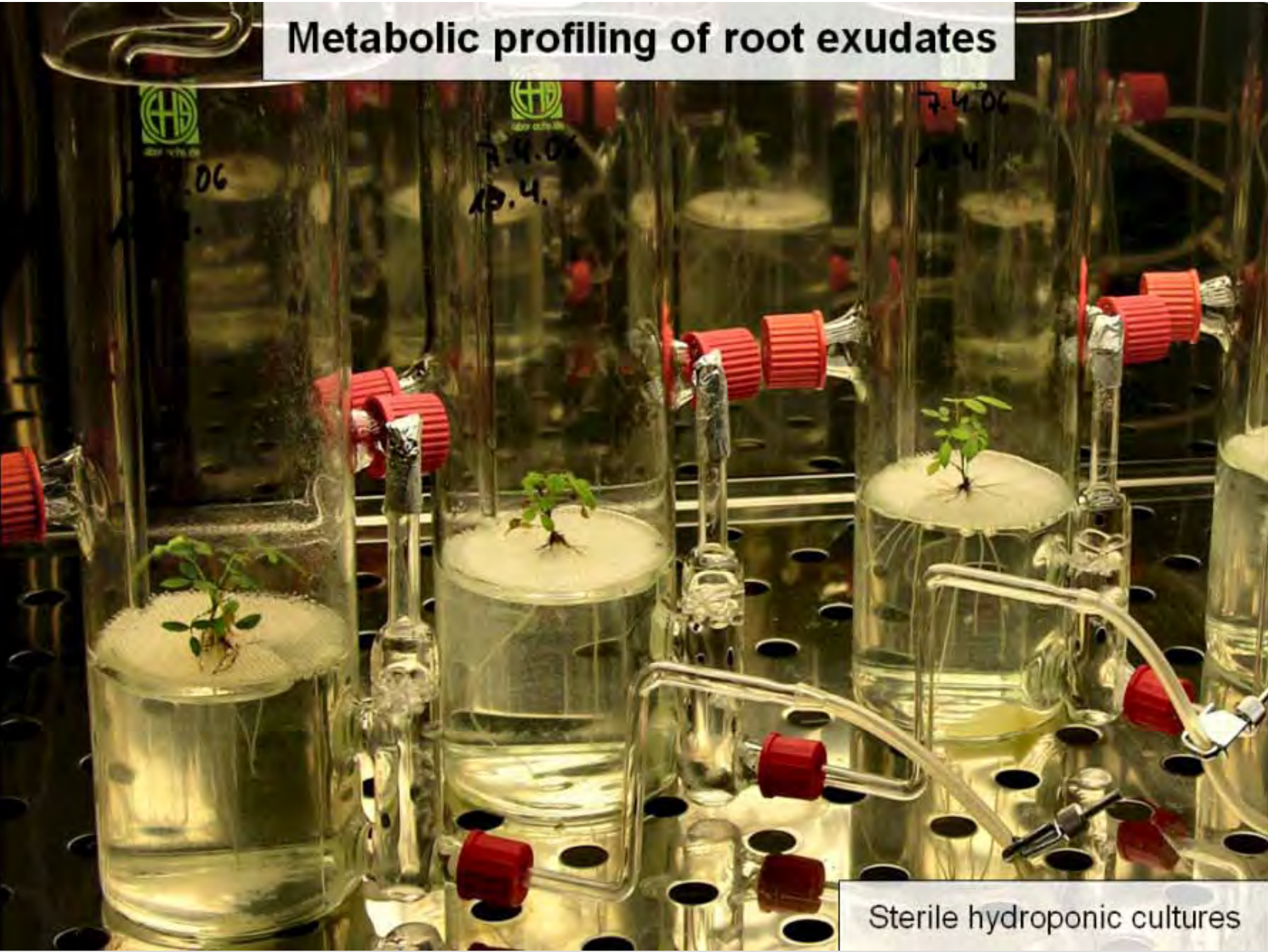
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Chemical interaction between *Brassica napus* and *Verticillium longisporum*

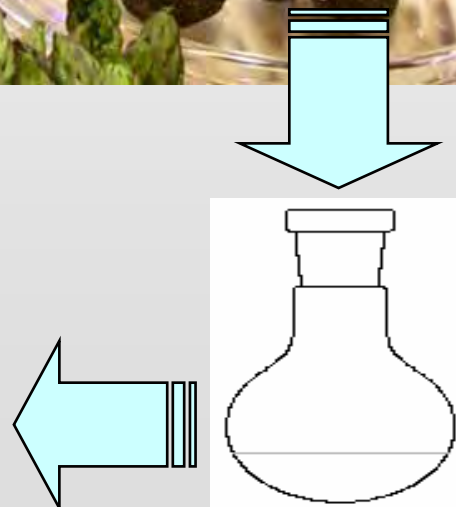
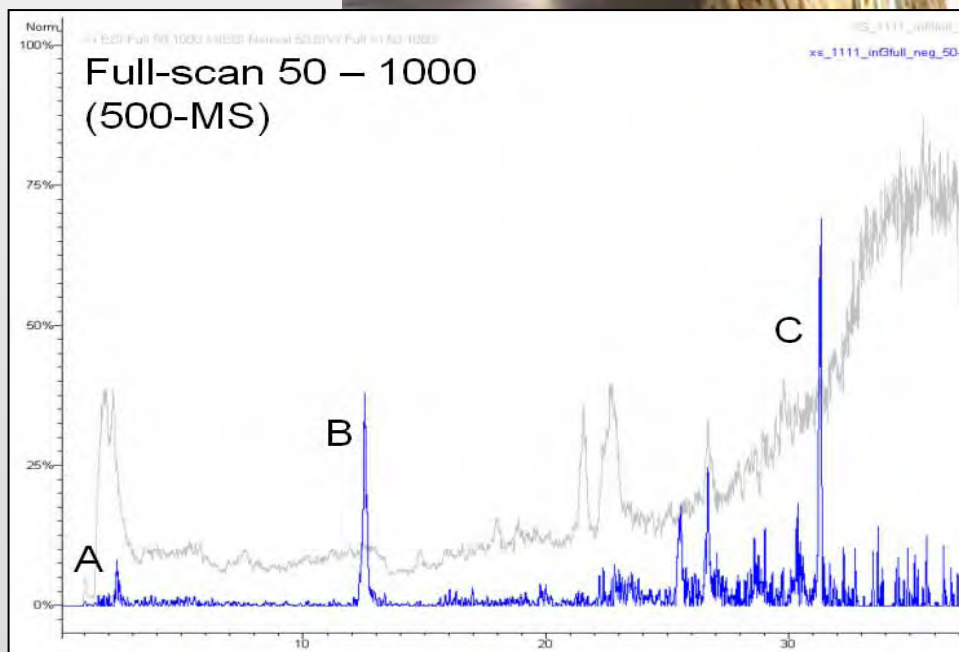
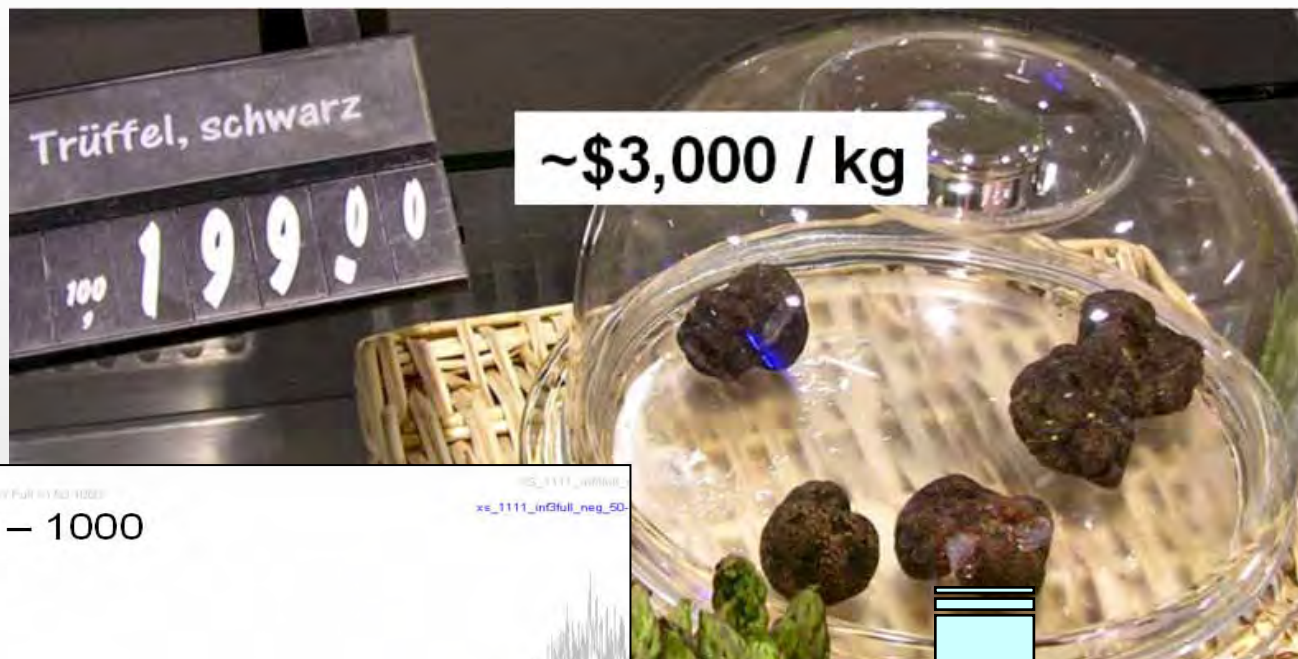


Metabolic profiling of root exudates



Sterile hydroponic cultures

Truffle metabolites in interactions with plants (Dr. R. Splivallo)

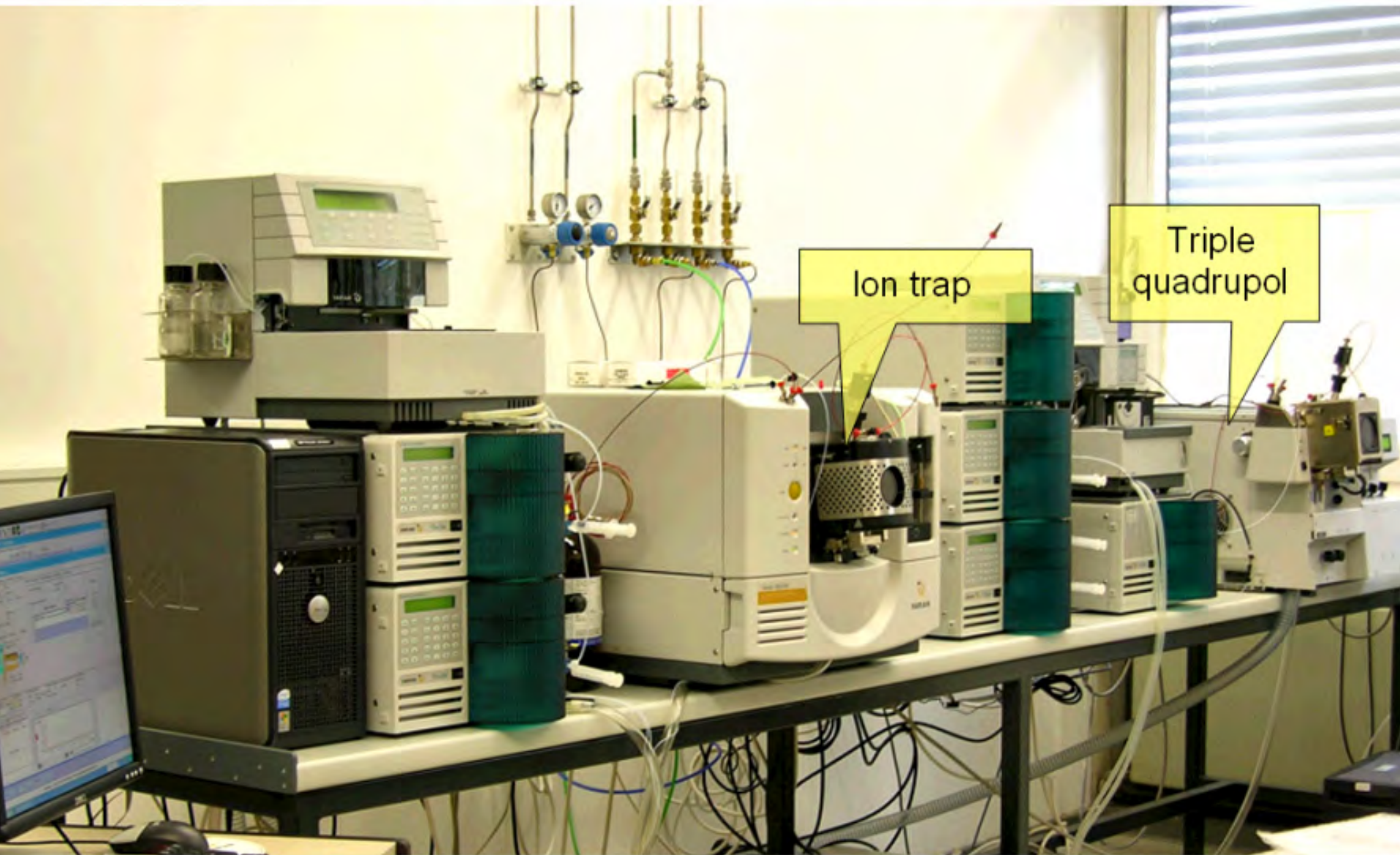




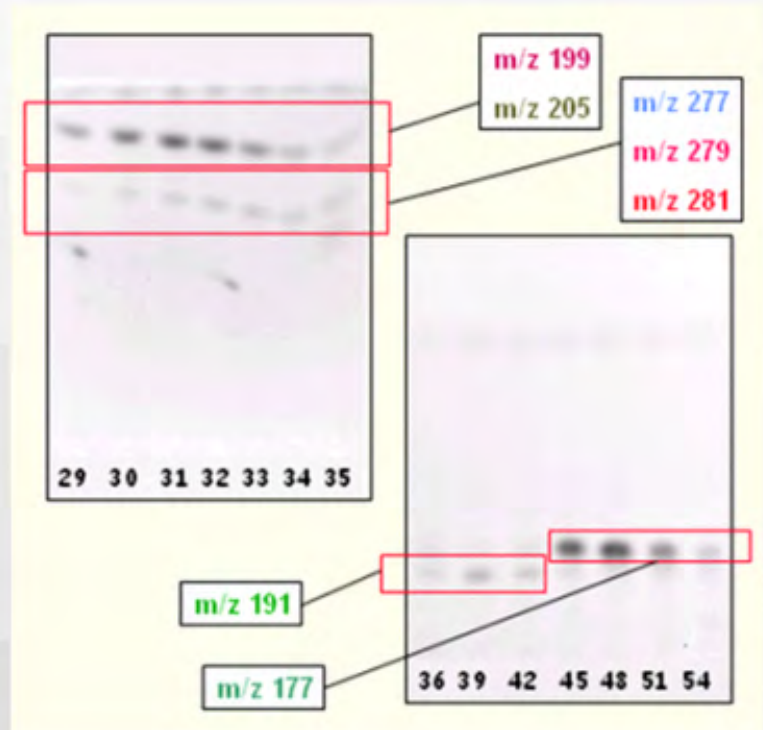
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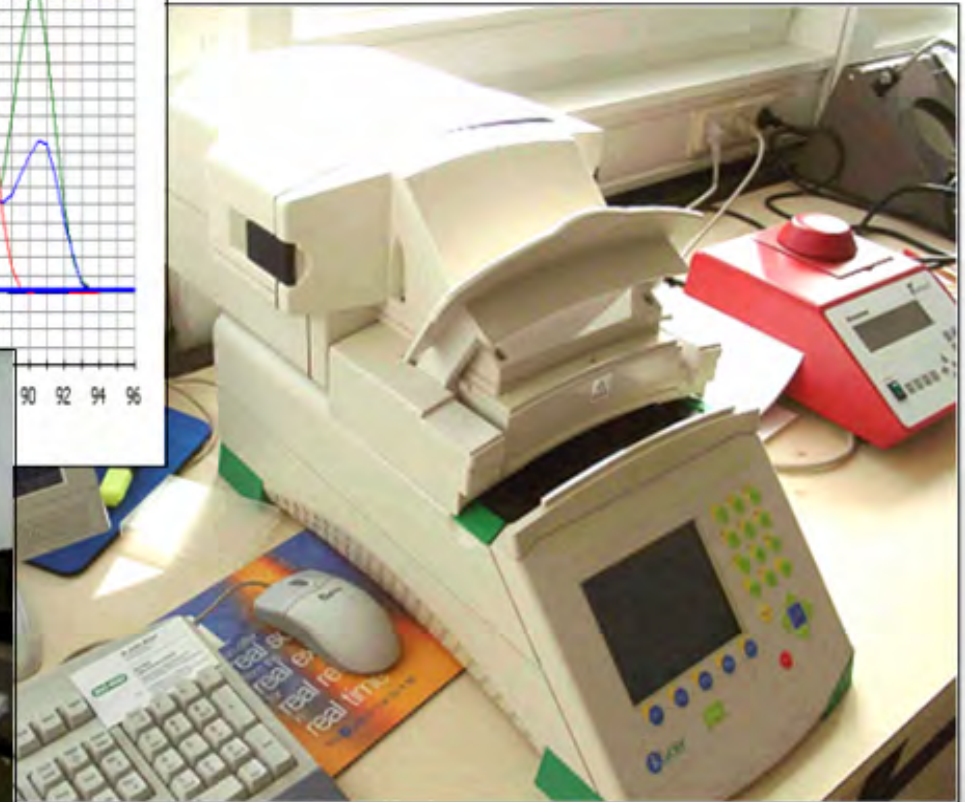
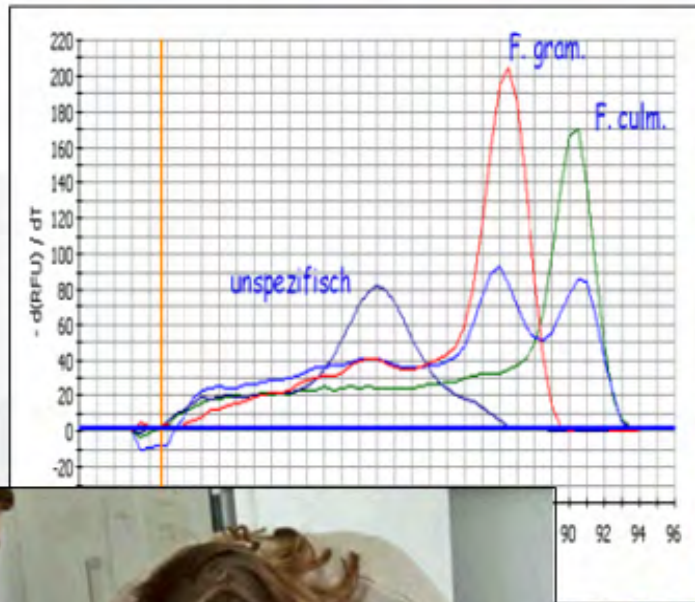
Chemical analysis and metabolic profiling



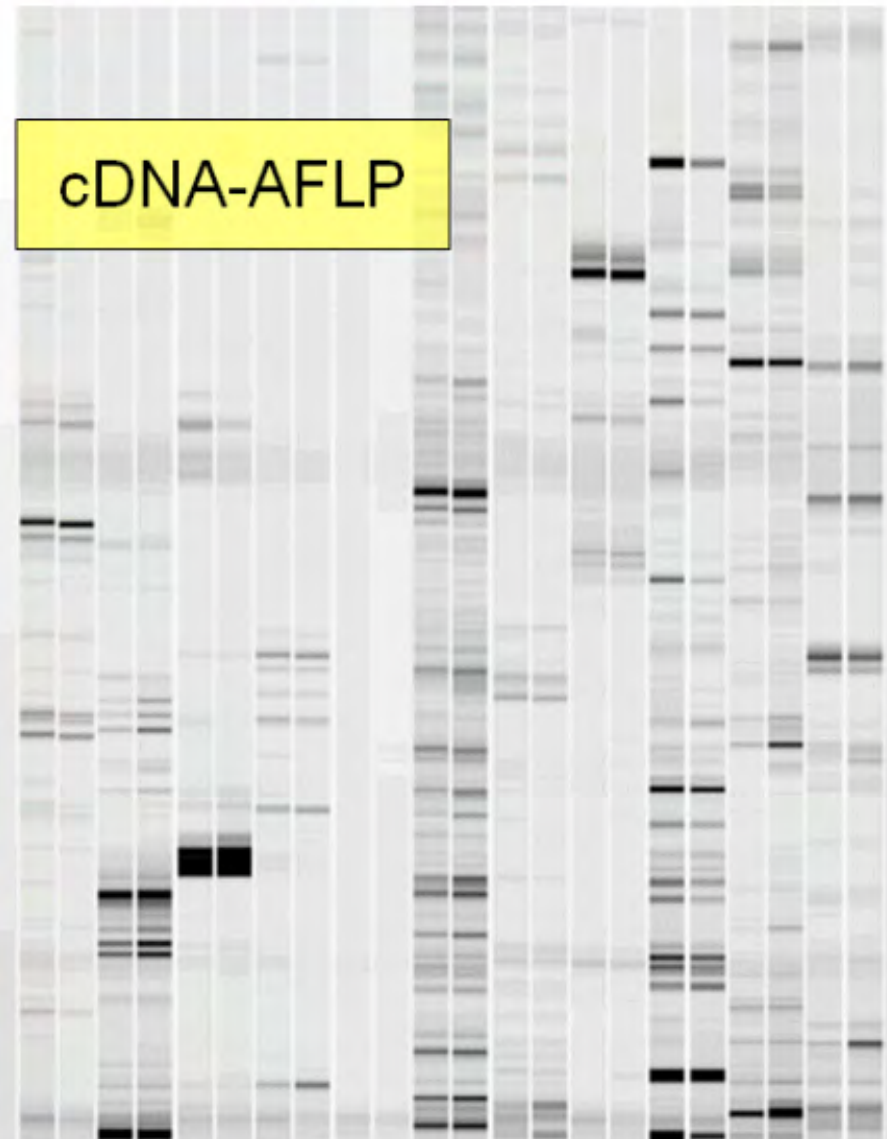
Natural product purification



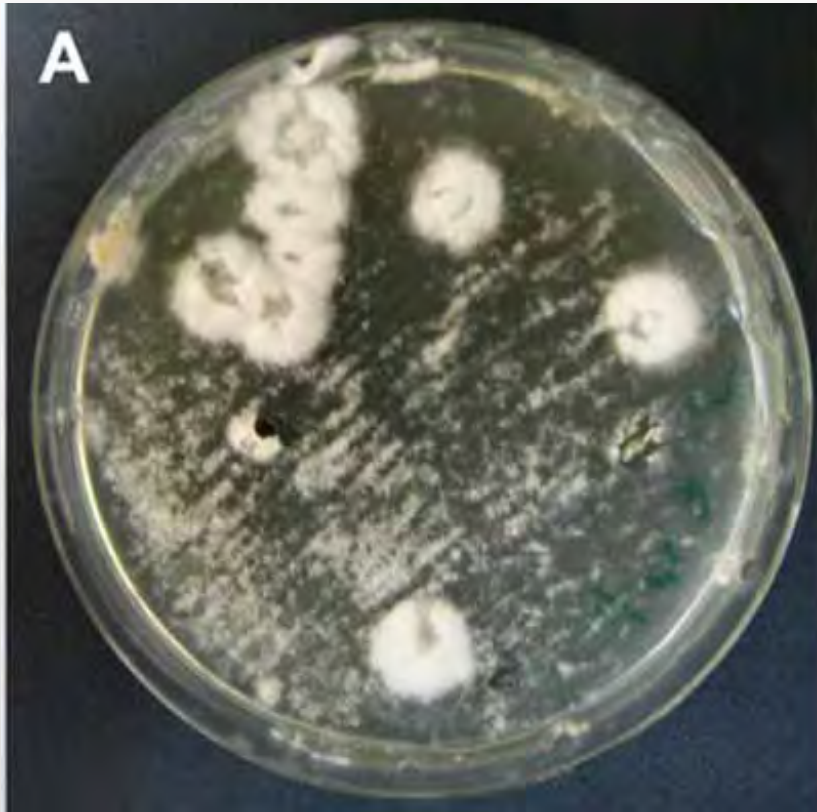
qPCR für diagnose, epidemiology and gene expression



Genetic diversity, microbial diversity and transcriptome analysis



Genetic engineering





Acknowledgment

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