Biofilm Formation by *Aspergillus*

Dr Gordon Ramage
Section of Infection & Immunity
Glasgow Dental School & Hospital
Faculty of Medicine

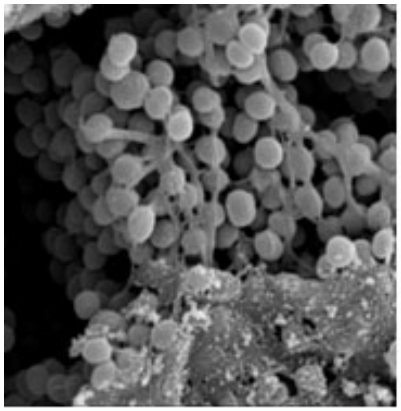
Outline

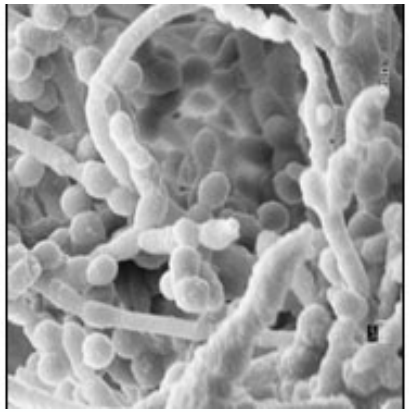
- Biofilms and their clinical significance?
- *Aspergillus* biofilm development
  - Phenotypic analysis
  - Genotypic analysis

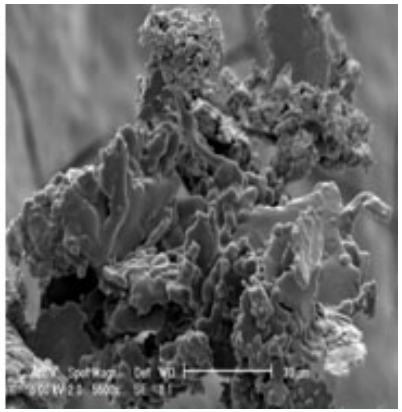
What are biofilms?

Biofilms Defined

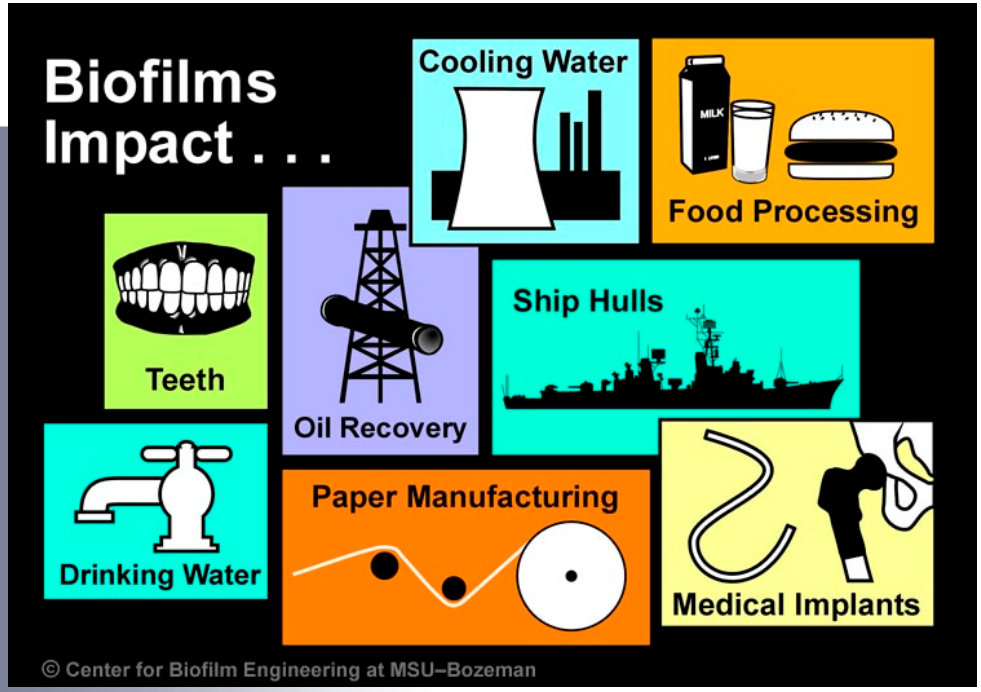
"….matrix-enclosed microbial populations adherent to <u>each other</u> and/or to <u>surfaces or interfaces</u>"



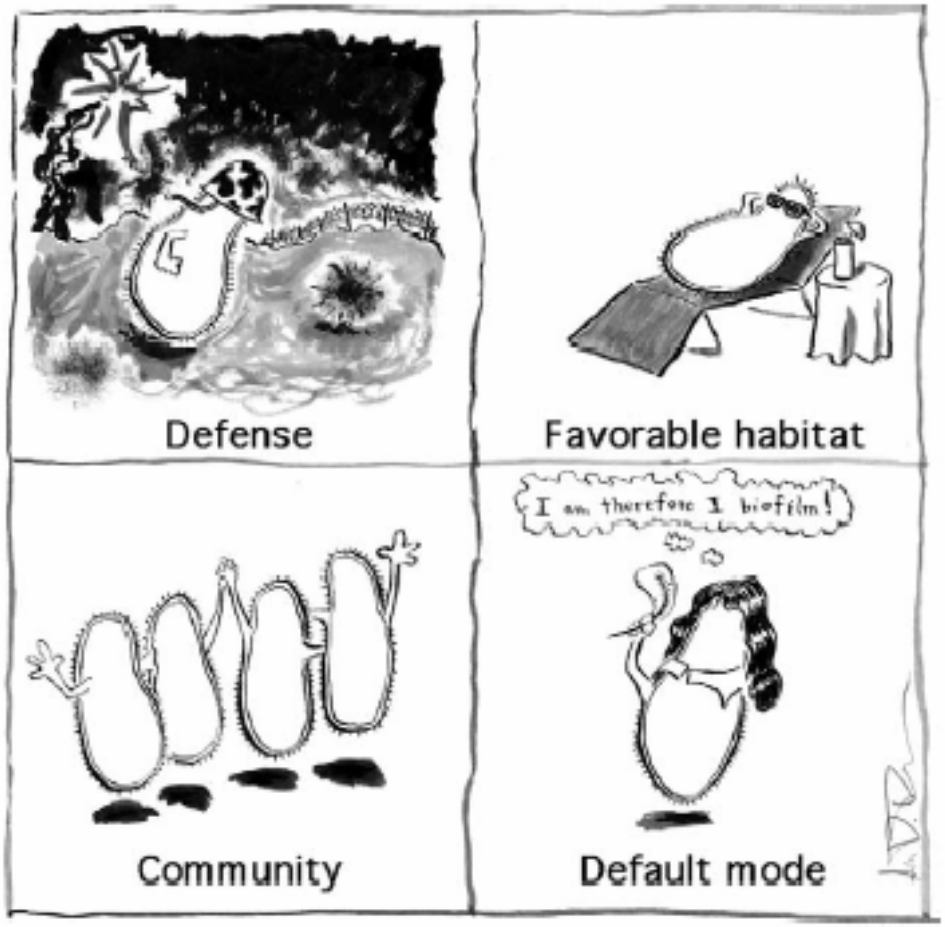




Ubiquitous

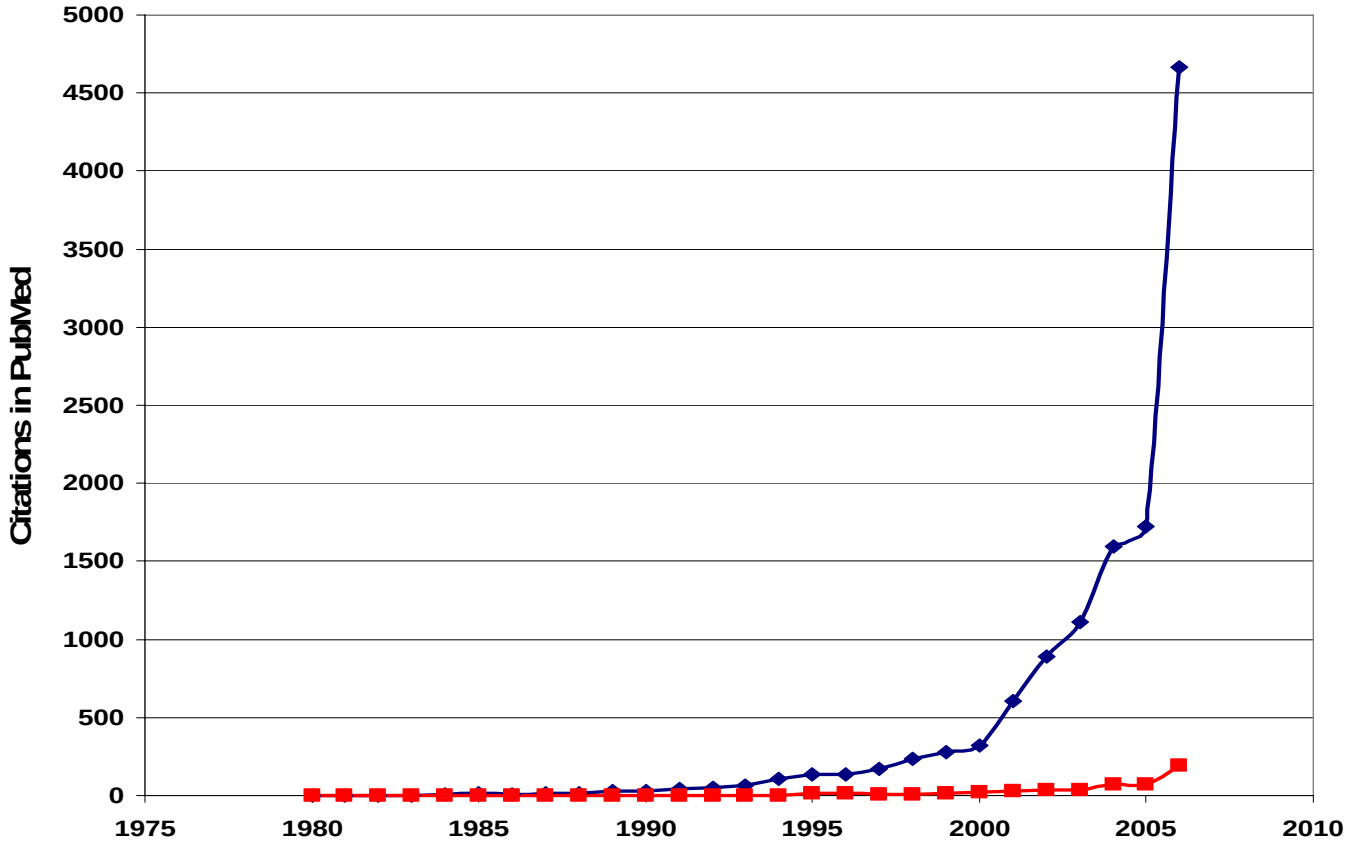

Biofilms Impact . . .
Cooling Water
Food Processing
MILK
Teeth
Oil Recovery
Ship Hulls
Drinking Water
Paper Manufacturing
Medical Implants
© Center for Biofilm Engineering at MSU–Bozeman

Functional Benefits



- Protected
- Nutrients
- Detoxification
- Co-ordinated

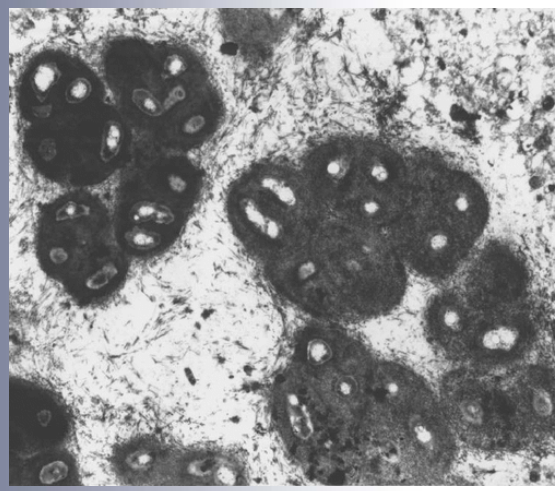
Biofilm Research Statistics



‘65% of all bacterial infections are biofilm related’

Pulmonary Biofilms

The Cystic Fibrosis Lung



CF sputum (Singh *et al*. Nature 2000. 407).

- Singh *et al* (2000) demonstrated the production of autoinducer molecules in sputum of CF patients
- Ratio of different auto-inducers (C4 to C12) was consistent with biofilm
- C4-HSL could serve as a biomarker of biofilms

Aspergillus and CF

Bakare et al 2003, Mycoses, 46, p10

Table 1 Funga
cystic fibrosis

Fungi

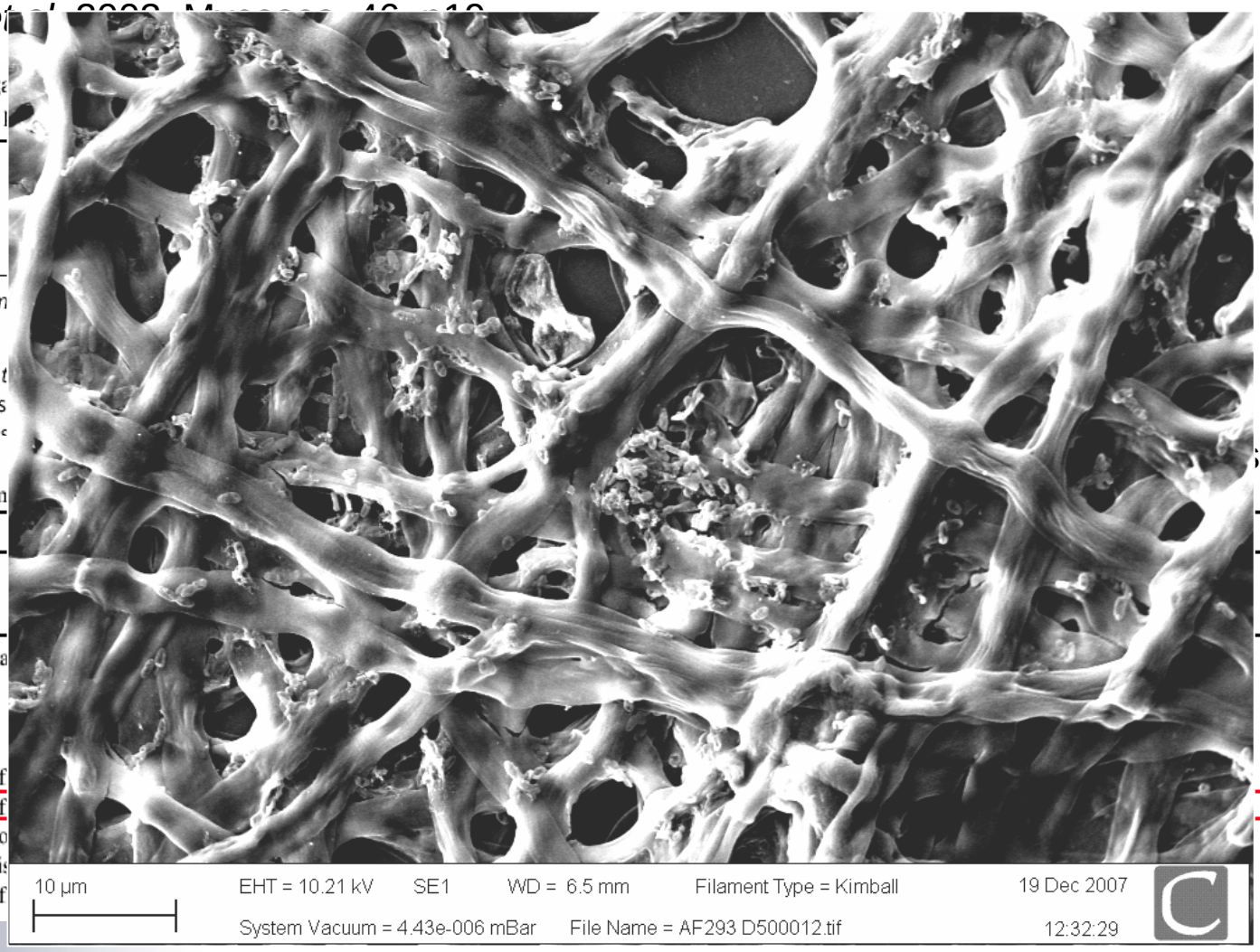
Aspergillus fum

Other moulds
Aspergillus t
Penicillium s
Chrysonilia s

Table 1
Baseline dem

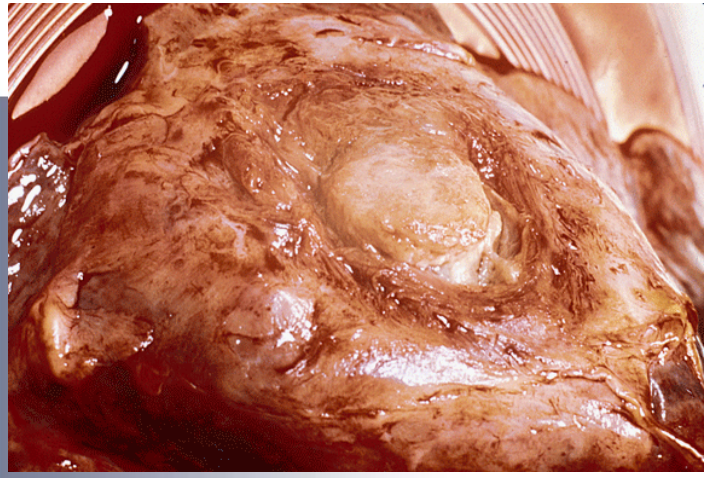
a)

	p value
Age (years) a	Ns
BMI *z*-score	Ns
BMI *z*-score	Ns
BMI *z*-score	Ns
Frequency of	Ns
Frequency of	0.01
Percent muco	<0.0001
Age at first is	Ns
Frequency of	<0.0001

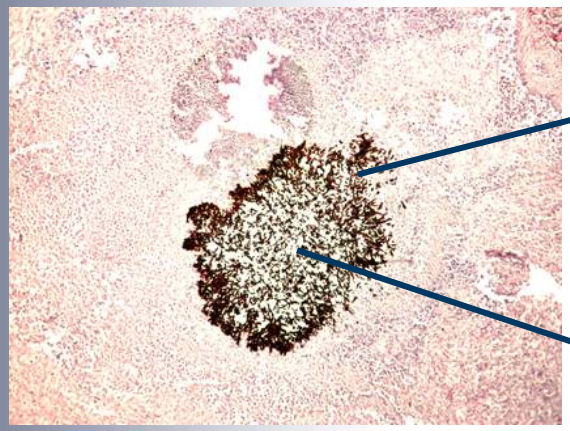


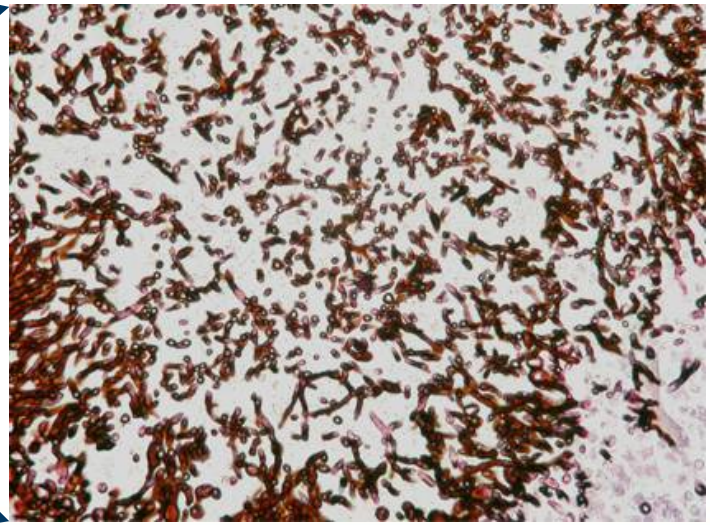
. 5, p9-15

Fungus Ball: Biofilms?



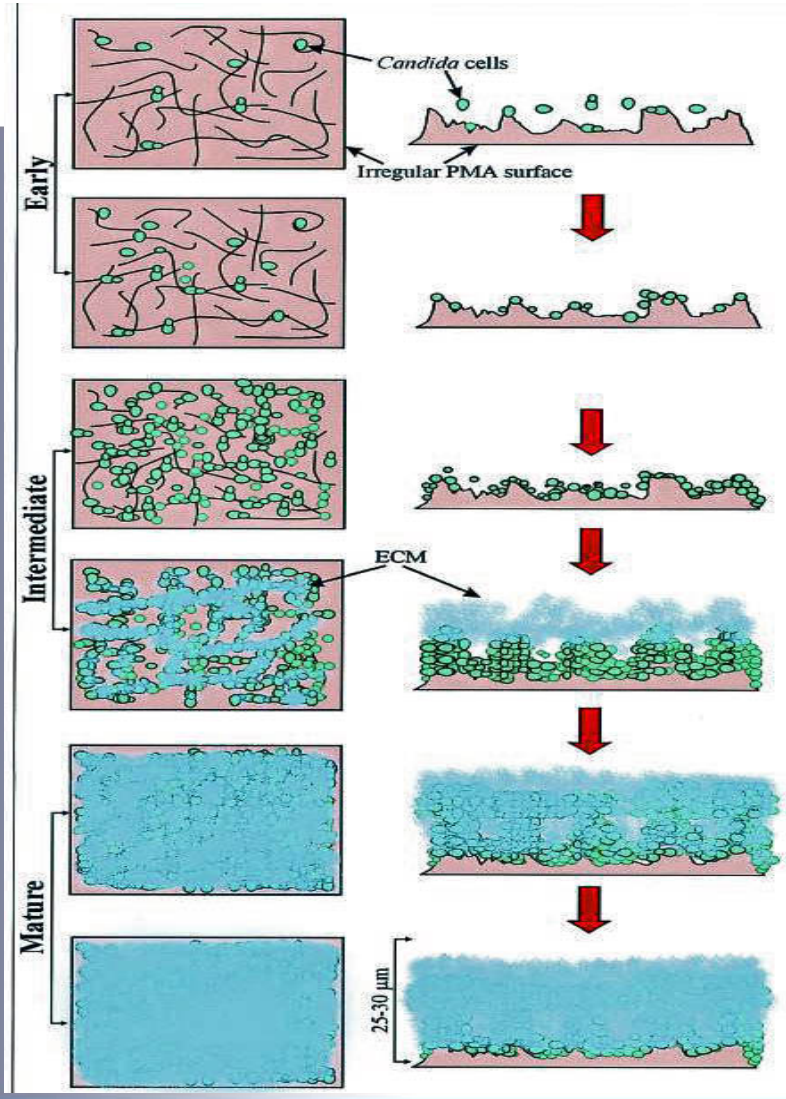
"….matrix-enclosed microbial populations adherent to <u>each other</u> and/or to <u>surfaces or interfaces</u>"





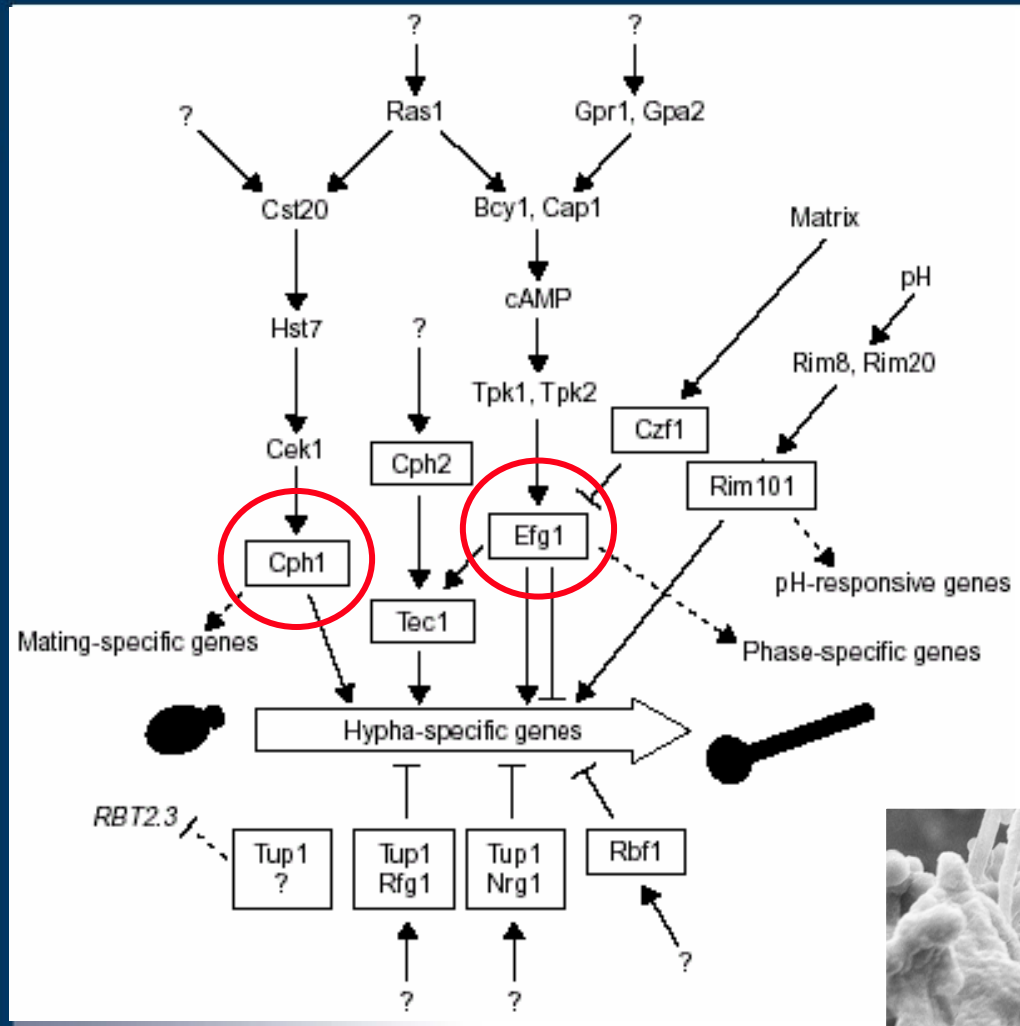
Biofilm Development

Development

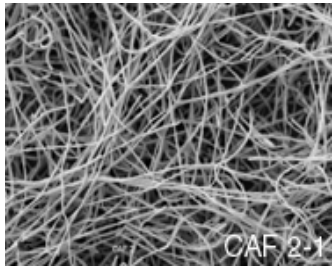


Chandra *et al*. J. Bact. 2001. 183

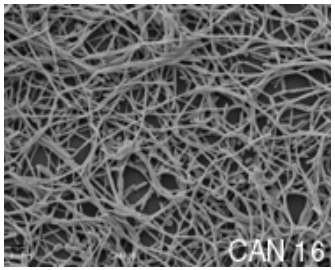
Hyphae and Fungal Biofilms

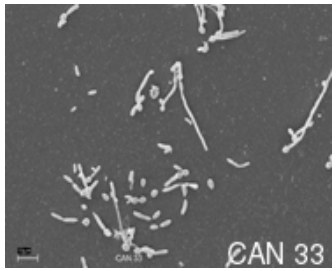


Wild type

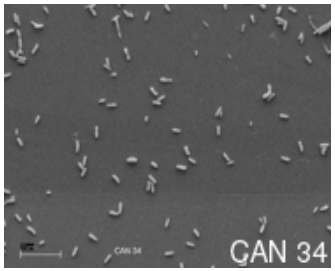


Δ cph1





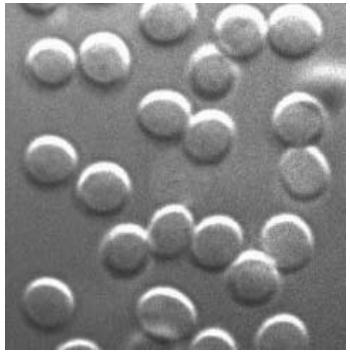
Δefg1



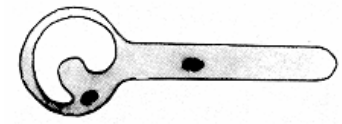
Δcph1 Δ efg1

Ramage *et al*. 2002. FEMS Micro Let.

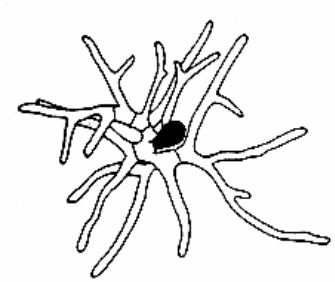
Aspergillus Life-Cycle



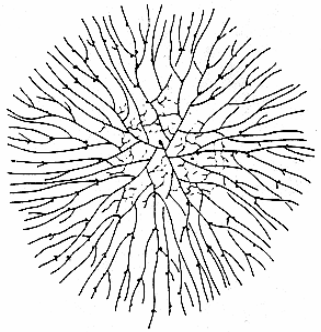
Spores inhaled



Germination



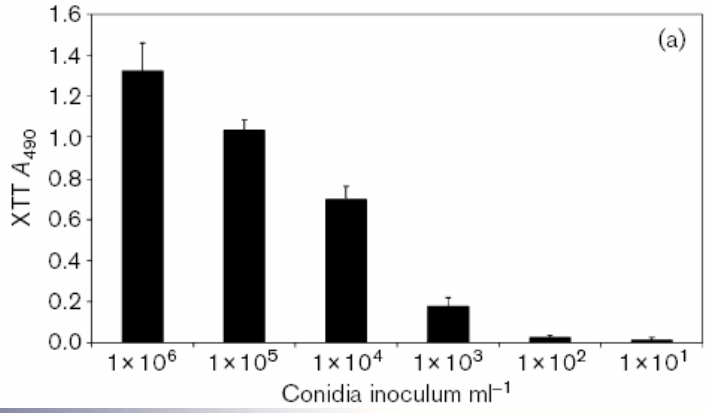
Hyphal elongation and branching

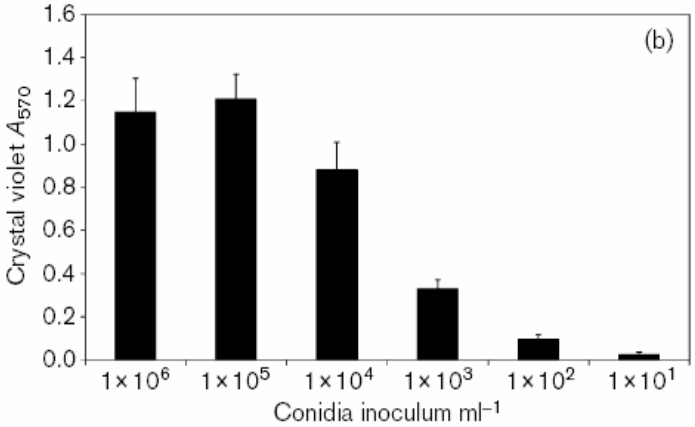


Mass of hyphae (plateau phase)

Biofilm Model

10^1 10^2 10^3 10^4 10^5 10^6

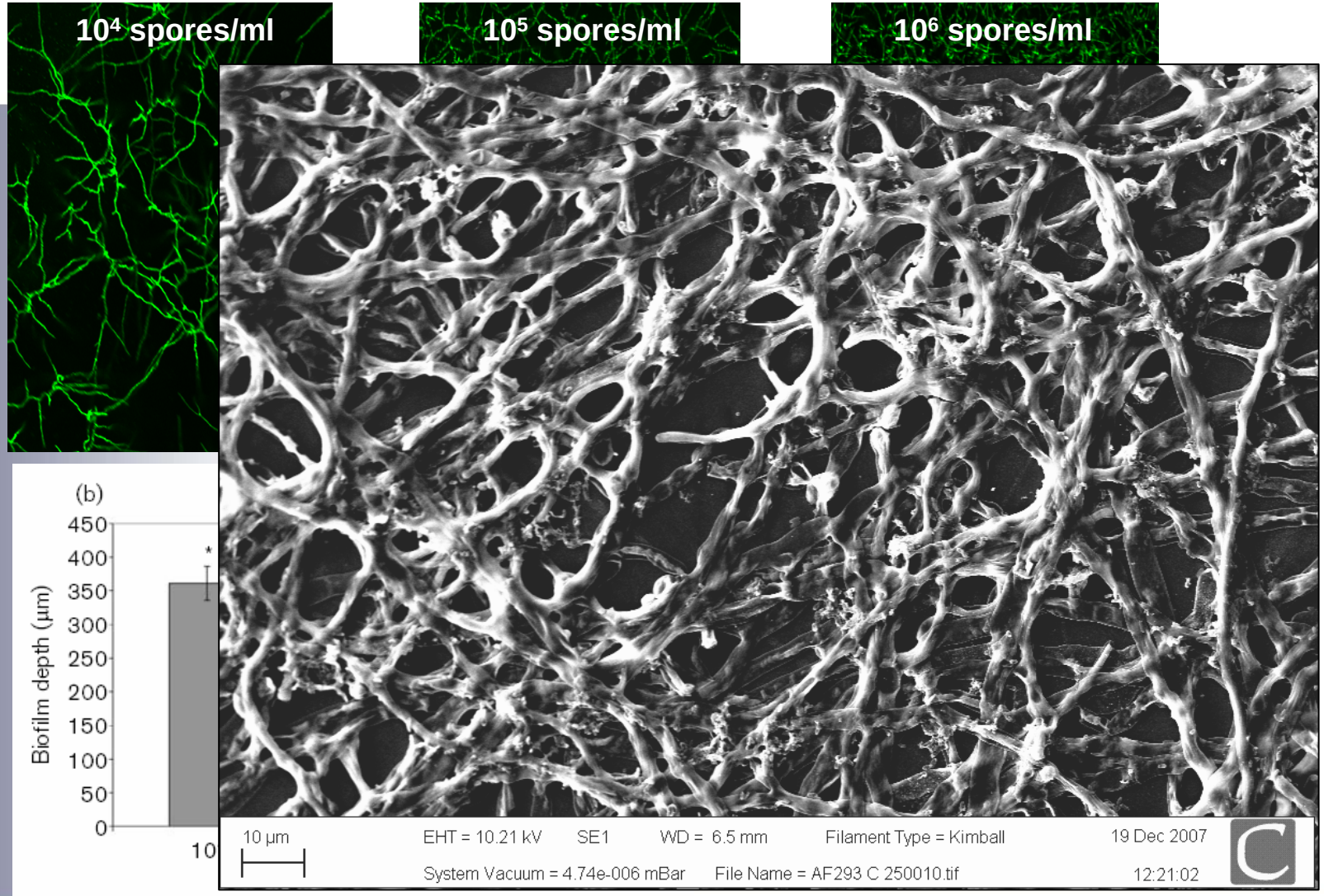




Metabolic activity

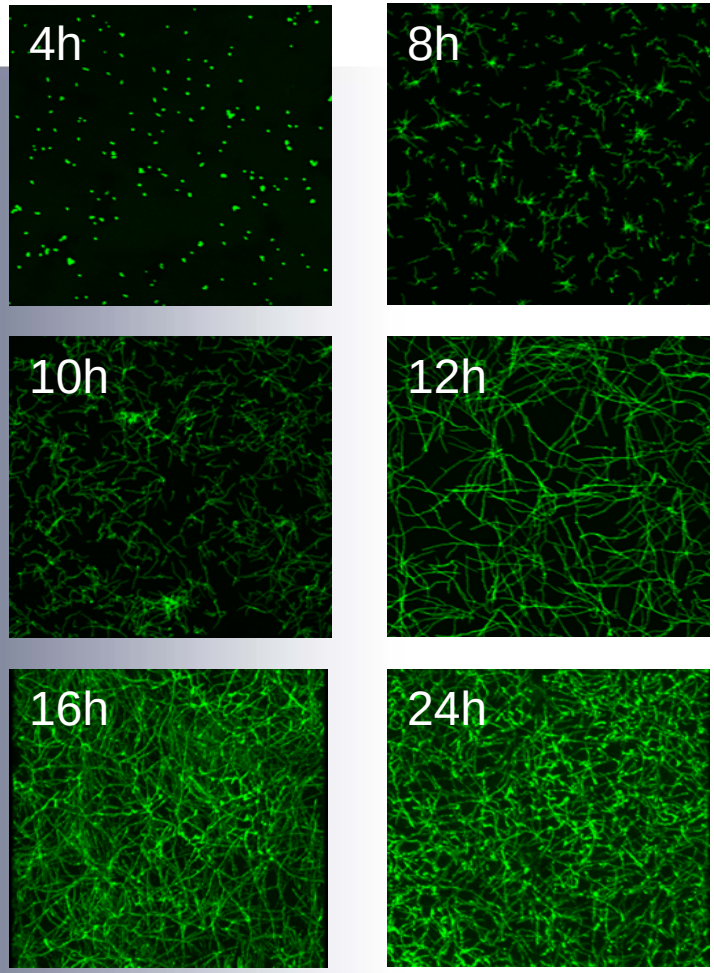
Biomass

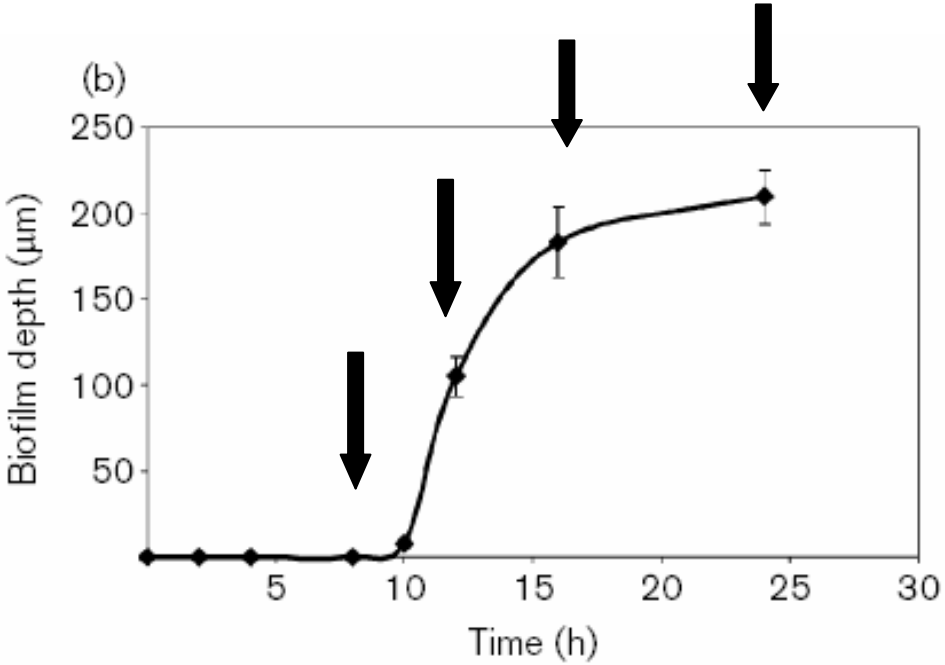
Seeding Density

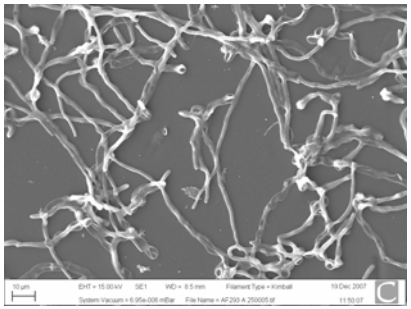


Mowat *et al*. 2007. J Med Micro. 56.

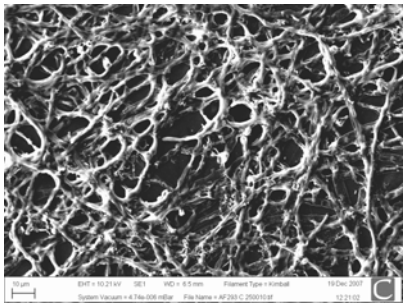
Biofilm Growth Kinetics





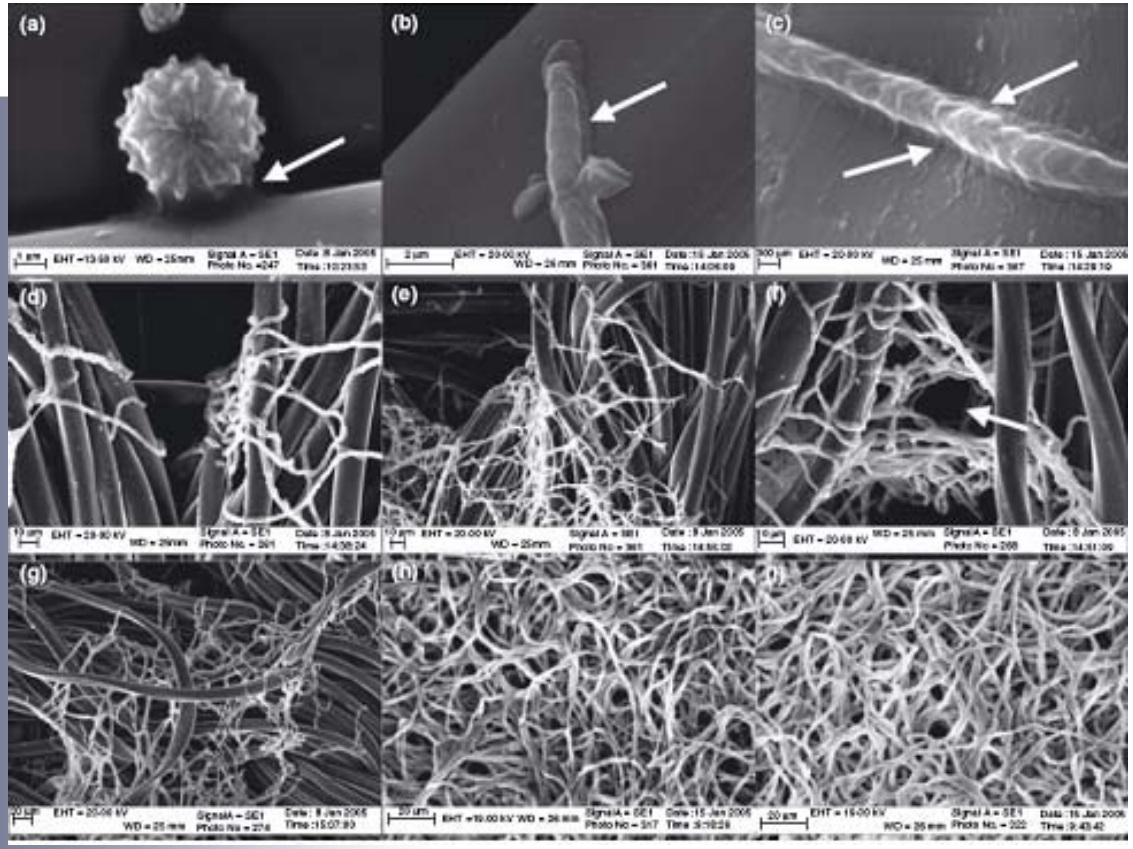


12 h



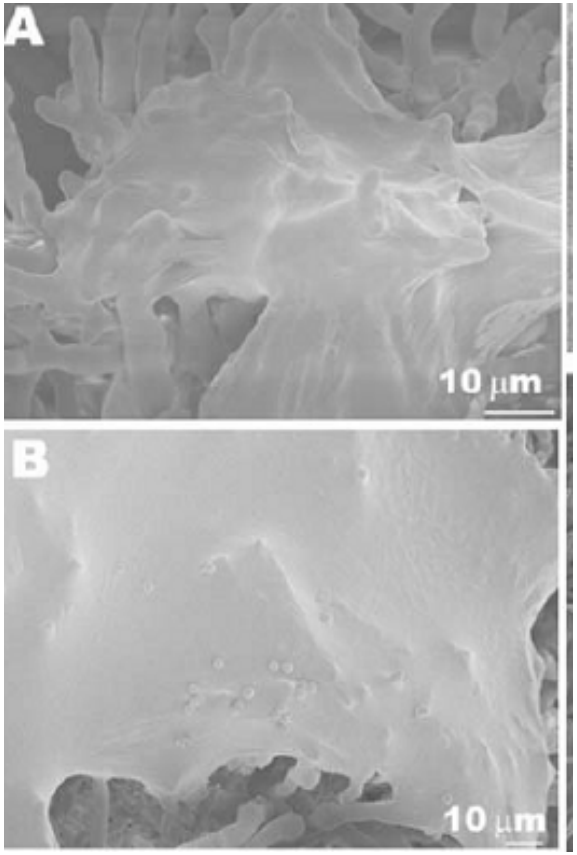
24 h

Aspergillus Matrix



G.K. Villena and M. Gutiérrez-Correa

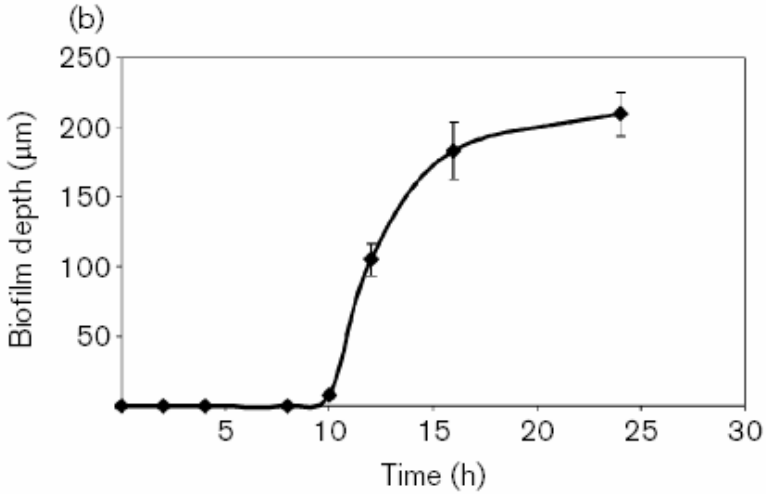
Letters in Applied Microbiology **45** (2007) 231–237

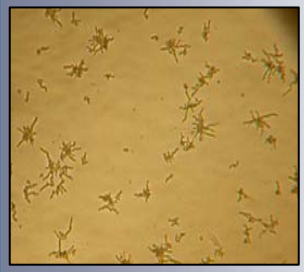


Beauvais et al. 2007. Cellular Microbiology. 9, p1588-1600

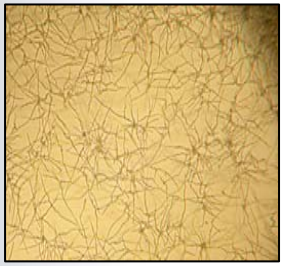
Transcriptional Profiling

Tissue Culture Model

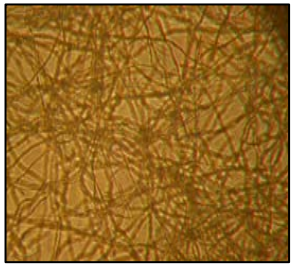




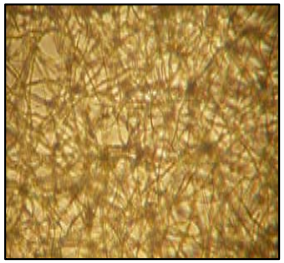
8h



12h



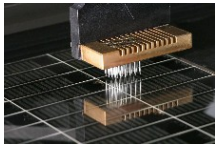
18h



24h

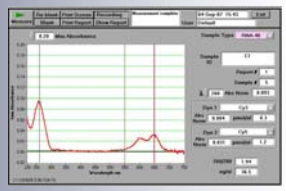
Microarray Studies at Genopole

A. fumigatus arrays covers 95% of genome, (9985 spots)



RNA quality control (Agilent Bioanalyser)

Cy3/ Cy5 Labelling (Promega kits)



Labelling checked using Nanodrop

Overnight automated hybridisation

Scan arrays

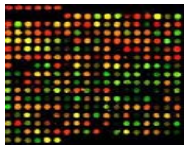
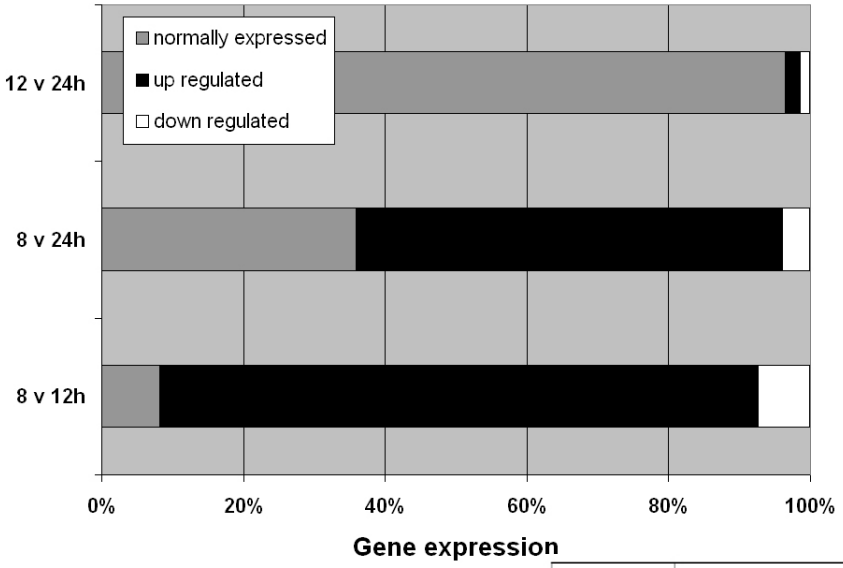
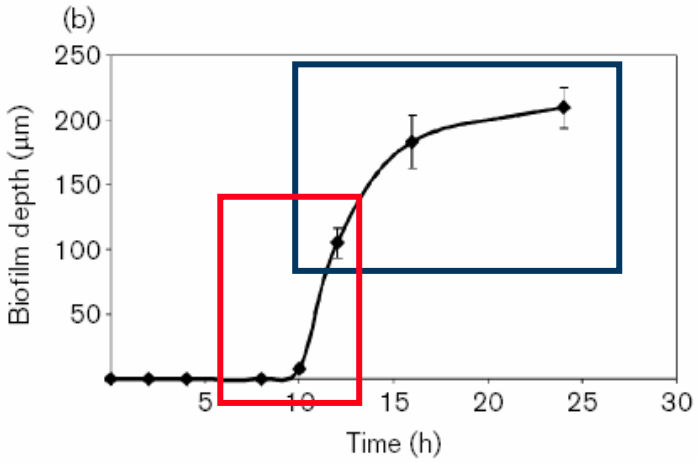


Image analysis

Data analysis Using Biopuce Software

Transcriptional Alterations

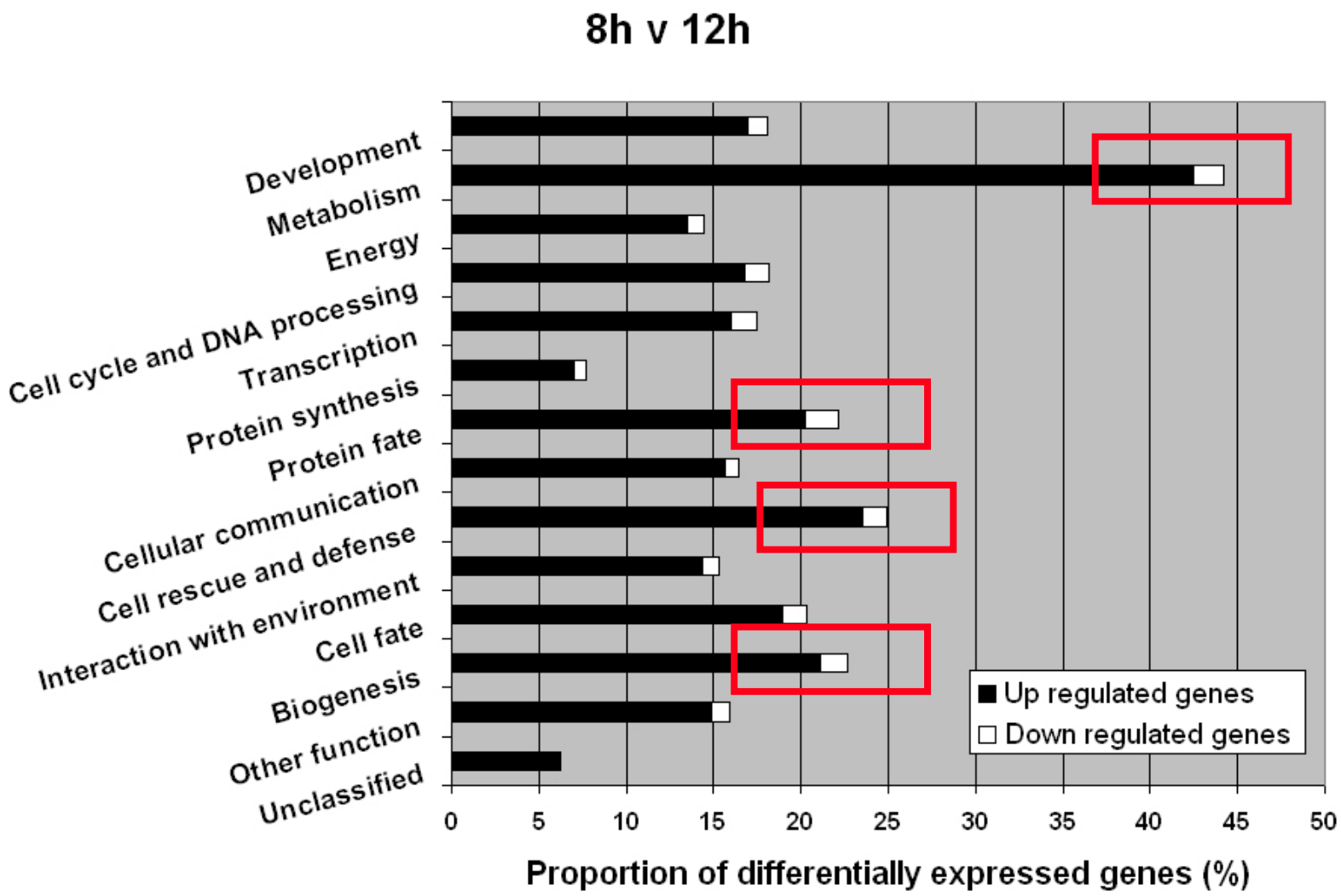




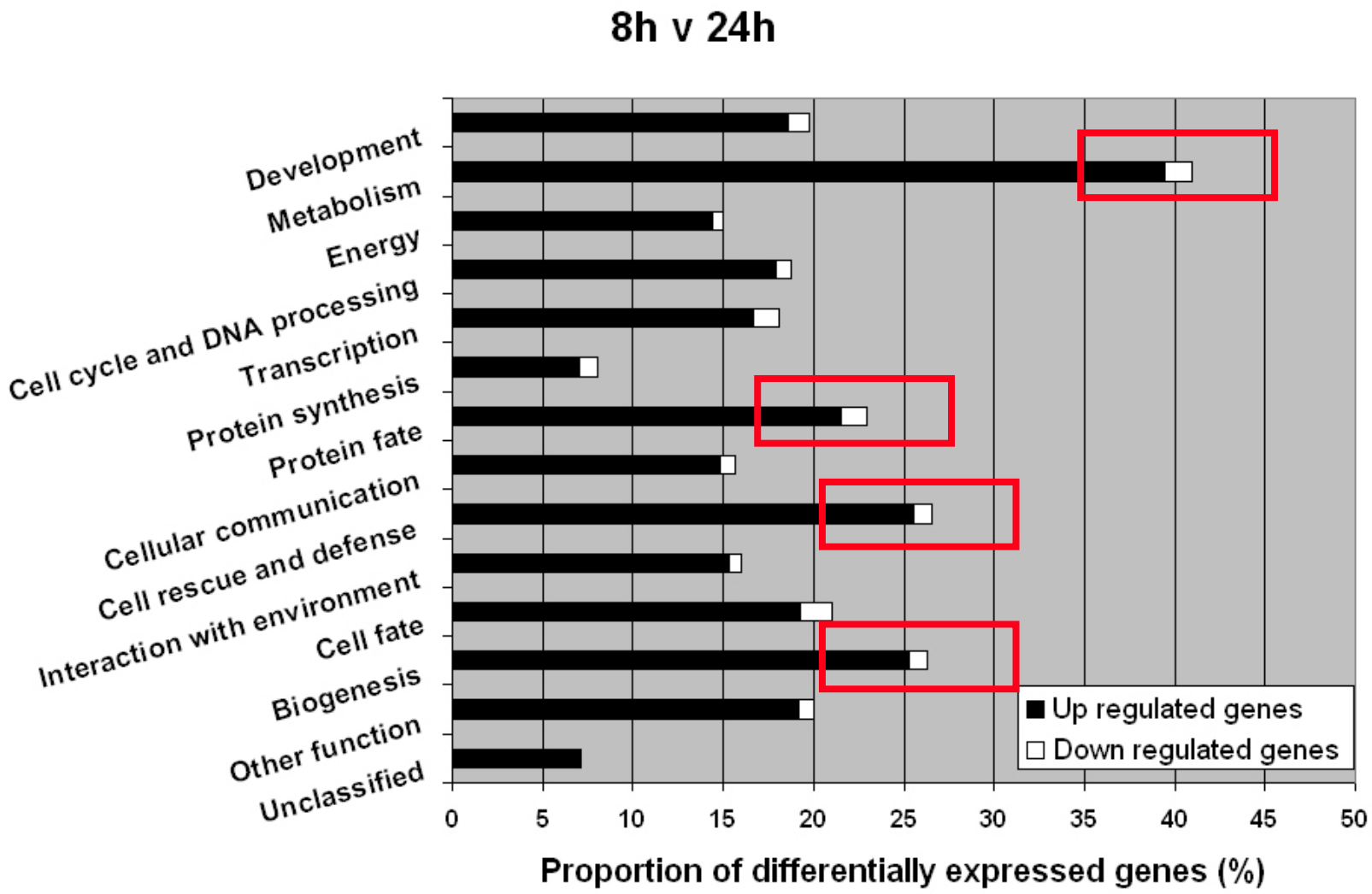
~ 30%

	8h			12h			24h		
	Norm	≥2	≤2	Norm	≥2	≤2	Norm	≥2	≤2
8h				8.2 (599)	7.3 (140)	84.5 (1611)	35.9 (599)	1.6 (65)	60.2 (1003)
12h	8.2 (156)	84.5 (1611)	7.3 (140)				96.4 (1317)	1.4 (19)	2.2 (30)
24h	35.9 (599)	60.2 (1003)	3.5 (65)	96.4 (1317)	2.2 (30)	1.4 (19)			

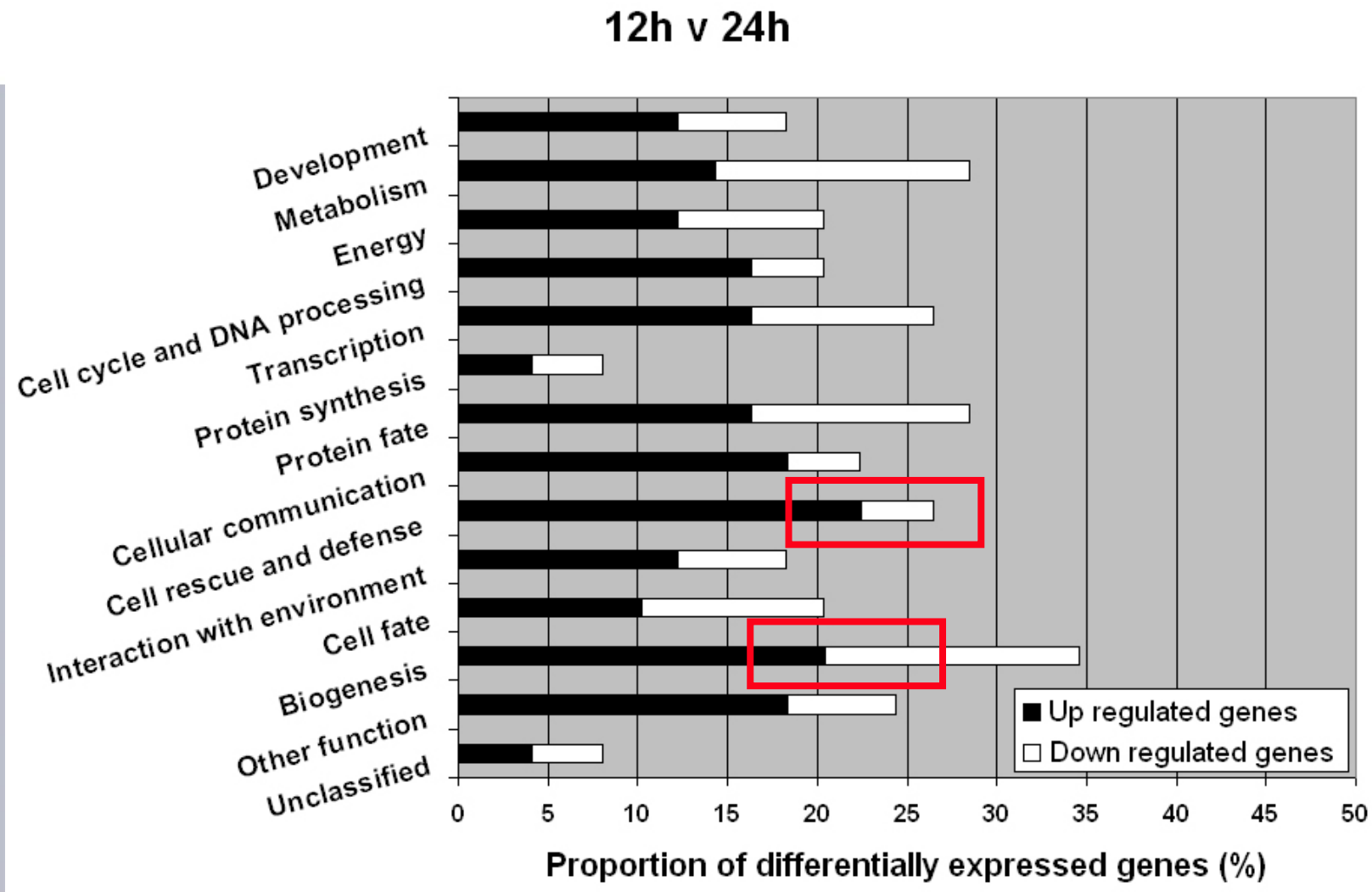
Active growth and differentiation



Active growth and differentiation



Stabilisation



Genes of Interest

Class	Gene name	Function	Putative	8 v 12	8 v 24	12 v 24
Cell wall structure	Rho2	GTP-binding	Yes	0.37	0.79	2.12
	Rho4	GTP-binding	Yes	2.01	2.38	1.18
	GpaA	Vegetative growth		2.65	3.41	1.23
	GpaB	Vegetative growth		3.27	2.66	0.81
	ChsE	Chitin synthase activator		2.89	3.08	1.06
	Gel3	Glucanosyltranferase		2.19	1.39	0.63
Immunity	PskP	Polyketide synthetase		2.49	2.76	1.11
	RodA	Conidial hydrophobin		2.43	1.84	0.76
	Cat2	Catalase peroxidase		2.64	2.78	1.05
	SodA	Superoxide dismutase		2.40	1.93	0.8
	Mrd2	ABC transporter		0.37	0.63	1.70
	Mdr4	ABC transporter		1.98	2.17	1.10

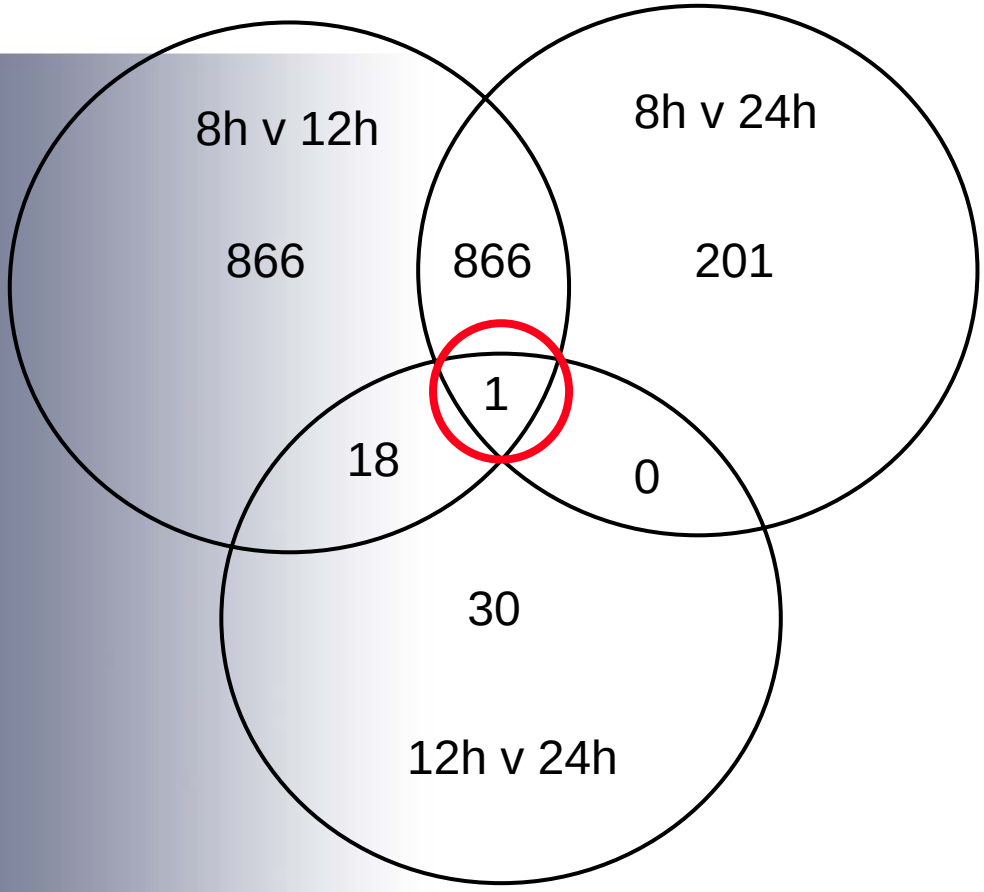
Antifungal Resistance – *mdr?*

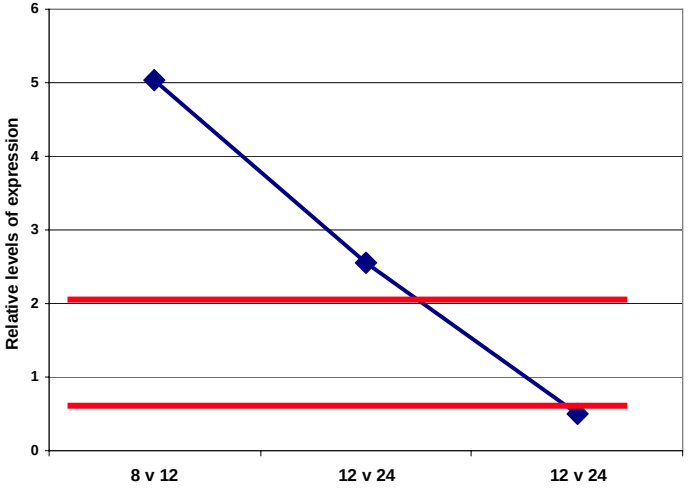
	Vfend®			Ambisome®			Cancidas®		
	PMIC	**SMIC 50**	**SMIC 90**	**PMIC**	**SMIC 50**	**SMIC 90**	**PMIC**	**SMIC 50**	**SMIC 90**
Af293	0.25	16	>256	1.0	0.125	32	0.25	128	>256
YHCF1	0.25	16	>256	1.0	0.125	16	0.25	128	>256
YHCF2	1.0	128	>256	0.25	<0.125	8	0.25	64	>256
YHCF3	0.5	32	>256	1.0	<0.125	32	0.25	64	>256
YHCF4	0.25	16	>256	0.5	1	16	0.25	64	>256

Other Genes of Interest

Class	Gene name	Function	Putative	8 v 12	8 v 24	12 v 24
Other	Fos-1	Sensor histidine kinase-regulator		2.21	1.46	0.67
	PkaR	cAMP protein kinase		3.35	2.82	0.84
	LysF	Homoaconitase		2.31	1.84	0.80
	Asp F2	Major allergen		3.11	2.70	0.87
	SirT	Pyridine oxidoreductase	Yes	5.03	2.55	0.51
	KexB	Endoprotease		0.35	0.33	0.94
	RasB	GTPase		2.21	2.37	1.07

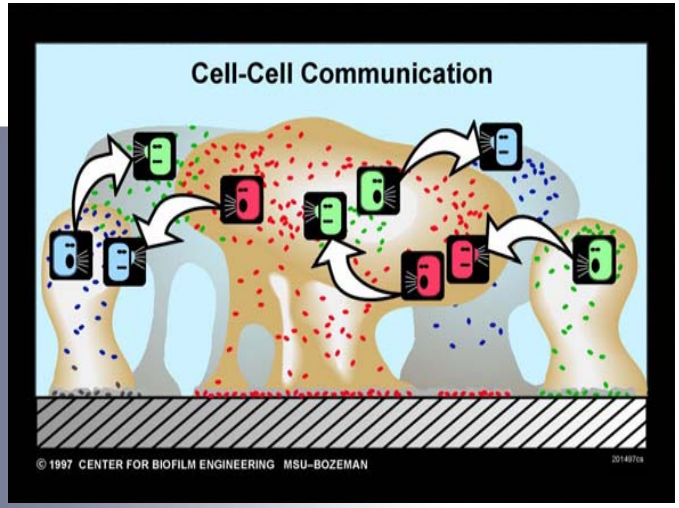
Cell Survival – Ageing

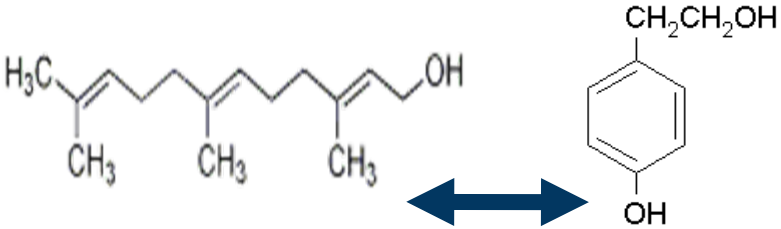


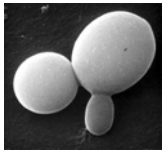


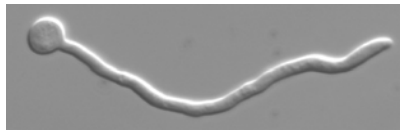
GilT (SirT)

Cell Cell Communication

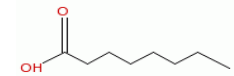




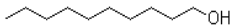




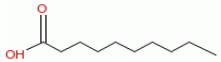
Fos-1



Octanoic acid



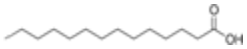
Decanol



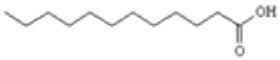
Decanoic acid

Dodecanol

Tetradecanol



Tetradecanoic acid
(Myrisitc acid)



Dodecanoic acid (Lauric acid)

Conclusions

- *Aspergillus* can form biofilms
- Complex and recalcitrant to antifungals
- Early phase of biofilm development is transcriptionally active

Acknowledgements

Glasgow

- **Miss Eilidh Mowat**
- Dr Craig Williams
- Mr John Butcher
- Mrs Helen Kennedy

Toulouse

- Prof J-M Francois & colleagues