มหาวิทยาลัยมหิดล ปัญญาของแผ่นดิน
Mahidol University Wisdom of the Land

Phenotypic analysis of *Aspergillus fumigatus* strain lacking the NAD-dependent formate dehydrogenase encoding gene *fdh*

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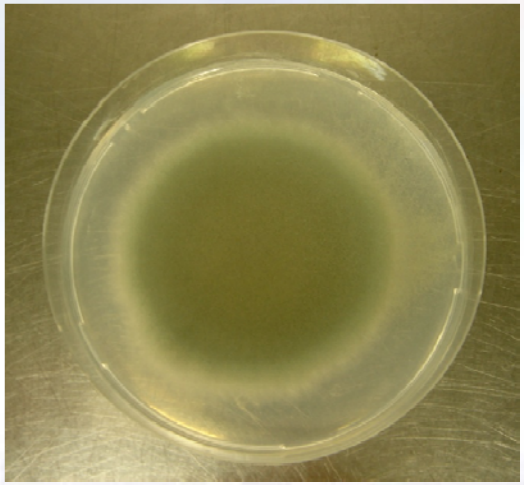
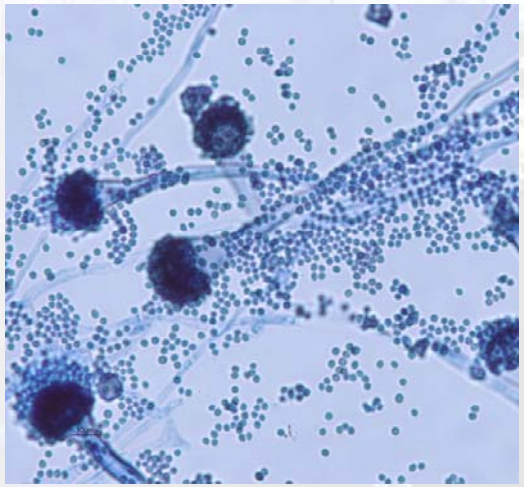
Department of Microbiology
Faculty of Medicine Siriraj Hoshital

12th December 2013

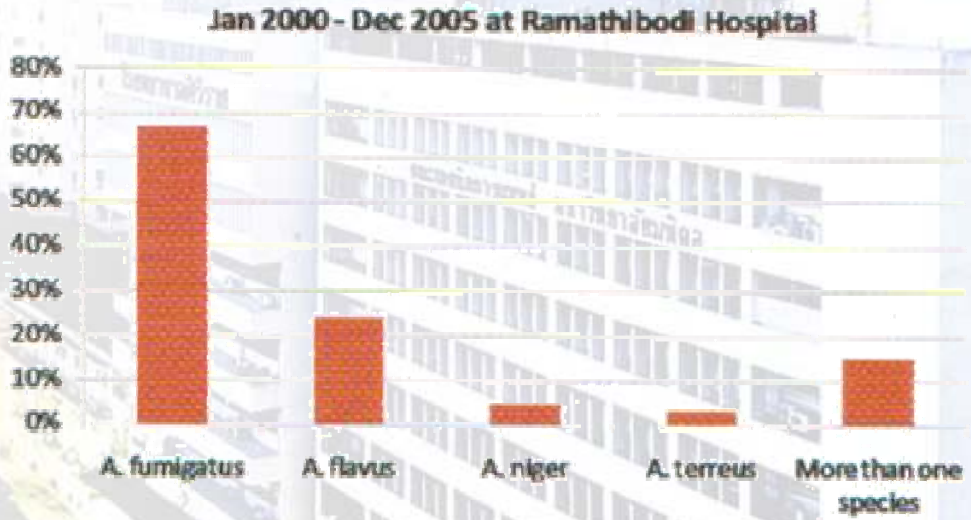
Aspergillus fumigatus



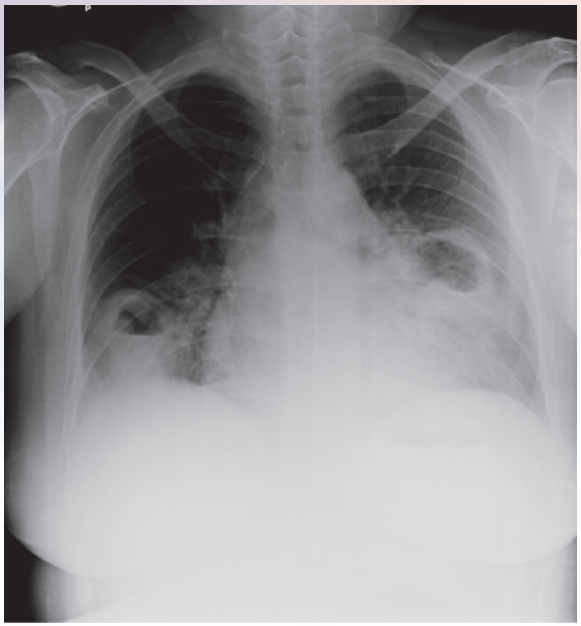


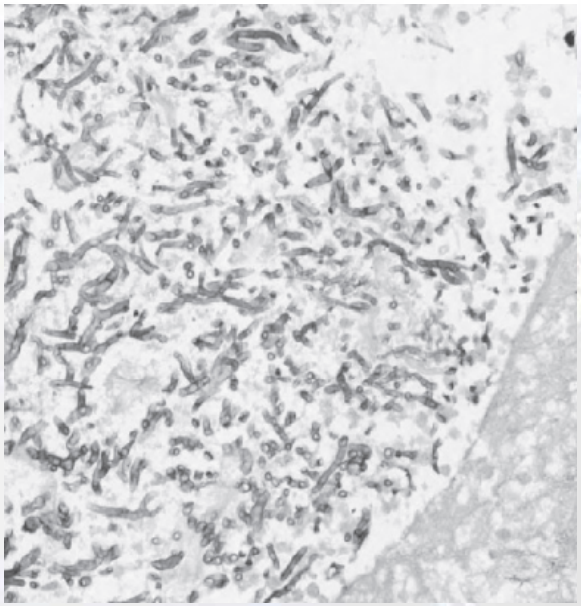
- Phylum Ascomycota
- The most common pathogenic *Aspergillus* species

Epidemiology in Thailand



Kiertiburanakul S et al. *J Med Assoc Thai* 2007;90(5):895-902





Rittayamai N, et al. Thai journal of tuberculosis, chest disease and critical care. 2009;30(3):166-71.

- Common cause of invasive pulmonary aspergillosis in immunosuppressed patients
- Mortality rate of 50% to 95%

Steinbach WJ, et al. Journal of Infection. 2012;65(5):453-64.

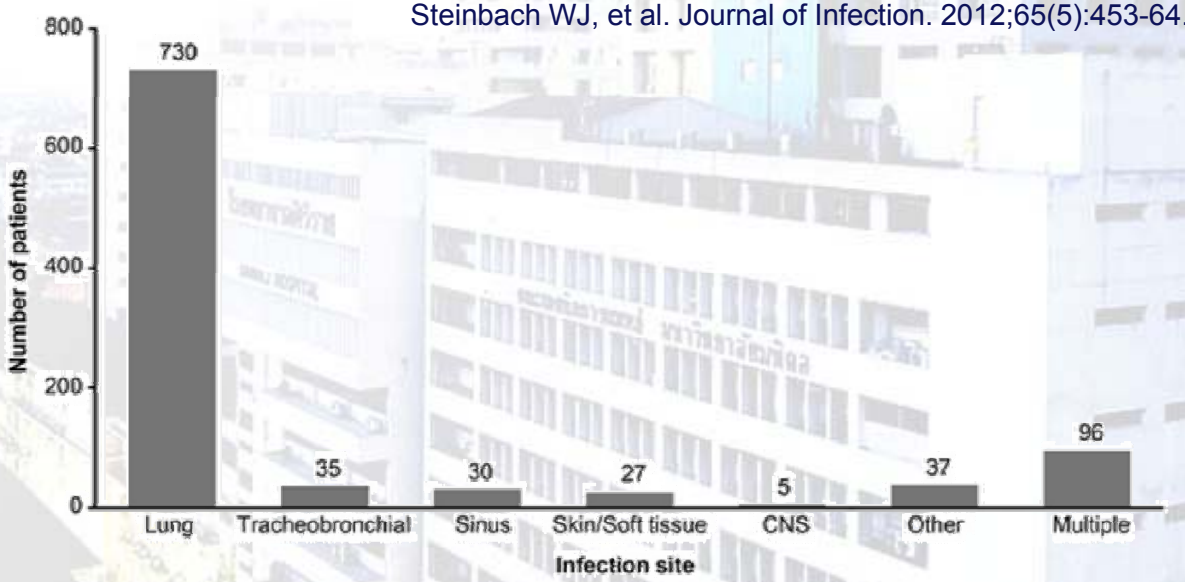
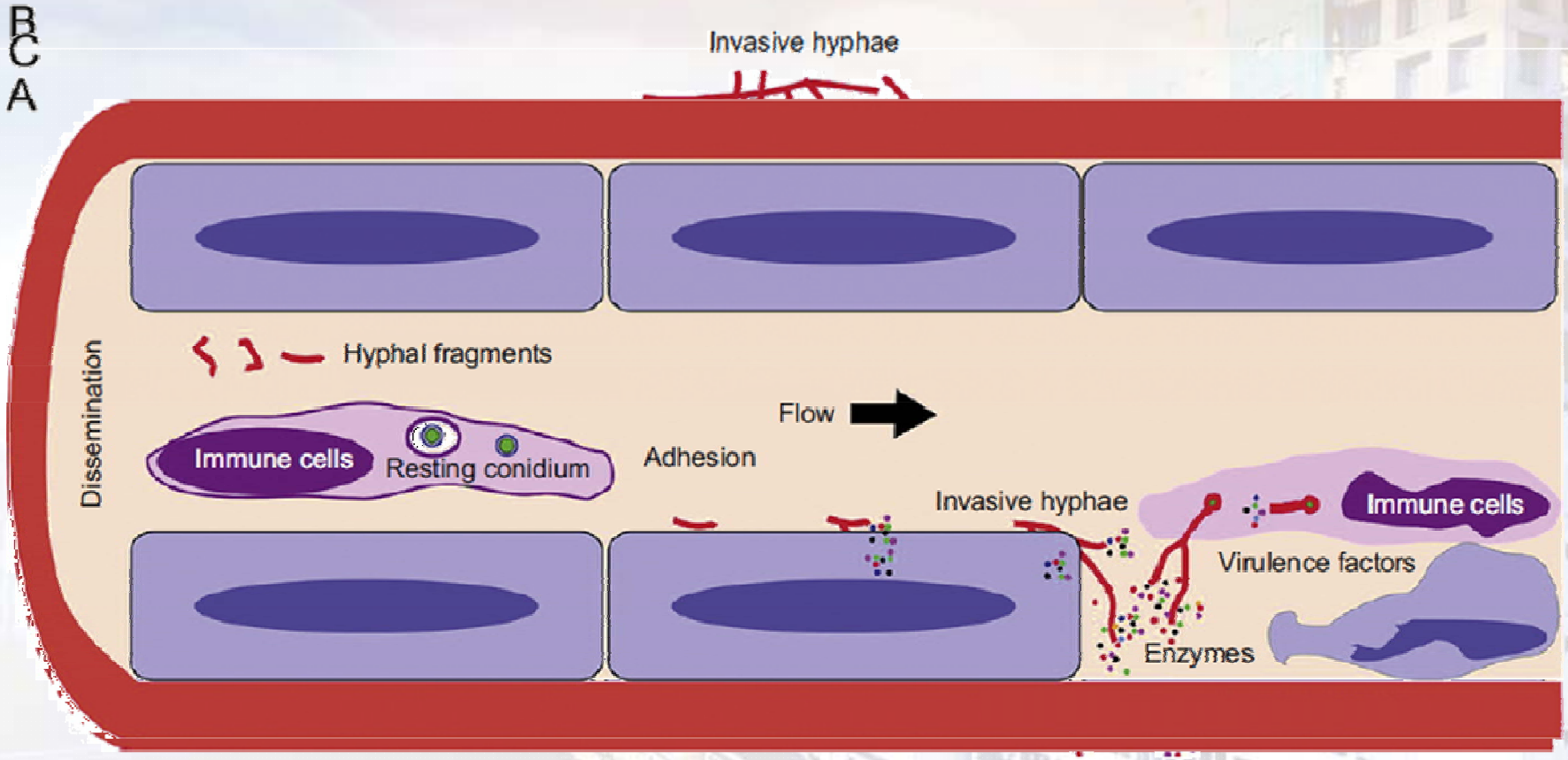


Figure 1 Invasive aspergillosis patients: breakdown by infection site.

Invasive aspergillosis development



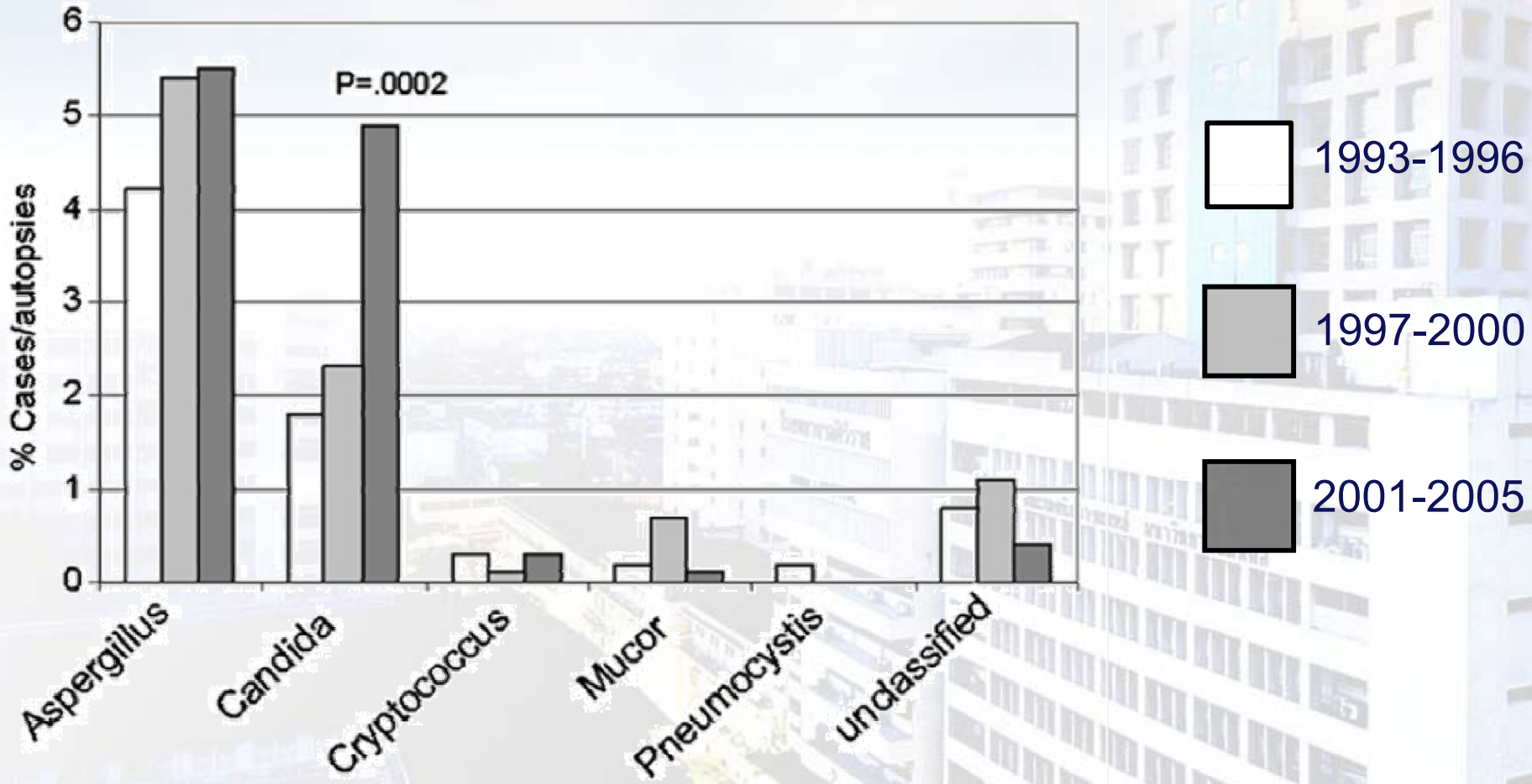
Invasion of blood vessels and dissemination of hyphal fragments

Adhesion of conidia to the damaged epithelium

Germination of conidia

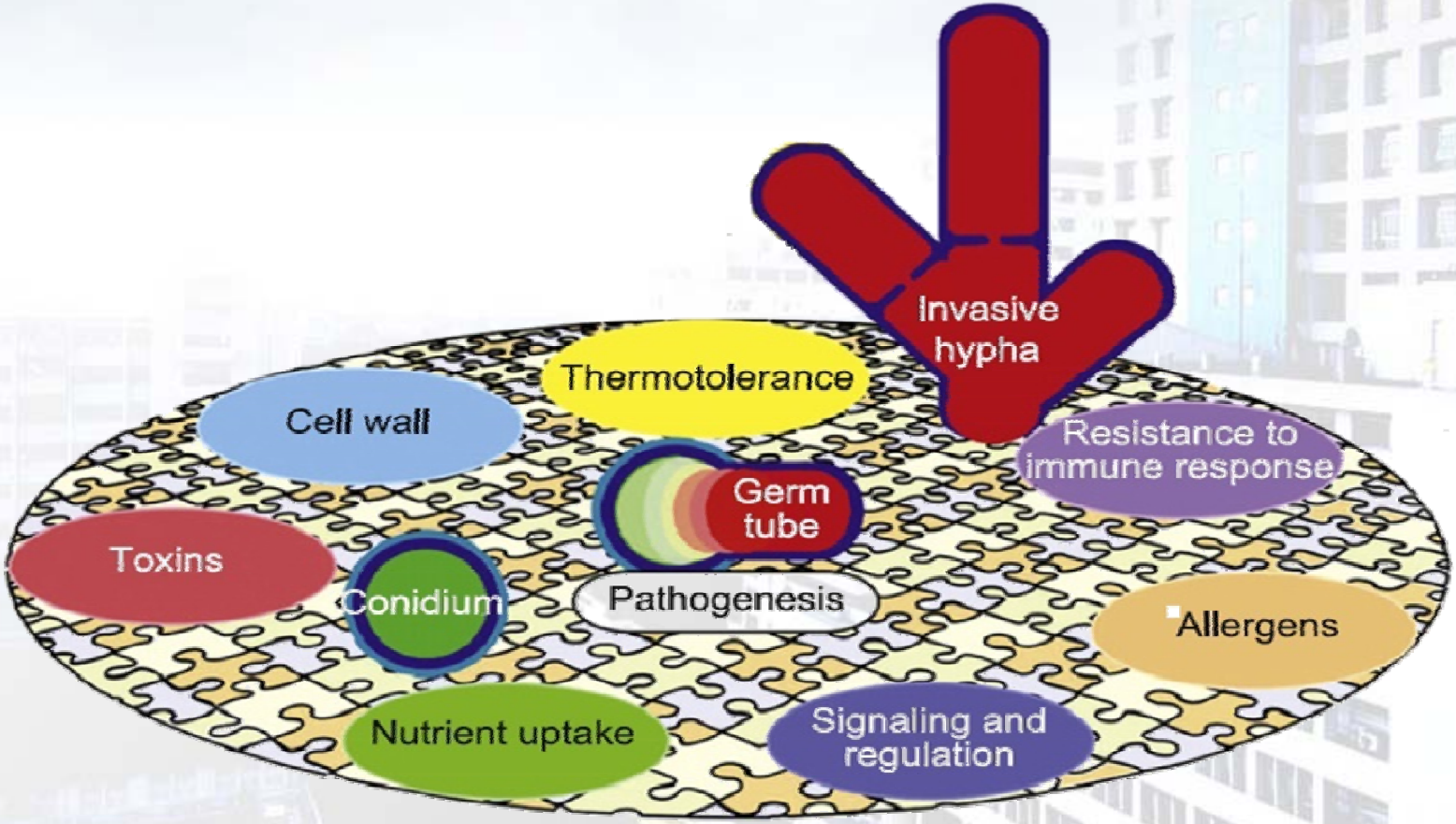
Abad A, et al. Revista Iberoamericana de Micología. 2010;27(4):155-82.

Prevalence of fungal pathogens



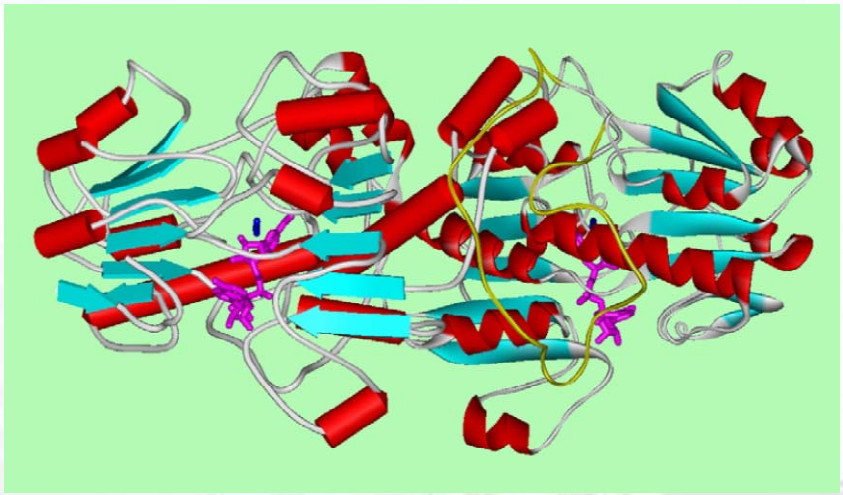
Lehrnbecher T, et al. Journal of Infection. 2010;61:259-65.

Factors that cause *A. fumigatus* to be a successful pathogen



Abad A, et al. Revista Iberoamericana de Micología. 2010;27(4):155-82.

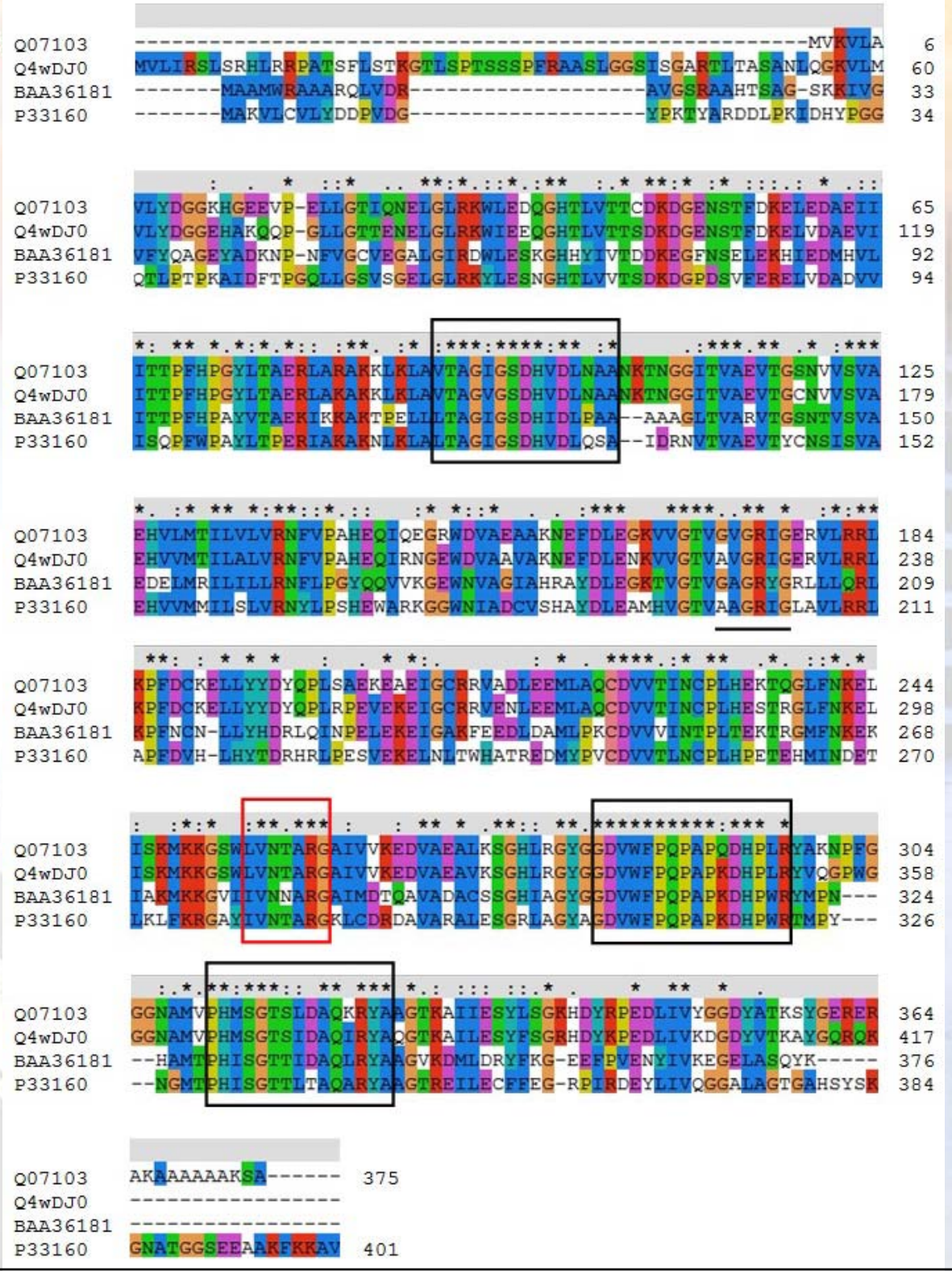
NAD^+-dependent formate dehydrogenase (NAD^+-dependent FDH)



- Widespread enzyme in mammals, plants, bacteria, yeasts, and fungi
- Plays a crucial role in respiratory chain and oxidative phosphorylation

$$HCOO^- + NAD^+ \longrightarrow CO_2 + NADH$$

Popov VO, Tishkov VI. Protein Structure: Kaleidoscope of Structural Properties and Functions. 2003:441-3.



Q07103 = *Neurospora crassa*

Q4wDJ0 = *Aspergillus fumigatus*

BAA36181 = *Hordeum vulgare* (Barley)

P33160 = *Pseudomonas* sp. 101

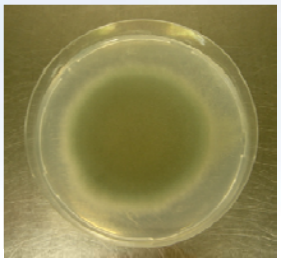
Previous studies of NAD^+-dependent FDH

- In barley, *fdh* was expressed under anaerobic conditions and iron deficiency. (Suzuki K, et al. Plant Physiol. 1998;116:725-32.)
- In potato, FDH transcripts is strongly increased under various stresses including hypoxia, chilling, drought, dark and wounding. (Hourton- Cabassa C, et al. Plant Physiol. 1998;116:627-35.)
- In *Neurospora crassa*, *fdh* mRNA is found only during conidiation and early germination and is not detectable during mycelial growth. (Chow CM, et al. J Bacteriol. 1993;175(12):3703-9.)
- In *A. fumigatus*, the expression of *fdh* also increased significantly 48h after biofilm production. (Bruns S. et al. Proteomics. 2010;10(17):3097-107.)

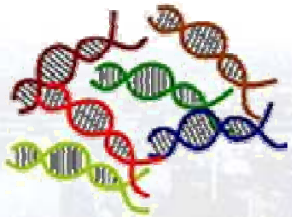
Objective

To clarify the physiological role of *fdh* in *Aspergillus fumigatus*

Generation of Δ*fdh* strain



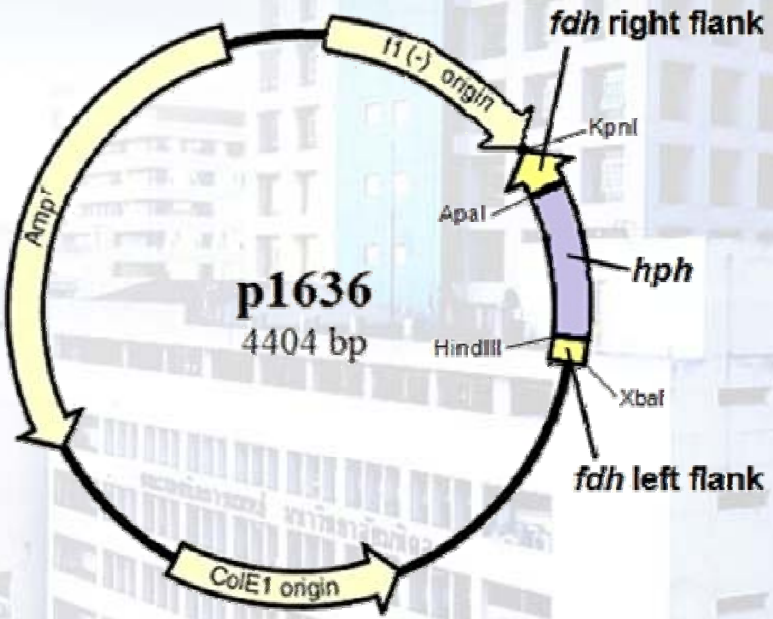
A. fumigatus WT
strain AF293

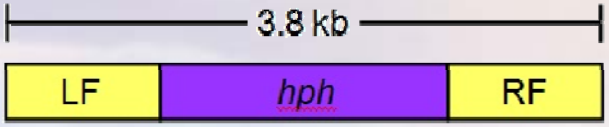


Extraction

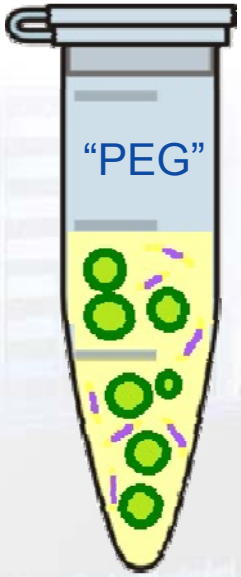
PCR

***fdh* gene (AFUA_6G04920)**

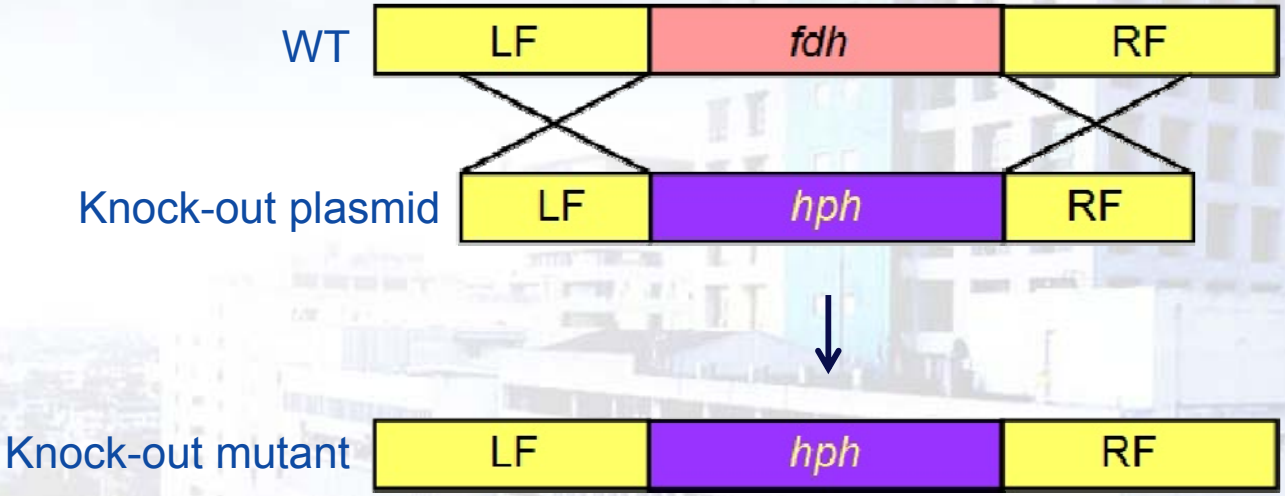




Final product

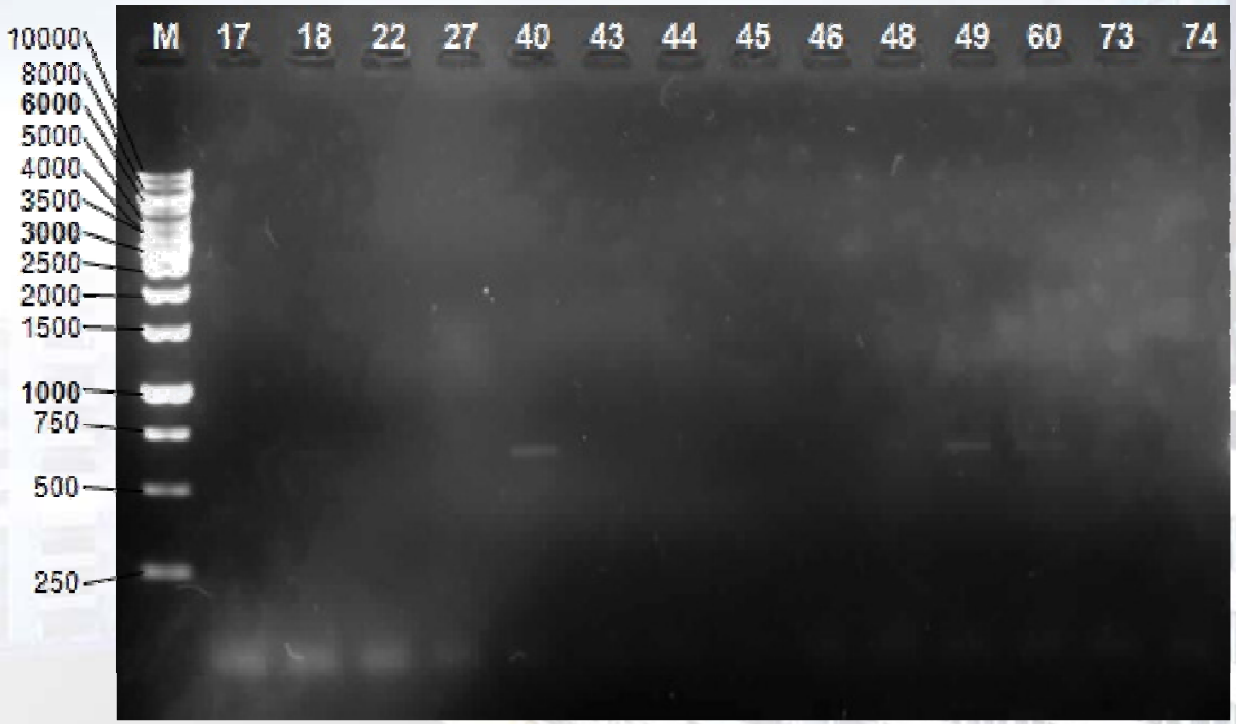


Protoplast transformation



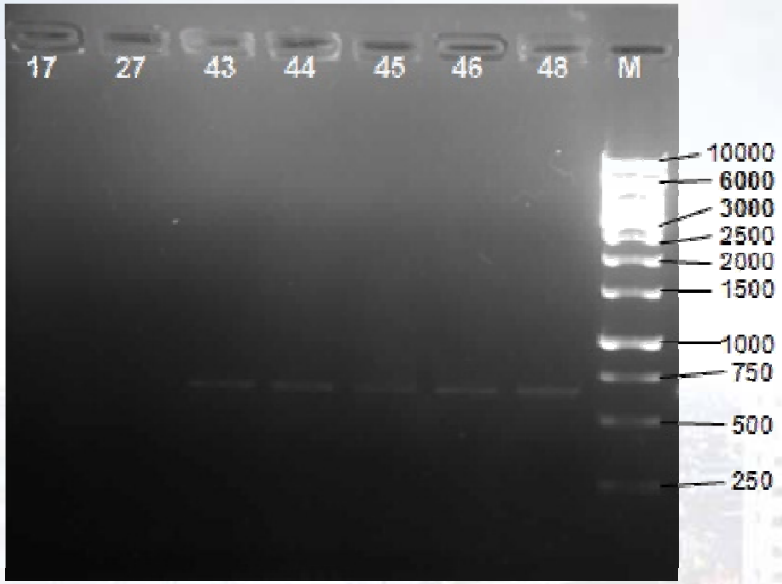
Homologous recombination

Screening and confirmation of Δ*fdh* strain

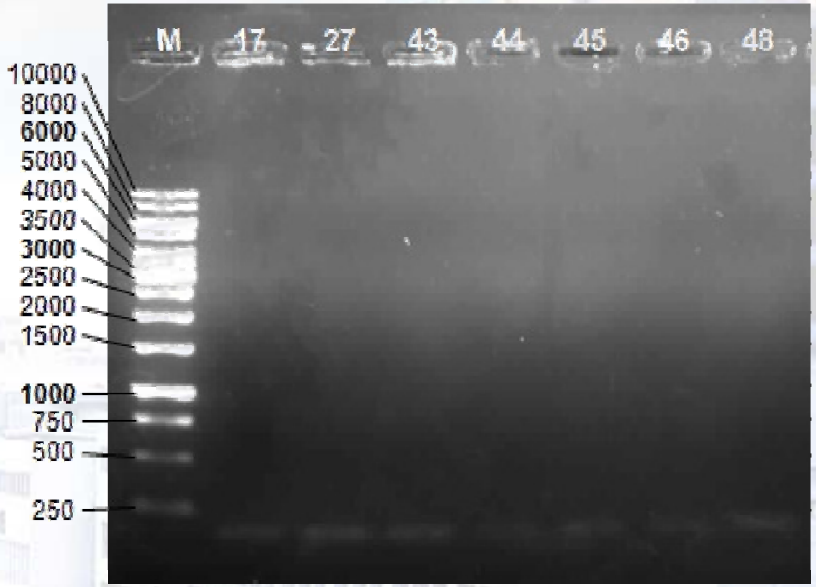


Colony PCR (*A. fumigatus*)

PCR



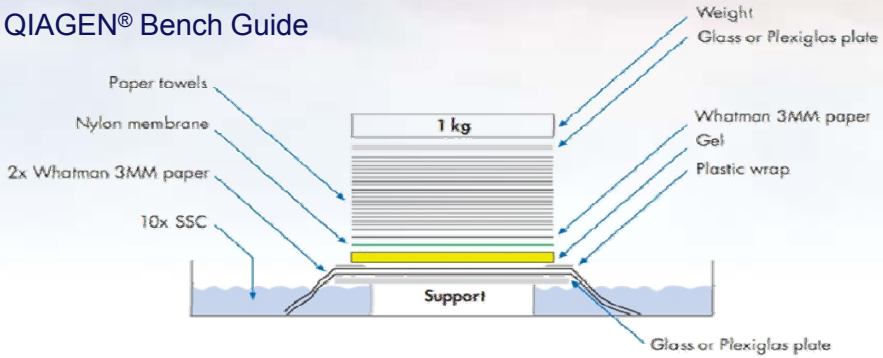
fdh ORF

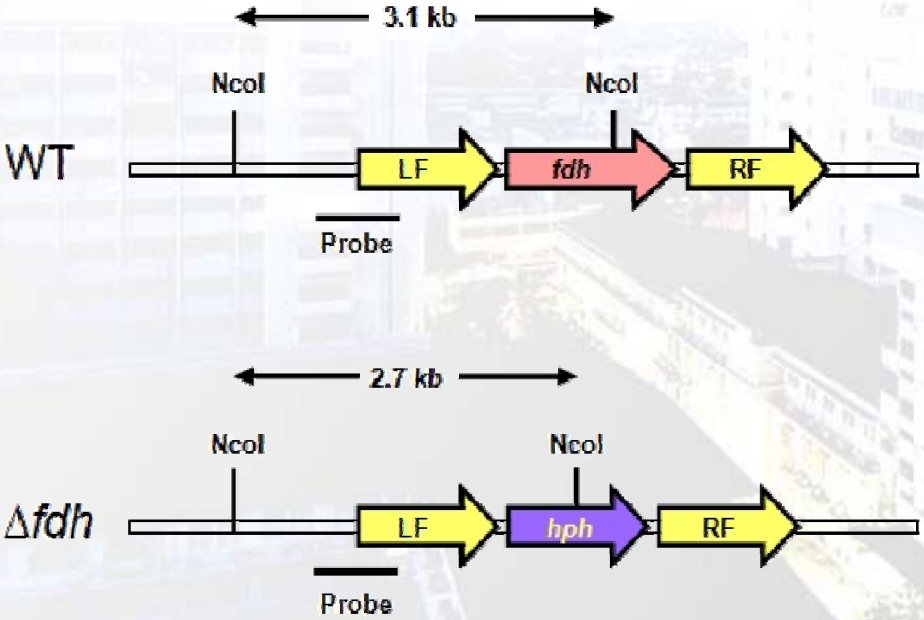


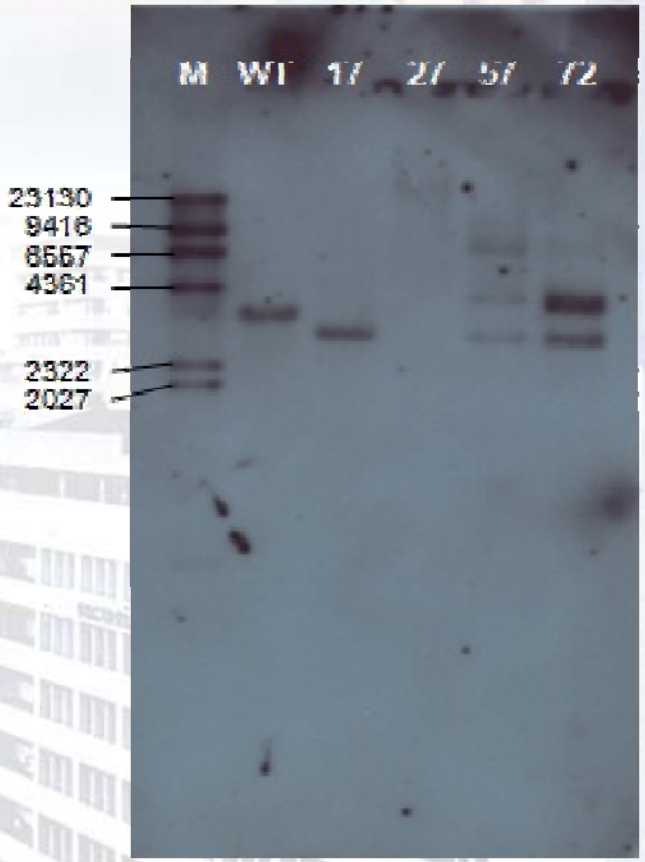
Tubulin gene

Southern blot analysis

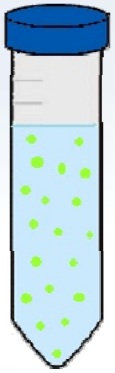
QIAGEN® Bench Guide







Radial hyphal growth analysis



10^6 conidial/ml of
WT or
Δ*fdh* strains

200 mM $CaCl_2$

0.8 M NaCl

1 M Sorbital

200 mM $MgCl_2$

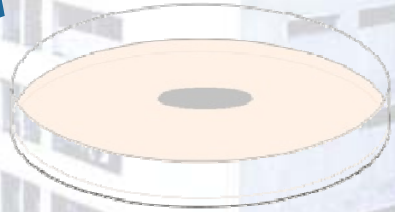
0.6 M KCl

10 mM $MnCl_2$

GMM pH4

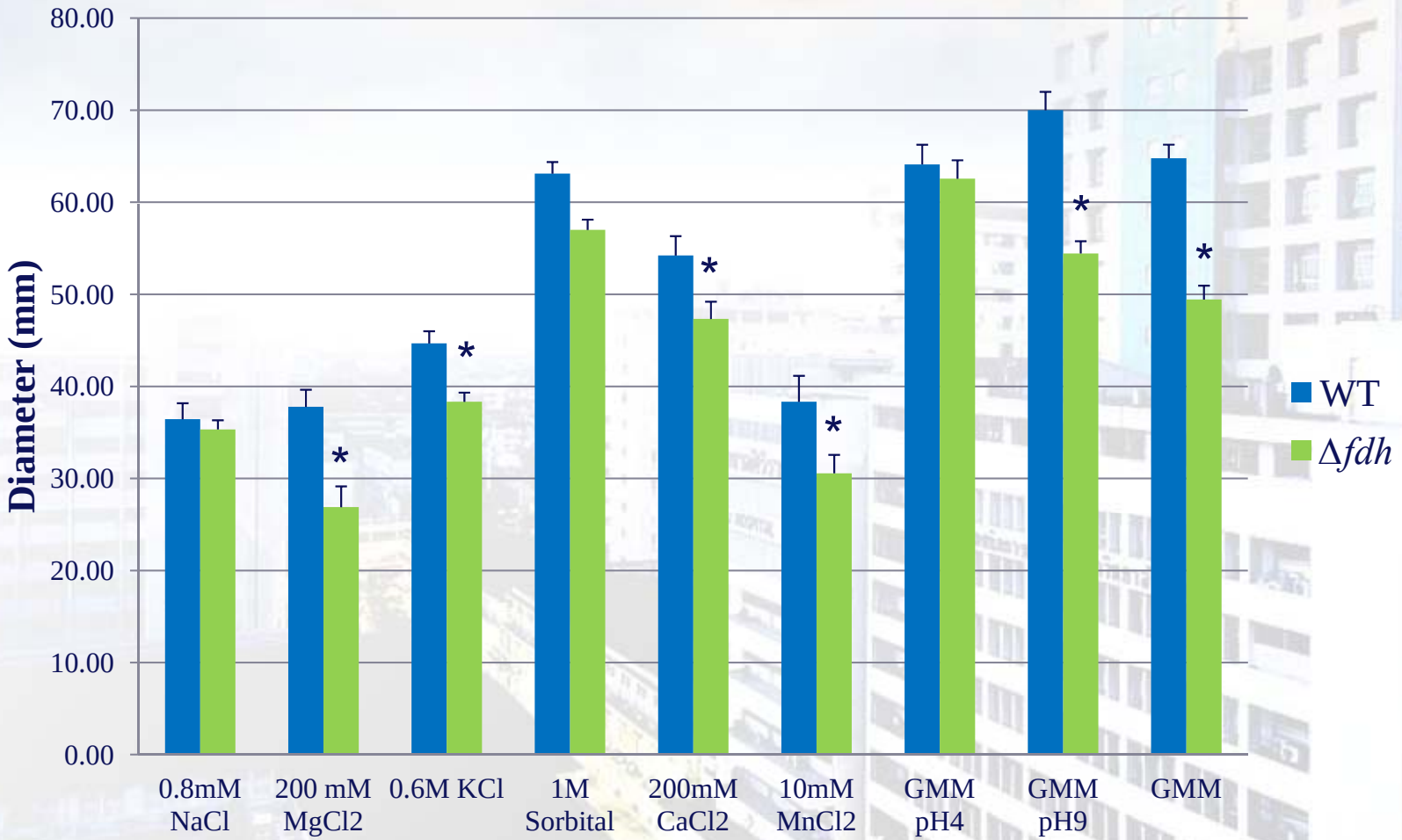
GMM

GMM pH9



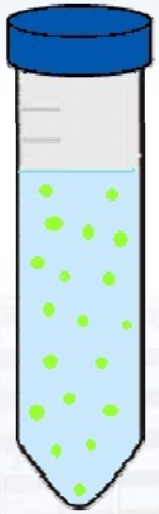
Every 24 h for
5 days

Radial hyphal growth of wild-type and Δ*fdh* under various unfavorable conditions

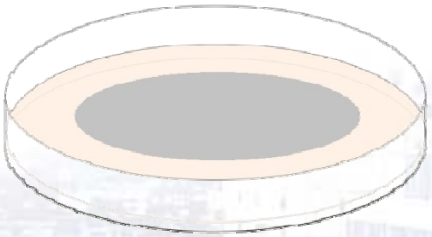


A p-value ≤ 0.05 was considered statistically significant.

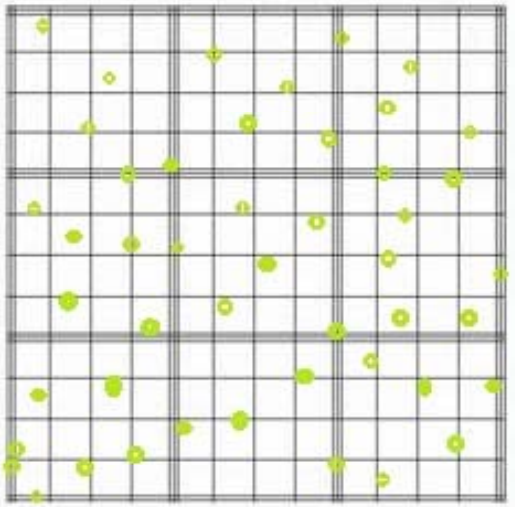
Conidiation analysis



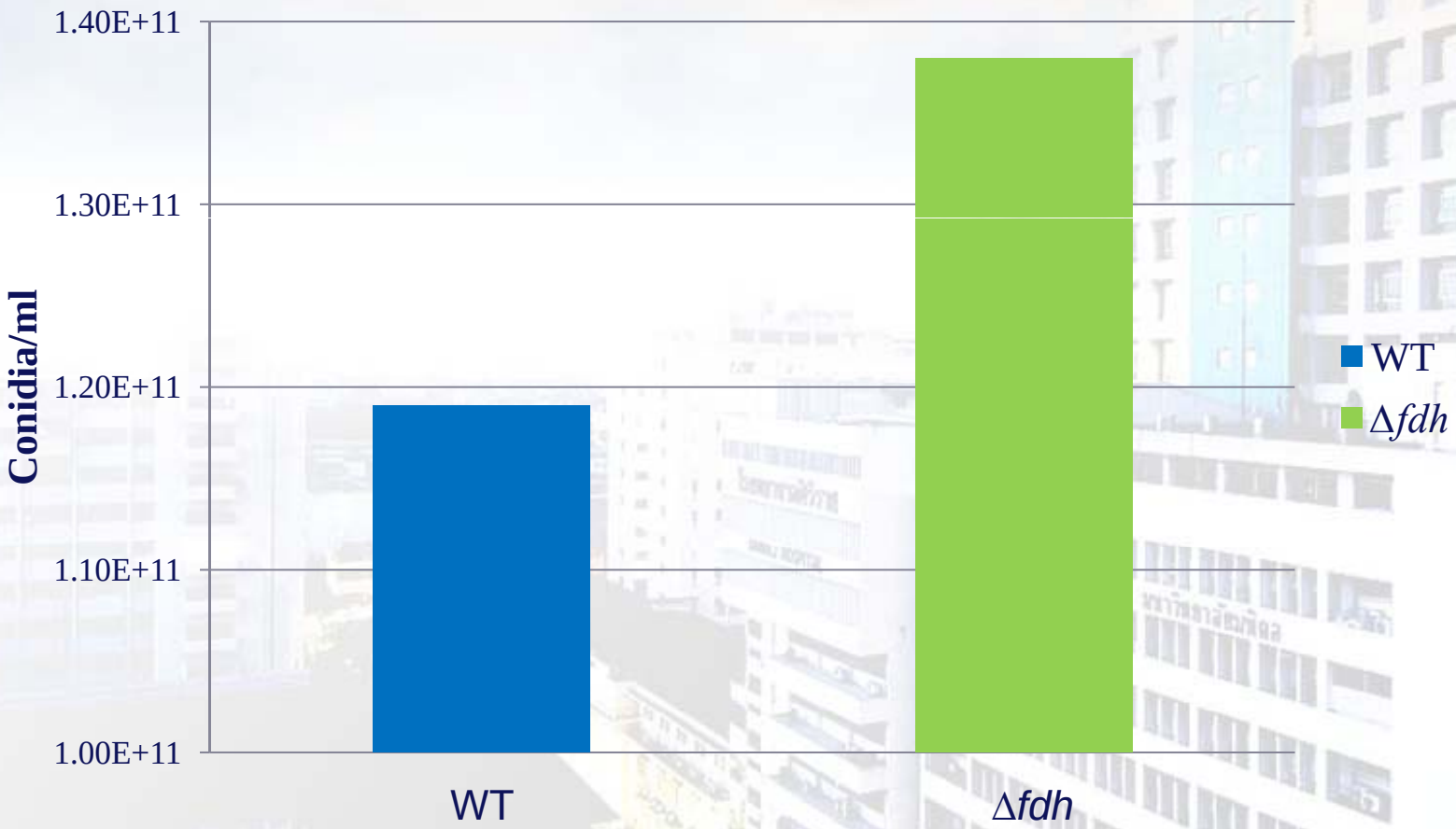
10^6 conidial/ml of
WT or
Δ*fdh* strains



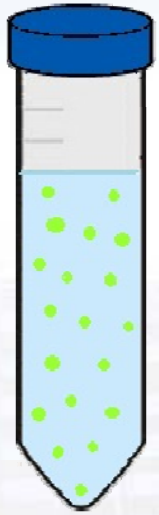
GMM agar, 37˚C
for 72 h



Conidiation of wild-type and Δ*fdh*

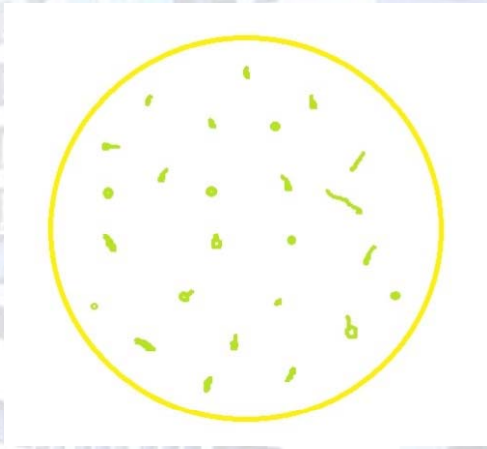


Germination analysis



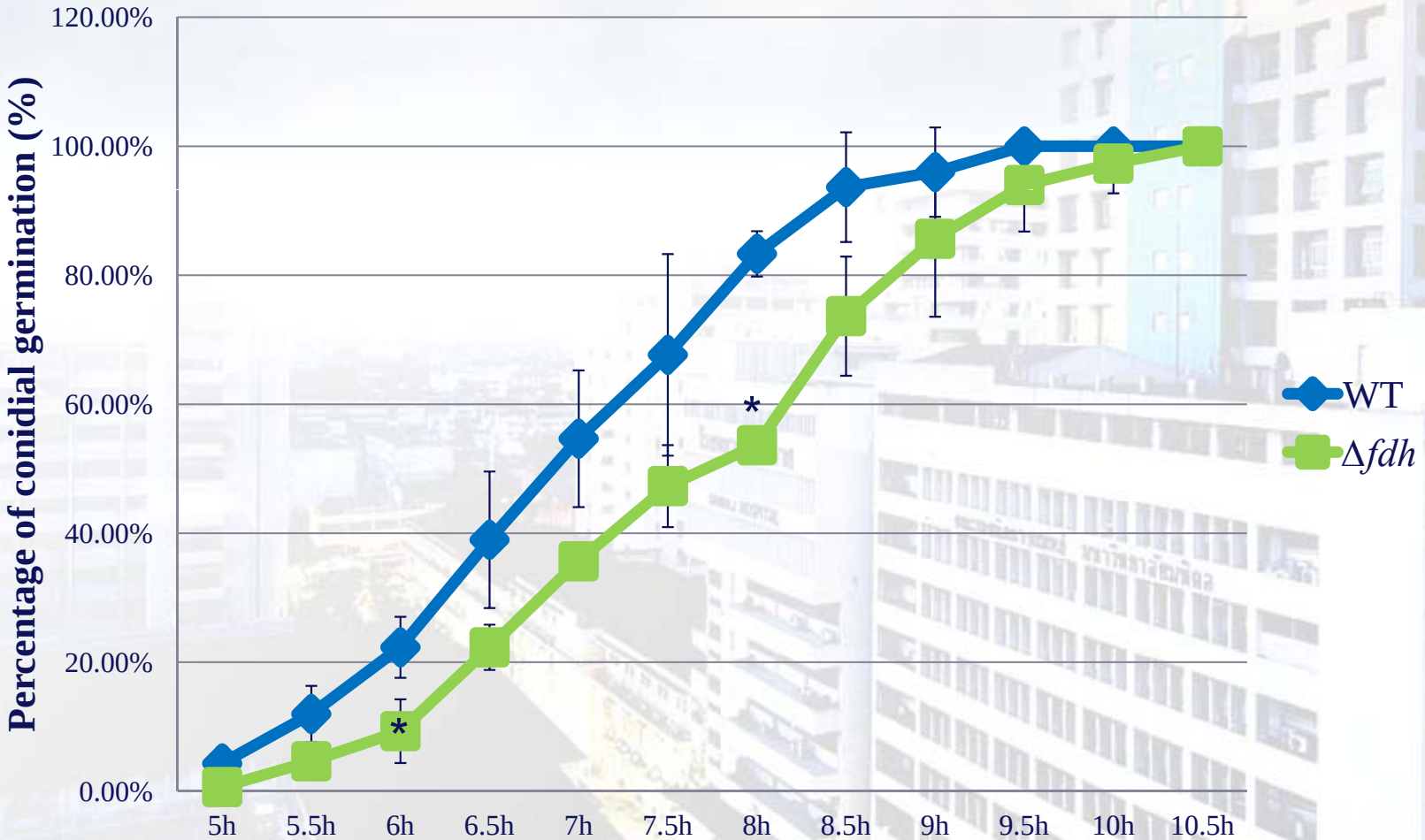
10^7 conidial/ml of
WT or
Δ*fdh* strains

37˚C, 300 rpm
for 5 h



Determined every 30 min
until 100% germination

Percentage of germination of wild-type and Δ*fdh* over the experimental time period



A p-value ≤ 0.05 was considered statistically significant.

References

1. Kiertiburanakul S, Thibbadee C, Santanirand P. Invasive Aspergillosis in a Tertiary-Care Hospital in Thailand. J Med Assoc Thai. 2007;90(5):895-902.

2. Rittayamai N, Tscheikuna J. Invasive Pulmonary Aspergillosis with Cerebral Dissemination in Chronic Refractory Idiopathic Thrombocytopenic Purpura. Thai journal of tuberculosis, chest disease and critical care. [Case report]. 2009;30(3):166-71.

3. Steinbach WJ, Marr KA, Anaissie EJ, Azie N, Quan S-P, Meier-Kriesche H-U, et al. Clinical epidemiology of 960 patients with invasive aspergillosis from the PATH Alliance registry. Journal of Infection. 2012;65(5):453-64.

4. Abad A, Victoria Fernández-Molina J, Bikandi J, Ramírez A, Margareto J, Sendino J, et al. What makes Aspergillus fumigatus a successful pathogen? Genes and molecules involved in invasive aspergillosis. Revista Iberoamericana de Micología. 2010;27(4):155-82

5. Lehrnbecher T, Frank C, Engels K, Kriener S, Groll AH, Schwabe D. Trends in the postmortem epidemiology of invasive fungal infections at a university hospital. Journal of Infection. 2010;61:259-65.

References

6. Suzuki K, Itai R, Nakanishi K, Nishizawa NK, Yoshimura E, Mori S. Formate Dehydrogenase, an Enzyme of Anaerobic Metabolism, Is Induced by Iron Deficiency in Barley Roots. Plant Physiol. 1998;116:725-32.

7. Hourton- Cabassa C, Ambard-Bretteville F, Morean F, Davy de Virville J, Rémy R, Colas des Francs-Small C. Stress Induction of Mitochondrial Formate Dehydrogenase in Potato Leaves. Plant Physiol. 1998;116:627-35.

8. Chow CM, RajBhandary UL. Developmental regulation of the gene for formate dehydrogenase in Neurospora crassa. J Bacteriol. 1993;175(12):3703-9.

9. Schmit JC, Brody S. Biochemical Genetics of *Neurospora crassa* Conidial Germination. Bacteriol Rev. 1976;40(1):1-41.

10. Bruns S, Seidler M, Albrecht D, Salvenmoser S, Remme N, Hertweck C, et al. Functional genomic profiling of *Aspergillus fumigatus* biofilm reveals enhanced production of the mycotoxin gliotoxin. Proteomics. 2010;10(17):3097-107.

Acknowledgement

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Siriraj Hospital, Mahidol University

Thank you for your attention

Plant Physiol. (1998) 116: 725–732

Formate Dehydrogenase, an Enzyme of Anaerobic Metabolism, Is Induced by Iron Deficiency in Barley Roots[1]

Kazuya Suzuki, Reiko Itai, Koichiro Suzuki, Hiromi Nakanishi, Naoko-Kishi Nishizawa, Etsuro Yoshimura, and Satoshi Mori*

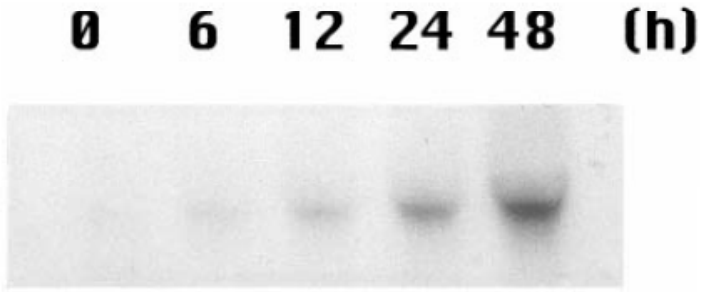


Figure 8. Expression of *Fdh* under anaerobic conditions. Each lane was loaded with 10 μg of RNA. Total RNA was isolated after 0, 6, 12, 24, and 48 h of anaerobic treatment.

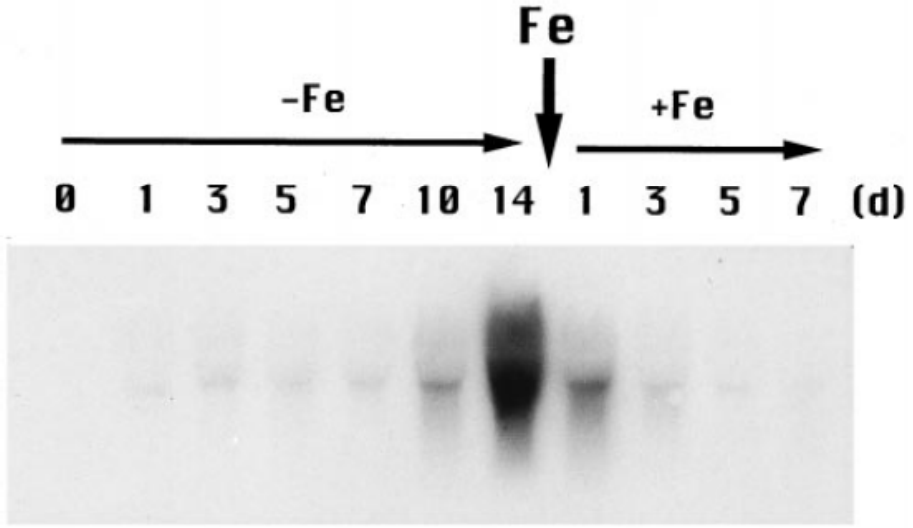


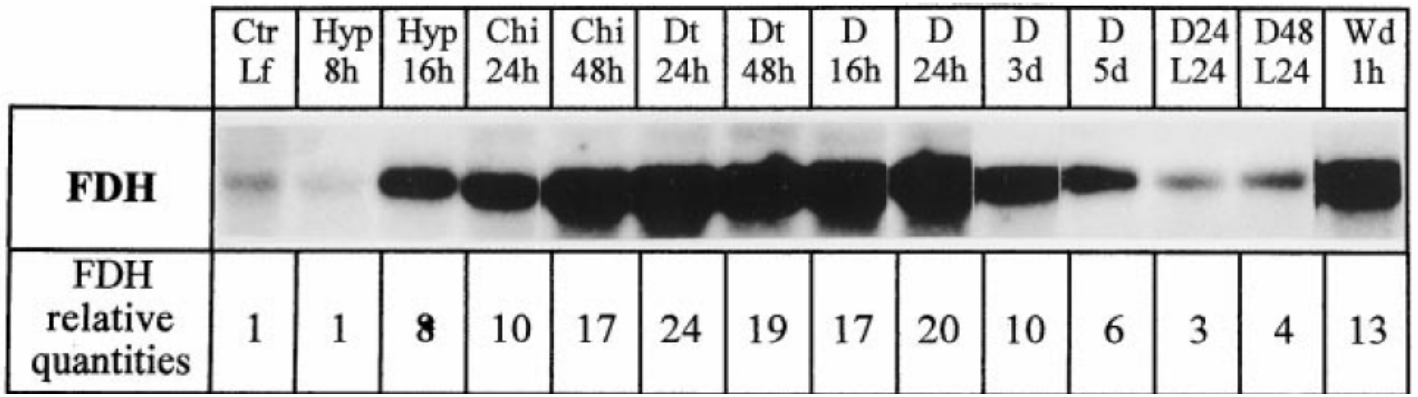
Figure 7. Expression of *Fdh* during Fe deficiency in barley roots. Each lane was loaded with 10 μg of RNA. Total RNA was isolated after 0, 1, 3, 5, 7, 10, and 14 d of Fe deficiency and 1, 3, 5, and 7 d after Fe resupply.

Suzuki K, et al. Plant Physiol. 1998;116:725-32.

Plant Physiol. (1998) 116: 627–635

Stress Induction of Mitochondrial Formate Dehydrogenase in Potato Leaves[1]

Cécile Hourton-Cabassa, Françoise Ambard-Bretteville, François Moreau, Jacques Davy de Virville, René Rémy, and Catherine Colas des Francs-Small*



	Ctr Lf	Hyp 8h	Hyp 16h	Chi 24h	Chi 48h	Dt 24h	Dt 48h	D 16h	D 24h	D 3d	D 5d	D24 L24	D48 L24	Wd 1h
FDH														
FDH relative quantities	1	1	8	10	17	24	19	17	20	10	6	3	4	13

Figure 4. RNA expression of FDH and SHMT in potato leaves under various stress treatments. Each lane was loaded with 10 μg of RNA. Ctr Lf, Control leaves; Hyp 8h and Hyp 16h, 8 and 16 h of hypoxia, respectively; Chi 24h and Chi 48h, 24 and 48 h of chilling, respectively; Dt 24h and Dt 48h, 24 and 48 h of drought treatment, respectively; D 16h, D 24h, D 3d, and D 5d, 16 h, 24 h, 3 d, and 5 d of dark treatment, respectively; D24 and L24, 24 h of dark treatment followed by 24 h under a 16-h photoperiod; D48 and L24, 48 h of dark treatment followed by 24 h under a 16-h photoperiod; Wd 1h, 1 h after wounding. The autoradiographs were scanned using the control leaf sample as the reference for both probes, and the figures were corrected according to the RNA loadings.

Hourton- Cabassa C, et al. Plant Physiol. 1998;116:627-35.

JOURNAL OF BACTERIOLOGY, June 1993, p. 3703–3709
0021-9193/93/123703-07$02.00/0

Vol. 175, No. 12

Developmental Regulation of the Gene for Formate Dehydrogenase in *Neurospora crassa*

C. MING CHOW AND UTTAM L. RAJBHANDARY*

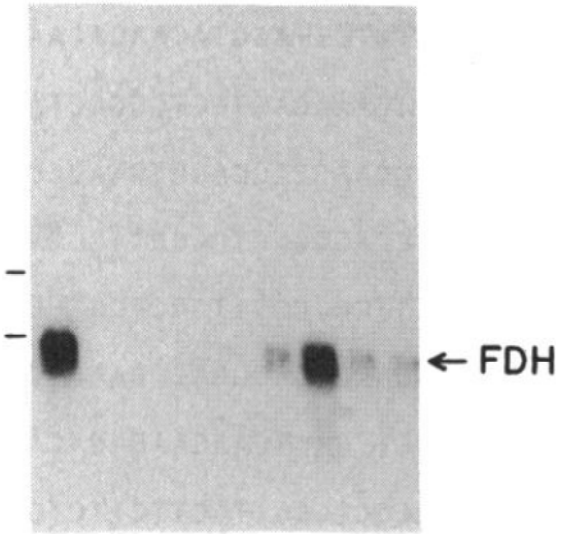


FIG. 2. Northern blot analysis of *N. crassa* total RNA. Membranes were probed for mRNA for FDH

Proteomics 2010, *10*, 3097–3107 DOI 10.1002/pmic.201000129

RESEARCH ARTICLE

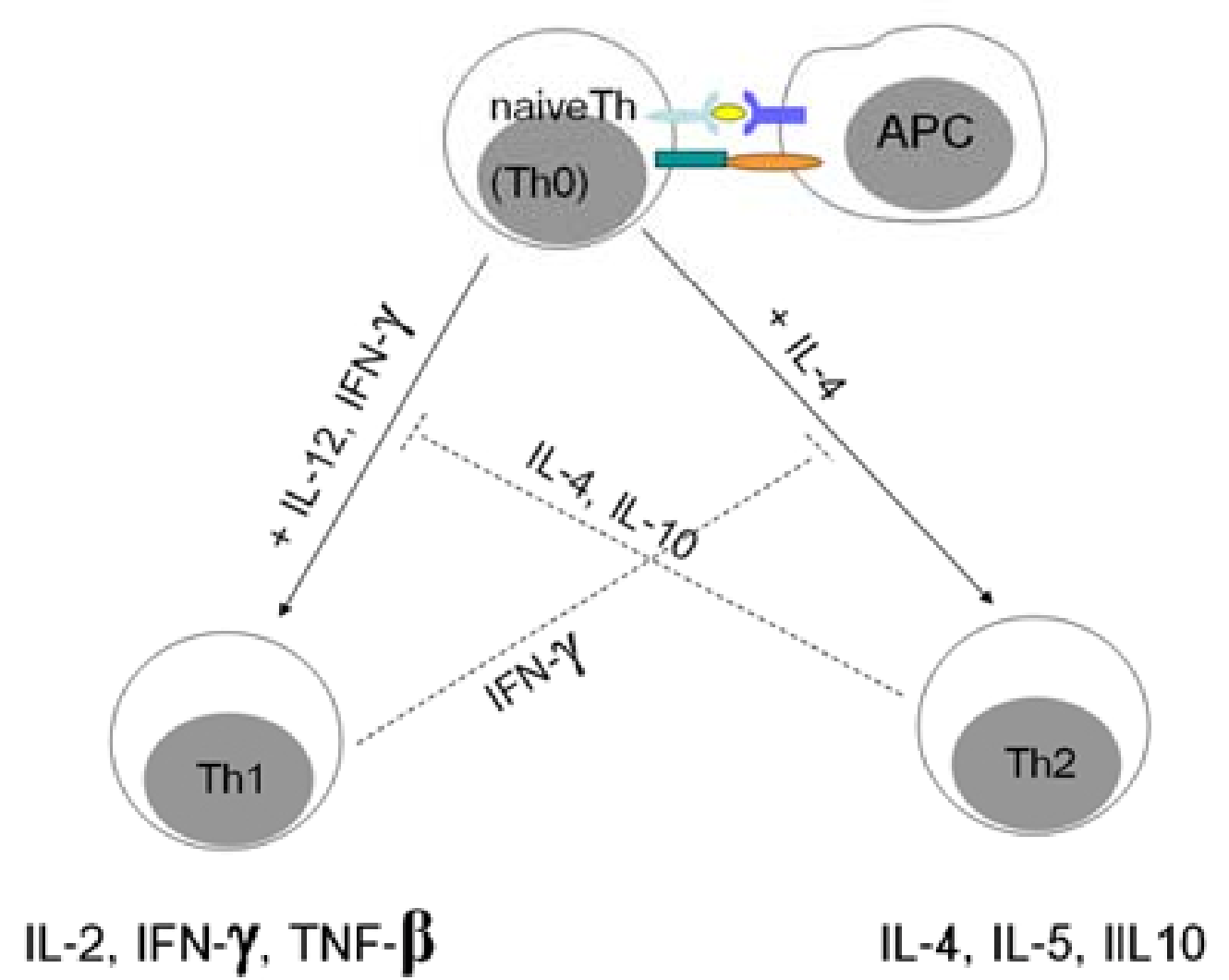
Functional genomic profiling of *Aspergillus fumigatus* biofilm reveals enhanced production of the mycotoxin gliotoxin

Sandra Bruns[1,2], Marc Seidler[3]*, Daniela Albrecht[4], Stefanie Salvenmoser[3], Nicole Remme[5], Christian Hertweck[5], Axel A. Brakhage[1,2], Olaf Kniemeyer[1,2]** and Frank-Michael C. Müller[3]*

Table 1. Biofilm- (biofilm *versus* planktonic grown) and time-dependent (48 *versus* 24 h), differentially synthesized proteins of *A. fumigatus*

Putative function	Fold change[a)]			
	Biofilm-dependent chances		Time-dependent changes in biofilm	
	24+/24–	48+/48–	48+/24+	48–/24–
Formate catabolic process				
803 NAD-dependent formate DH (AFUA_6G04920)	n.s.	n.s.	13.0	2.7
807 "–"	n.s.	n.s.	11.4	3.1
814 "–"	n.s.	n.s.	34.0	2.9
815 "–"	n.s.	n.s.	20.7	2.5
817 "–"	n.s.	n.s.	3.2	0.9

Th1/Th2 cytokines



http://www.cai.md.chula.ac.th/lesson/lesson5201/html/05th.html

Characteristic pattern of *A. fumigatus*

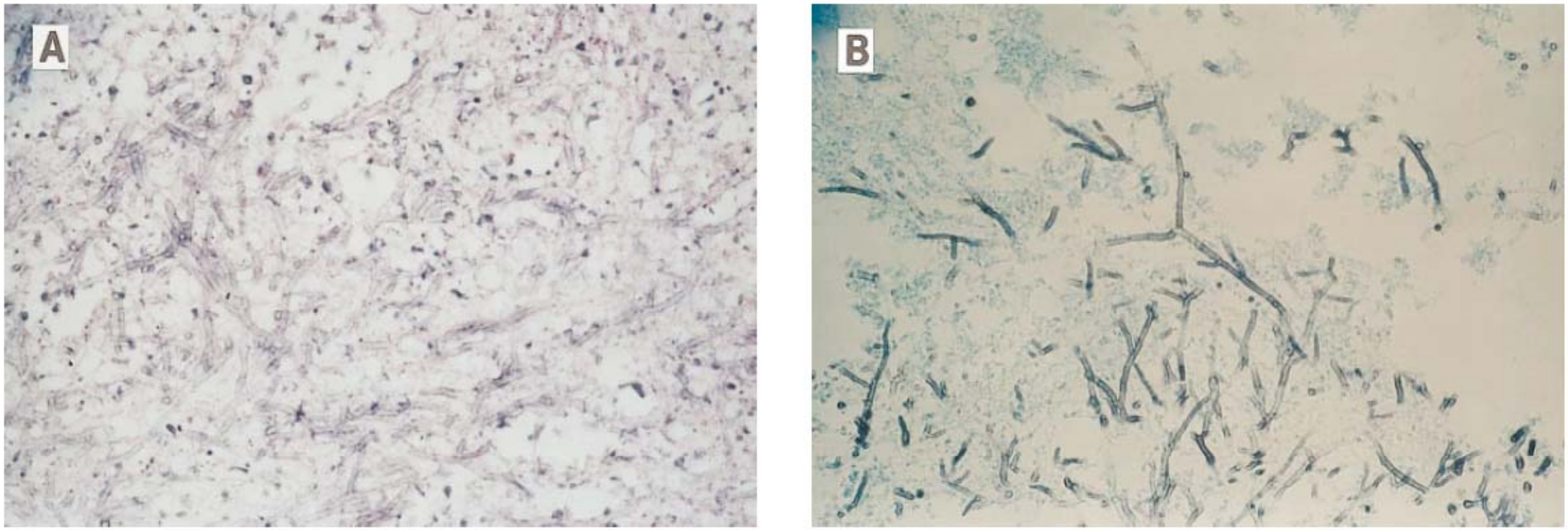


Figure 2. *A,* histological appearance under high power of an aspergillus brain abscess showing an inflammatory cell infiltrate surrounding *Aspergillus* hyphae. (Stain, hematoxylin-eosin; original magnification, ×400.) *B,* Section of the same abscess showing the characteristic radiating pattern of *Aspergillus* hyphae with septae and branching at 45° angles. (Stain, methenamine silver; original magnification, ×400.)

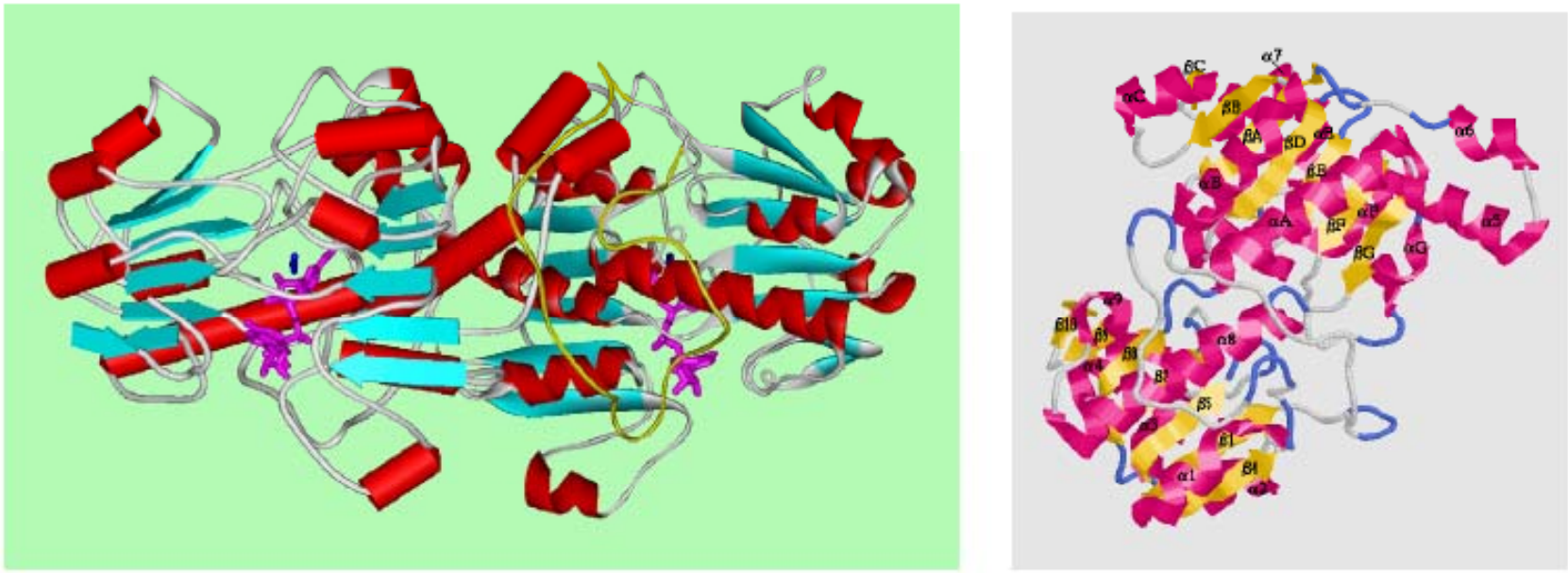
Denning DW. CID. 1998;26:781-805.

Chest radiograph of AIDS patient with invasive pulmonary aspergillosis

Figure 3. Chest radiograph of a patient with late-stage AIDS and probable invasive pulmonary aspergillosis, illustrating diffuse bilateral disease. The patient had cytomegalovirus retinitis and was receiving chemotherapy for Kaposi's sarcoma; he was symptomatic with a high fever and cough, and cultures of both sputum and bronchoalveolar lavage fluid yielded *Aspergillus fumigatus*.

Denning DW. CID. 1998;26:781-805.

Structure of NAD^+-dependent FDH



A B

Figure 2. Structure of PseFDH. A) FDH ternary complex with NAD^+ (magenta) and formate (blue) occupying azide binding site. α-helices are depicted as red cylinders (left subunit) or helices (right subunit) while β-strands as cyan arrows (left) or strips (right). A long loop comprising a/a residues 12-47 present in bacterial FDHs but absent in the enzymes from other species is shown in yellow. B) Representation of the structure of the FDH subunit. Numbering of structural elements from [17].

Popov VO, Tishkov VI. Protein Structure: Kaleidoscope of Structural Properties and Functions. 2003:441-3.