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FUNGAL AND BACTERIAL PHYSIOLOGY - REVIEW

Professor Luiz R. Travassos and the study of surface structures of fungal pathogens

Marcio L. Rodrigues[1,2] · Daniela S. Alviano[2] · Lucimar F. Kneipp[3] · Celuta S. Alviano[2] · Eliana Barreto-Bergter[2] · Leonardo Nimrichter[2]

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Abstract
Brazilian medical mycology considerably expanded in the last decades due to the efforts of several pioneers who started and expanded mycology during the twentieth century. In this manuscript, we highlight some of the contributions of one of these pioneers: Professor Luiz R. Travassos, who started his career in the field of microbiology in the 1960s. We will discuss his contributions to the areas of medical mycology and glycobiology, with a focus on glycosphingolipids, sialic acids, and surface enzymes.

Keywords Luiz R. Travassos · Fungi · Glycoconjugates · Cell surface

Introduction

The awareness of the medical importance of fungal diseases increased considerably after the AIDS pandemic in the early 1980s, although fungal pathogens are still widely neglected [1]. In the 1970s, the situation was incomparably worse. In the late 1960s and early 1970s, studies on the surface structures of fungal pathogens were driven by a young, visionary Luiz R. Travassos, who recognized not only the importance of fungal pathogens as infectious agents but also an underappreciated molecular class at that time: glycoconjugates. In the next sections, we will illustrate some of Travassos' contributions to the fields of medical mycology and microbial glycobiology.

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All authors contributed similarly to this manuscript.

✉ Marcio L. Rodrigues
marcio.rodrigues@fiocruz.br

Daniela S. Alviano
danialviano@micro.ufrj.br

Lucimar F. Kneipp
lucimar@ioc.fiocruz.br

Celuta S. Alviano
alviano@micro.ufrj.br

Eliana Barreto-Bergter
eliana.bergter@micro.ufrj.br

Leonardo Nimrichter
nimrichter@micro.ufrj.br

[1] Instituto Carlos Chagas, Fundação Oswaldo Cruz (Fiocruz), Curitiba, Brazil

[2] Instituto de Microbiologia Paulo de Góes (IMPG), Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

[3] Laboratório de Taxonomia, Bioquímica e Bioprospecção de Fungos (LTBBF), Instituto Oswaldo, Rio de Janeiro, Brazil

A pioneer of modern medical mycology in Brazil

In the 1970s, Professor Travassos led in Rio de Janeiro several innovative initiatives in the field of medical mycology, including efforts that resulted in seminal contributions to the field of glycobiology mainly using *Sporothrix* and *Ceratocystis* as experimental models [2–6]. His findings of structural and functional aspects of fungal glycans greatly influenced other areas of glycobiology in Brazil.

The initial period of studies of the compositional and structural properties of the fungal cell wall was prolific in many ways. The establishment of collaborations between Professors Travassos, Philip Gorin (Canada), and Kenneth Lloyd (USA) in the 1970s not only resulted in the generation of fundamental knowledge of the composition and structural arrangements of the fungal cell wall, but also changed

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the way fungal surface components were being analyzed. Through these interactions, the use of nuclear magnetic resonance for the study of cell wall glycans of fungi was introduced in Brazil. Dr. Gorin came from Canada to Brazil in 1976 as a visiting professor at the Federal University of Rio de Janeiro (1976–1977) to analyze polysaccharides and glycocomplexes from fungi and protozoa. Later, he moved to Curitiba, Brazil and, in 1983, became a full professor at the Department of Biochemistry of the Federal University of Paraná where he initiated a series of studies that helped the consolidation of glycobiology in that region. Throughout this period, Dr. Gorin continued to actively collaborate with Professor Travassos and his students. According to a Pubmed search (https://pubmed.ncbi.nlm.nih.gov/), this collaboration produced 13 scientific articles. Most importantly, this whole exciting scenario attracted young scientists to glycobiology who Professor Travassos guided to become leaders in their model systems. To name a few of them, we highlight Drs. Lucia Mendonça-Previato and José Oswaldo Previato, in addition to Eliana Barreto-Bergter and Celuta S. Alviano, who are authors in this manuscript.

After moving to São Paulo in the early 1980s, Professor Travassos initiated a series of studies in the *Paracoccidioides* model that opened another large new field within South American medical mycology. These seminal findings will be discussed in other articles of this special issue. Of note, Professor Travassos authored 125 mycology articles out of 242 scholarly outputs from his whole career reported on Pubmed. In this group of 125 mycology articles, 71 focused on the *Paracoccidioides* model. These numbers can slightly vary according to the database used for article search, but they demonstrate unequivocally how Professor Travassos contributed to and influenced a whole field. We will describe in the next sections a series of studies led by Professor Travassos in non-*Paracoccidoides* fungal models.

The cellular distribution of fungal glycolipids and its resonance on the discovery of fungal extracellular vesicles

Glycosphingolipids are typical components of the membranes of eukaryotic cells. Glycosylceramides (also known as cerebrosides or ceramidemonohexosides) represent the most well-studied class of neutral glycosphingolipids produced by fungal cells [7].

Glycosylceramides consist of D-glucose (Glc) or D-galactose (Gal) residues linked by a β1-1′-glycosidic bond to ceramide composed of D-erythro-sphingosine and long-chain fatty acid [8]. These lipids (glucosylceramide, GlcCer; galactosylceramide, GalCer) are produced by most fungal pathogens, where they play key roles in growth, differentiation, and immunogenicity [9]. Consequently, glycosylceramides are now considered as promising targets for novel antifungals [10] and therapeutic antibodies [11]. Eliana Barreto-Bergter, one of the authors in the present manuscript and Professor Travassos' former student, led a series of studies resulting in the full structural characterization of fungal glycosylceramides [7]. Other authors in this manuscript collaborated with her in several of these studies [12–16], which illustrates how Travassos' former students closely interacted and still collaborate.

For many years, it was widely accepted that fungal glycosylceramides were plasma membrane components. This view changed a couple of decades ago with the direct participation of Professor Travassos. In the *Cryptococcus neoformans* model, a collaboration between Drs. Travassos and Barreto-Berger resulted in the identification of human antibodies against GlcCer that were able to control fungal growth [13]. To exert these effects against a plasma membrane component, the antibodies would necessarily have to cross the fungal cell wall, a thick external layer that encages the plasma membrane [17]. That would be an unlikely event, considering the relatively large molecular weight of antibodies and the reduced porosity of the cell wall. At that time, after a brainstorm discussion with Arturo Casadevall during the 14th Congress of the International Society of Human and Animal Mycology (Buenos Aires, Argentina, 2000), Professor Travassos and Dr. Casadevall raised the hypothesis that, as targets of antifungal antibodies, cryptococcal GlcCer had to be a cell wall component. In fact, lipid extraction of cell wall samples resulted in the chemical identification of GlcCer [13]. Transmission electron microscopy using immunogold labeling demonstrated extensive antibody binding to the cryptococcal wall, revealing a previously unknown cellular site for fungal lipids [13]. This observation was in accordance with the antifungal activity of antibodies to GlcCer [13] and was the basis for the future development of antifungals targeting GlcCer [10].

Of note, the antibodies reacted with unique structures at the cryptococcal cell wall. As originally stated by the authors, "points of transport of presumed glucosylceramide-containing vesicles from the plasma membrane to the cell wall" were detected [13]. This observation was the experimental basis for the formulation of the hypothesis that fungal cells were able to produce vesicles that could cross the cell wall and reach the extracellular space. In fact, extracellular vesicles containing GlcCer were initially detected in cultures of *C. neoformans* and *P. brasiliensis* [18, 19]. These findings were expanded to at least 20 fungal species during the following 15 years [20]. We see the discovery of fungal extracellular vesicles as a direct consequence of the unique views of Professor Travassos, who foresaw the possibility that fungal lipids could be transitory cell wall components participating in exportation events in fungal cells. Fungal EVs are now candidates for vaccine development [21–24],

which connects a fundamental discovery with a clearly translational potential.

Sialic acids and surface enzymes

The studies of fungal pathogens initiated by Professor Travassos in the early 1970s were extended to other projects focused on surface-located structures participating in the interaction with the host. In the *S. schenckii* model, an early study suggested the presence of cell wall-associated acidic amino acids [25]. These initial findings evolved to the investigation of the presence of sialic acids on the surface of fungal pathogens, resulting from a collaboration between Drs. Travassos and Celuta S. Alviano. Of note, Dr. Alviano, also an author in this manuscript, was a PhD student under the supervision of Dr. Travassos. Together, they described the presence of sialic acids in *S. schenckii* [26], *Fonsecaea pedrosoi* [27], *P. brasiliensis* [28], *C. neoformans* [29], *Candida albicans* [30], and *Mucor polymorphosporus* [31]. These findings made possible the collaboration between the Alviano and Travassos laboratories with the group of Roland Schauer (1936–2019) in Germany. Dr. Schauer, also known as "Mr. Sialic Acid" [32], was a world leader in the field of sialic acids who visited Drs. Alviano and Travassos in Brazil in 1998 (Fig. 1). His collaboration with the Travassos and Alviano laboratories resulted in three publications in this field [33–35]. The group's expertise on the analysis of surface structures of fungal cells evolved to studies on the functions of pigments [36], inducers of differentiation [37], and surface enzymes [38–40], as detailed below.

During the decades of work at the Federal University of São Paulo, Professor Travassos maintained collaborations with several groups in Rio de Janeiro, where he was born as a person and as a scientist. He used to travel to Rio on a regular basis to discuss collaborative projects and to follow the progress of his former students. In this scenario, Professor Travassos initiated a tripartite collaboration involving his group in São Paulo and the laboratories of Celuta S. Alviano and Jose Roberto Meyer-Fernandes at the Federal University of Rio de Janeiro. They combined their expertise in mycology and biochemistry to identify an ecto-phosphatase in *F. pedrosoi*, as initially revealed by transmission electron microscopy and biochemical assays [39, 40]. Driven by Dr. Travassos' enthusiasm in these face-to-face meetings in Rio, the initial observation of surface ecto-phosphatase activity in *F. pedrosoi* evolved to functional studies. The group found that under conditions of inorganic phosphate deprivation, the enzymatic activity was approximately 130-fold increased [39]. Fungal cells (conidial forms) with higher ecto-phosphatase activity had a greater capacity to adhere to mammalian cells (fibroblasts and epithelial cells). The confirmation that this property resulted from increased enzyme activity came from the reduction in adhesion under conditions of enzymatic inhibition [39]. Ecto-phosphatases were involved not only in adhesion processes but also in fungal physiology, as concluded from the distinct levels of enzyme activity in the different morphotypes of *F. pedrosoi* [40].

Professor Travassos' legacy to the study of surface structures of fungal pathogens

The six of us, authors in this manuscript, were PhD students or post-doctoral fellows under the supervision of Professor Travassos, from the 1970s to the 2000s. All of us became independent principal investigators in different Brazilian institutions, and we are now at diverse career levels. Most importantly, all of us still study surface structures of fungal pathogens, with several students and young investigators under our supervision. This is a clear illustration of how

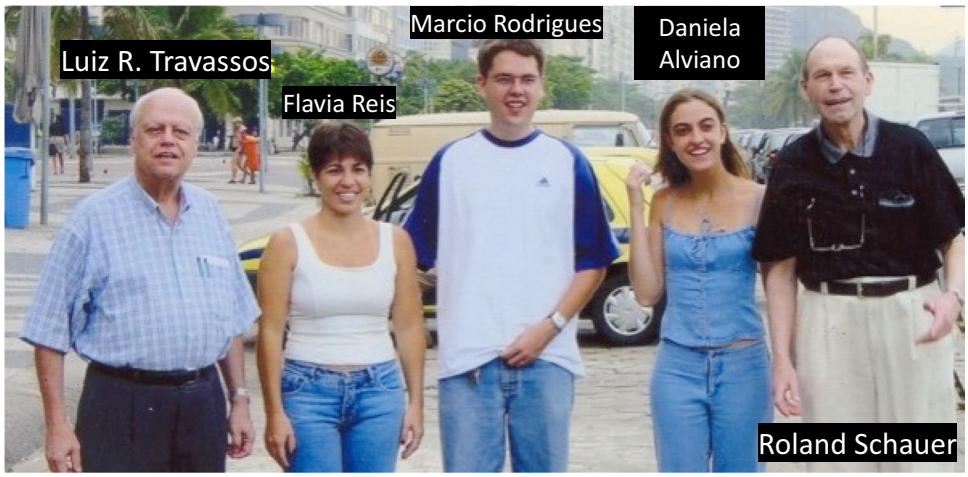

Fig. 1 Visit of Dr. Roland Schauer to Rio de Janeiro, Brazil, in 1998. This picture was taken in the Copacabana beach, after a visit to Rio guided by Dr. Travassos. Besides Drs. Travassos and Schauer, Flavia Reis, Marcio Rodrigues, and Daniela Alviano—all of them PhD students at that time—participated in Dr. Schauer's visit

Professor Travassos impacted medical mycology in Brazil and, at a more personal level, our careers and lives.

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Declarations

Conflict of interest The authors declare no competing interests.

References

1. Rodrigues ML, Nosanchuk JD (2020) Fungal diseases as neglected pathogens: a wake-up call to public health officials. *PLoS Negl Trop Dis* 14(2):e0007964. https://doi.org/10.1371/journal.pntd.0007964
2. Gorin PAJ, Haskins RH, Travassos LR, Mendonca-Previato L (1977) Further studies on the rhamnomannans and acidic rhamnomannans of Sporothrix schenckii and Ceratocystis stenoceras. *Carbohydr Res* 55(1):21–33. https://doi.org/10.1016/S0008-6215(00)84440-7
3. Travassos LR, Mendonça-Previato L (1978) Synthesis of monorhamnosyl L-rhamno-D-mannans by conidia of Sporothrix schenckii. *Infect Immun* 19(1):1–4. https://doi.org/10.1128/iai.19.1.1-4.1978
4. Travassos LR, Mendonça-Previato L, Gorin PA (1978) Heterogeneity of the rhamnomannans from one strain of the human pathogen Sporothrix schenckii determined by 13C nuclear magnetic resonance spectroscopy. *Infect Immun* 19(3):1107–1109. https://doi.org/10.1128/iai.19.3.1107-1109.1978
5. Mendonça-Previato L, Gorin PA, Travassos LR (1980) Galactose-containing polysaccharides from the human pathogens Sporothrix schenckii and Ceratocystis stenoceras. *Infect Immun* 29(3):934–939. https://doi.org/10.1128/iai.29.3.934-939.1980
6. Travassos LR, Gorin PAJ, Lloyd KO (1974) Discrimination between *Sporothrix schenckii* and *Ceratocystis stenoceras* Rhamnomannans by proton and carbon-13 magnetic resonance spectroscopy. *Infect Immun* 9(4):674–680. https://doi.org/10.1128/iai.9.4.674-680.1974
7. Barreto-Bergter E, Pinto MR, Rodrigues ML (2004) Structure and biological functions of fungal cerebrosides. *An Acad Bras Cienc* 76(1)
8. Reza S, Ugorski M, Suchański J (2021) Glucosylceramide and galactosylceramide, small glycosphingolipids with significant impact on health and disease. *Glycobiology* 31(11):1416–1434. https://doi.org/10.1093/glycob/cwab046
9. del Poeta M, Nimrichter L, Rodrigues MLML, Luberto C (2014) Synthesis and biological properties of fungal glucosylceramide. *PLoS Pathog* 10(1):e1003832. https://doi.org/10.1371/journal.ppat.1003832
10. Mor V, Rella A, Farnou AMAM et al (2015) Identification of a new class of antifungals targeting the synthesis of fungal sphingolipids. *mBio* 6(3):e00647. https://doi.org/10.1128/mBio.00647-15
11. Rodrigues ML, Shi L, Barreto-Bergter E et al (2007) Monoclonal antibody to fungal glucosylceramide protects mice against lethal Cryptococcus neoformans infection. *Clinical and Vaccine Immunology* 14(10):1372–1376. https://doi.org/10.1128/CVI.00202-07
12. Nimrichter L, Barreto-Bergter E, Mendonça-Filho RR et al (2004) A monoclonal antibody to glucosylceramide inhibits the growth of Fonsecaea pedrosoi and enhances the antifungal action of mouse macrophages. *Microbes Infect* 6(7). https://doi.org/10.1016/j.micinf.2004.03.004
13. Rodrigues ML, Travassos LR, Miranda KR et al (2000) Human Antibodies against a purified Glucosylceramide from Cryptococcus neoformans inhibit cell budding and fungal growth. *Infect Immun* 68(12):7049–7060. https://doi.org/10.1128/IAI.68.12.7049-7060.2000
14. da Silva AFC, Rodrigues ML, Farias SE, Almeida IC, Pinto MR, Barreto-Bergter E (2004) Glucosylceramides in Colletotrichum gloeosporioides are involved in the differentiation of conidia into mycelial cells. *FEBS Lett* 561((1-3)):137–1743. https://doi.org/10.1016/S0014-5793(04)00156-5
15. Nimrichter L, Rodrigues ML, Barreto-Bergter E, Travassos LR (2008) Sophisticated functions for a simple molecule: the role of glucosylceramides in fungal cells. *Lipid Insights* 2:LPI-S1014
16. Pinto MR, Rodrigues ML, Travassos LR, Haido RMT, Wait R, Barreto-Bergter E (2002) Characterization of glucosylceramides in Pseudallescheria boydii and their involvement in fungal differentiation. *Glycobiology* 12(4)
17. Nimrichter L, Rodrigues ML, Rodrigues EG, Travassos LR (2005) The multitude of targets for the immune system and drug therapy in the fungal cell wall. *Microbes Infect* 7(4). https://doi.org/10.1016/j.micinf.2005.03.002
18. Rodrigues ML, Nimrichter L, Oliveira DL et al (2007) Vesicular polysaccharide export in Cryptococcus neoformans is a eukaryotic solution to the problem of fungal trans-cell wall transport. *Eukaryot Cell* 6(1):48–59. https://doi.org/10.1128/EC.00318-06
19. Vallejo MC, Matsuo AL, Ganiko L et al (2011) The pathogenic fungus Paracoccidioides brasiliensis exports extracellular vesicles containing highly immunogenic α-galactosyl epitopes. *Eukaryot Cell* 10(3):343–351. https://doi.org/10.1128/EC.00227-10
20. Piffer AC, Kuczera D, Rodrigues ML, Nimrichter L (2021) The paradoxical and still obscure properties of fungal extracellular vesicles. *Mol Immunol* 135:137–146. https://doi.org/10.1016/j.molimm.2021.04.009
21. Rizzo J, Wong SSW, Gazi AD et al (2021) Cryptococcus extracellular vesicles properties and their use as vaccine platforms. *J Extracell Vesicles* 10(10):e12129. https://doi.org/10.1002/jev2.12129
22. Vargas G, Rocha JDB, Oliveira DL et al (2015) Compositional and immunobiological analyses of extracellular vesicles released by Candida albicans. *Cell Microbiol* 17(3). https://doi.org/10.1111/cmi.12374
23. Honorato L, Bonilla JJA, Piffer AC, Nimrichter L (2021) Fungal extracellular vesicles as a potential strategy for vaccine development. *Fungal Extracell Vesicles: Biol Roles 432*:121–138. https://doi.org/10.1007/978-3-030-83391-6_10
24. Vargas G, Honorato L, Guimarães AJ et al (2020) Protective effect of fungal extracellular vesicles against murine candidiasis. *Cell Microbiol* 22(10). https://doi.org/10.1111/cmi.13238
25. Benchimol M, de Souza W, Travassos LR (1979) Distribution of anionic groups at the cell surface of different *Sporothrix schenckii* cell types. *Infect Immun* 24(3):912–919. https://doi.org/10.1128/iai.24.3.912-919.1979
26. Oda LM, Kubelka CF, Alviano CS, Travassos LR (1983) Ingestion of yeast forms of Sporothrix schenckii by mouse peritoneal macrophages. *Infect Immun* 39(2):497–504. https://doi.org/10.1128/iai.39.2.497-504.1983
27. Souza ET, Silva-Filho FC, de Souza W, Alviano CS, Angluster J, Travassos LR (1986) Identification of sialic acids on the cell surface of hyphae and conidia of the human pathogen *Fonsecaea*

pedrosoi. *Med Mycol* 24(2):145–154. https://doi.org/10.1080/02681218680000211
28. Soares RM, Alviano CS, Angluster J, Travassos LR (1993) Identification of sialic acids on the cell surface of hyphae and yeast forms of the human pathogen Paracoccidioides brasiliensis. *FEMS Microbiol Lett* 108(1):31–34. https://doi.org/10.1016/0378-1097(93)90483-I
29. Rodrigues ML, Rozental S, Couceiro JN, Angluster J, Alviano CS, Travassos LR (1997) Identification of N-acetylneuraminic acid and its 9-O-acetylated derivative on the cell surface of Cryptococcus neoformans: influence on fungal phagocytosis. *Infect Immun* 65(12):4937–4942. https://doi.org/10.1128/iai.65.12.4937-4942.1997
30. Soares RM, Rosangela MDA, Alviano DS, Angluster J, Alviano CS, Travassos LR (2000) Identification of sialic acids on the cell surface of Candida albicans. Biochim Biophys Acta - Gen Subj 1474(2):262–268. https://doi.org/10.1016/S0304-4165(00)00003-9
31. Almeida CA, de Campos-Takaki GM, Portela MB, Travassos LR, Alviano CS, Alviano DS (2013) Sialoglycoproteins in morphological distinct stages of mucor polymorphosporus and their influence on phagocytosis by human blood phagocytes. *Mycopathologia* 176(3-4):183–189. https://doi.org/10.1007/s11046-013-9692-6
32. Varki A (2020) Roland Schauer (1936–2019): A tribute to "Mr. sialic acid". *Glycobiology* 30(3):132–133. https://doi.org/10.1093/glycob/cwaa007
33. Alviano CS, Travassos LR, Schauer R (1999) Sialic acids in fungi: a minireview. *Glycoconj J* 16(9):545–554. https://doi.org/10.1023/A:1007078106280
34. Esquenazi D, Rozental S, Alviano CS, Travassos LR, Schauer R (2003) Sialic acids are absent from the dermatophytes Trichophyton mentagrophytes and Trichophyton rubrum. Die Dermatophyten Trichophyton mentagrophytes und Trichophyton rubrum sind Sialinsaure-negativ. *Mycoses* 46(5-6):197–202. https://doi.org/10.1046/j.1439-0507.2003.00873.x
35. Rodrigues ML, Dobroff AS, Couceiro JNDS, Alviano CS, Schauer R, Travassos LR (2003) Sialylglycoconjugates and sialyltransferase activity in the fungus Cryptococcus neoformans. *Glycoconj J* 19(3). https://doi.org/10.1023/A:1024245606607
36. Alviano DS, Franzen AJ, Travassos LR et al (2004) Melanin from *Fonsecaea pedrosoi* induces production of human antifungal antibodies and enhances the antimicrobial efficacy of phagocytes. *Infect Immun* 72(1):229–237. https://doi.org/10.1128/IAI.72.1.229-237.2004
37. Alviano DS, Kneipp LF, Lopes AH et al (2003) Differentiation of Fonsecaea pedrosoi mycelial forms into sclerotic cells is induced by platelet-activating factor. *Res Microbiol* 154(10):689–695. https://doi.org/10.1016/j.resmic.2003.09.002
38. Itallo C Jr, Rodrigues ML, Alviano CS, Travassos LR, Meyer-Fernandes JR (2005) Characterization of an ecto-ATPase activity in Cryptococcus neoformans. *FEMS Yeast Res* 5(10). https://doi.org/10.1016/j.femsyr.2005.04.005
39. Kneipp LF, Rodrigues ML, Holandino C et al (2004) Ectophosphatase activity in conidial forms of Fonsecaea pedrosoi is modulated by exogenous phosphate and influences fungal adhesion to mammalian cells. *Microbiology (N Y)* 150(10). https://doi.org/10.1099/mic.0.27405-0
40. Kneipp LF, Palmeira VF, Pinheiro AAS et al (2003) Phosphatase activity on the cell wall of *Fonsecaea pedrosoi*. *Med Mycol* 41(6):469–477. https://doi.org/10.1080/10683160310001615399