# *Aspergillus* and Aspergillosis

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The genus *Aspergillus* contains about 180 validly described saprophytic species. Nearly 10 percent of these species can cause a wide spectrum of infectious disease including life threatening invasive aspergillosis, colonization of the sinus and respiratory organs as well as allergic diseases. Cases of life threatening invasive aspergillosis have been steadily rising throughout the world. While prophylactic antifungal drugs have reduced the mortality due to invasive aspergillosis in immunosuppressed and immunodeficient patients, the overall case fatality rate remains well above 50% making it one of the most difficult microbial diseases to manage. *A. fumigatus* is by far the most common cause of invasive aspergillosis regardless of the underlying conditions of patients. Old concepts regarding the identification of *Aspergillus* species have strictly been based on morphological characteristics which have often been problematic due to their variability. The new classification concept employs phenotypic characteristics with multigene DNA sequences. The new method allowed differentiation of genetically distinct but morphologically similar sister species of *A. fumigatus*. The recently described *A. lentinus* is one such example which had previously been identified as *A. fumigatus*. Clinical diagnosis of invasive aspergillosis without waiting for isolation of culture has also made significant progress during the past 10 years. This lecture will focus on recent developments in the diagnosis of aspergillosis and the biological characteristics of *A. fumigatus* which renders it to be the primary cause of invasive aspergillosis.
**[Kor J Med Mycol 2007; 12(1): 31-56]**

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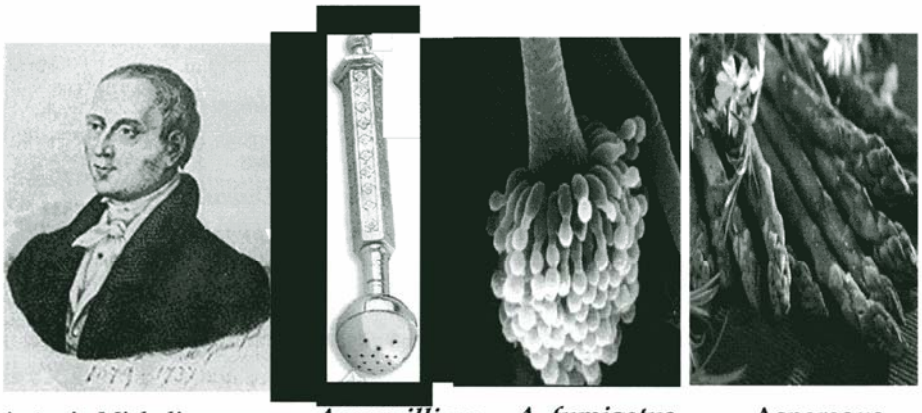
## Where does the name *Aspergillus* come from?



Antonio Micheli (1679-1736) *Aspergillium* *A. fumigatus* Asparagus

## Aspergillosis

- Etiologic agents : About 20 species of the genus *Aspergillus*
- Type of disease : Allergic response
  Colonization of air spaces
  Invasive aspergillosis

## Invasive Aspergillosis

**Portal of entry:**

**Bronchial Tree, G.I.Tract, Paranasal Sinuses, Indwelling Catheters**

**Manifestations:**

**Pneumonia, G.I. ulcerations, Brain abscess, Liver abscess, Renal lesions, Dissemination**

**Invades Blood Vessels:**

- **Mimicks Pulmonary Embolism**
- **Budd-Chiari syndrome**
- **Renal Papillary Necrosis**

## *Aspergillus* species recovered from clinical cases

***A. fumigatus*** **60%-90%** ***A. flavus*** **up to 30%**

***A. niger, A. terreus, A. nidulans***

***A. oryzae, A. restrictus, A. amstelodami***

***A. ustus, A. avenaceus, A. versicolor***

***A. sydowi, A. clavatus, A. carneus, A. caesiellus***

NCB1-Genomic Projects

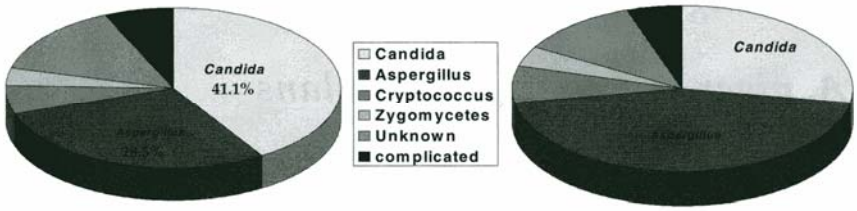
http://www.ncbi.nlm.nih.gov/genomes/leuks.cgi?p3=11:Fungi&taxgroup=11:Fungi|12:

This site contains all the microbial genomics information.

## *Aspergillus genomes*

| Species | Chrom. | Genome size | No. genes | Importance |
|---|---|---|---|---|
| *A. fumigatus* | 8 | 28 Mb | 9,926 | Pathogenic |
| *A. oryzae* | 8 | 36 Mb | 12,074 | Sake, Miso, Soy sauce |
| *A. nidulans* | 8 | 30 Mb | 9,627 | Genetic model |
| *A. terreus* | 8 (?) | 35 Mb | 10,406 | Pathogenic |

## Proportons of causative agents for severe mycosis in 1989 and 1994 in Japan

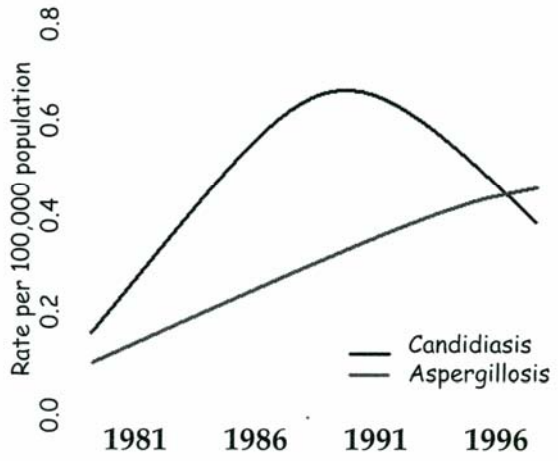



(n=863) (n= 629)

**1989** **1994**

Yamazaki et al: J. Clin. Microbiol. 37: 1732-1738

## Changing incidence of fatal invasive aspergillosis and candidiasis in non-HIV patients in USA




McNeil et al, Clin Infect Dis 2001;33:641

## Treatment cost of Aspergillosis in Europe

**500 million Euro/year**

**30,000 Euro/person**

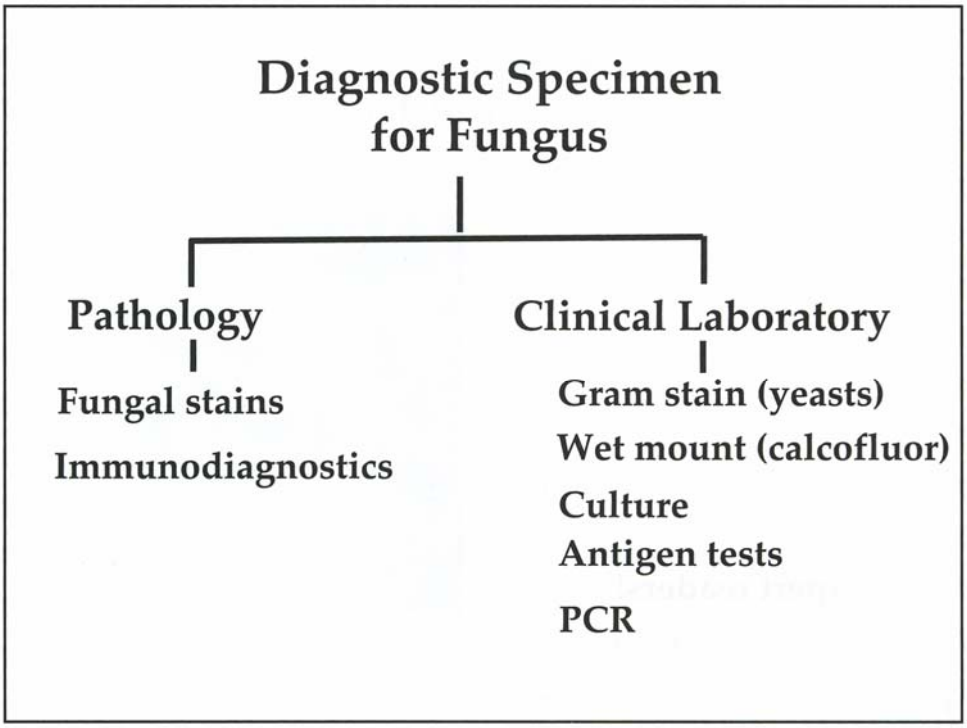
## Recent examples of the frequency of invasive aspergillosis

| Underlying condition | Incidence | Reference |
|---|---|---|
| CGD | 25 – 40% | Denning, 1998 |
| Allogeneic HSCT | 11 – 15% | Grow/Marr, 2002 |
| Lung transplantation | 6.2 – 12.8% | Minari/Singh, 2002/2003 |
| Heart-lung transplant. | 11% | Duchini, 2002 |
| Small bowel transplant. | 11% | Duchni, 2002 |
| Acute myeloid leukemia | 8% | Corn et al, 2002 |
| Acute lymphatic leukemia | 6.3 % | Corn et al, 2002 |
| AIDS | 2.9% | Libanore, 2002 |

## Early diagnosis of invasive aspergillosis is important

| Treatment start | <10 d | >11 d |
|---|---|---|
| Mortality | 40% | 90% |

Von Eiff et al, Respiration 1995;62:241-7.

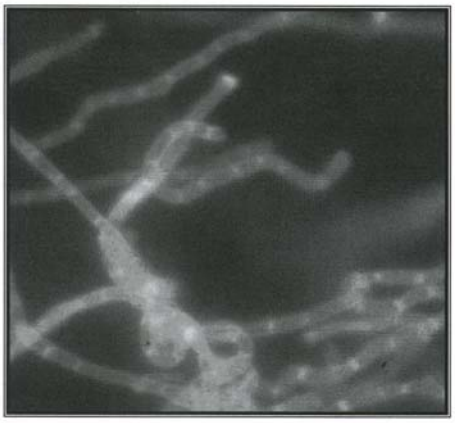



## Diagnosis of Invasive Aspergillosis

1. **Cultural identification based on morphology**
   (Culture of sputum and BAL samples may be insufficiently sensitive)
2. **Histopathological sections: Size, morphology of hyphae**
   (Biopsies may not always be feasible in patients with a severe underlying cond.)
3. **Detection of surrogate marker antigens**
   - **galactomannan (Platelia Aspergillus)**
   - **1,3-β-D-glucan (fungitell; Fungi-Tec)**
4. **PCR based identification using 18S rDNA primer sets specific for *Aspergillus***

## Microscopy in the Clinical Lab:
## The Critical Tools

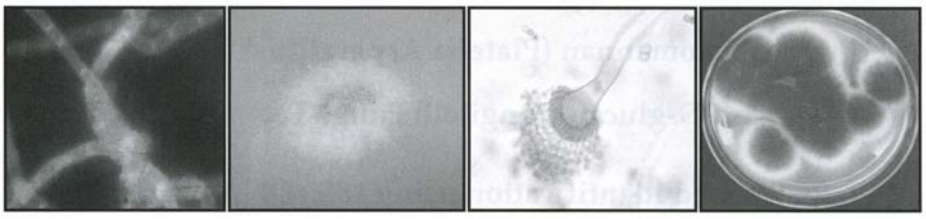
- Fluorescent dye with KOH
  - Blankophor
  - Calcofluor
  - Uvitex 2B
- Calibrated micrometer
- Expert readers!



Nancy McClenny

## Rapid Identification of *A. fumigatus* (morphology)

±1 hr → 24-48 hours at 35-37° C → 3-7 days



Nancy McClenny

## Detection of surrogate marker antigens

**Galactomannan (ELISA): Reaction with EB-A2** (rat IgM)
**Platelia *Aspergillus* (Bio-Rad)**

- **Useful only for blood, BAL, CSF, urine**
- **Best OD cut-off 0.5**
- **Sensitivity varied between 33% to 100% with high specificity for proven cases**
- **False positives higher in children/antibiotics**
- **False negatives with antifungal prophylaxis**

## Detection of surrogate marker antigens

**1,3-β-D-glucan**

**Fungitell (Associates of Cape Cod)**

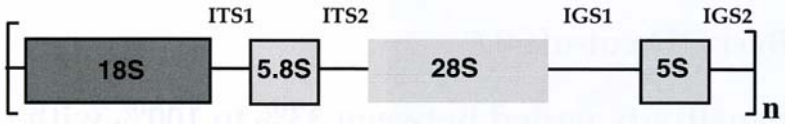
**Fungi-Tec (Seikagaku Co.)**

- **Detected by activation of factor G, a coagulation enzyme of horseshoe-crab**
- **Cut off level of 20 pg/ml to 60 pg/ml depending on the kit**
- **60- 100% sensitivity with 87 to 90 % specificity in patients with proven/probable invasive fungal disease**
- **Sensitivity higher in patents with proven candidiasis and aspergillosis not receiving antifungal Rx**
- **False-positive BG activity in hemodialysis with cellulose membrane, intravenous immunoglobulin treatments, exposure to gauze etc.**

## PCR assay for detection of invasive aspergillosis

- Amplify genus specific rDNA sequence in variable regions of 18S RNA by PCR

rRNA organization




- Whole blood or serum samples
- No interlaboratory consensus has been reached for an optimal method

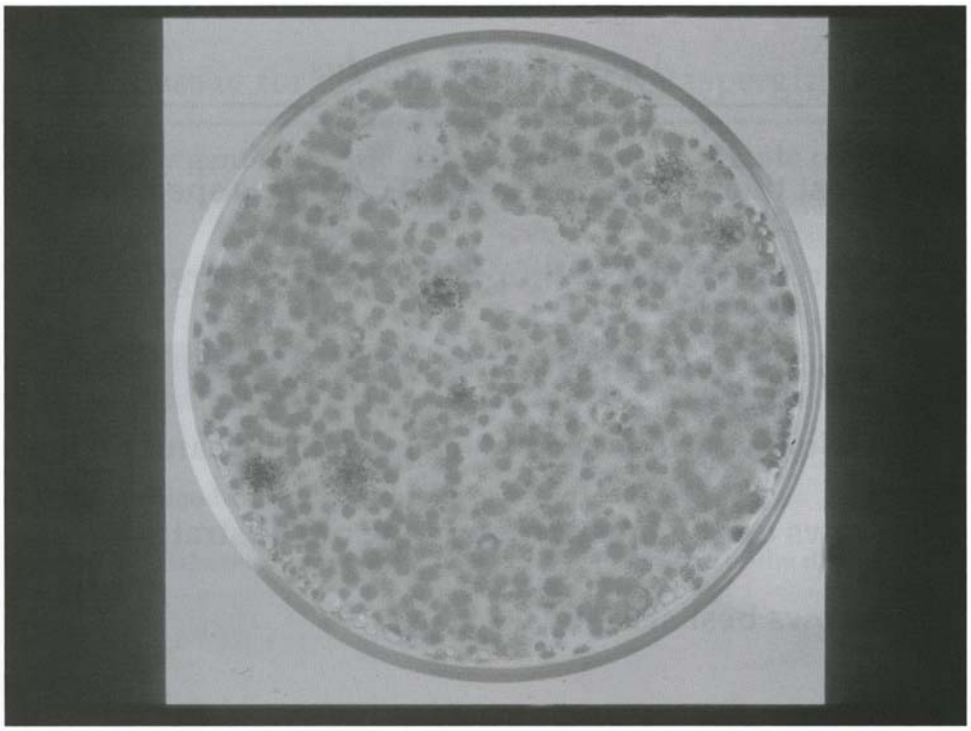
## Why is *A. fumigatus* most common among the species causing Aspergillosis ?

## *Aspergillus fumigatus*

- Most thermotolerant of all *Aspergillus* species
- Spores are most hydrophobic of all *Aspergillus* sp.
- Spore size optimal for inhalation (2-3.5 um)
- Produces various secondary metabolites and toxins
- Grows on the most broad range of substrates
- Grows best at 37°C

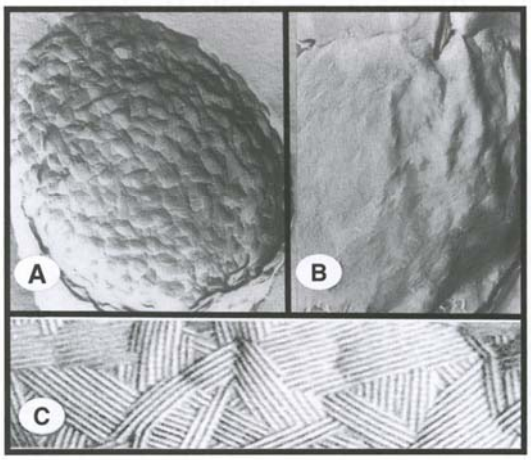
## Cloud of fungal spores

(Disturbed compost pile, Maryland)



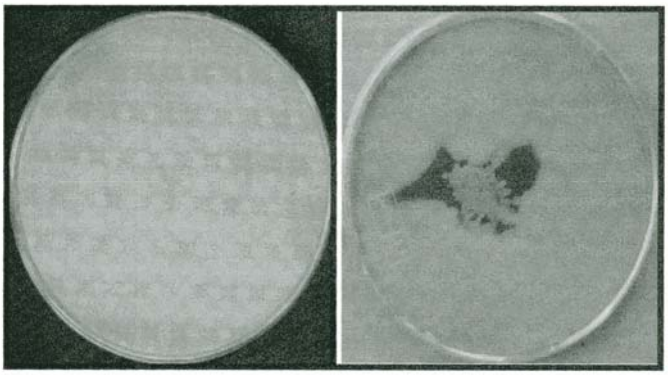
## *Aspergillus fumigatus* conidia with and without *HYP1* gene

- Hyp1=RodA encodes spore coat protein, a layer of regularly arranged rodlets



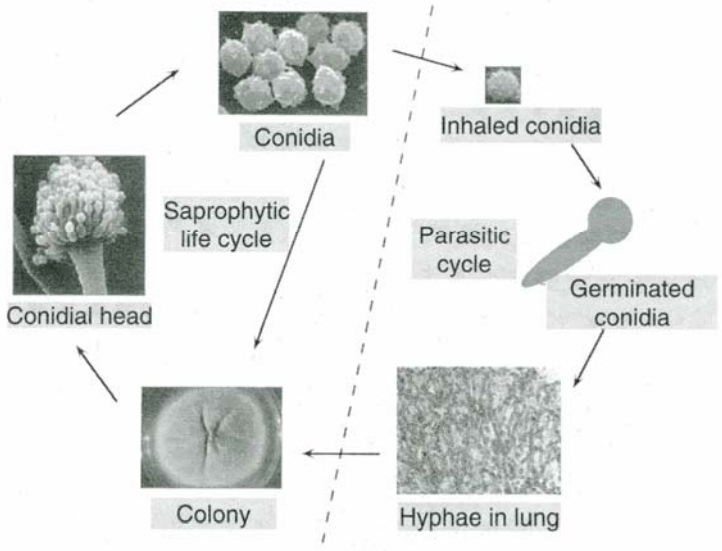

- Hyp1 protein is resistant to chemical degradation and plays a role in resistance to phagocytic defenses

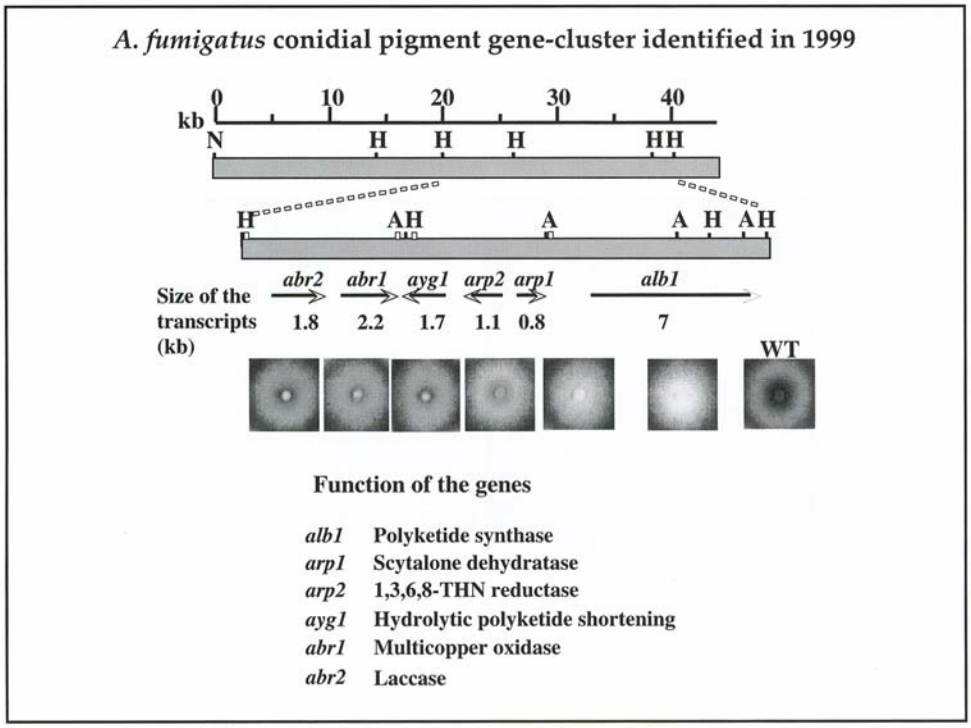
**Conidial dispersions by the wild type but not by the *hyp1* mutant**

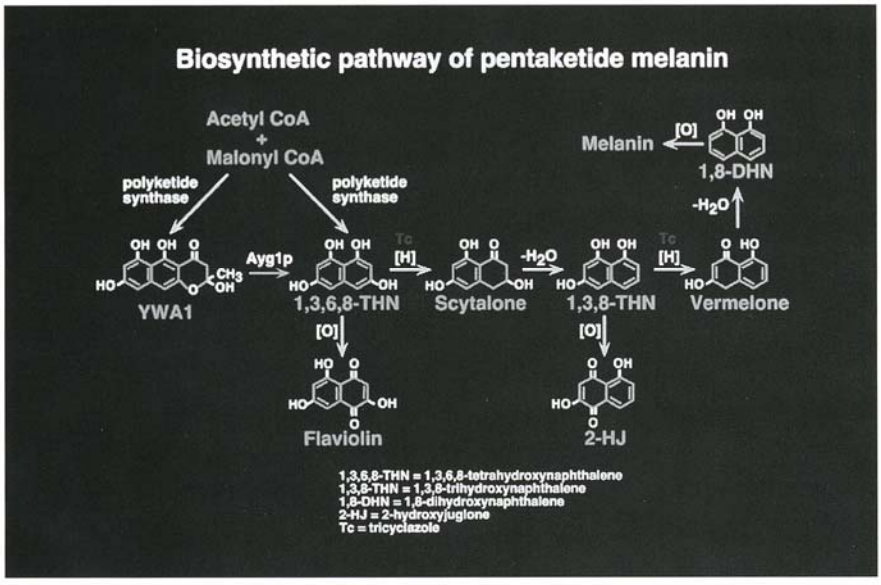


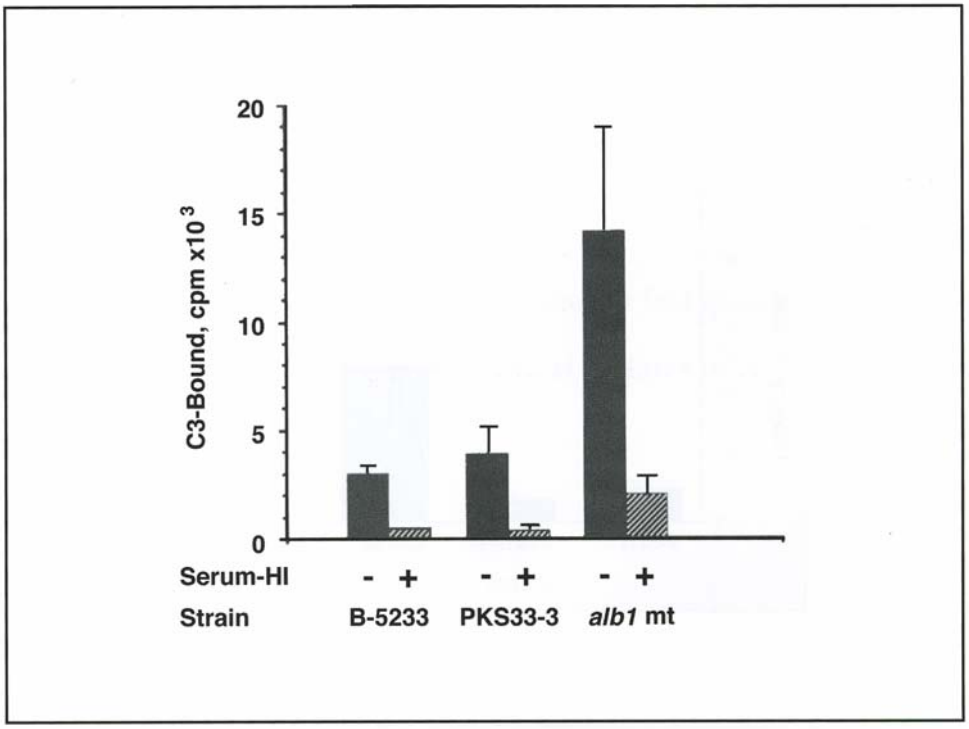
B-5233 B-5233*hyp1Δ*

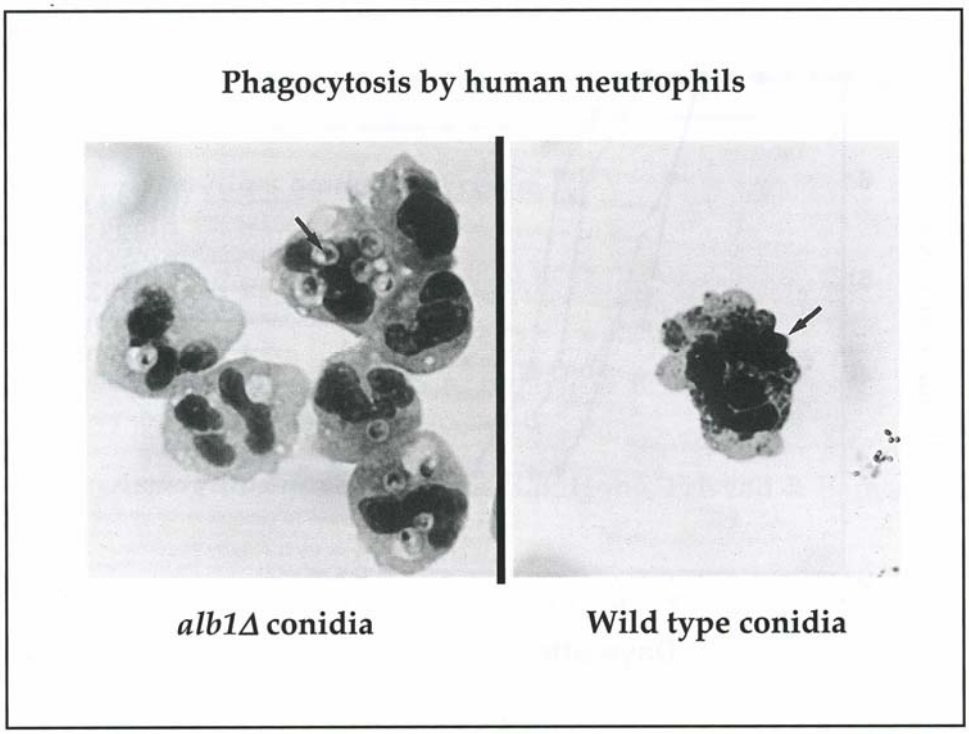
**Saprophytic and parasitic life cycles of *A. fumigatus***

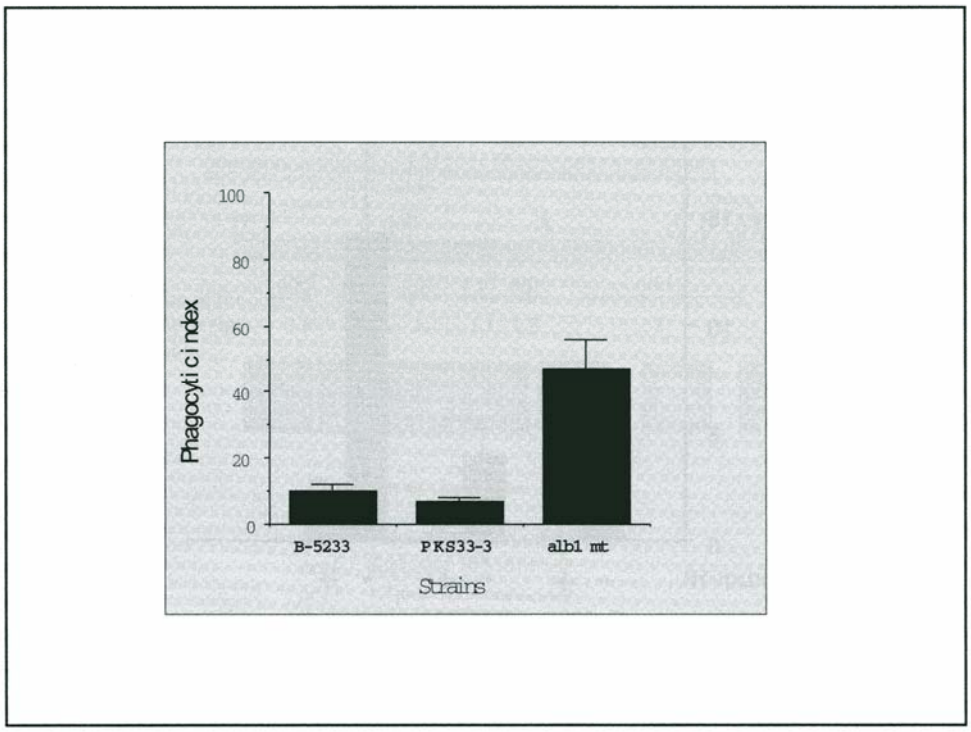


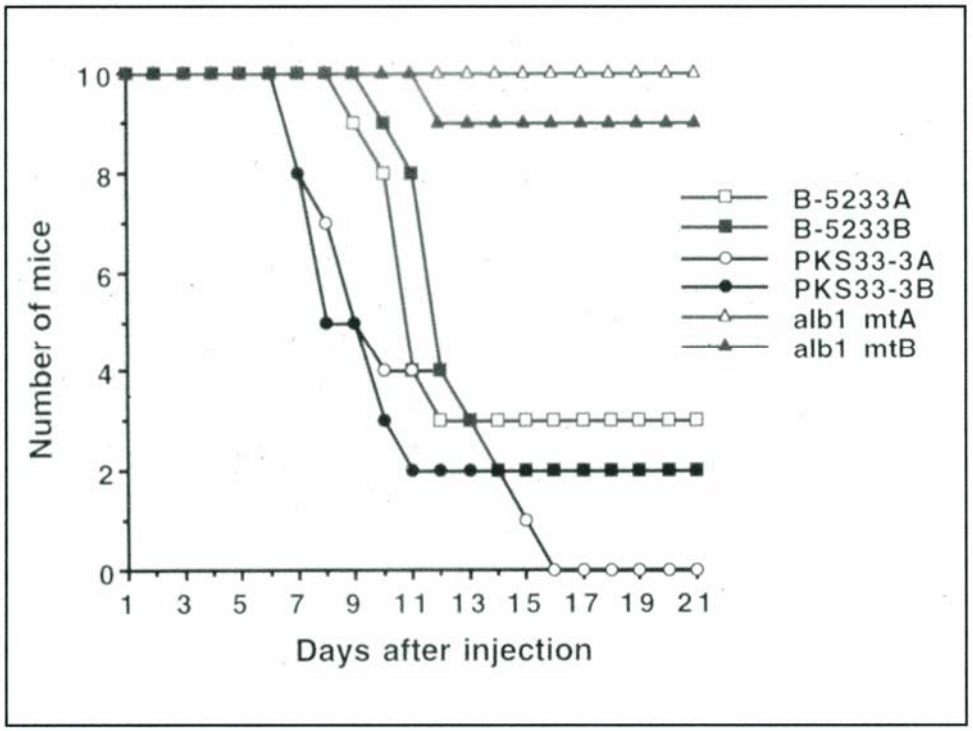

A. fumigatus conidial pigment gene-cluster identified in 1999
kb
0
10
20
30
40
N
H
H
H
HH
H
AH
A
A H AH
Size of the transcripts (kb)
abr2
abr1
ayg1
arp2
arp1
alb1
1.8
2.2
1.7
1.1
0.8
7
WT
Function of the genes
alb1 Polyketide synthase
arp1 Scytalone dehydratase
arp2 1,3,6,8-THN reductase
ayg1 Hydrolytic polyketide shortening
abr1 Multicopper oxidase
abr2 Laccase


Biosynthetic pathway of pentaketide melanin
Acetyl CoA
+
Malonyl CoA
polyketide synthase
polyketide synthase
YWA1
Ayg1p
1,3,6,8-THN
[H]
Tc
Scytalone
-H2O
1,3,8-THN
[H]
Tc
Vermelone
Melanin
[O]
1,8-DHN
-H2O
[O]
Flaviolin
[O]
2-HJ
1,3,6,8-THN = 1,3,6,8-tetrahydroxynaphthalene
1,3,8-THN = 1,3,8-trihydroxynaphthalene
1,8-DHN = 1,8-dihydroxynaphthalene
2-HJ = 2-hydroxyjuglone
Tc = tricyclazole


20
15
10
5
0
C3-Bound, cpm x10 3
Serum-HI
- + - + - +
Strain
B-5233
PKS33-3
alb1 mt


Phagocytosis by human neutrophils
alb1Δ conidia
Wild type conidia


100
80
60
40
20
0
Phagocytic index
B-5233
PKS33-3
alb1 mt
Strains


10
8
6
4
2
0
Number of mice
1 3 5 7 9 11 13 15 17 19 21
Days after injection
B-5233A
B-5233B
PKS33-3A
PKS33-3B
alb1 mtA
alb1 mtB

**Are there morphologically similar but genetically distinct strains among clinical isolates of *A. fumigatus* ?**

**Anamorphic species closely resembling *A. fumigatus***

* ***Aspergillus fumigatus*** **Fresen.**

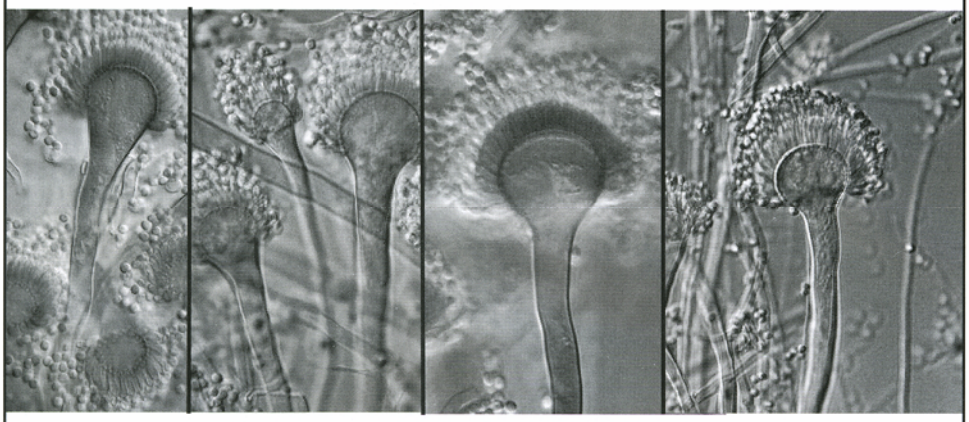
***Aspergillus fumigatiaffinis*** **S.B. Hong,**

* ***Aspergillus lentulus*** **S.A. Balajee & K.A. Marr**

***Aspergillus novofumigatus*** **S.B. Hong, Frisvad & Samson**

* Isolated from clinical cases

## Microscopical similarities of *Aspergillus fumigatus, A. lentulus, A. novofumigatus* and *A. fumigatiaffinis*

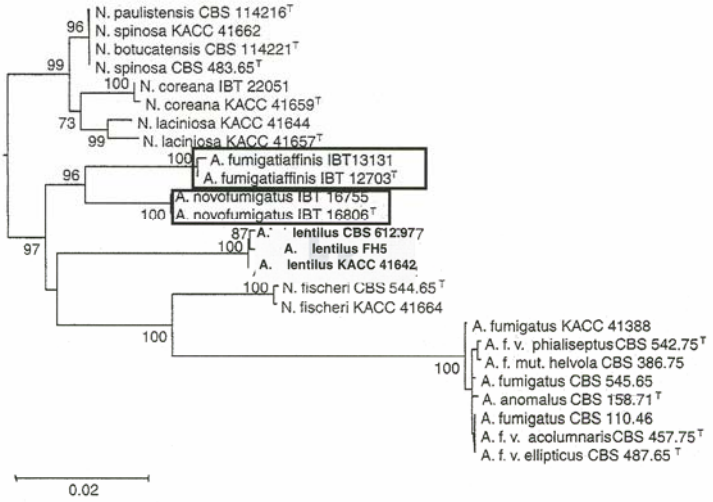


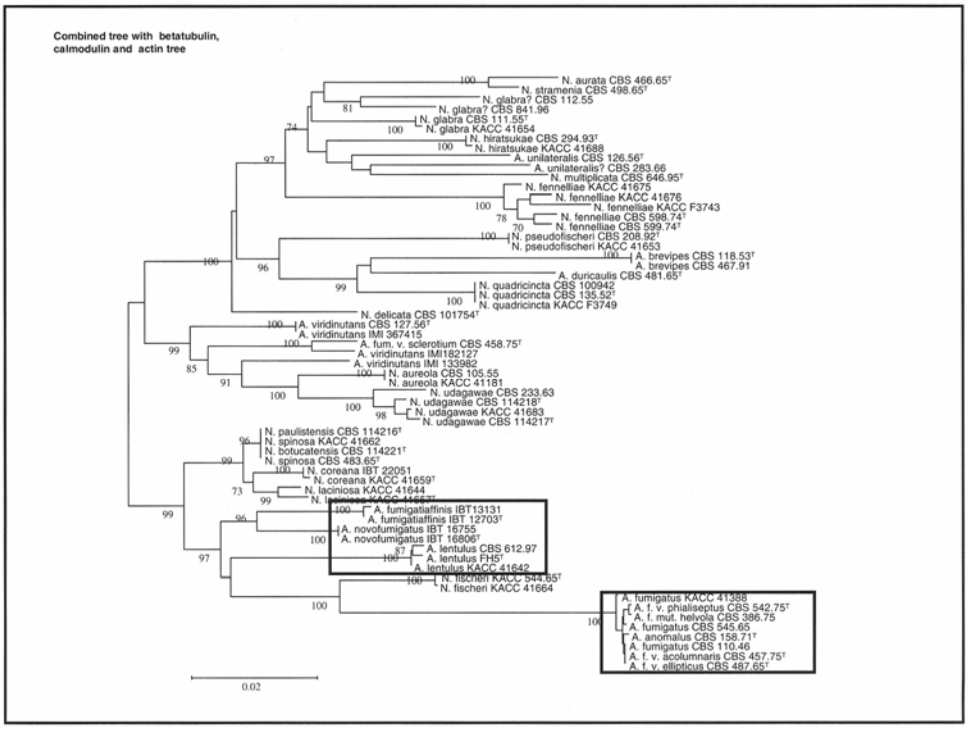
*fumigatus* *lentulus* *novofumigatus* *fumigatiaffinis*

Growth at 50° but not at 10° C

Growth not at 50° but at 10 °C

## Genetic diversity between sister species of *A. fumigatus*






**Mycotoxins of *A. fumigatus* and its closely related species**

| Toxins | *A. fumigatus* | *A. lentulus* | *A. fumigatiaffinis* | *A. novofumigatus* |
|---|---|---|---|---|
| Indolalkaloids | - | - | - | + |
| Auranthine | - | + | + | - |
| Fumagillin | + | - | - | - |
| Fumigaclavines | + | - | + | - |
| Fumigatin | +/- | - | - | - |
| Fumitremorgins | + | - | - | - |
| Gliotoxin | + | - | - | - |
| Helvolic acid | + | - | + | + |

## Fungal secondary metabolites

**Non-ribosomal peptide synthase (NRPS)**

b-lactam antibiotics

Cyclosporin- immunosuppressant

Echinocandin- antifungal drug

Gliotoxin- toxin

**Polyketides (PKS)**

Lovastatin- cholesterol lowering agent

Aflatoxin- carcinogen

**Indole alkaloids**

Ergotamine- migraine treatment

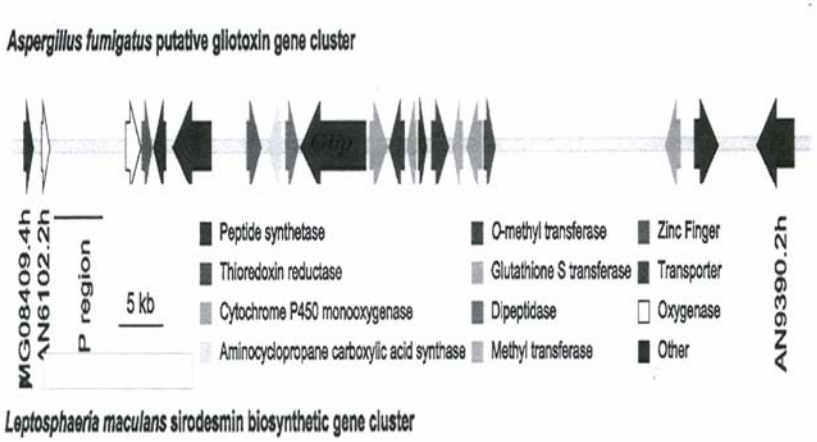
- Control of post partum bleeding

**Terpenes**

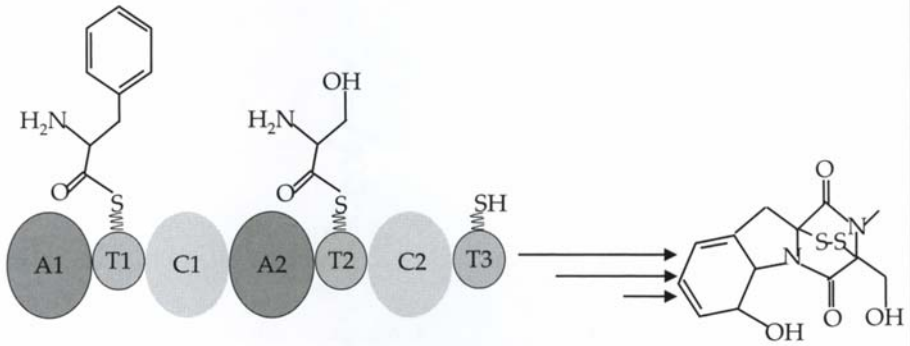
Trichodiene- toxin

Aristolochene- toxin

## Gliotoxin gene cluster found in *Aspergillus fumigatus* genomic data base



**GliP, a Multimodular Nonribosomal Peptide Synthetase in *Aspergillus fumigatus*, Makes the Diketopiperazine Scaffold of Gliotoxin**

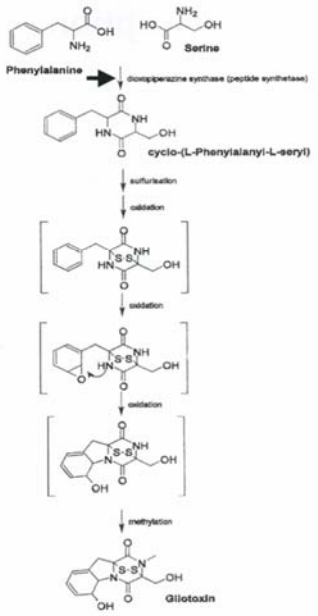


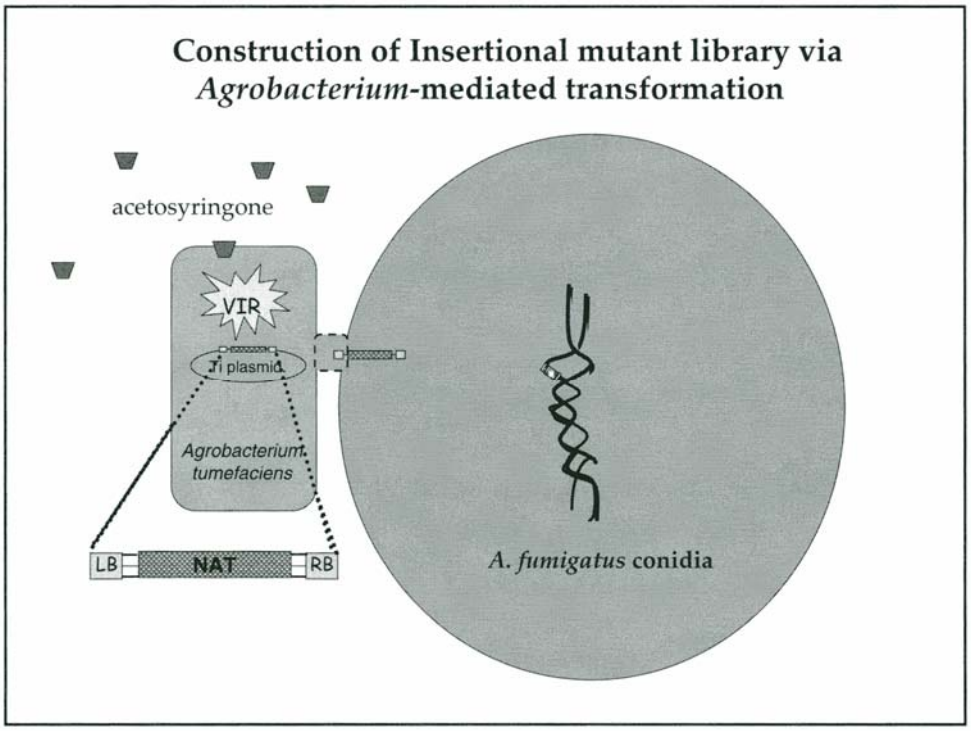

A : Adenylation domain

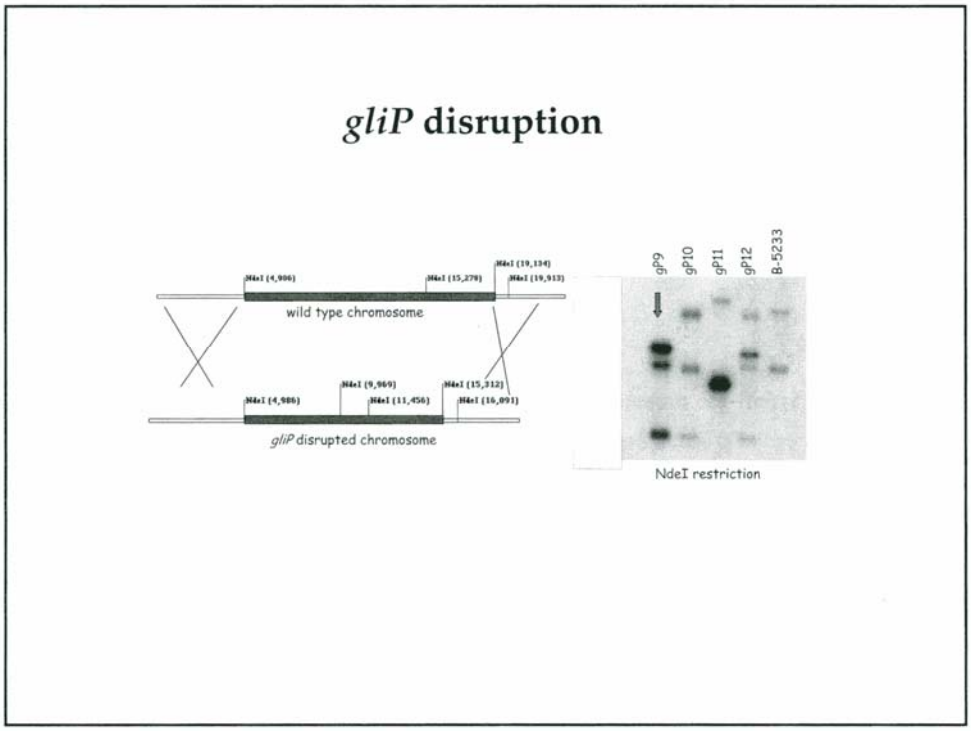
T : Peptidyl carrier

C : Condensation domain

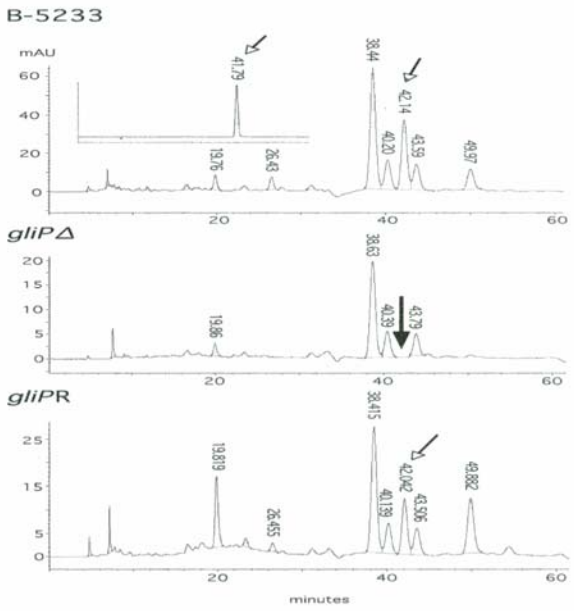
## Putative biosynthetic pathway of gliotoxin



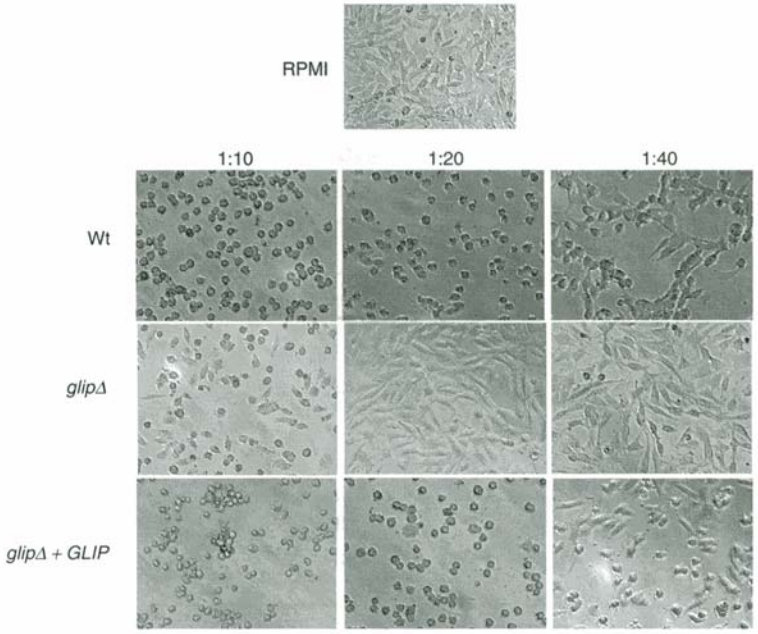

Construction of Insertional mutant library via
Agrobacterium-mediated transformation
acetosyringone
VIR
Ti plasmid
Agrobacterium
tumefaciens
LB
NAT
RB
A. fumigatus conidia


gliP disruption
wild type chromosome
gliP disrupted chromosome
gP9
gP10
gP11
gP12
B-5233
NdeI restriction

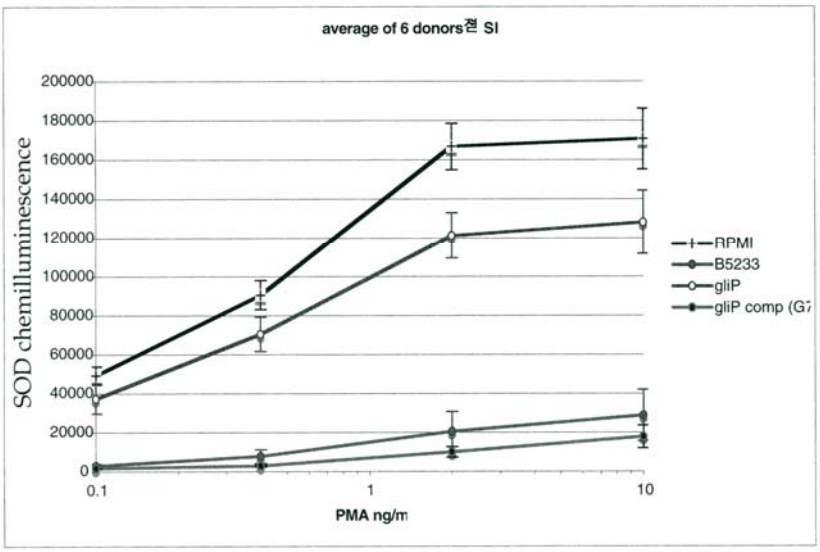
**HPLC analysis of gliotoxin in the culture filtrate of the *glipΔ* strain**



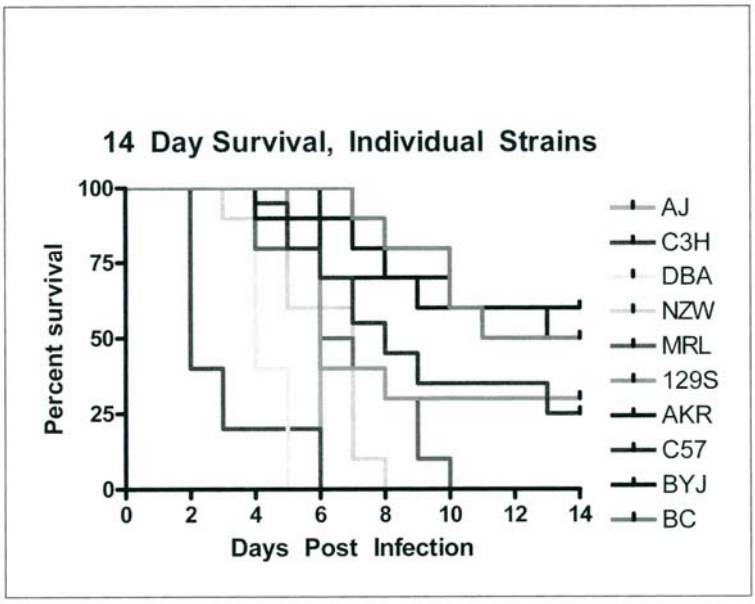

**Effect of *A. fumigatus* culture supernatant on MEF cells**



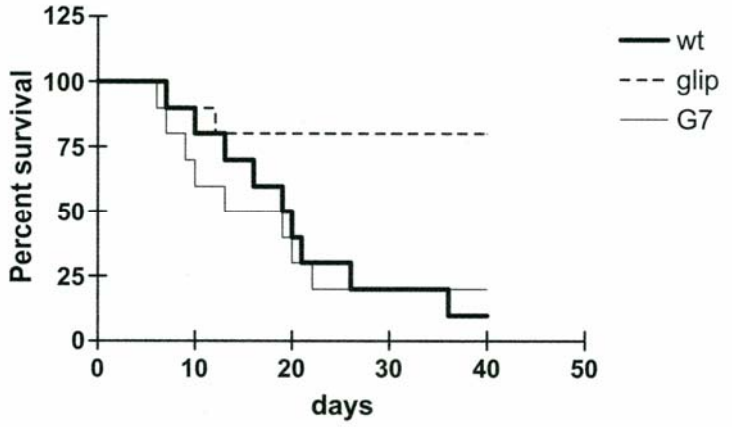
## Effect of gliotoxin on the oxidative burst of human neutrophils




## Host genetic background and susceptibility to aspergillosis

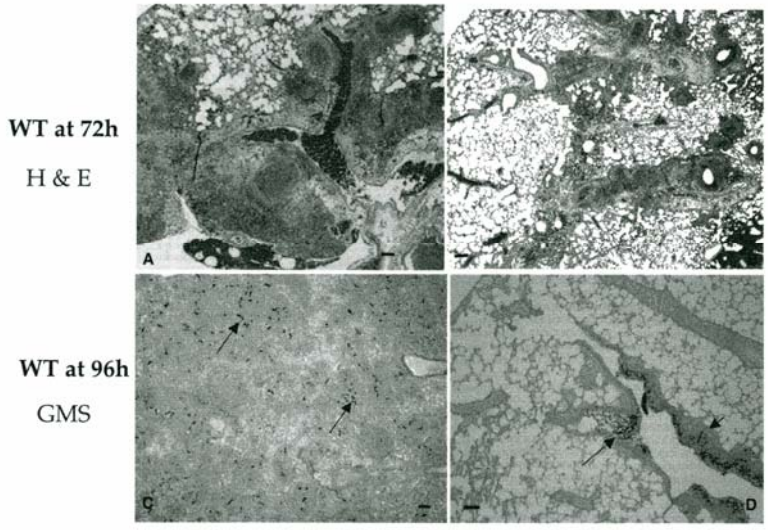


**Effect of gliotoxin on the virulence of *A. fumigatus* in 129/Sv mice**




P= 0,005, wt vs glip

**Histopathology of mouse lungs**



## Conclusions

- Invasive aspergillosis is on the rise world-wide
- Early diagnosis is pivotal in saving lives of IA patients
- *A. fumigatus* is the most common cause of IA
- Routine laboratory diagnosis may misidentify the species morphologically similar to *A. fumigatus*
- Surrogate antigen markers may assist in monitoring
- Secondary metabolites such as gliotoxin are important virulence determinants of *A. fumigatus*


## Acknowledgements

| *MMS/LCID* | *Clin. Pathol. Unit/LHD/NIAID* | *Max-Planck-Institut für Immunbiologie* |
|---|---|---|
| **Janyce Sugui** | **John Gallin** | **Markus Simmon** |
| **Yun C. Chang** | **Kol Zarember** | **Julian Pardo** |
| **Huei-Fung Tsai** | | |