



# Melaninas fúngicas: importância na biologia celular e na patogênese

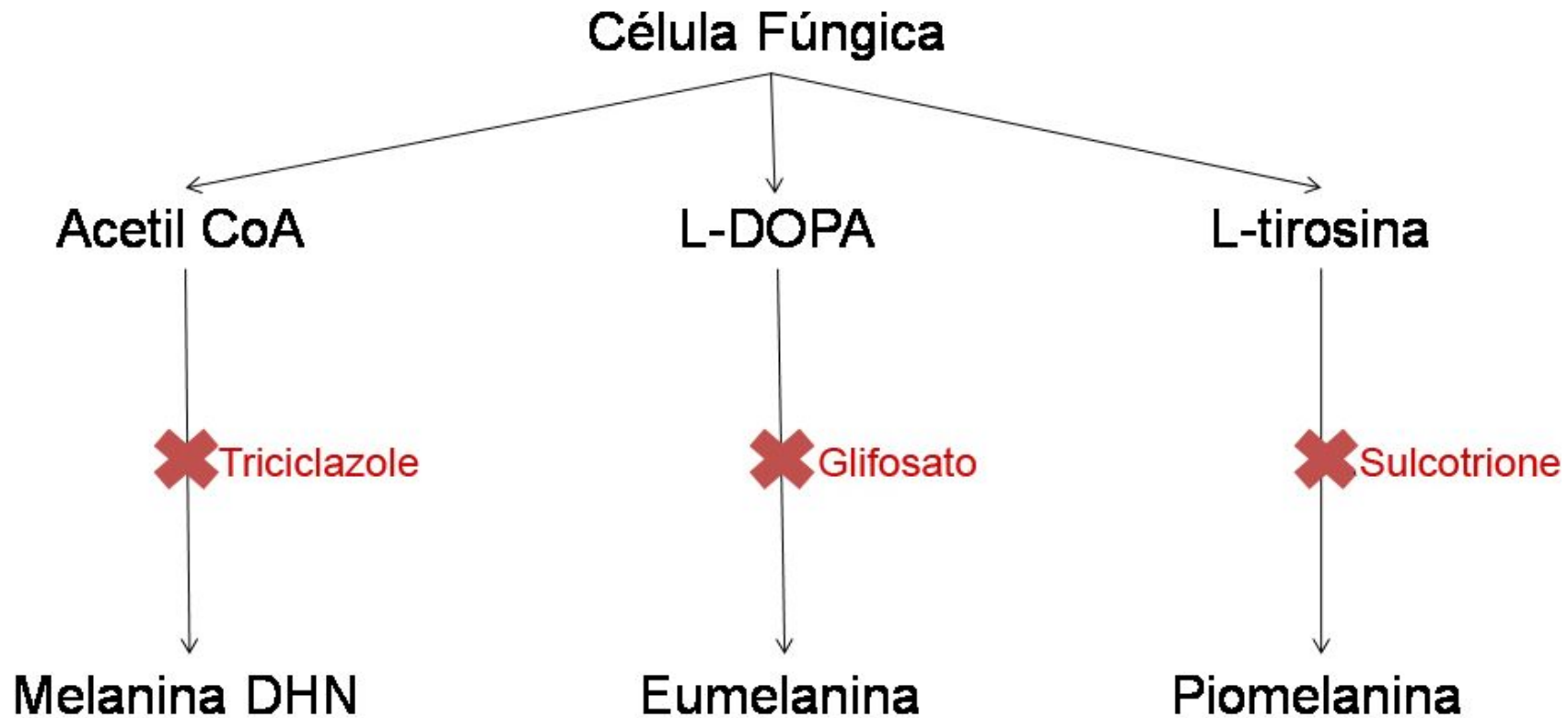
**Rodrigo de Almeida Paes**

rodrigo.paes@ini.fiocruz.br

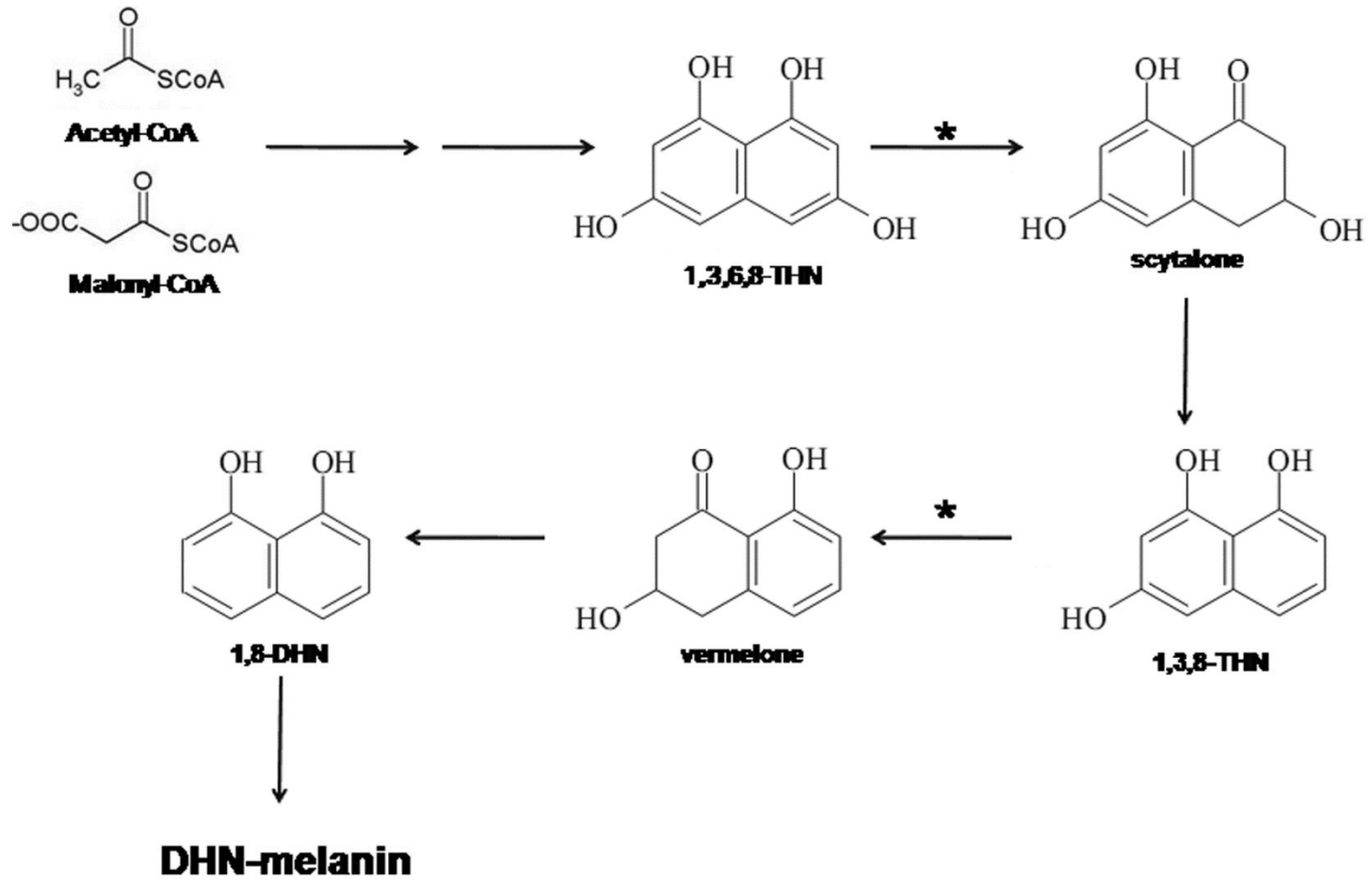
**Fundação Oswaldo Cruz  
Instituto Nacional de Infectologia Evandro Chagas  
Rio de Janeiro – RJ**



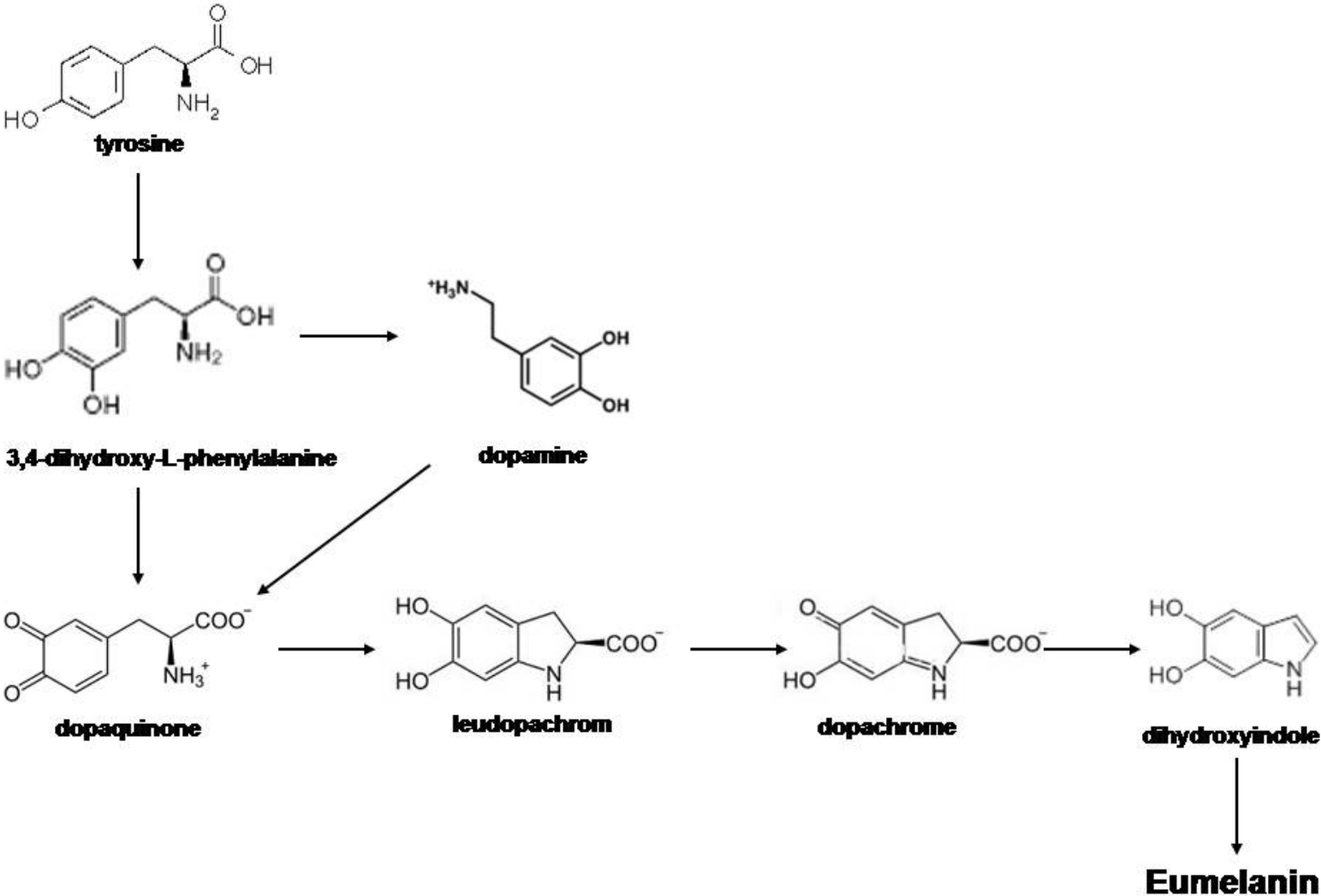
# Tipos de melanina



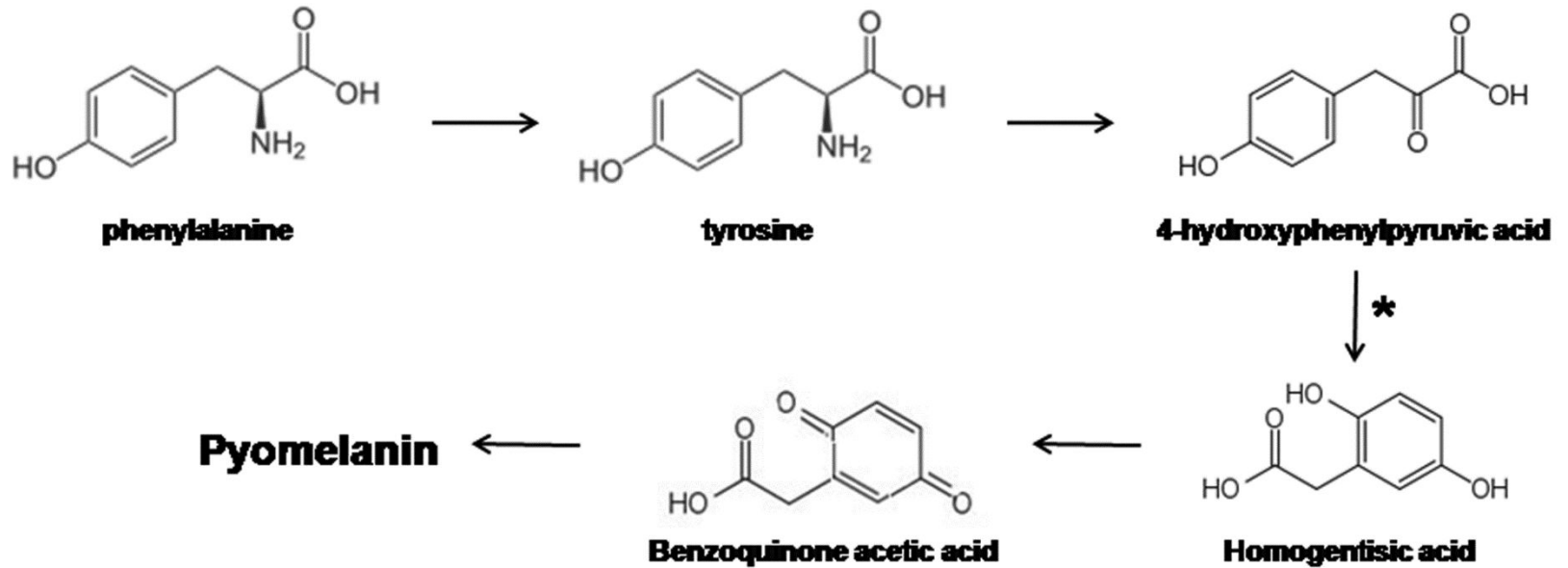
# Melanina DHN



# Eumelanina



# Piomelanina





# Fungos produtores de melanina

- *Cryptococcus neoformans*
  - *Fonsecaea pedrosoi*
  - *Aspergillus fumigatus*
  - *Sporothrix schenckii*
  - *Histoplasma capsulatum*
  - *Candida albicans*
  - *Paracoccidioides brasiliensis*
  
  - E muitos outros...
-



# O modelo *C. neoformans*

- Eumelanina
- Proteção contra:
  - Luz ultravioleta
  - Enzimas proteolíticas
  - Frio e calor
  - Fagocitose
  - Anfotericina B
- Ação comprovada na virulência



# Melaninas fúngicas

## Rio de Janeiro - Fiocruz

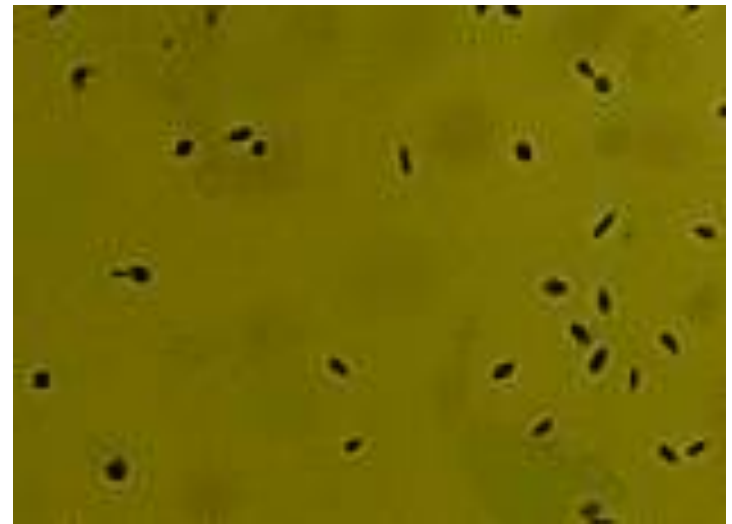
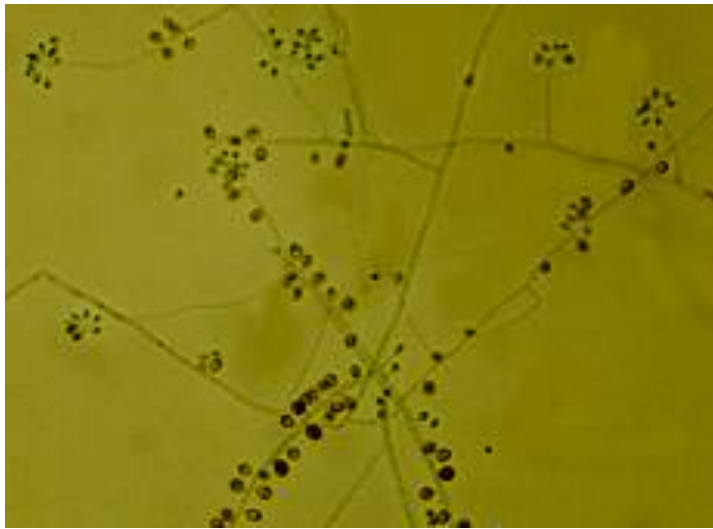


- Complexo *Sporothrix schenckii*
- *Histoplasma capsulatum*
- *Trichosporon* sp.
- *Candida glabrata*



# *Sporothrix schenckii*

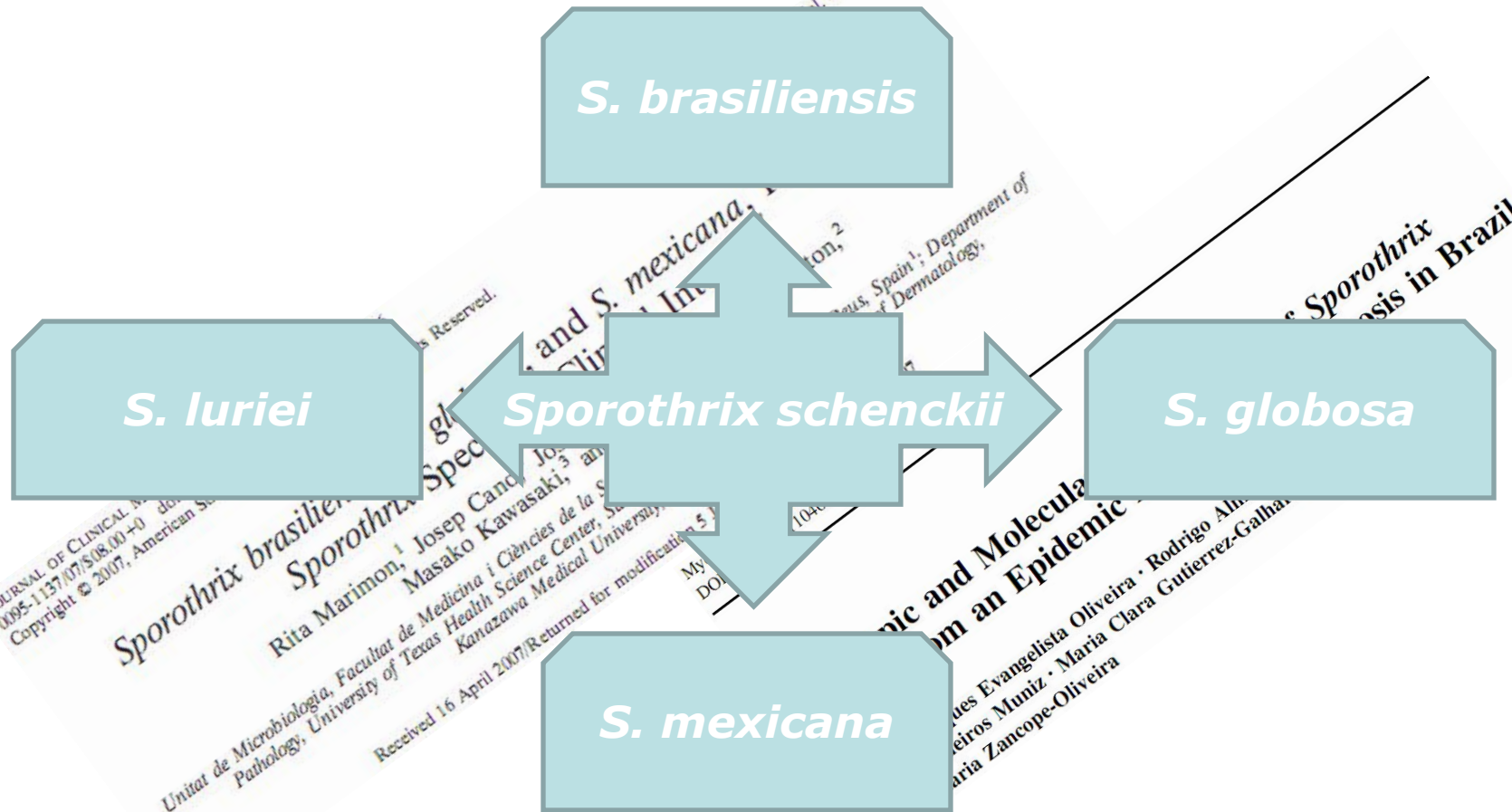
- Fungo patogênico, causador da esporotricose
- Fungo dimórfico



- Habitat natural: madeira ou solo



# Novas espécies de *Sporothrix*



JOURNAL OF CLINICAL MICROBIOLOGY, Vol. 45, No. 10  
0095-1137/07/\$08.00+0  
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*Sporothrix brasiliensis* and *S. mexicana*, Rita Marimon,<sup>1</sup> Josep Candolin,<sup>2</sup> Masako Kawasaki,<sup>3</sup> and Unitat de Microbiologia, Facultat de Medicina i Ciències de la Salut, Pathology, University of Texas Health Science Center, San Antonio, Texas, Kanazawa Medical University, Kanazawa, Japan  
Received 16 April 2007/Returned for modification 5 May 2007/DOI: 10.1128/JCM.01040-07

*Sporothrix schenckii* from an Epidemic in Brazil  
Rosa Evangelista Oliveira · Rodrigo Almeida Muniz · Maria Clara Gutierrez-Galha · Maria Zancoppe-Oliveira

# Esporotricose

- Micosse subcutânea
- Adquirida por inoculação traumática do fungo na pele
- Casos raros de infecção por inalação de conídios
- Quadro clínico variado
- Afeta o homem e diversos mamíferos



# Endemia de Esporotricose no Rio de Janeiro







INSTITUT PASTEUR

Microbes and Infection 11 (2009) 554–562



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Original article

# Growth conditions influence melanization of Brazilian clinical *Sporothrix schenckii* isolates

Rodrigo Almeida-Paes<sup>a,c,\*</sup>, Susana Frases<sup>d</sup>, Paulo Cezar Fialho Monteiro<sup>a</sup>,  
Maria Clara Gutierrez-Galhardo<sup>b</sup>, Rosely Maria Zancopé-Oliveira<sup>a,1</sup>, Joshua D. Nosanchuk<sup>c,d,1</sup>

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<sup>b</sup> Dermatologia Infecçiosa, Instituto de Pesquisa Clínica Evandro Chagas, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

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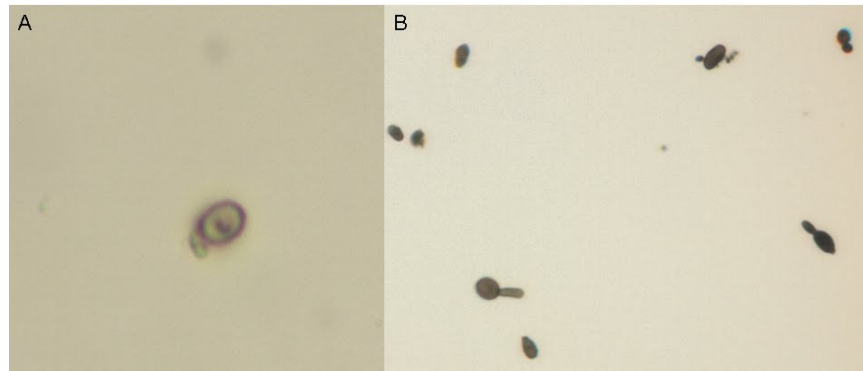
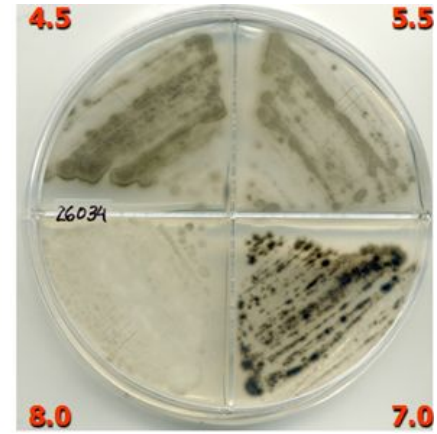
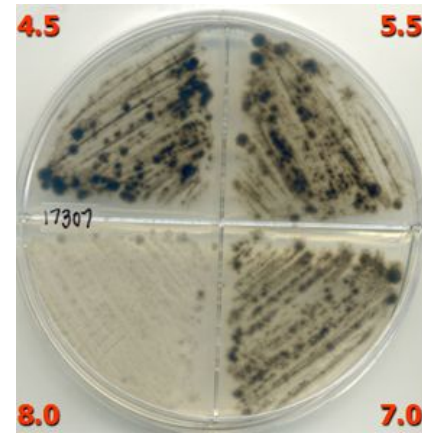
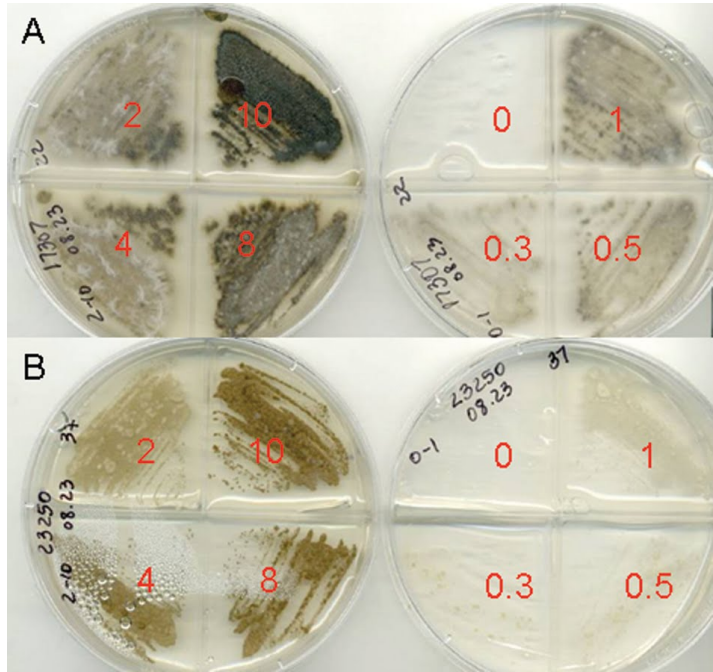
# Melanina DHN

- Condições de melanização

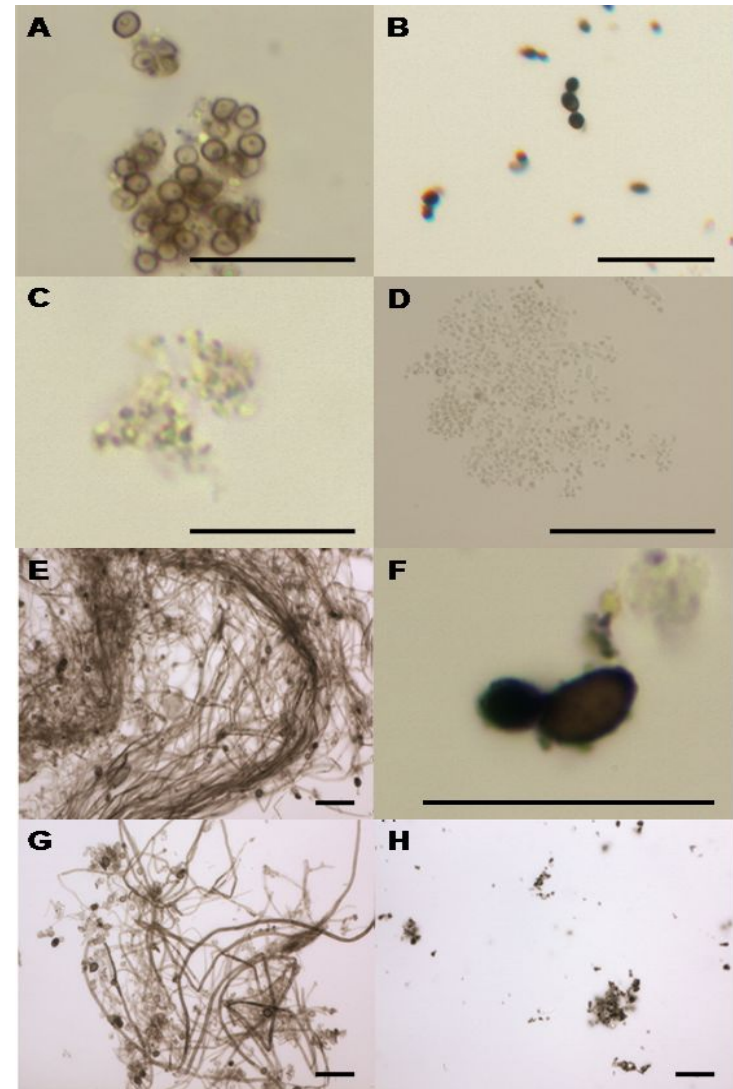
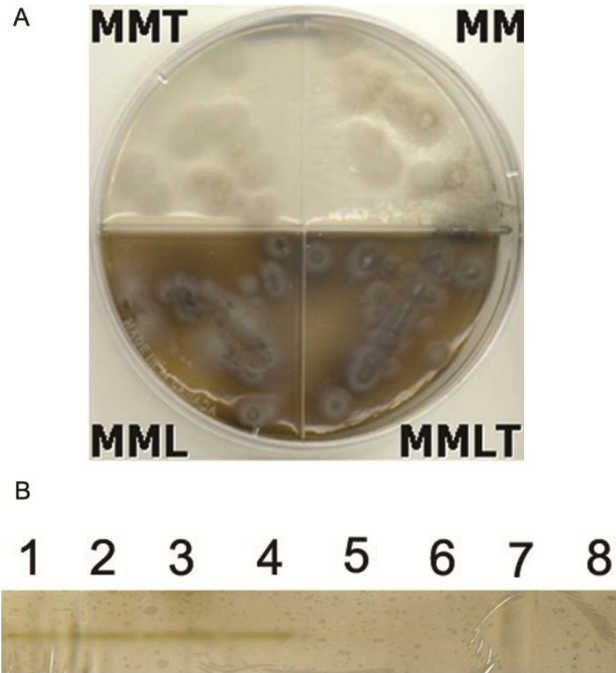
Profile of melanization and precedence of 26 *S. schenckii* strains used in this study.

Strain	Days to start melanization			Degree of melanization <sup>a</sup>			pH	Glucose enhancement (°C)
	Sab <sup>b</sup>	MM <sup>c</sup>	MMLD <sup>d</sup>	Sab	MM	MMLD		
United States ATCC 10212	NM <sup>e</sup>	NM	6	–	–	++	None	No
Espírito Santo State, Brazil								
IPEC 23249	NM	NM	10	–	–	+	None	37
IPEC 23250	NM	NM	12	–	–	+	None	37
IPEC 23251	9	7	4	+	+	+	Acid	No
IPEC 23252	NM	NM	15	–	–	+	None	37
IPEC 23253	3	2	2	+++	+++	+++	A/N <sup>f</sup>	No
Rio de Janeiro State, Brazil								
IPEC 17307	7	2	2	+++	+++	+++	A/N	22 and 30
IPEC 17331	7	2	2	++	+++	+++	Acid	No
IPEC 17521	15	11	12	+	+	++	A/N	No
IPEC 17585	8	3	3	+++	+++	+++	A/N	No
IPEC 17608	13	6	7	+++	+++	+++	Acid	22 and 30
IPEC 17692	30	6	6	+	+	+	Neutral	No
IPEC 17786A	16	10	2	+	+	+++	A/N	No
IPEC 17786B	NM	NM	12	–	–	+	None	No
IPEC 17920	2	2	2	++	+++	+++	A/N	No
IPEC 18202	2	2	2	++	+++	+++	A/N	22 and 30
IPEC 18782A	18	4	2	+	+++	+++	A/N	22 and 30
IPEC 18782B	NM	NM	12	–	–	+	None	No
IPEC 22582	15	5	3	+	++	+++	Neutral	22 and 30
IPEC 25374	29	14	6	+	+	++	Neutral	No
IPEC 25644	9	6	6	+++	+++	++	A/N	No
IPEC 25758	7	2	2	+	+++	+++	A/N	22 and 30
IPEC 25976	9	3	8	++	++	++	A/N	No
IPEC 26034	10	3	3	+++	+++	+++	Neutral	No
IPEC 26156	17	10	9	+	+++	+++	Acid	22 and 30
IPEC 26449	2	3	3	+++	+++	+++	Neutral	22 and 30

# Melanina DHN

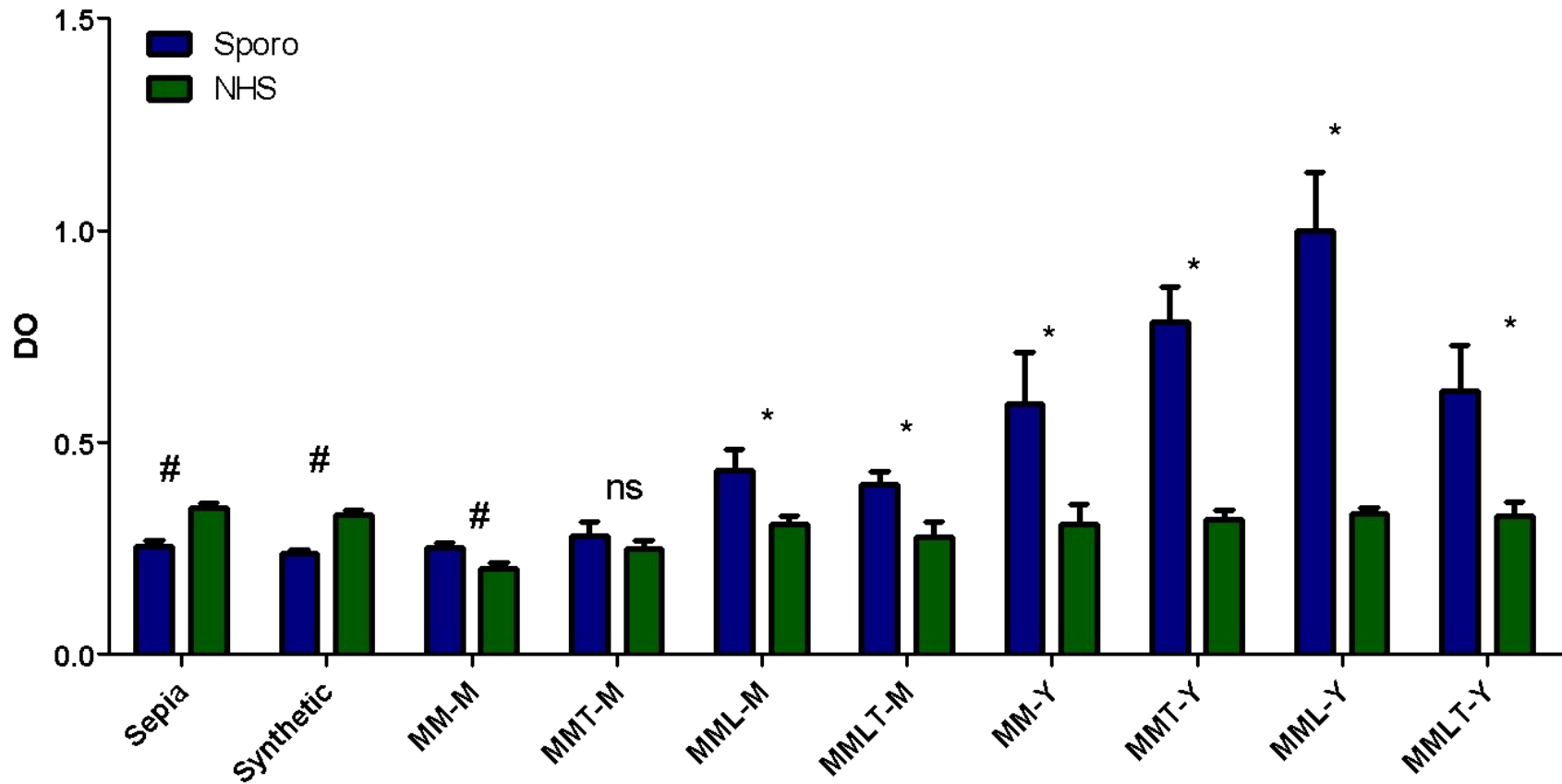


# Melanina L-DOPA





# Melanina L-DOPA



**Phenotypic characteristics associated with virulence of clinical isolates from the *Sporothrix* complex**

**Running title:** Virulence attributes of *Sporothrix* spp.

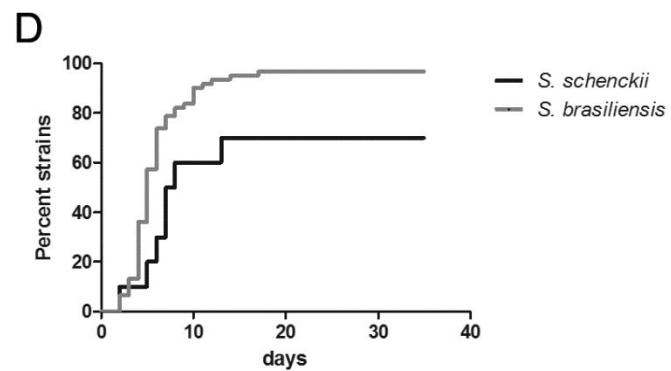
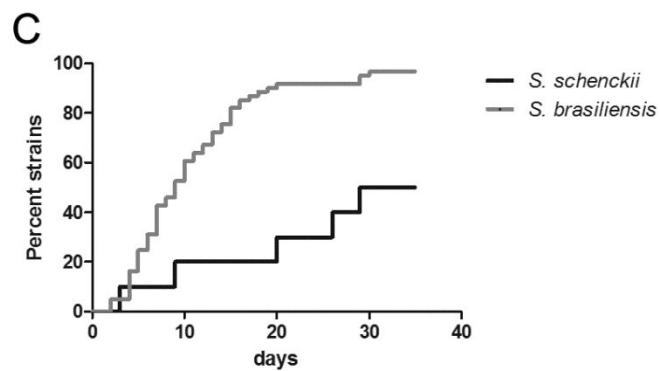
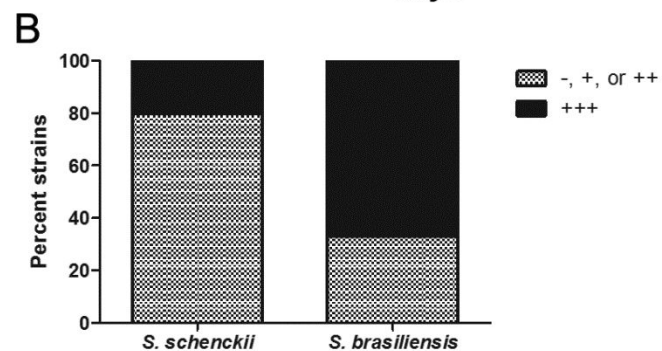
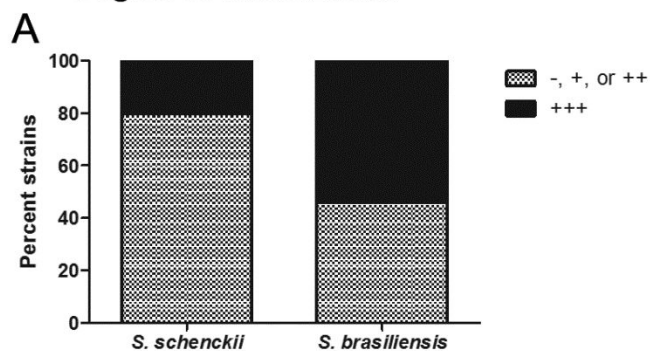
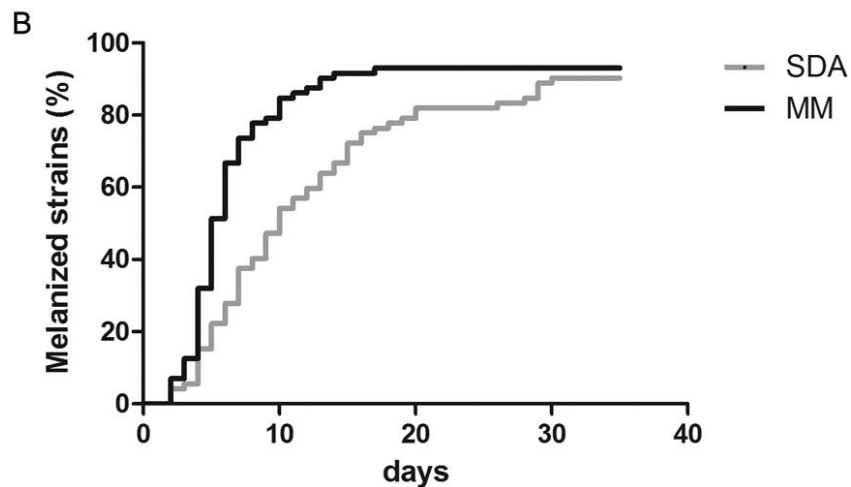
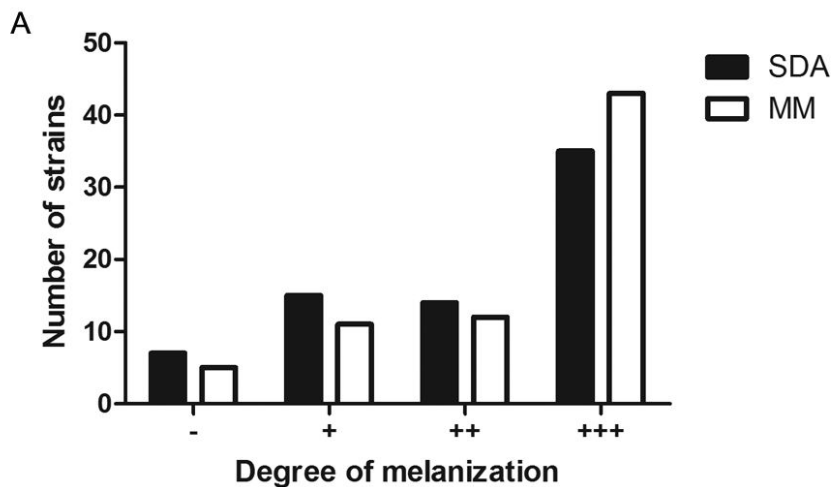
Rodrigo Almeida-Paes<sup>1\*</sup>, Luã Cardoso de Oliveira<sup>1</sup>, Manoel Marques Evangelista de Oliveira<sup>1</sup>,  
Maria Clara Gutierrez-Galhardo<sup>2</sup>, Joshua Daniel Nosanchuk<sup>3,4</sup>, Rosely Maria Zancopé-Oliveira<sup>1</sup>

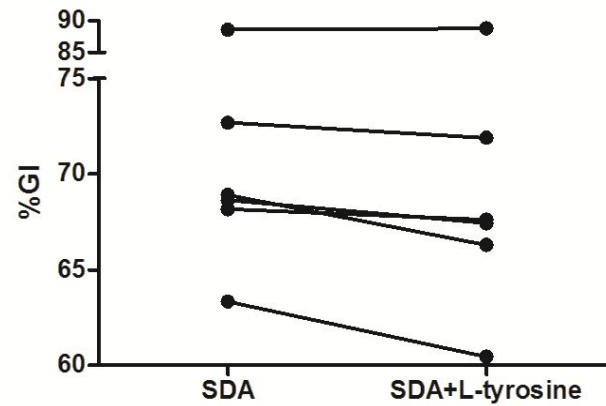
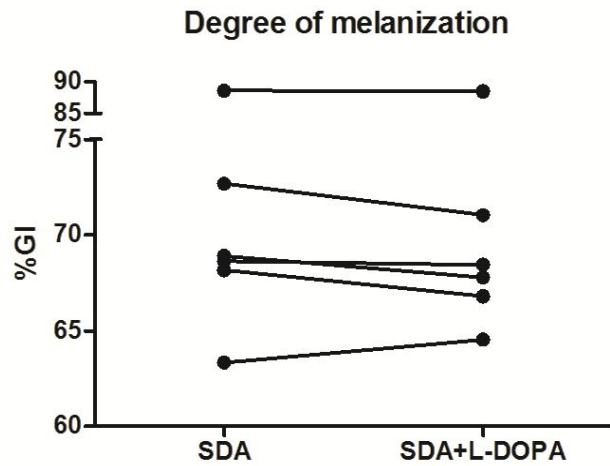
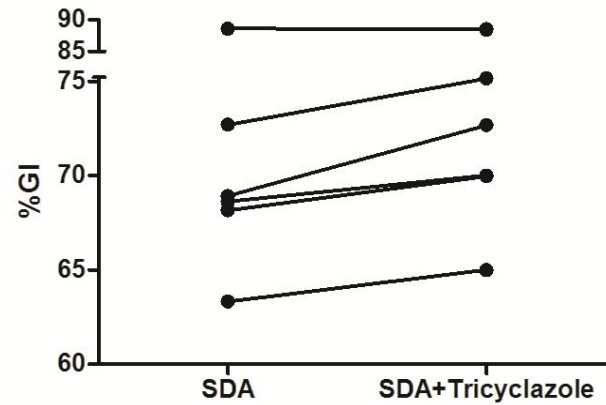
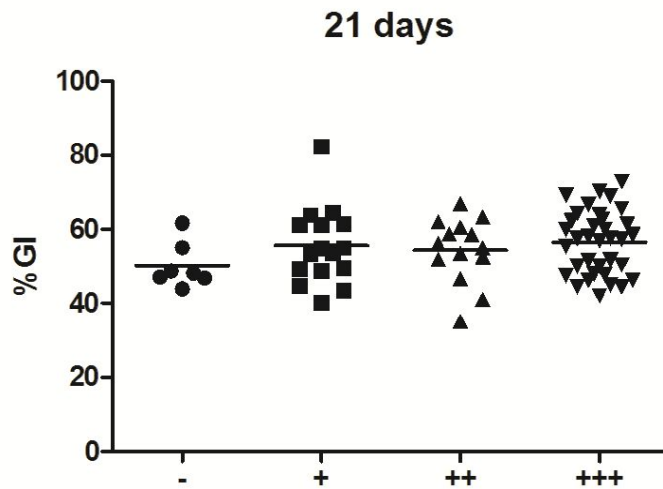
<sup>1</sup> Laboratório de Micologia, and <sup>2</sup> Laboratório de Dermatologia Infecciosa. Instituto Nacional de Infectologia Evandro Chagas, Fundação Oswaldo Cruz, Avenida Brasil, 4365, Manguinhos, 21045-900, Rio de Janeiro, Brazil.

<sup>3</sup> Departments of Medicine [Division of Infectious Diseases] and <sup>4</sup> Microbiology and Immunology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, 10461, Bronx, NY, USA.

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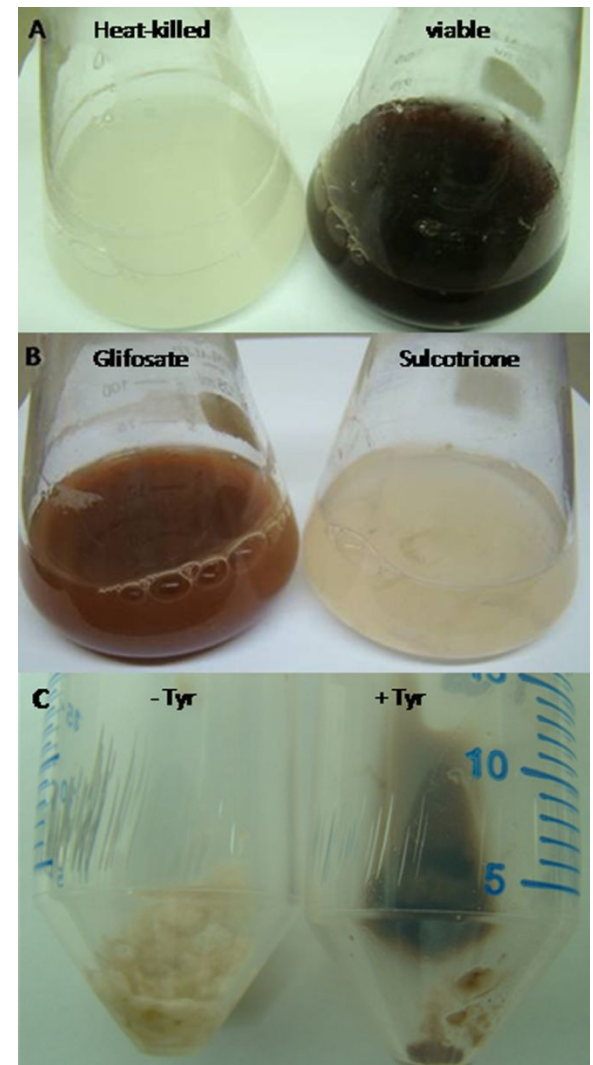
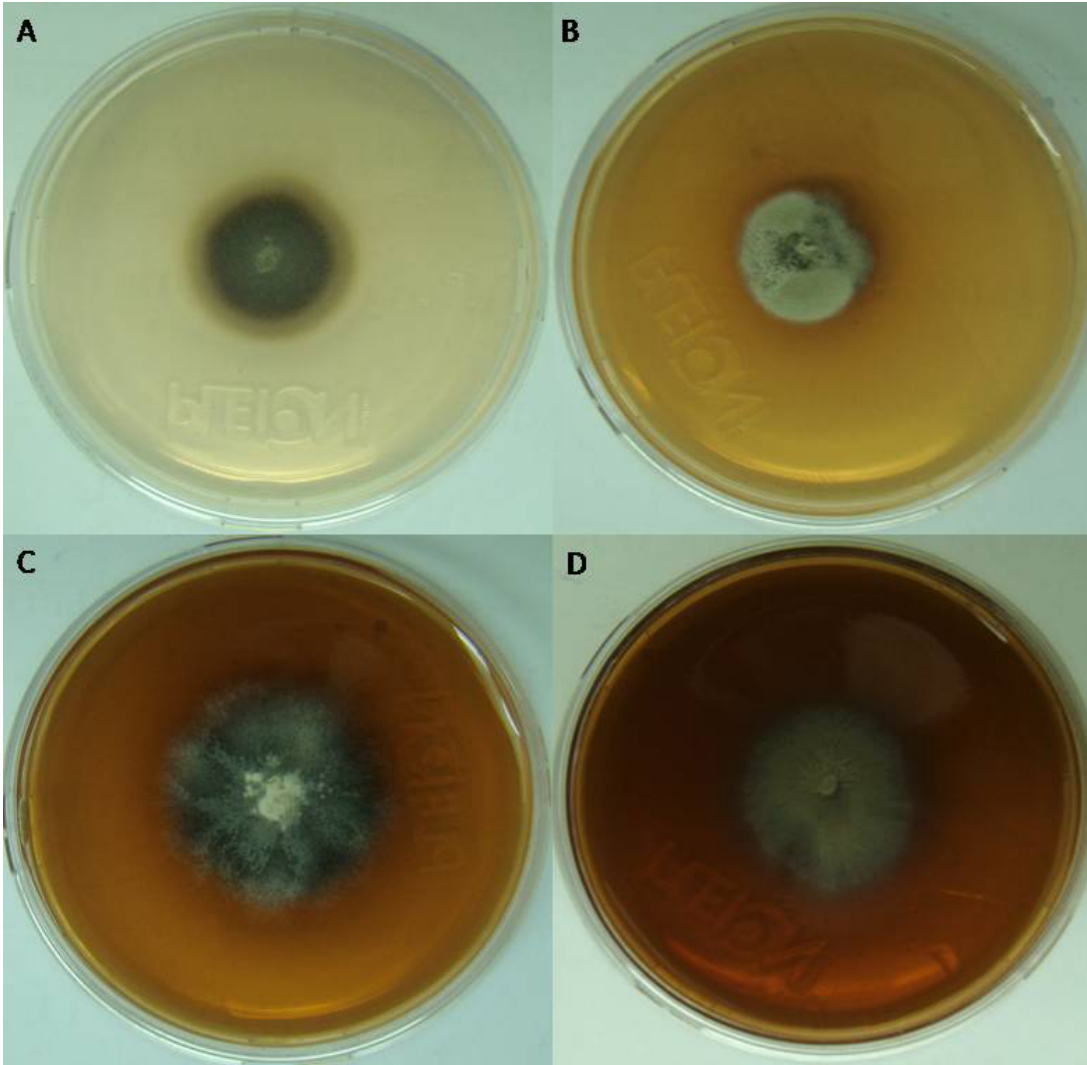
# Biosynthesis and Functions of a Melanoid Pigment Produced by Species of the *Sporothrix* Complex in the Presence of L-Tyrosine

Rodrigo Almeida-Paes,<sup>a</sup> Susana Frases,<sup>b,f</sup> Glauber de Sousa Araújo,<sup>b,f</sup> Manoel Marques Evangelista de Oliveira,<sup>a</sup> Gary J. Gerfen,<sup>c</sup> Joshua D. Nosanchuk,<sup>d,e</sup> and Rosely Maria Zancopé-Oliveira<sup>a</sup>

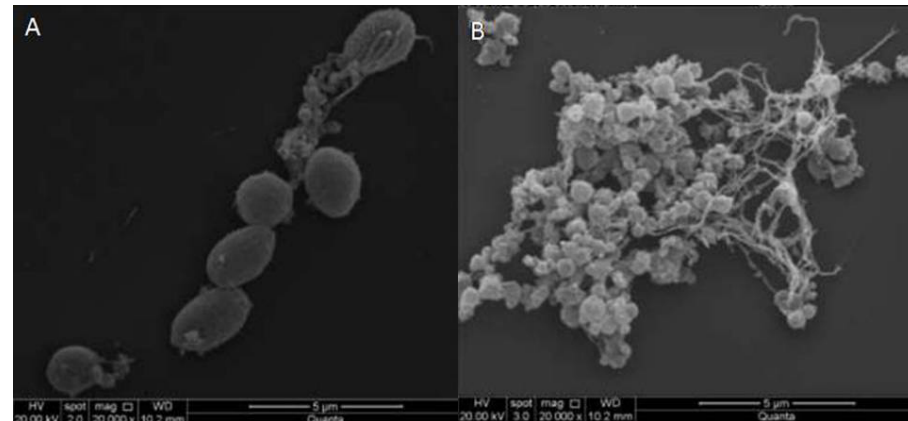
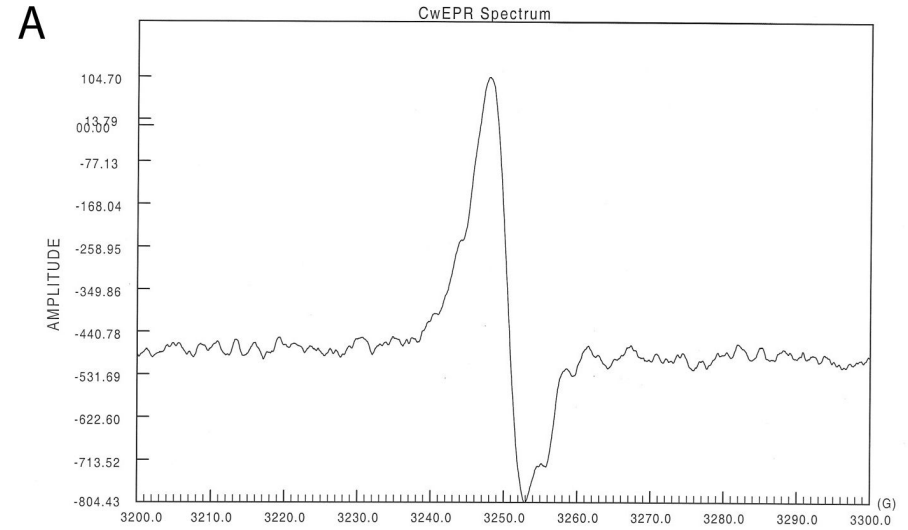
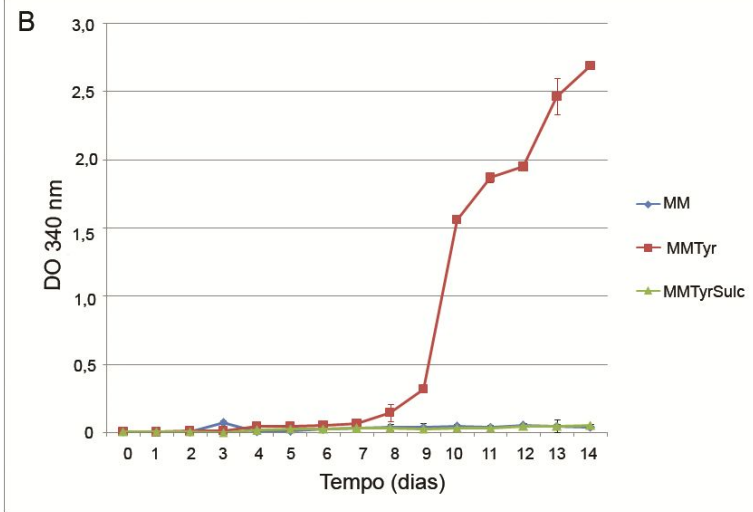
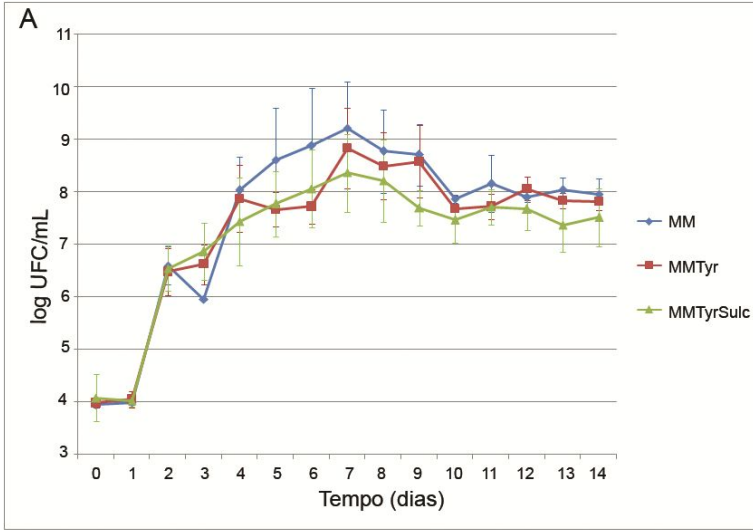
Laboratório de Micologia, Instituto de Pesquisa Clínica Evandro Chagas, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil<sup>a</sup>; Instituto de Biofísica Prof. Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil<sup>b</sup>; Departments of Physiology and Biophysics,<sup>c</sup> Medicine, Division of Infectious Diseases,<sup>d</sup> Microbiology and Immunology,<sup>e</sup> Albert Einstein College of Medicine, Yeshiva University, Bronx, New York, USA; and Laboratório de Biotecnologia, Instituto Nacional de Metrologia, Normalização e Qualidade Industrial, Rio de Janeiro, Brazil<sup>f</sup>



# Piomelanina



# Piomelanina



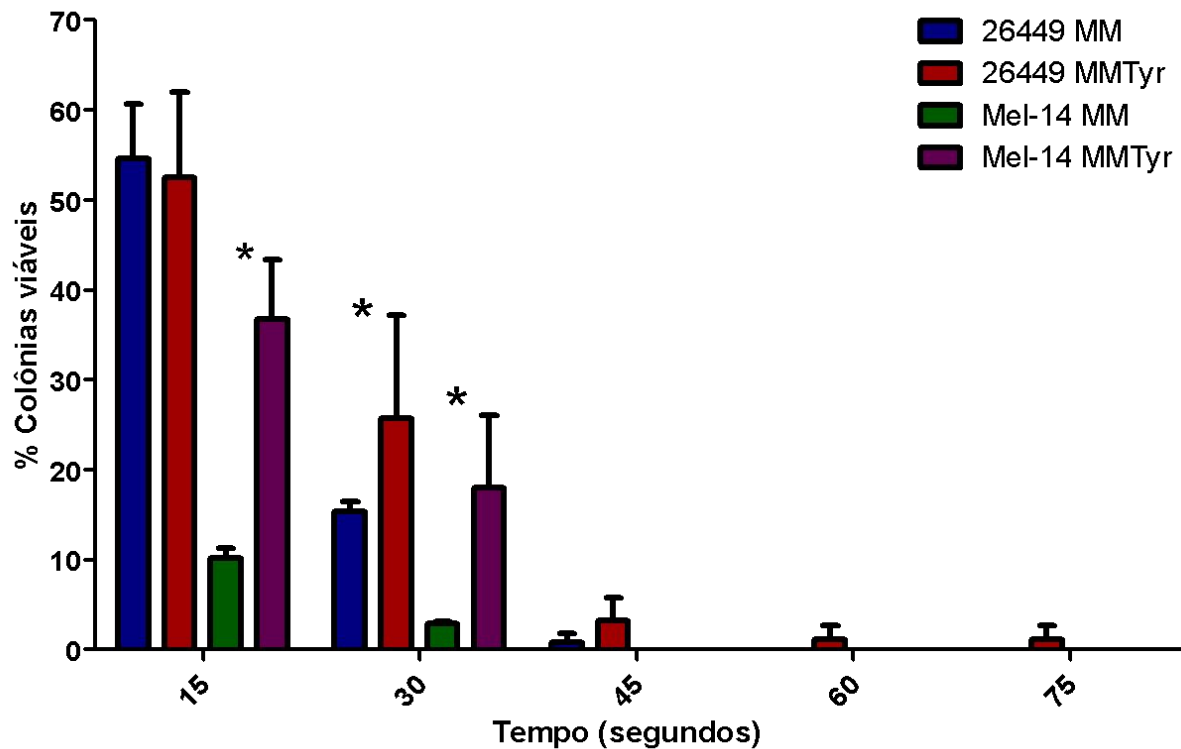


# Papel biológico



Luz UV

NO



Tempo (horas)	MM	MM Tir <sup>a</sup>	MM Tir Sulc <sup>b</sup>
1	22,90±6,39%	64,56±36,30%	23,65±10,80%
2	5,55±1,47%	21,41±7,54%	7,01±1,27%
3	2,51±0,93%	7,32±2,42%	1,34±0,41%
4	0,51±0,12%	2,78±1,95%	0,50±0,39%

Original article

## Melanin biosynthesis in *Madurella mycetomatis* and its effect on susceptibility to itraconazole and ketoconazole

Wendy W.J. van de Sande <sup>a,\*</sup>, Johan de Kat <sup>a</sup>, Jojanneke Coppens <sup>b</sup>, Abdalla O.A. Ahmed <sup>c</sup>,  
Ahmed Fahal <sup>d</sup>, Henri Verbrugh <sup>a</sup>, Alex van Belkum <sup>a</sup>



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Microbes and Infection 8 (2006) 197–205

Microbes and  
Infection

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Original article

## Melanin in the dimorphic fungal pathogen *Paracoccidioides brasiliensis*: effects on phagocytosis, intracellular resistance and drug susceptibility

Marcelo B. da Silva <sup>a</sup>, Alexandre F. Marques <sup>a</sup>, Josh D. Nosanchuk <sup>b</sup>, Arturo Casadevall <sup>b</sup>,  
Luiz R. Travassos <sup>c</sup>, Carlos P. Taborda <sup>a,\*</sup>

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 2002, p. 3394–340

0066-4804/02/\$04.00+0 DOI: 10.1128/AAC.46.11.3394–3400.2002

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## Melanization of *Cryptococcus neoformans* and *Histoplasma capsulatum* Reduces Their Susceptibilities to Amphotericin B and Caspofungin

David van Duin, <sup>1</sup> Arturo Casadevall, <sup>1,2</sup> and Joshua D. Nosanchuk <sup>1,\*</sup>

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1994, p. 2648–2650

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Vol. 38, No. 11

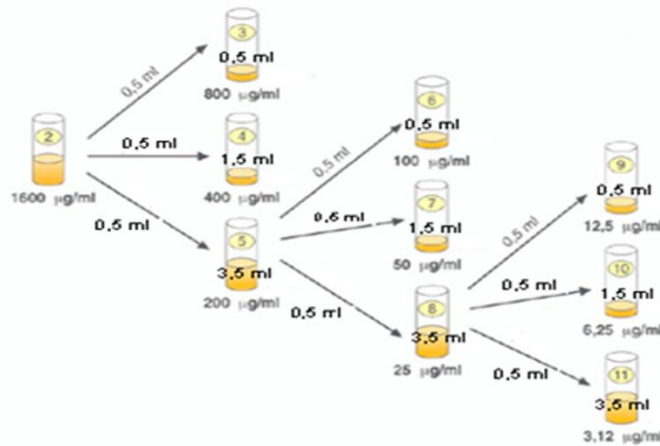
## NOTES

### Growth of *Cryptococcus neoformans* in Presence of L-Dopa Decreases Its Susceptibility to Amphotericin B

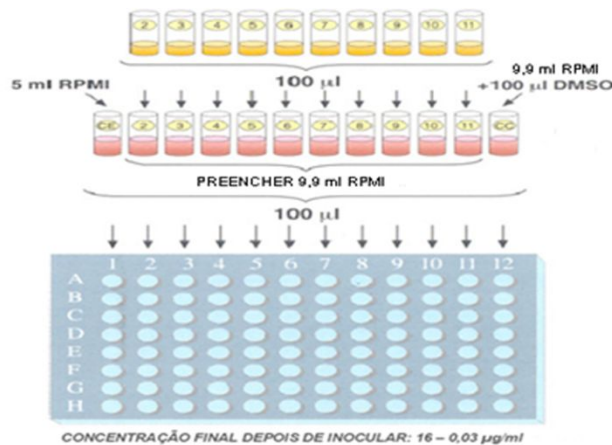
YULIN WANG <sup>1</sup> AND ARTURO CASADEVALL <sup>1,2,\*</sup>

# Susceptibilidade a antifúngicos

A



B



- Fármacos:
  - Itraconazol
  - Cetoconazol
  - Anfotericina B
  - Terbinafina
- Nenhum tipo de melanina afetou a CIM de seis isolados de *Sporothrix* testados
  - Melanizados pré-ensaio
  - Melanizados durante o ensaio

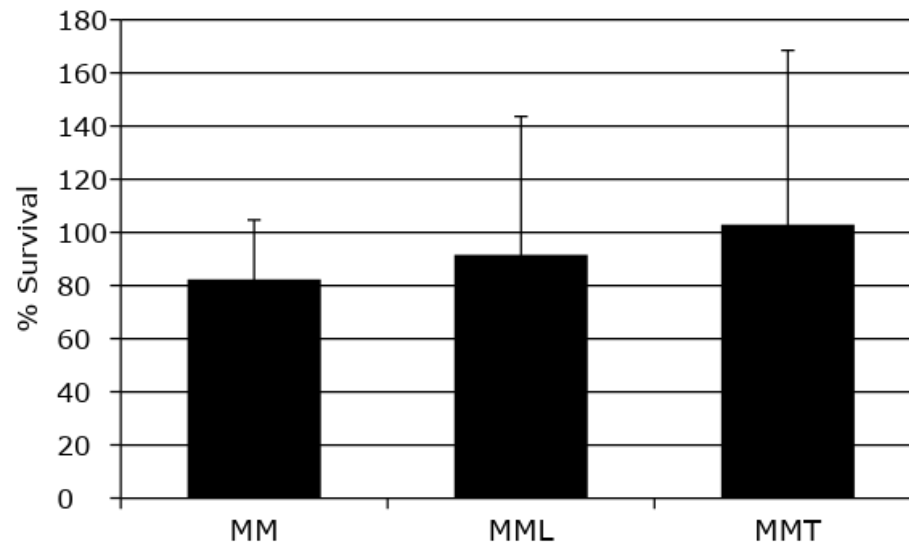
# Susceptibilidade a antifúngicos

- Proteção contra efeito fungicida

Tempo (h)	MM		MM com L-DOPA		MM com L-tirosina	
	0,5 AnfoB	2,0 AnfoB	0,5 AnfoB	2,0 AnfoB	0,5 AnfoB	2,0 AnfoB
2	12,51±5,67	1,17±0,51	6,98±0,29	4,88±1,55	70,04±36,17	25,81±7,98
6	0,55±0,14	0	1,8±1,27	0	8,47±4,44	0
24	0,14±0,08	0	2,02±0,40	0	4,70±3,82	0

# Suscetibilidade a antifúngicos

- Proteção contra efeito fungistático
  - Terbinafina







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1 SHORT COMMUNICATION

2  
3 **L-Dihydroxyphenylalanine induces melanin production by**  
4 **1,2 members of the genus *Trichosporon***

5  
6 Maria Helena Galdino Figueiredo de Carvalho<sup>1</sup>, Fábio B. dos Santos<sup>1</sup>, Joshua D. Nosanchuk<sup>2</sup>,  
7 Rosely M. Zancope-Oliveira<sup>1</sup> & Rodrigo Almeida-Paes<sup>1</sup>

8 <sup>1</sup>Laboratório de Micologia, Instituto de Pesquisa Clínica Evandro Chagas, Fundação Oswaldo Cruz, Manguinhos, Rio de Janeiro, Brazil; and

9 **3,4** <sup>2</sup>Departments of Medicine [Division of Infectious Diseases] and Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY, USA

10  
11  
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13  
14 **Correspondence:** Rodrigo Almeida-Paes,  
15 Laboratório de Micologia do Instituto de  
16 Pesquisa Clínica Evandro Chagas, Fundação  
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22 Received 2 April 2014; revised 6 May 2014;  
23 accepted 6 June 2014.

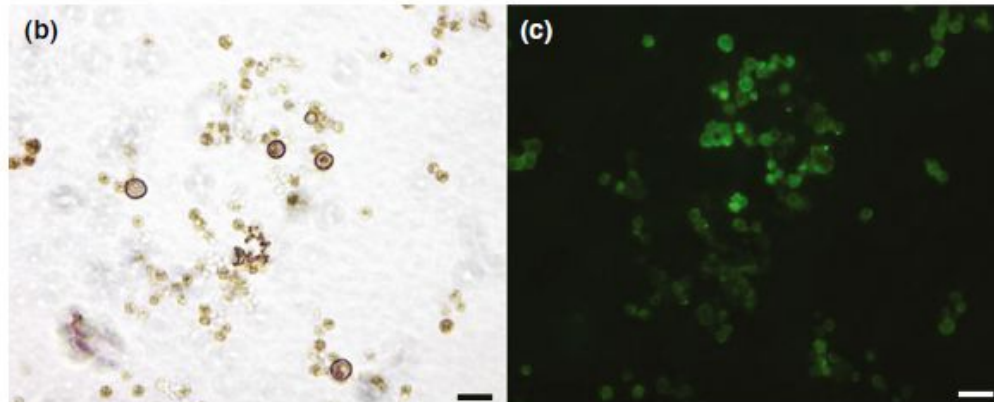
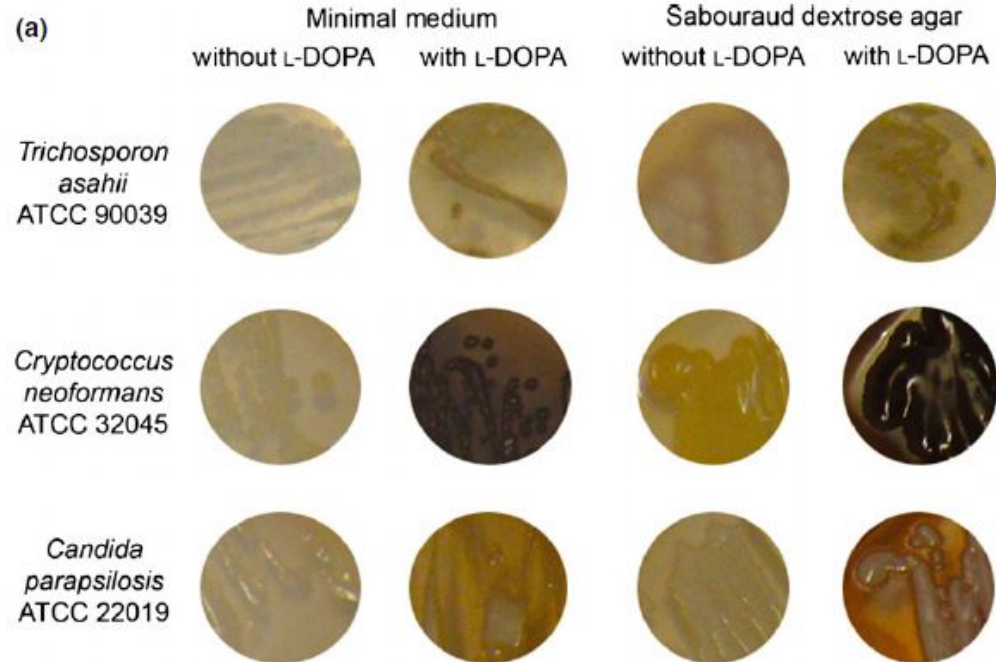
24 DOI: 10.1111/1567-1364.12174

25  
26  
27 Editor: Richard Calderone

**Abstract**

Melanization of members of the genus *Trichosporon* is poorly described. In this study, six strains, including two clinical isolates, from four different species (*Trichosporon asahii*, *T. asteroides*, *T. inkin*, and *T. mucoides*) were grown in culture media with or without L-dihydroxyphenylalanine (L-DOPA). Each strain produced a brownish pigment compatible with melanin when cultured in presence of L-DOPA, suggesting that these species are able to produce eumelanin. L-tyrosine was not able to elicit any type of pigment production on cultures. As eumelanin is produced by several fungi during parasitism, this pigment may contribute to *Trichosporon* virulence.

# Trichosporon spp.







# *Histoplasma capsulatum*



**Table 1. Absorbance readings of cultures**

	Reading spectrophotometer (340 nm)
Blank (only Ham's F12)	0,149 nm
Ipec 01/12 (Ham's F12)	0,228 nm
Ipec 01/12 (Ham's F12 + L-tyrosine)	2,969 nm
Ipec 01/12 (Ham's F12 + L-tyrosine + sulcotrione)	0,394 nm

**Figure 1. Ipec strain 01/12 after 14 days of cultivation. Left in Ham's F12 and right in Ham's F12 with L-tyrosine.**

# Conclusões

- A melanização dos membros do complexo *Sporothrix* é um processo dinâmico que pode ser modulado por diversos fatores, desde pH, temperatura e concentração de fonte de carbono até interações inter-microbianas que estes fungos possam realizar na natureza ou em parasitismo. Pelo menos três tipos diferentes de melanina podem ser produzidos: melanina DHN, melanina derivada de L-DOPA e piomelanina, derivada da L-tirosina.





# Conclusões

- Os diferentes tipos de melanina podem proteger *S. schenckii* e outras espécies relacionadas como *S. brasiliensis* de agressões na natureza como luz UV e de toxinas produzidas por bactérias vivendo no mesmo ambiente que o fungo. Em parasitismo, a piomelanina é capaz de proteger o fungo de radicais livres de oxigênio e principalmente nitrogênio, além de conferir maior resistência à ação fungicida de fármacos antifúngicos como a anfotericina B, característica compartilhada, em menor proporção, pela melanina derivada de L-DOPA.



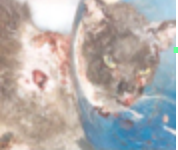
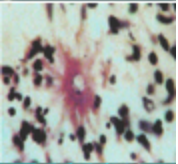
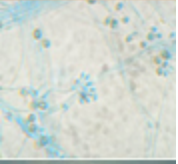
# Conclusões

- Outros fungos dimórficos e emergentes são capazes de produzir algum tipo de melanina. O papel biológico destes pigmentos (eumelanina / piomelanina) serão futuramente investigados.



# No futuro...

- *Paracoccidioides brasiliensis*
- *Fonsecaea pedrosoi*
- Outras espécies de *Candida*
- Outras espécies de *Sporothrix*
  - *S. pallida*: eumelanina e piomelanina



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