

## **Educational Workshop**

### **EW09 How to diagnose and treat bacterial and fungal biofilm infections**

Arranged with the ESCMID Study Group for Biofilms (ESGB)

**Convenors:**      **Cornelia Lass-Flörl (Innsbruck, AT)**  
                         **Thomas Bjarnsholt (Copenhagen, DK)**

**Faculty:**            **Gordon Ramage (Glasgow, United Kingdom)**  
                         - no handout available  
                         **Mariana Henriques (Braga, Portugal)**  
                         **Thomas Bjarnsholt (Copenhagen, Denmark)**  
                         - no handout available  
                         **Claus Moser (Copenhagen, Denmark)**  
                         - no handout available



# Henriques - Fungal biofilm treatment



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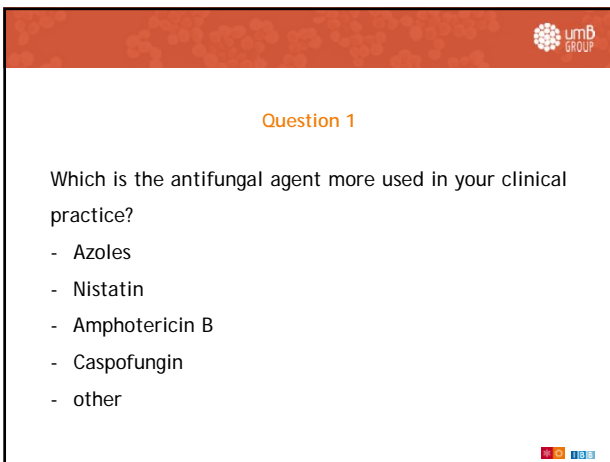
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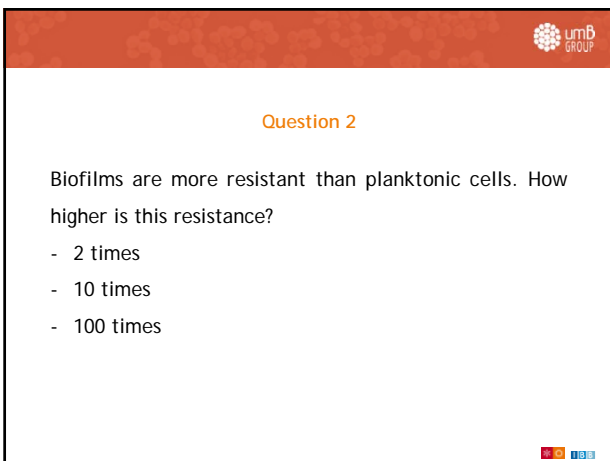
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# Henriques - Fungal biofilm treatment



**Fungal Biofilm Resistance** 

Table I.1. Minimum inhibitory concentrations (MICs) for planktonic cells and biofilms of *Candida tropicalis*

C. glabrata strains	MIC FLU (mg/L)	MIC AmB (mg/L)
ATCC 2001	>1250	>2
AE2	625-1250	>2
D1	625-1250	>2
534784	312.5-625	1-2
585626	625-1250	1-2
513100	50-312.5	0.4-0.5
562123	40-50	0.3-0.5



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**Fungal Biofilm Resistance** 

**Caspofungin**

Species	Plank MIC (mg/L)	Biof MIC (mg/L)
<i>Candida glabrata</i> ATCC 2001	0,0001	3
<i>Candida glabrata</i> 562123	0,0001-0,0005	0,5
<i>Candida glabrata</i> 534784	0,0005	1-1,5
<i>Candida glabrata</i> AE2	0,0005	0,5-1
<i>Candida glabrata</i> D1	0,0005	3
<i>Candida glabrata</i> 513100	0,0005	2-2,5
<i>Candida glabrata</i> 585626	0,0005	2-2,5
<i>Candida albicans</i> SC5314	0,0075-0,015	2,5-3
<i>Candida parapsilosis</i> ATCC	0,005-0,0075	2-2,5
<i>Candida tropicalis</i> ATCC 750	0,005-0,0075	2-2,5



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**Fungal Biofilm Resistance** 

**WHY?**





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# Henriques - Fungal biofilm treatment

**Question 3**

Which is the factor that has more influence on biofilm resistance?

- High cell density
- Altered cell phenotype
- Biofilm Matrix

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**Fungal Biofilm Resistance**

**Cell Density**

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**Fungal Biofilm Resistance**

**Cell Density**

Antifungal agent

Biofilm formation      Pre-formed biofilm

Figure. Total biomass quantification. Biofilms subject to different concentrations of Fluconazole

Fonseca et al., Biofouling: The Journal of Bioadhesion and Biofilm Research, 30(4), 447-457, 2014

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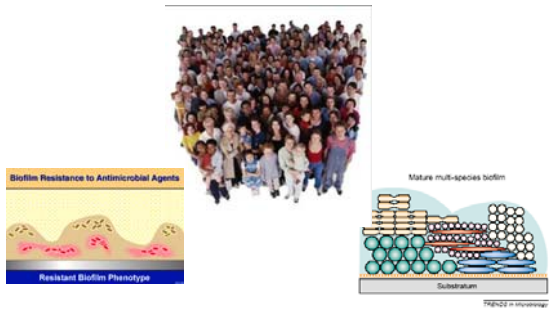
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# Henriques - Fungal biofilm treatment

**Fungal Biofilm Resistance** 

**Cell alterations**




**Biofilm Resistance to Antimicrobial Agents**

**Resistant Biofilm Phenotype**

**Mature multi-species biofilm**

**Substratum**

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**Fungal Biofilm Resistance** 

**Cell alterations**

Gene	Protein	Function
<i>SNQ2</i>	Plasma membrane ATP-binding cassette (ABC) transporter	Multidrug resistance Pdr1p-mediated azole resistance
<i>CDR1</i>	Plasma membrane ATP-binding cassette (ABC) transporter	Resistance to azoles Expression regulated by Pdr1p
<i>PDR1</i>	Zinc finger transcription factor	Activator of drug resistance genes via pleiotropic drug response elements (PDRE) Regulates drug efflux pumps Controls multi-drug resistance
<i>ERG3</i>	Δ-5,6 sterol desaturase C-5 sterol desaturase	Predicted transmembrane domain and endoplasmic reticulum (ER) binding motif
<i>ERG6</i>	C-24 sterol methyltransferase;	Mutation confers resistance to amphotericin B and nystatin and increased sensitivity to azoles
<i>ERG11</i>	Cytochrome P-450 lanosterol 14α-demethylase	Synthesis of ergosterol Target enzyme of azole antifungal drugs



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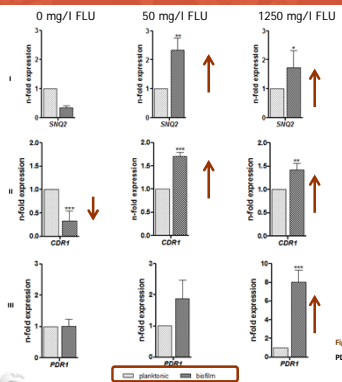
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**Fungal Biofilm Resistance** 



**0 mg/l FLU**    **50 mg/l FLU**    **1250 mg/l FLU**

**I** n-fold expression of *SNQ2*

**II** n-fold expression of *CDR1*


**III** n-fold expression of *PDR1*

Legend: █ planktonic    █ biofilm

In the absence of FLU, CDR1 is underexpressed in biofilms  
 ✓ In the presence of FLU genes are overexpressed in biofilm cells

Figure. Mean values of n-fold expression levels of SNQ2 (I), CDR1 (II) and PDR1 (III) genes in *C. glabrata* 562123 treated with Fluconazole.

Fonseca et al., Biofouling: The Journal of Bioadhesion and Biofilm Research, 30(4), 447-457, 2014



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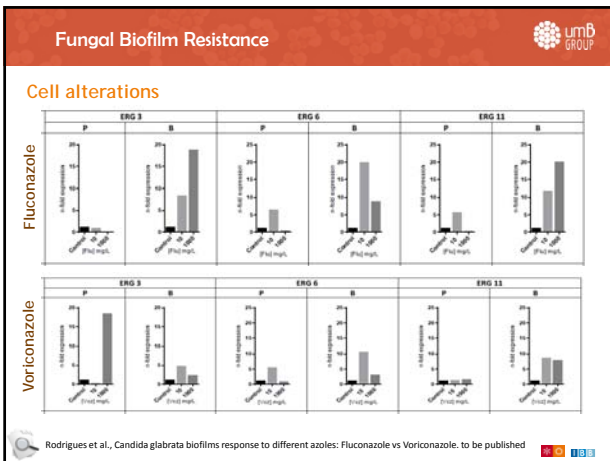
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# Henriques - Fungal biofilm treatment




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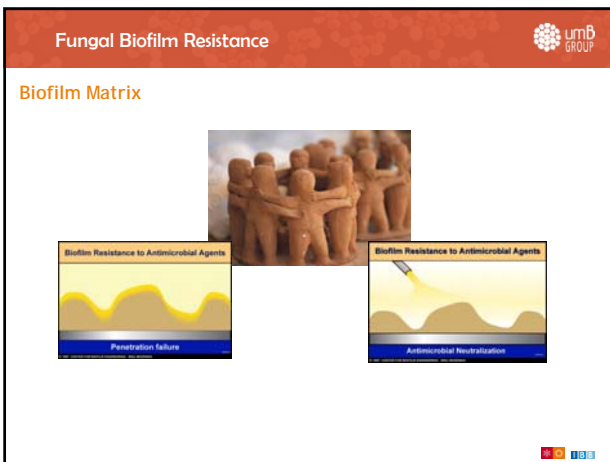
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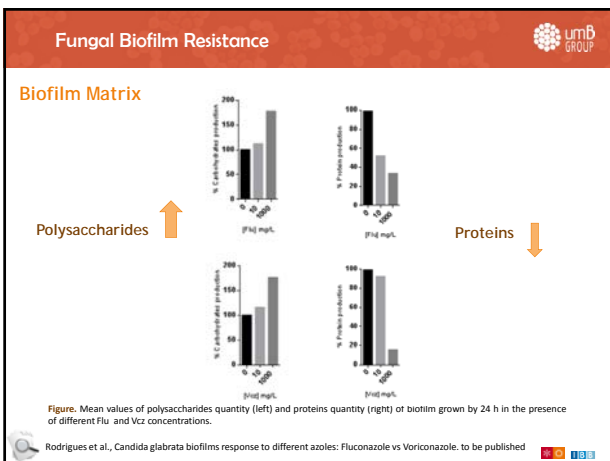
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# Henriques - Fungal biofilm treatment



**Question 4**

Do you know new alternatives for fungal biofilm treatments?

- Yes
- No



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**Fungal biofilms treatment: new alternatives**

New alternatives

- Silver nanoparticles
- Natural plants
- PDT



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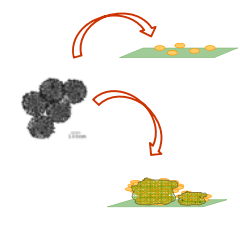
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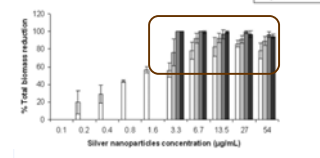
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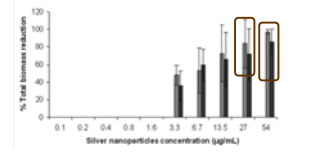
**Fungal Biofilm Treatment**

**Silver Nanoparticles**






Legend:  
 □ C. albicans ATCC 9002  
 □ C. albicans ZNCL459  
 ■ C. glabrata ATCC 90030  
 ■ C. glabrata ZP1



Monteiro et al., Biofouling: The Journal of Bioadhesion and Biofilm Research, 27(7), 711-719, 2011



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# Henriques - Fungal biofilm treatment

**Fungal Biofilm Treatment** 

**Silver Nanoparticles**




SN  
13.5 µg/ml  
27 µg/ml

Nistatin  
13.5 µg/ml  
216 µg/ml

Chlorhexidine  
9 µg/ml  
37.5 µg/ml

*C. albicans*  
*C. glabrata*

Monteiro et al., Mycoses, 56(6), 672-680, 2013 

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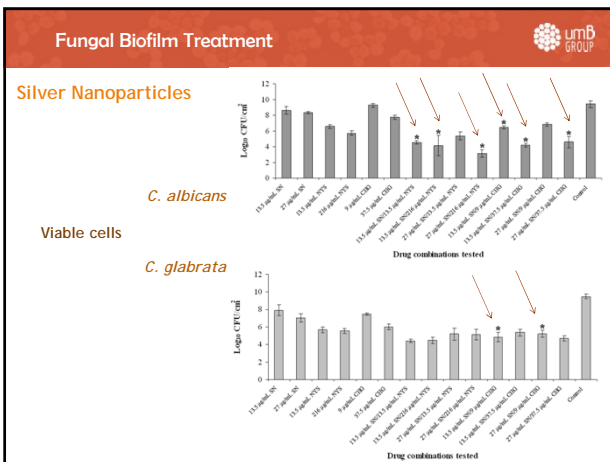
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**Fungal Biofilm Treatment** 

**Natural Plants**



*Origanum vulgare*

*Thymus vulgaris*

*Salvia officinalis*

Martins et al., Food Chemistry, (In Press), 2014 

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# Henriques - Fungal biofilm treatment

**Fungal Biofilm Treatment**

	<i>Origanum vulgare</i> L.			<i>Salvia officinalis</i> L.			<i>Thymus vulgaris</i> L.		
	Infusion	Decoction	Hydroalcoholic extract	Infusion	Decoction	Hydroalcoholic extract	Infusion	Decoction	Hydroalcoholic extract
<i>C. albicans</i> ATCC 90028	-	-	-	-	-	-	-	-	-
<i>C. albicans</i> 575541	-	-	-	+	+	-	+	+	-
<i>C. albicans</i> 557834	+	+	-	-	-	-	-	-	-
<i>C. albicans</i> 558234	-	-	-	-	-	-	-	-	-
<i>C. glabrata</i> ATCC 2001	-	-	-	-	-	-	-	-	-
<i>C. glabrata</i> D1	-	-	-	-	-	-	-	-	-
<i>C. glabrata</i> 513200	-	-	-	-	-	-	-	-	-
<i>C. parapsilosis</i> ATCC 22019	-	-	-	+	+	-	-	-	-
<i>C. parapsilosis</i> ATC2	-	-	-	-	-	-	-	-	-
<i>C. parapsilosis</i> AD	-	-	++	-	-	++	++	+	+
<i>C. parapsilosis</i> 491861	+	+	-	-	+	-	+	+	-
<i>C. parapsilosis</i> 513143	-	+	-	-	-	-	-	-	+
<i>C. tropicalis</i> ATCC 750	-	-	-	++	+++	++	-	-	-
<i>C. tropicalis</i> AG1	-	-	-	-	-	-	++	+++	++
<i>C. tropicalis</i> T5	-	+	+	-	-	-	-	-	+
<i>C. tropicalis</i> T2.2	-	-	-	-	+	+	-	-	-

(-) absence of halo, 0.0 mm; (+) weak halo, 0.3-0.7 mm; (++) moderate halo, 0.8-1.0 mm; (+++) stronger halo, greater than 1.0 mm

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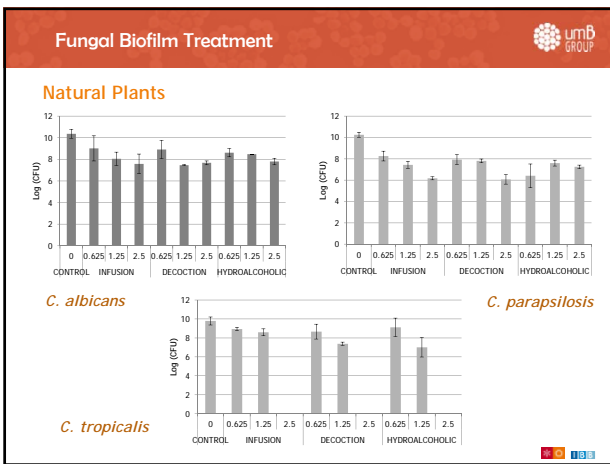
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**Fungal Biofilm Treatment**

**Natural Plants**

*Croton nepetaefolius* - Brazilian plant  
 Casbane diterpene - ethanolic extract

Carneiro, et al. *Molecules*, 16(1), 190-201, 2011

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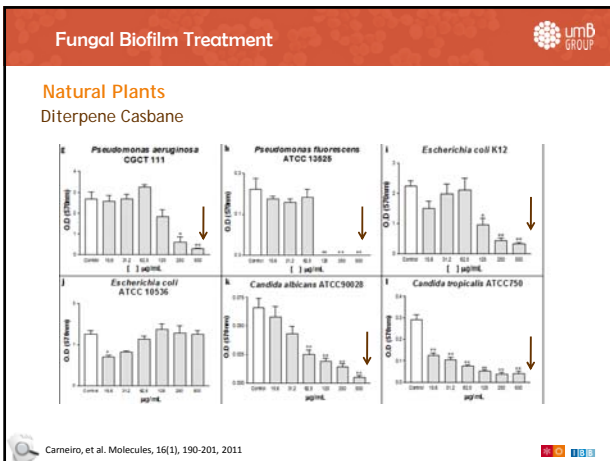
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# Henriques - Fungal biofilm treatment




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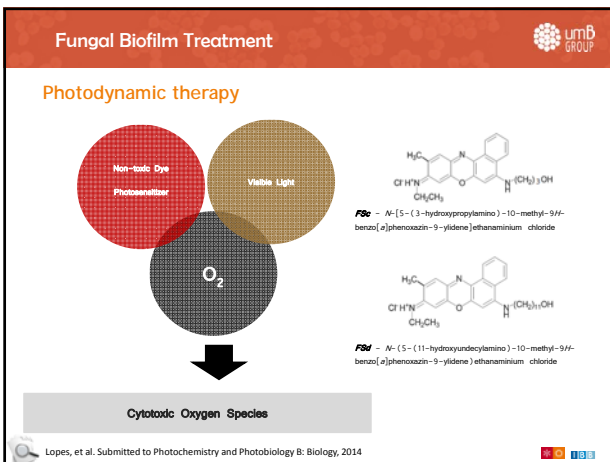
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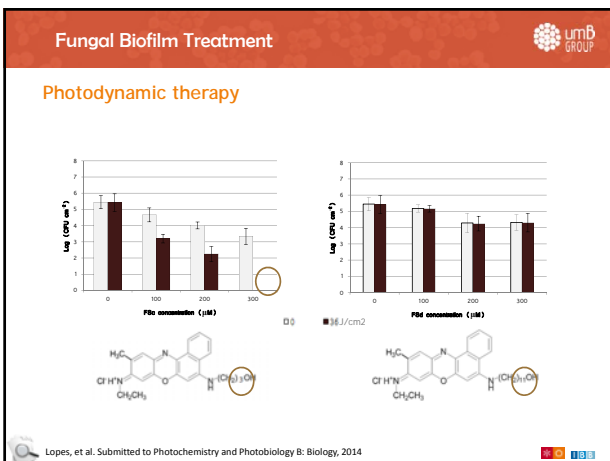
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# Henriques - Fungal biofilm treatment

**Fungal Biofilm Treatment** 

**Photodynamic therapy**

Initial Concentration of dye ( $\mu\text{M}$ )	Concentration of Dye on Supernatant ( $\mu\text{M}$ )								
	Dye retained on Biofilm Matrix ( $\mu\text{M}$ )				Dye absorbed by cells ( $\mu\text{M}$ )				
	3 h		18 h		3 h		18 h		
<b>F8c</b>	100	0,54	0,03	0,89	0,25	1,49	0,43	3,46	0,44
	300	2,22	0,21	8,65	1,03	1,98	0,54	13,52	4,90
<b>F8d</b>	100	0,48	0,31	1,25	0,21	0,13	0,02	0,70	0,04
	300	0,61	0,27	1,61	0,04	0,14	0,03	0,64	0,19

Lopes, et al. Submitted to Photochemistry and Photobiology B: Biology, 2014 

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**Conclusions**

- ✓ Better diagnostic
- ✓ Improved treatments
- ✓ Evaluation of antifungal activity on biofilms



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**Acknowledgments** 

- ✓ This was only possible with the contribution of the Candida biofilm group



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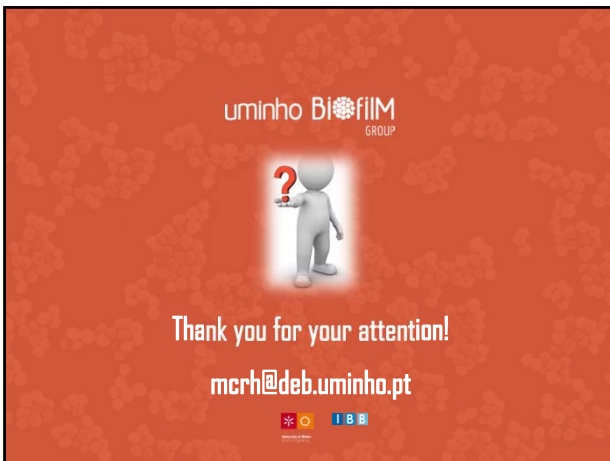
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# Henriques - Fungal biofilm treatment



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