



University of Hamburg



A Secreted Lipase as a new Virulence Factor of *Fusarium graminearum*

Siegfried Salomon

Dept. Molecular Phytopathology and Genetics

Biozentrum Klein Flottbek

Ohnhorststr. 18

D-22609 Hamburg Germany

The ascomycete *Fusarium graminearum* is a plant pathogenic fungus



Name of the sexual state: *Gibberella zeae*

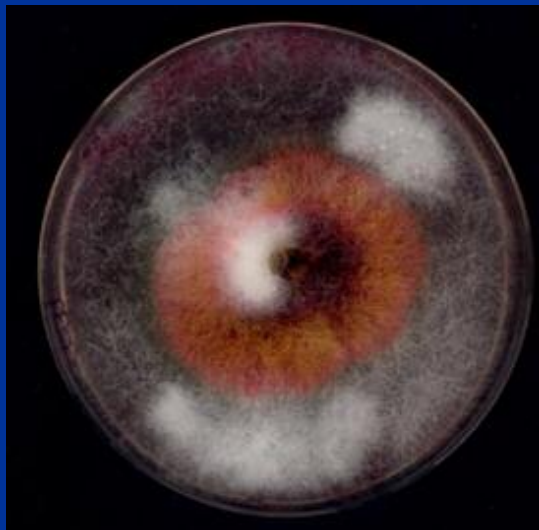
Fusarium graminearum sequencing project (2003)

at the Whitehead Institute for Biomedical Research/MIT

total length 36093143 bp

The entire genome sequence has been deposited in GenBank with accession AACM00000000

Release 2: Automated annotation, preliminary genome analysis, and integration with genetic map



Fusarium graminearum



Conidia of *Fusarium graminearum*

Fusarium graminearum

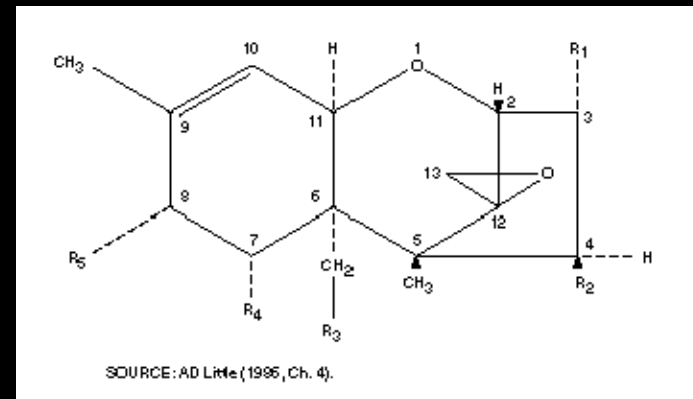


Disease: Fusarium head blight (FHB)

first described just over a century ago and considered a major threat to wheat, maize, and barley during the early years of this century



The fungus produces mycotoxins that pose a serious food safety hazard



General structure of **trichothecene toxins**



Do secreted lipases represent a new class of virulence factors ?

Strategies for infection of host (cells)

- Appressoria
 - Direct penetration through Stoma
 - Wound infection
 - Secretion of hydrolytic enzymes
 - Cutinases
 - Esterases
 - Phospholipases
 - Lipases**



Rapid detection of lipase activity on RhodaminB - olive oil agar plates

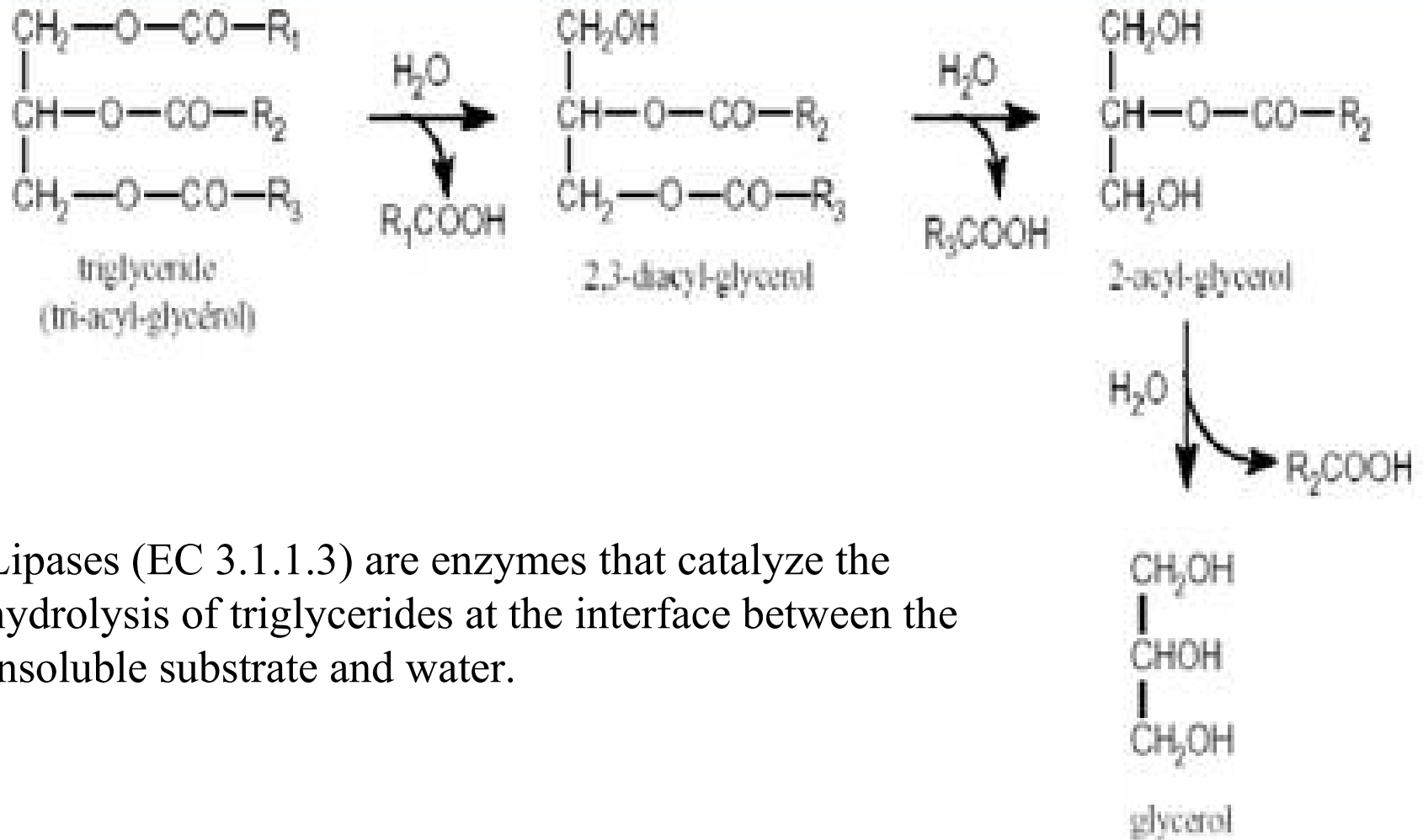
<i>Acremonium crysogenum</i>	+
<i>Aspergillus fumigatus</i>	++
<i>Aspergillus parasiticus</i>	++
<i>Cochliobolus heterostrophus</i>	0
<i>Fusarium graminearum</i>	+++
<i>Fusarium solani</i>	+++
<i>Magnaporthe grisea</i>	+
<i>Penicillium cyrsogenum</i>	+
<i>Penicillium digitatum</i>	+
<i>Penicillium expansum</i>	0
<i>Penicillium italicum</i>	0
<i>Penicillium oxallicum</i>	++
<i>Pyrenophora avenae</i>	+++
<i>Pyrenophora tritici-repentis</i>	+++
<i>Pyrenophora teres</i>	+++
<i>Candida albicans</i>	++
<i>Candida parapsilosis</i>	++
<i>Pichia pastoris</i>	0

High level lipolytic activity
of *F. graminearum*





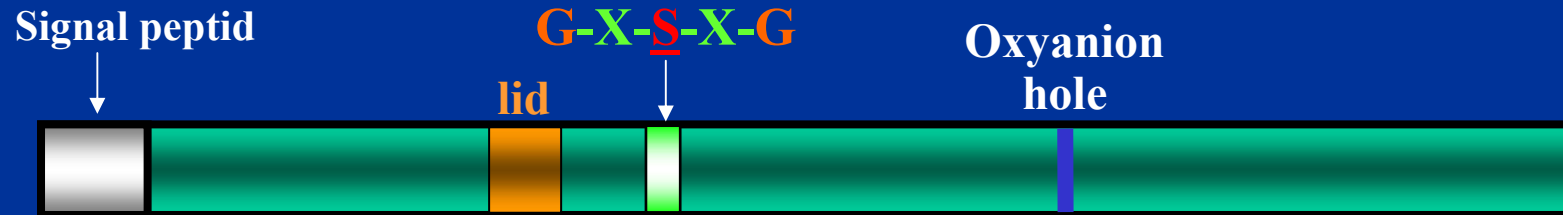
Lipases



Lipases (EC 3.1.1.3) are enzymes that catalyze the hydrolysis of triglycerides at the interface between the insoluble substrate and water.



Conserved AS-motives of secreted lipases



The active site for lipases is in a well conserved motif **Gly-X-Ser-X-Gly**, where X can be His, Leu, or Tyr.

<i>Nectria haematococca</i>	TGFLEAWEEVAANI KAAVS AAKTANPT FKF VVT TGHS SLG GAV ATVAAAYLRKDGFPFDLYT
<i>Aspergillus niger</i>	GGYY IGWISVQDQVESLVKQQASQYPDYAL TVT TGHS SLG ASMA ALTAAQLSATYDNRVRLYT
<i>Aspergillus oryzae</i>	LGFWTAWKVVDRDI IKTLDELKPEHSDYK IVV VGH SLG AAI ASLAAADLRTKNYDAIILYA
<i>Aspergillus tubingensis</i>	GGYY IGWISVQDQVESLVQQVVSQFPDYAL TVT TGHS SLG ASLA ALTAAQLSATYDNIIRLYT
<i>Fusarium heterosporum</i>	TGFLDAWEEVAANV KAAVS AAKTANPT FKF VVT TGHS SLG GAV ATI AAAAYLRKDGFPFDLYT
<i>Neurospora crassa</i>	TGFYTAWREVATKV TAAVQ SAKAA YPS YSIG GV TGHS SLG GAV ATVAAAYLRKAGYTADLYT
<i>Penicillium camembertii</i>	LGFWSSWKLVRDDI IKELKEVVAQNPNYEL VVV VGH SLG AAV ATLAATDLRGKGYPSAKLY
<i>Rhizopus niveus</i>	AGFLSSYEQVVNDYFPVVQEQLTAHPTYK VI VT TGHS SLG GAQ ALLAGMDLYQREPRLS PKN
<i>Rhizopus arrhizus</i>	AGFLSSYEQVVNDYFPVVQEQLTANPTYK VI VT TGHS SLG GAQ ALLAGMDLYQREPRLS PKN
<i>Rh.oryzae</i>	AGFLSSYEQVVNDYFPVVQEQLTAHPTYK VI VT TGHS SLG GAQ ALLAGMDLYQREPRLS PKN
<i>Rhizomucor miehei</i>	KGFLDSYGEVQNELVATVLDQFKQYPSYK VA VT TGHS SLG GAT ALLCALDLYQREEGLSSSN
<i>Thermomyces lanuginosus</i>	DGFTSSWRSVADTLRQKVEDAVREHPDYR V V T TGHS SLG GAL ATVAGADLRGNGYDIDVFS
<i>Yarrowia lipolytica</i>	NGFIQSYNNTYNQIGPKLDSVIEQYDPYQ IA V T TGHS SLG GAA ALLFGINLKVNGHDPLVVT
<i>Saccharomyces cerevisiae</i>	FLRFTETLGMDVFKKME SILESFPEYR IV V T TGHS SLG GA ALASLAGIELKIRGFDPPLVLT
<i>Schizosaccharomyces pombe</i>	EVQDDSRYYASLDIFYSVKELYPDQAQ I W L T TGHS SLG GAT AALMGLSFGIPTVTFEAPG
<i>Candida ernobii</i>	YETLKQFSDEVFPVKE LKEGNYSYQ V V T TGHS SLG GAL TTLAGIEFLLMGYDPLVIS
<i>Caenorhabditis elegans</i>	VEMYKDILEFGFDASLEKVVQEYPSYS M L I T TGHS SLG GAM ATI FSLHVALKYPQKKTSL
<i>Arabidopsis thaliana</i> (triacylglycerol lipase homolog)	QNATTLYAYYTVRRHLKEILDQNPTSK F I L T TGHS SLG GAL AILFTAVLVMHDEEQMLE



Cloning and characterization of a new lipase gene: FGL1

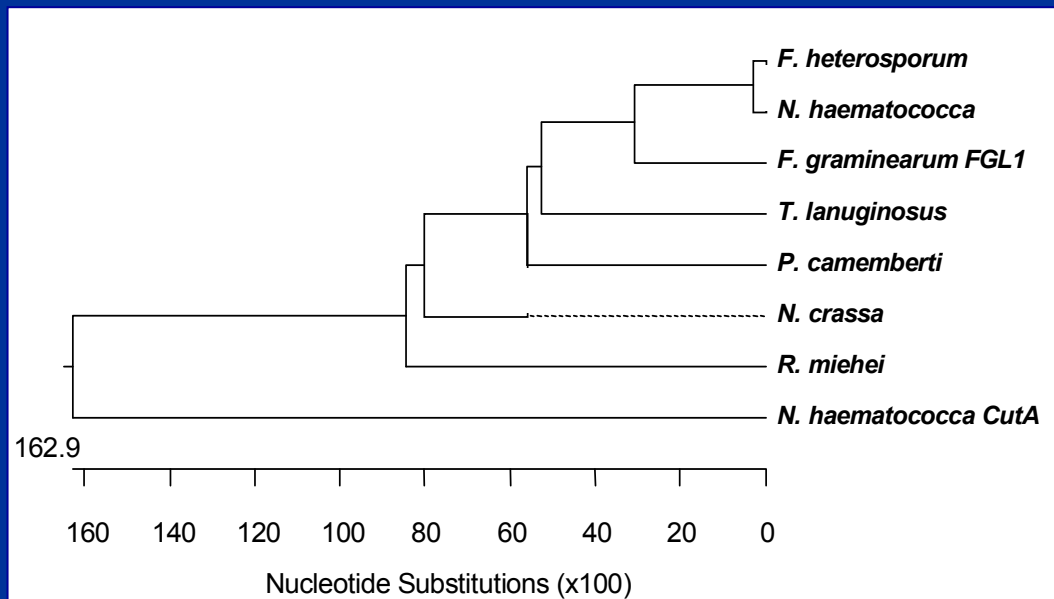
FGL1:

ORF: 1056 bp

two introns of 52 bp and 58 bp

encoded protein: 352 amino acids

molecular weight: 37.3 kDa

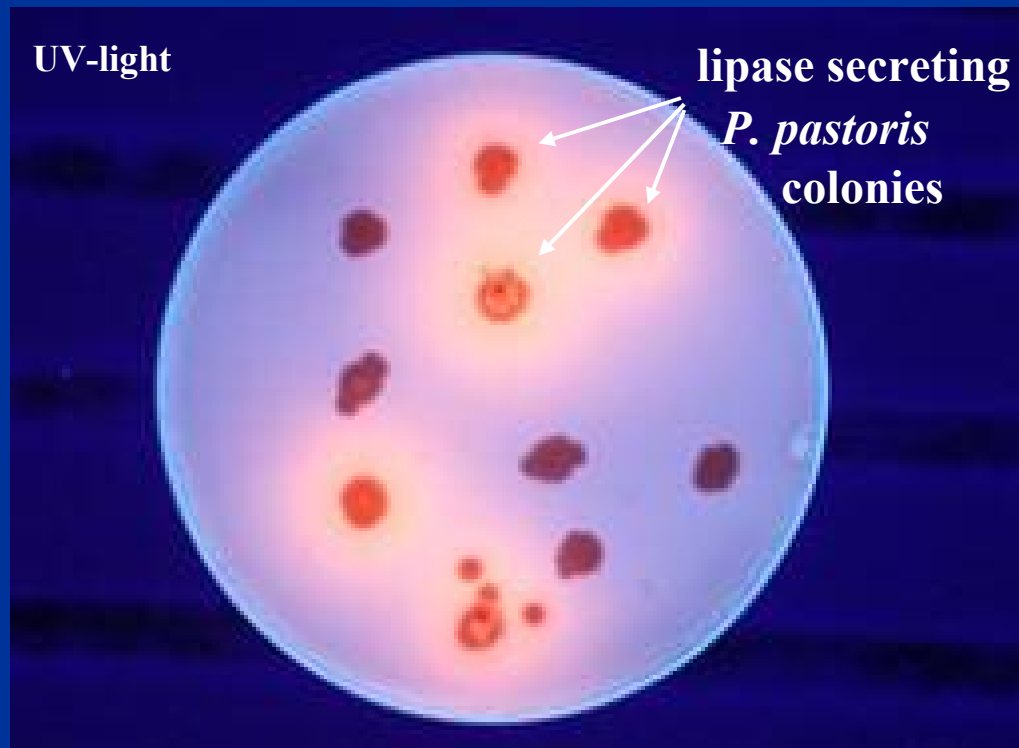
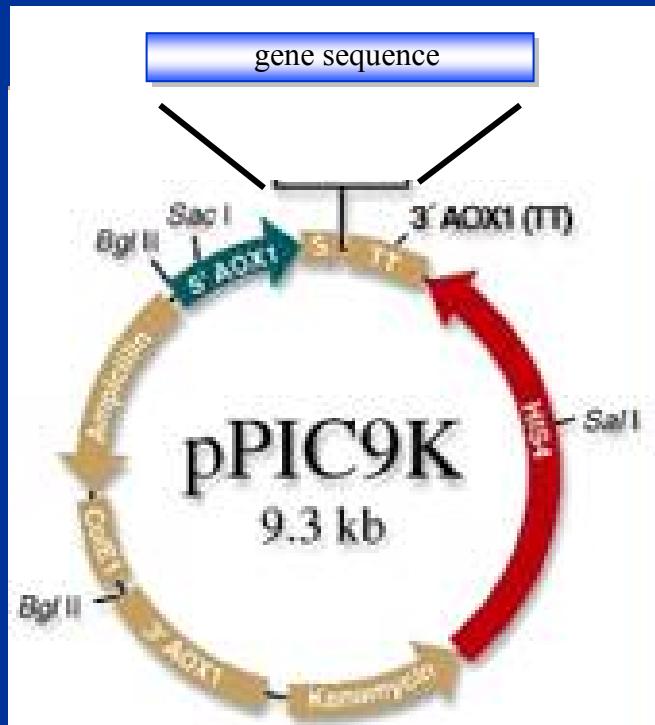


***FGL1* shows highest similarity to known lipases of *F. heterosporum* (66.2 %) and *N. haematococca* (65.2 %)**



Characterization of new lipase genes *via Pichia pastoris* expression system

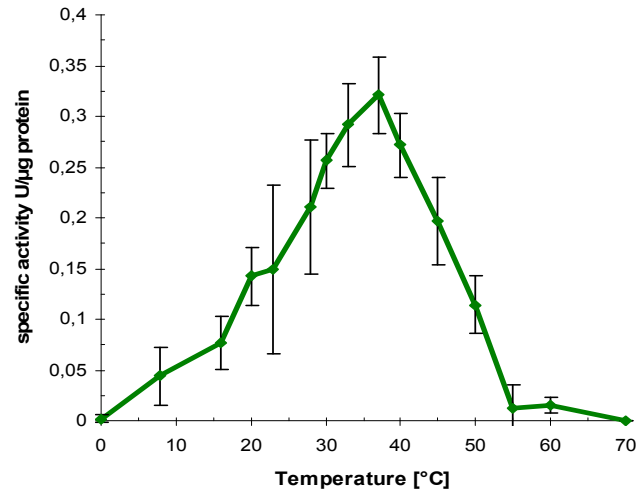
For detailed biochemical characterization of putative lipases, we use the *Pichia pastoris* expression system [Invitrogen].



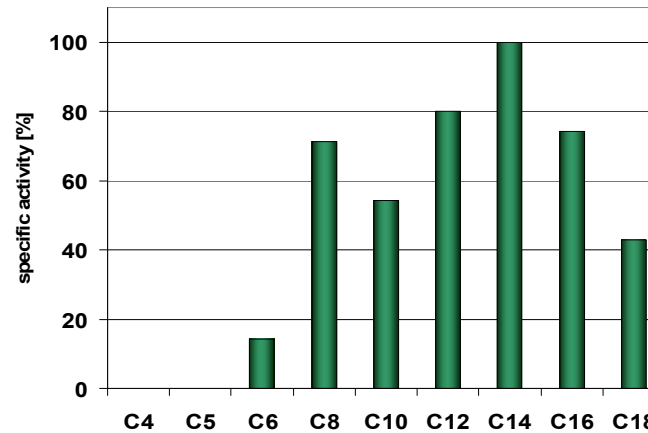


Biochemical characterization of lipases

Temperature-dependant activity



Determination of substrate specificity



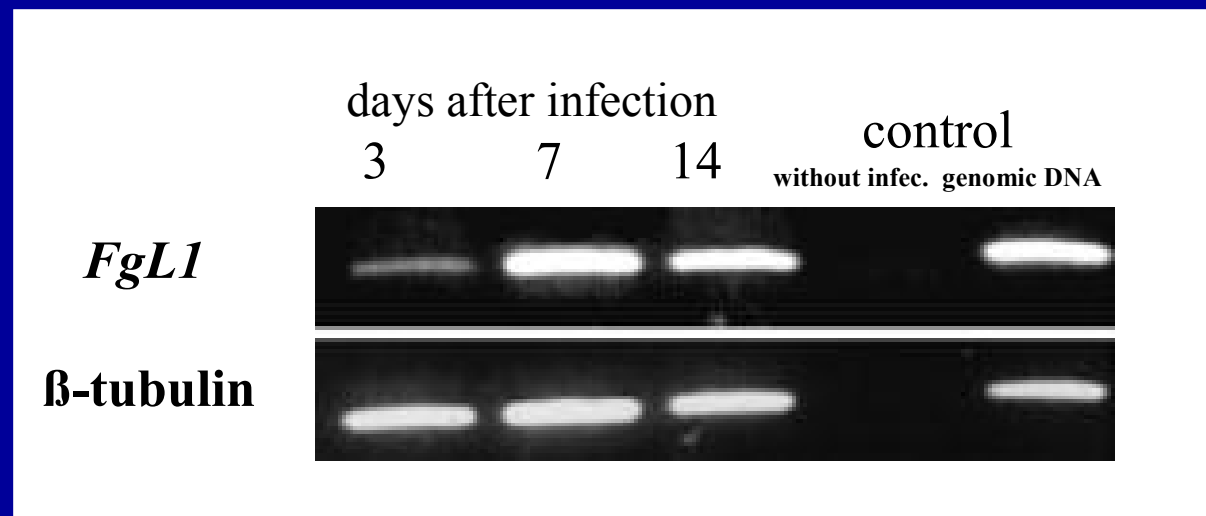
Substrates:

- C4: p-nitrophenyl-butyrate
- C5: pNP-valerate
- C6: pNP-hexanoate
- C8: pNP-octanoate
- C10: pNP-decanoate
- C12: pNP-dodecanoate
- C14: pNP-myristate
- C16: pNP-palmitate
- C18: pNP-stearate

Fusarium graminearum
FGL1



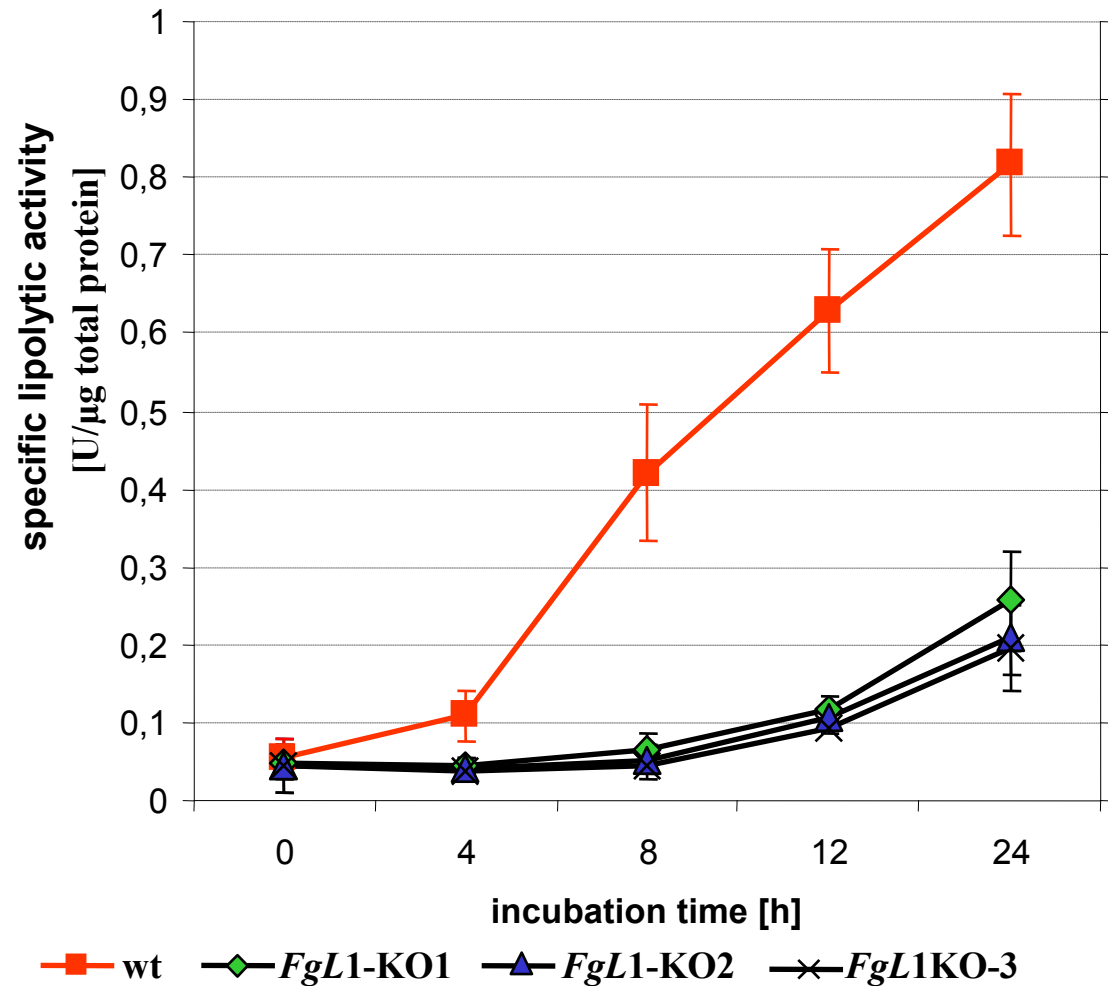
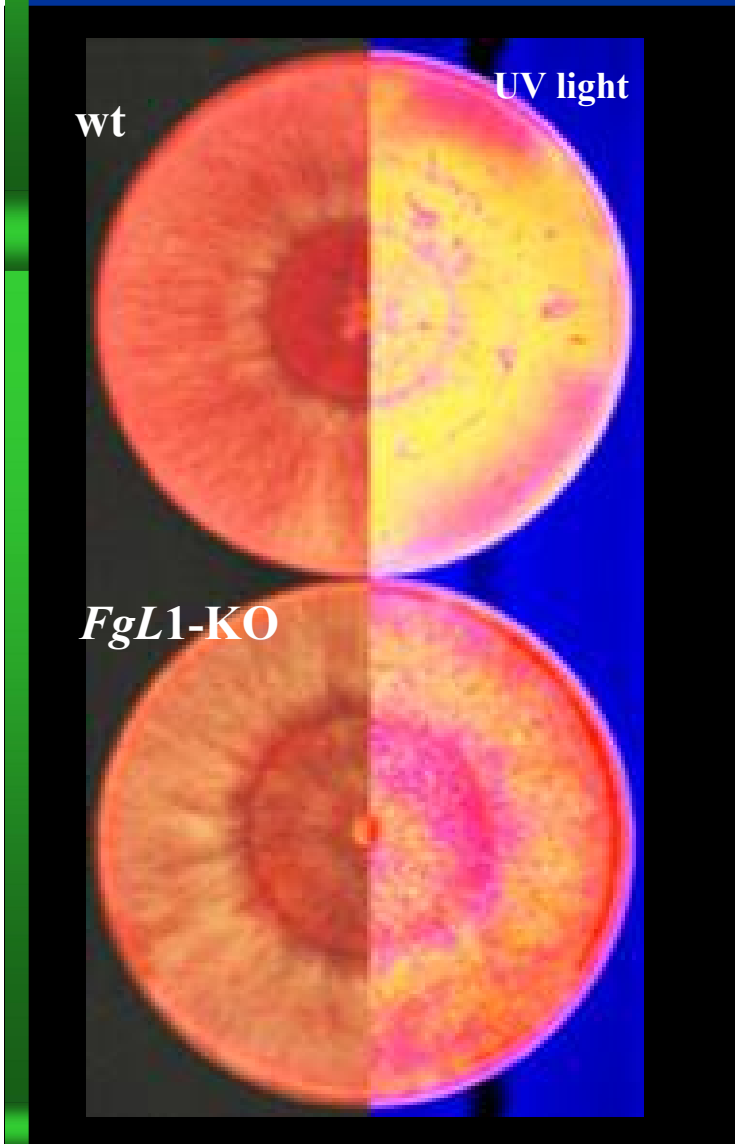
The *FgL1* gene is expressed during infection of wheat spikes





FgL1-KO mutants of *F. graminearum*

Investigation of residual extracellular lipolytic activity



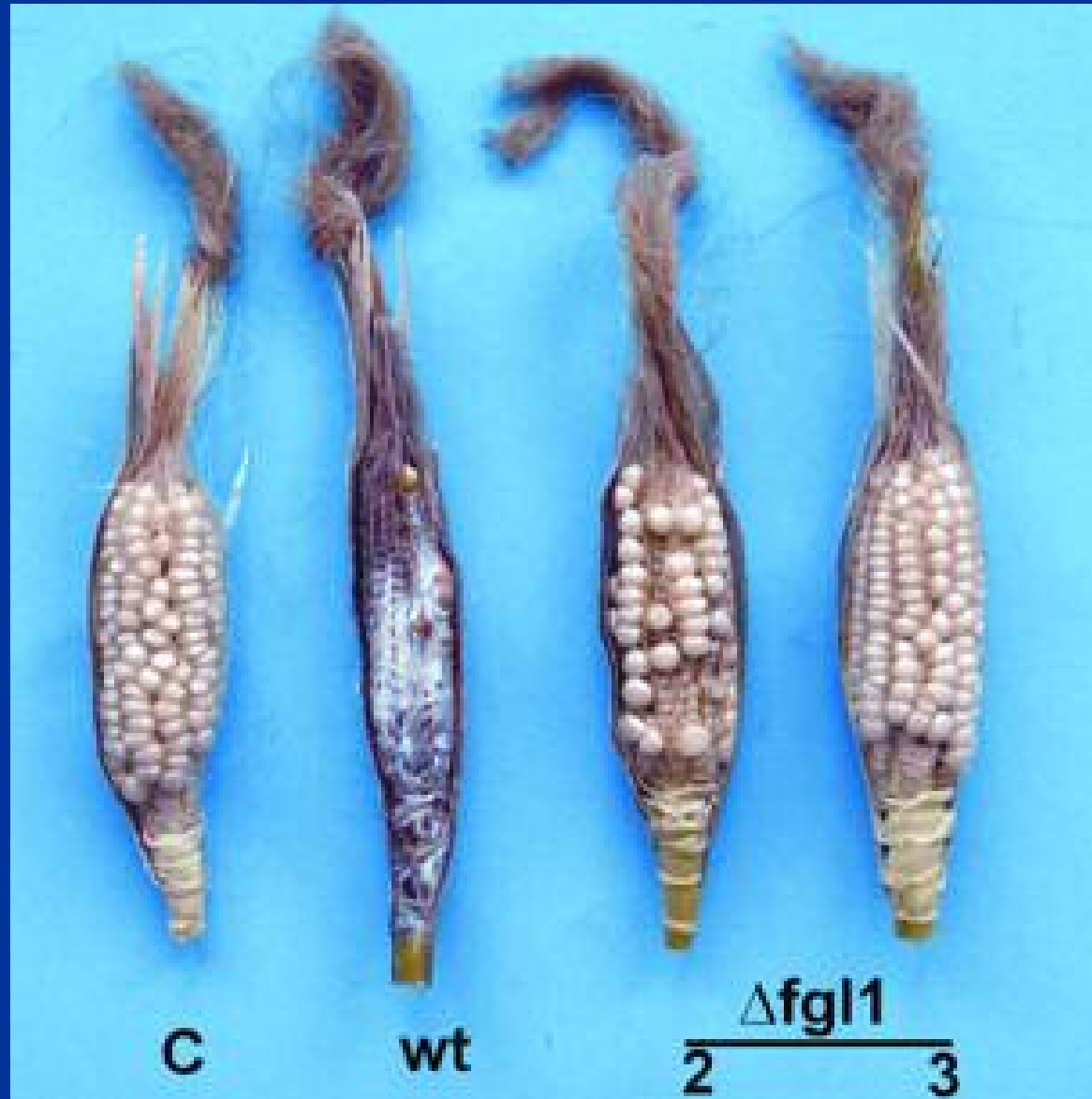


Fusarium graminearum virulence assay: wheat



(→ infected spikelet)

Virulence assay on maize (zea mays) ears

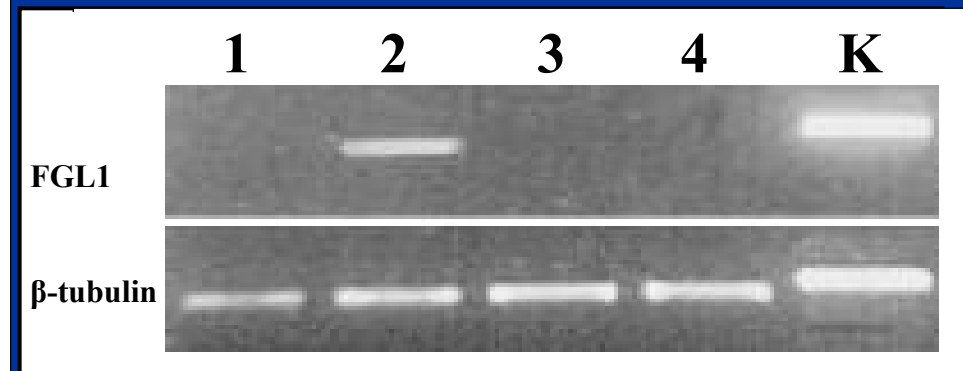
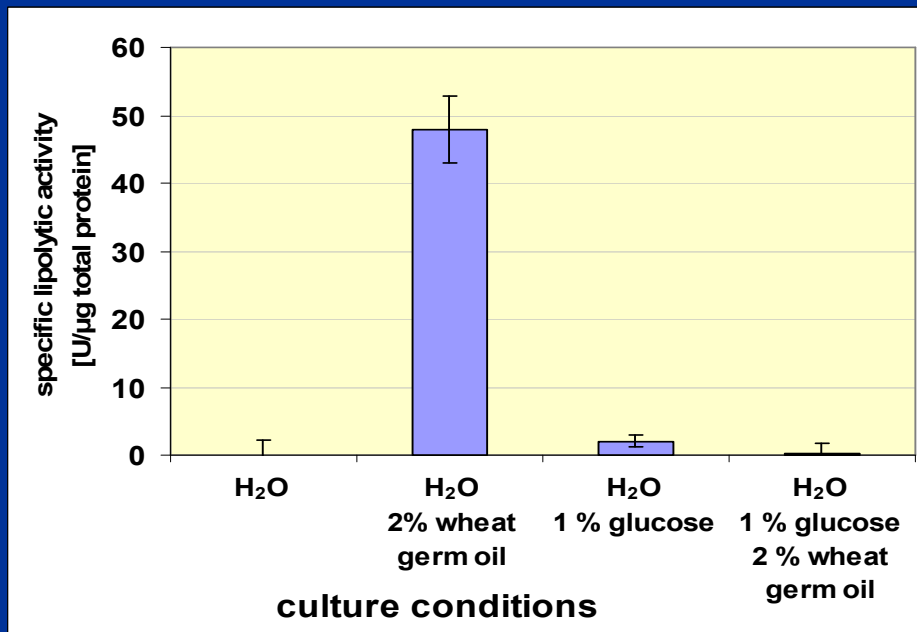


Induction/repression of lipolytic activity



The transcription of the *FgL1* gene is regulated:

- plant oil induces expression
- glucose represses the gene



Lipolytic activity of supernatants

Incubation: 4 h at 28°C;

measuring method: pNPP lipase assay

Expression analysis via RT-PCR.

1) H₂O

2) 2 % wheat germ oil

3) 1 % glucose,

4) 1 % glucose + 2 % wheat germ oil;

K) genomic *F. graminearum* DNA

Working plan: characterization of unknown FgL1 regulating genes

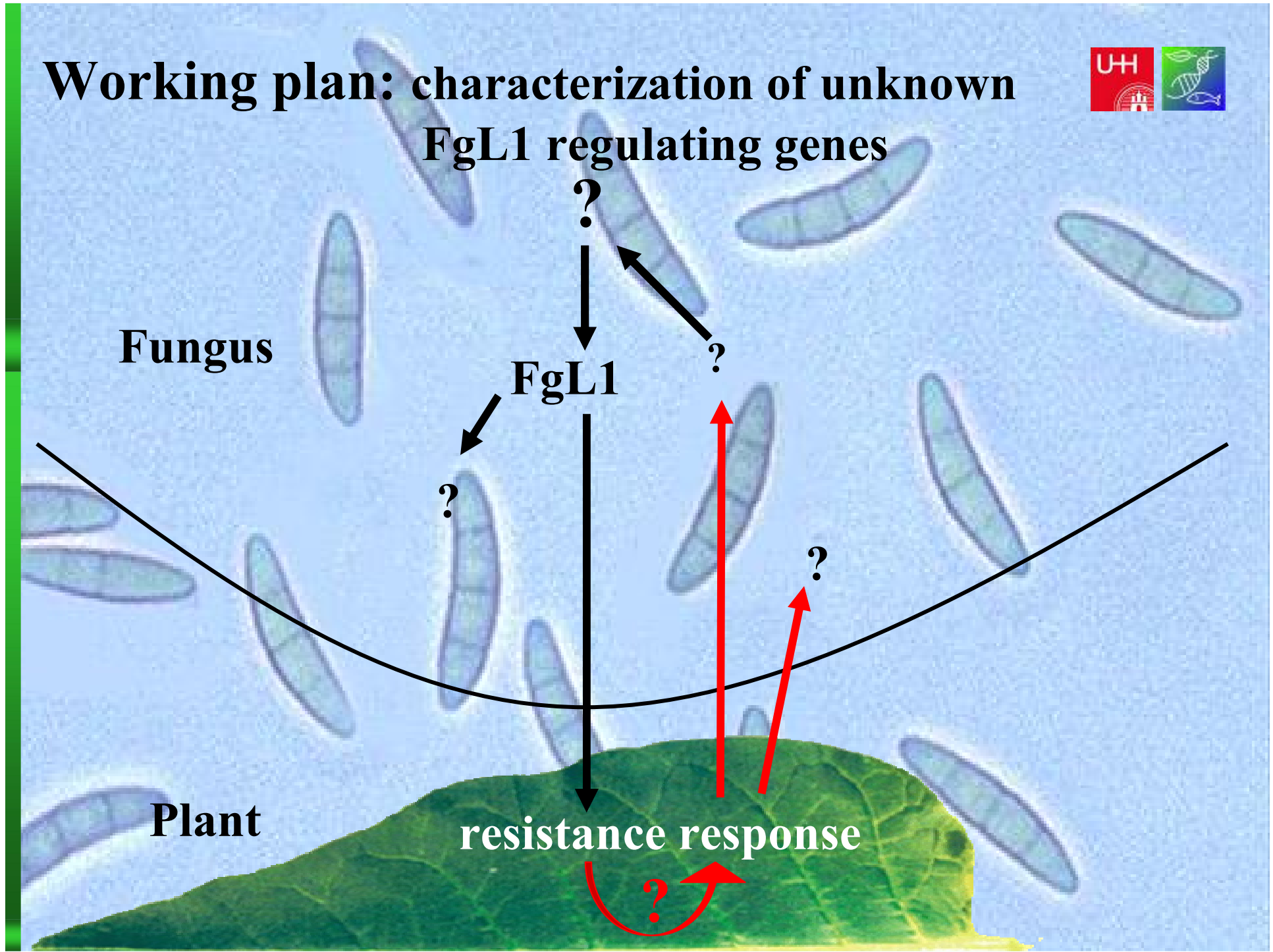


Fungus

FgL1

Plant

resistance response



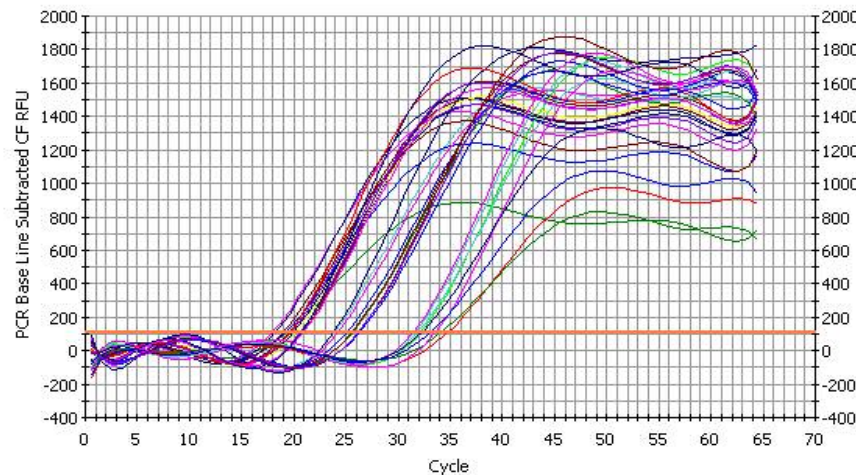
Future steps:



1. Characterization of unknown FgL1 regulating genes
2. Find candidate genes of plant defense



Real-Time PCR based expression analyses
of lipid induced candidate genes in plants and fungi



Microarray technique
- expression profiling



A *F. graminearum* Affymetrix chip will be usable until the end of this year

Summary



- **Lipases of pathogenic fungi are expressed during infection of host cells**
- **Southern blot analysis and screening of the sequence data from the *F. graminearum* genome project revealed only one secreted lipase**
- **Disruption of the *FgL1* gene leads to a strong reduction of extracellular lipolytic activity**
- **FgL1 knock out mutants are strongly reduced in virulence**

Future steps:

- **The role of more regulatory genes have to be investigated (Real-Time PCR, Microarray technique)**
- **A commercial *Fusarium graminearum* Affymetrix chip will be usable until the end of this year**



„AMP III - Lipase Group“

Wilhelm Schäfer

Siegfried Salomon

Attila Gácsér

Frank Stehr

Christian Haase

Cathrin Kröger

Nadine Borchert

Inga M. Melzer

Susanne Riecken

Sandra Gerstenbruch

Tijana Zivkovic

Frederik Holst

Nils Kruse