

This article was downloaded by: [UNAM Ciudad Universitaria]

On: 29 December 2014, At: 17:10

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Biofouling: The Journal of Bioadhesion and Biofilm Research

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/gbif20>

In vitro characterization of *Trichophyton rubrum* and *T. mentagrophytes* biofilms

C.B. Costa-Orlandi^a, J.C.O. Sardi^a, C.T. Santos^a, A.M. Fusco-Almeida^a & M.J.S. Mendes-Giannini^a

^a Department of Clinical Analysis, Clinical Mycology Laboratory, School of Pharmaceutical Sciences, UNESP - Universidade Estadual Paulista, Araraquara, SP 14801-902, Brazil
Published online: 23 May 2014.



[Click for updates](#)

To cite this article: C.B. Costa-Orlandi, J.C.O. Sardi, C.T. Santos, A.M. Fusco-Almeida & M.J.S. Mendes-Giannini (2014) In vitro characterization of *Trichophyton rubrum* and *T. mentagrophytes* biofilms, *Biofouling: The Journal of Bioadhesion and Biofilm Research*, 30:6, 719-727, DOI: [10.1080/08927014.2014.919282](https://doi.org/10.1080/08927014.2014.919282)

To link to this article: <http://dx.doi.org/10.1080/08927014.2014.919282>

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at <http://www.tandfonline.com/page/terms-and-conditions>

***In vitro* characterization of *Trichophyton rubrum* and *T. mentagrophytes* biofilms**

C.B. Costa-Orlandi*, J.C.O. Sardi, C.T. Santos, A.M. Fusco-Almeida and M.J.S. Mendes-Giannini*

Department of Clinical Analysis, Clinical Mycology Laboratory, School of Pharmaceutical Sciences, UNESP – Universidade Estadual Paulista, Araraquara, SP 14801-902, Brazil

(Received 18 February 2014; accepted 22 April 2014)

Dermatophytes are fungi responsible for a disease known as dermatophytosis. Biofilms are sessile microbial communities surrounded by extracellular polymeric substances (EPS) with increased resistance to antimicrobial agents and host defenses. This paper describes, for the first time, the characteristics of *Trichophyton rubrum* and *T. mentagrophytes* biofilms. Biofilm formation was analyzed by light microscopy, scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) as well as by staining with crystal violet and safranin. Metabolic activity was determined using the XTT reduction assay. Both species were able to form mature biofilms in 72 h. *T. rubrum* biofilm produced more biomass and EPS and was denser than *T. mentagrophytes* biofilm. The SEM results demonstrated a coordinated network of hyphae in all directions, embedded within EPS in some areas. Research and characterization of biofilms formed by dermatophytes may contribute to the search for new drugs for the treatment of these mycoses and might inform future revisions with respect to the dose and duration of treatment of currently available antifungals.

Keywords: *Trichophyton rubrum*; *T. mentagrophytes*; biofilm; dermatophytes; SEM; CLSM

Introduction

Dermatophytes are fungi that have the ability to invade keratinized structures of humans and animals, producing a condition called dermatophytosis (Weitzman & Summerbell 1995). There are three anamorphic genera: *Trichophyton*, *Microsporum* and *Epidermophyton*, which share certain microscopic features despite the taxonomic distance between them (Weitzman & Summerbell 1995; Moraes et al. 2001; Costa-Orlandi et al. 2012).

Soil is a natural reservoir of dermatophytes; keratins present in soil are used as nutrients, so these fungi are adapted to various environments. Keratin is a protein of high molecular weight, relatively insoluble and present in the skin, hair, nails and debris deposited in soil (Saenz 2001; Aquino et al. 2007; Macura et al. 2010; Achterman & White 2012). The prevalence of dermatophytes is variable but high in Latin America, causing infections in both humans and domestic animals (Pinheiro et al. 1997). The infection occurs when arthroconidia adhere to host skin, followed by germination and the invasion of keratinized structures by fungal hyphae. During invasion, keratinized tissues are digested by secretion of multiple endoproteases, the presence of which can determine fungal survival on the host as well as the clinical evolution of the infection, by providing nutrients, and triggering and modulating the immune response (Vermout et al. 2008; Baldo et al. 2012). The severity of the infection is related, in part, to the reaction of the host to the invading organism, in addition to other

factors, such as species or virulence of the infecting strain, host reaction to metabolic products produced by the fungus, anatomic site of infection, and local environmental factors (Soares et al. 2013). Endoproteases fall into two large protein families: the subtilisins (which are serine proteases) and the fungalysins (metalloproteases) (Monod 2008). Studies have demonstrated seven putative genes encoding serine proteases belonging to the subtilisin family (SUB) and five putative genes encoding metalloproteases belonging to the M36 family, which only exists in fungi (Zhang et al. 2013). The sequencing of seven dermatophyte genomes has recently been completed, and the sequences have been made publicly available. Seven genomes were found to encode high numbers of protease-encoding genes compared to related non-dermatophytic fungi. In particular, dermatophytes appear to have expanded sets of endopeptidases, exopeptidases, and secreted proteases (Achterman & White 2012).

This observation highlights the important role of protein degradation in the life cycle of dermatophytes (Achterman & White 2012). Many authors have investigated the virulence factors that are activated in dermatophytes exposed to environmental stresses such as growth on lipids (Peres et al. 2010; Maranhao et al. 2011), changes in pH (Silveira et al. 2010), and antifungal drugs (Peres et al. 2010).

Biofilms are structured sessile microbial communities that are organized by microorganisms adhering to a

*Corresponding authors. Email: carolbarceloscosta@gmail.com (C.B. Costa-Orlandi); giannini@fcfar.unesp.br (M.J.S. Mendes-Giannini)

surface as well as to each other *via* an extracellular polymeric matrix. Biofilms are of particular significance because it is now estimated that a significant proportion of all human microbial infections involve biofilm formation (Ramage et al. 2009). The formation of biofilms provides a number of advantages to microorganisms, including protection against the environment, microbial communication, increased virulence, improved metabolic cooperation and the emergence of community-based regulation of gene expression (Percival et al. 2012). In recent years, there has been an increasing appreciation of the role played by biofilms in human medicine, primarily because the microorganisms growing within a biofilm exhibit phenotypic characteristics that are substantially different from those exhibited when the same microorganism is present in planktonic form. Furthermore, the microorganisms in a biofilm community also have increased resistance to antimicrobial agents and increased protection against host defenses (Ramage et al. 2009).

Similar to bacteria, fungi are also able to form biofilms, which contribute to their clinical significance and the economic problems they cause, as they are eukaryotic organisms and are therefore more complex than bacteria. Moreover, most diseases caused by fungi are neglected, making diagnosis and treatment more difficult and resulting in high mortality rates. Biofilm formation has been identified in species of *Candida* (Pires, Montanari et al. 2011; Pires, Santos et al. 2011; Martins et al. 2012; Sardi et al. 2013), *Cryptococcus* (Ajesh & Sreejith 2012), *Malassezia* (Figueredo et al. 2012), *Trichosporon* (Colombo et al. 2011), *Saccharomyces* (Bojsen et al. 2012), *Aspergillus* (Muszkieta et al. 2013), *Histoplasma* (Pitangui et al. 2012) as well as *Paracoccidioides brasiliensis* (unpublished data) and others. The ability of dermatophytes to form biofilms has not been described, but it is believed that it is related to antifungal resistance (Vlassova et al. 2011). Thus, this study aimed to describe and characterize biofilms of *Trichophyton rubrum* and *T. mentagrophytes in vitro*.

Materials and methods

Dermatophyte cultivation

This study was performed with standard strains of *T. rubrum* (ATCC 28189) and *T. mentagrophytes* (ATCC 11481), from the collection of the Clinical Mycology Laboratory, Department of Clinical Analysis, School of Pharmaceutical Sciences, UNESP. All samples were cultured on Sabouraud dextrose agar (DIFCO – BD Biosciences, Sparks, MD, USA) with additional chloramphenicol (0.1%) or on Mycosel agar (DIFCO) and incubated at 28°C for up to 15 days (Costa-Orlandi et al. 2012).

Biofilm formation assay

A biofilm assay was performed based on the method described by Mowat et al. (2007) for *Aspergillus fumigatus* with some modifications. The strains of *T. rubrum* ATCC 28189 and *T. mentagrophytes* ATCC 11481 were grown on potato dextrose agar (DIFCO) and incubated at 28°C for seven days or until sporulation. The inoculum was prepared by covering the cultures with 5 ml of sterile saline at 0.85% or 0.01 M PBS (pH 7.2), counting the conidia on a hemocytometer, and adjusting to a final concentration of 1×10^6 CFU ml⁻¹. Then, 1,000 µl of inoculum were added to 24-well plates (TPP®, Trasadingen, Switzerland) containing previously sterilized coverslips. The plates were then incubated without agitation at 37°C for 3 h for biofilm pre-adhesion. After this time, the supernatant was gently removed from the wells, the cells were washed three times with sterile saline at 0.85% or 0.01 M PBS (pH 7.2) for removal of non-adherent cells, 1,000 µl of RPMI 1640 medium were added and the plates were incubated at 37°C for 72 h. Morphology was observed by light microscopy on an IN Cell Analyzer 2000 instrument (GE Healthcare Life Sciences, Little Chalfont, Buckinghamshire, UK), and image analysis was performed with the IN Cell Image Analysis Investigator software (GE Healthcare Life Sciences).

Quantification of biofilm mass by crystal violet staining

The method of quantifying dermatophytic biofilm mass was adapted from the technique described by Mowat et al. (2007). Biofilms were allowed to form in 96-well plates (TPP®) for 72 h before the culture medium was removed from each well and the adhered cells were washed three times with PBS. After drying at room temperature, 100 µl of 0.5% crystal violet solution were added to each well for 5 min. The wells were washed one more time with sterile water to remove excess stain and biofilms were decolorized by the addition of 100 µl of 95% ethanol solution to each well. The ethanol solution was gently homogenized with a pipette until the rest of the crystal violet was completely dissolved (~1 min). Finally, the solution from each well was transferred to a new 96-well plate and then read in an ELISA reader (Microplate Reader iMark™; BIO-RAD; Hercules, CA, USA) at a wavelength of 570 nm. The absorbance values should be proportional to the biomass of the biofilm, which is comprised of hyphae, extracellular material and dead cells (ie the greater the quantity of biological material, the greater the staining and absorbance value).

Crystal violet was also used for the quantification of *T. rubrum* biofilm formed as described in the biofilm formation section, in three different media: RPMI 1640 with L-glutamine, without sodium bicarbonate (Gibco®

by Life Technologies, Grand Island, NY, USA); Keratinocyte serum free medium (Gibco®) and Dulbecco's Modified Eagle Medium – DMEM (Gibco®) with bovine fetal serum (BFS). Biofilm morphology in the different media was observed by light microscopy on an IN Cell Analyzer 2000.

Quantification of the extracellular matrix by safranin staining

The extracellular matrix produced by the biofilm was quantified by safranin staining as described by Seidler et al. (2008). After biofilm formation for 72 h in 96-well plates, the extracellular matrix was stained with 50 µl of safranin solution for 5 min. Then, the plates were thoroughly washed until the supernatant stayed clear. Finally, the plates were read in an ELISA reader (Microplate Reader iMark™; BIO-RAD) at a wavelength of 492 nm.

Determination of the metabolic activity of the biofilm by the XTT reduction assay

The XTT reduction assay (2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[carbonyl (phenylamino)]-2H-tetrazolium hydroxide) is reproducible, sensitive, rapid, and is directly related to the metabolic activity of the biofilm. To conduct this assay, test solutions were prepared from stock – XTT (1 mg ml⁻¹ of PBS) and menadione (1 mM in ethanol). Biofilms were formed in different plates for different time points: 0, 3, 12, 24, 48, 72 and 96 h. Then, 50 µl of XTT solution plus 4 µl of a solution of menadione were added to each well of the 96-well plates before incubation at 37°C for 3 h. The activity of the fungal mitochondrial dehydrogenase reduces the tetrazolium salt XTT to formazan salts, resulting in a colorimetric change that correlates with cell viability. The colorimetric change was measured using an ELISA reader (Microplate Reader iMark™; BIO-RAD) at 490 nm. In all experiments, RPMI 1640 medium free of biofilm formation was included as a negative control (Mowat et al. 2007; Pitanguí et al. 2012).

Scanning electron microscopy (SEM)

The biofilms formed on coverslips were processed as described by Morris et al. (1997), Ells and Truelstrup Hansen (2006), and Pitanguí et al. (2012) with modifications. Briefly, the biofilms were washed three times with PBS to remove planktonic cells and then fixed with 800 µl of glutaraldehyde solution (Sigma-Aldrich, St Louis, MO, USA) at 2.5% in sterile distilled water for 60 min. After three PBS washes, the specimens were dehydrated with an increasing gradient of ethanol solutions (50% to 100%) at room temperature. The samples were dried using the critical point method in a Samdri

780A desiccator (Rockville, MD, USA) using CO₂. The samples were mounted on aluminum and silver cylinders and placed in a high vacuum evaporator (Denton Vacuum Desk V, Jeol, Moorestown, NJ, USA) for gold coating. Topographic features of biofilms were analyzed under a scanning electron microscope (Jeol JSM-6610LV, Peabody, MA, USA) at the School of Dentistry of Araraquara, UNESP.

Confocal laser scanning microscopy (CLSM)

For confocal microscopy, a solution of CAAF (Concanavalin A [ConA], conjugated to Alexa Fluor 488; Molecular Probes, USA) and FUN 1 (Molecular Probes, Invitrogen, Eugene, OR, USA) was prepared. This mixture was added to the wells containing the biofilms on coverslips and the plates were incubated at 37°C for 45 min, protected from light. Then, the coverslips were washed with distilled water, carefully removed from the wells, covered with 4 µl of Fluoromount-G (Sigma-Aldrich), and deposited on microscope slides for observation under a confocal microscope (Leica TCS SP5, Leica Microsystems, Wetzlar, Germany) with the capture and image program LAS AF 1.8.2 build 1465 (Leica Microsystems) (Ramage et al. 2001; Kuhn et al. 2002; Chandra et al. 2005).

Statistical analysis

All experiments were performed in triplicate. Statistical analysis was performed using a *t*-test or one-way ANOVA with GraphPad Prism 5 software. *P*-values < 0.05 were considered statistically significant.

Results

Biofilm morphology

The morphology of the biofilm was evaluated by light microscopy, using an IN Cell Analyzer 2000 (Figure 1). The images demonstrate that both species were able to form biofilms, highlighting a denser mass in *T. rubrum* after 72 h (Figure 1d).

Biofilm quantification

The results of the measurement of biofilm mass of *T. rubrum* ATCC 28189 performed with crystal violet show higher biomass production compared with *T. mentagrophytes* ATCC 11481 (*p* < 0.01) (Figure 2a). The same results were observed for the polysaccharide structure and the extracellular matrix quantified by safranin staining as shown in Figure 2b (*p* < 0.0001). In all three media tested, there was substantial biomass production by *T. rubrum* biofilms with no significant difference between the RPMI medium, Keratinocyte

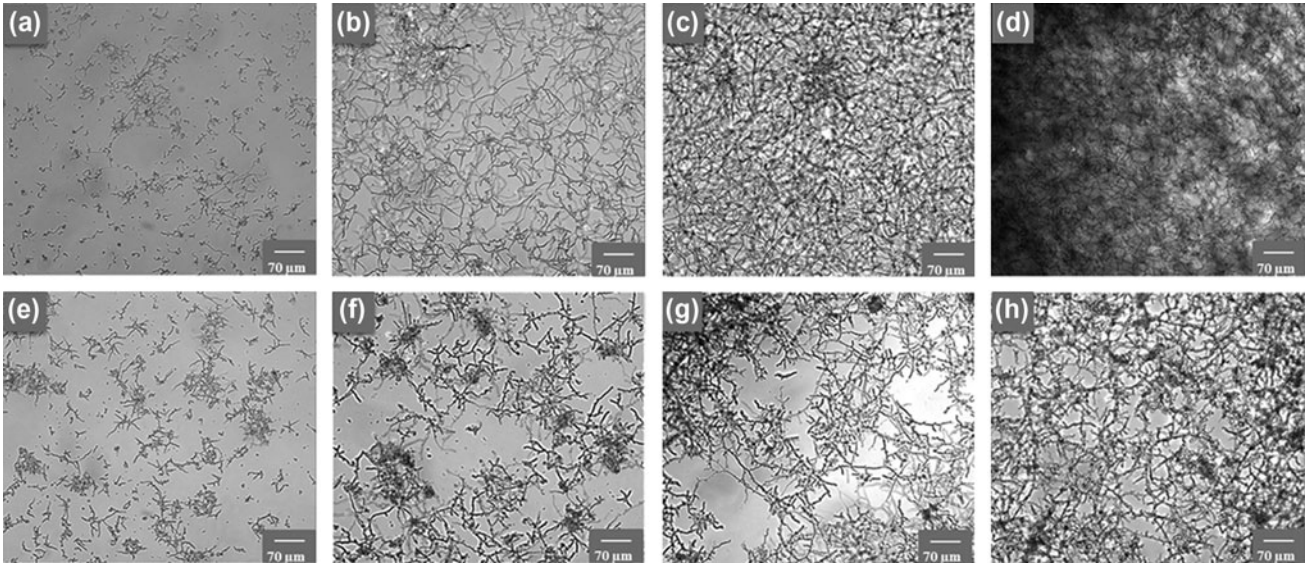


Figure 1. Analysis of the morphology of *T. mentagrophytes* ATCC 11481 and *T. rubrum* ATCC 28189 biofilms on the IN Cell Analyzer 2000. (a) *T. rubrum* biofilm after 12 h; (b) *T. rubrum* biofilm after 24 h; (c) *T. rubrum* biofilm after 48 h; (d) *T. rubrum* biofilm after 72 h; (e) *T. mentagrophytes* biofilm after 12 h; (f) *T. mentagrophytes* biofilm after 24 h; (g) *T. mentagrophytes* biofilm after 48 h; (h) *T. mentagrophytes* biofilm after 72 h.

serum free medium and DMEM with BFS ($p > 0.05$) (Figure 2c).

Determination of biofilm metabolic activity by the XTT reduction assay

The results show that the initial metabolic activity of the biofilms could be measured within 3 h. Over a period of 12–24 h there was a slight increase in metabolic activity, and by 48 h there was a significant increase in metabolic activity for both species. After 72 h, cells started to

detach and their metabolic activity tended to plateau, so this period was considered the ideal time for biofilm maturation. Both strains showed similar results and no significant difference was observed ($p > 0.05$) (Figure 3).

SEM

SEM analysis revealed that biofilm formation occurred in the presence of a highly organized extracellular matrix. Figure 4 shows images captured from different areas of a

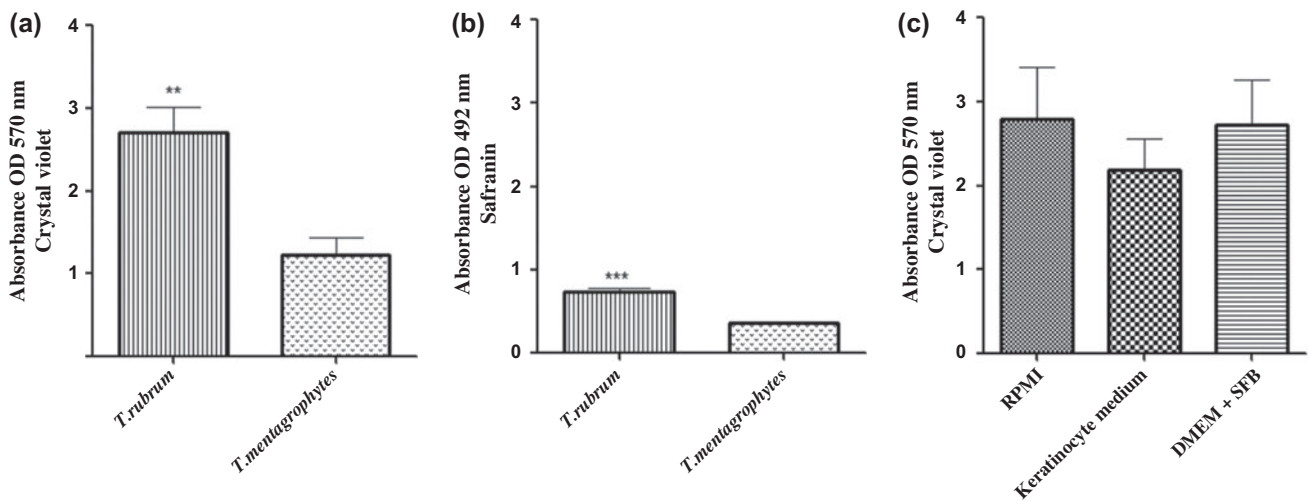


Figure 2. Quantification of biomass and extracellular matrix by staining with crystal violet (a) and safranin (b). *T. rubrum* ATCC 28189 shows a greater biomass ($p < 0.01$) and extracellular matrix production ($p < 0.0001$) when compared with *T. mentagrophytes* ATCC 11481. (c) shows a similar profile of *T. rubrum* biomass in different media, with no significant difference between them. ** = $p < 0.01$; *** = $p < 0.0001$.

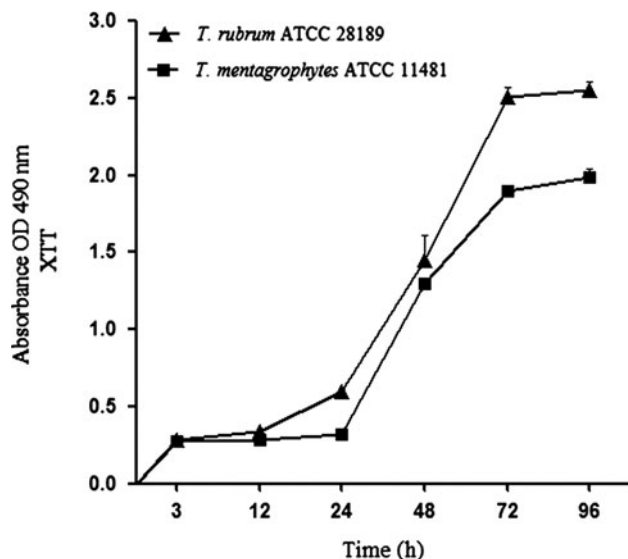


Figure 3. Kinetics of biofilm formation in *T. rubrum* and *T. mentagrophytes* in 96-well plates. The two strains showed similar results. No significant difference was observed ($p > 0.05$).

T. mentagrophytes biofilm (A) with tangled hyphae covered by the exopolymeric matrix (B, C and D). The same can be seen in Figure 5, which shows *T. rubrum* biofilm (A), with a large amount of extracellular matrix involving hyphae (B, C and D). By being denser and more compact, a larger amount of EPS was found in the *T. rubrum* biofilm compared with the *T. mentagrophytes* biofilm.

CLSM

The results of CLSM showed mature biofilm formation in both species. The fungal mass was compared by sections (Figure 6). In Figure 7, ConA stained the fungal cell wall and the extracellular matrix green, whereas FUN 1 stained the active cells red. The biofilm thickness of *T. rubrum* and *T. mentagrophytes* was 249.67 μm and 134.82 μm , respectively.

Discussion

The pathogenesis of mycosis is directly related to the interaction and adhesion of pathogenic fungi to host tissues (Vermout et al. 2008). Its mechanisms can vary according

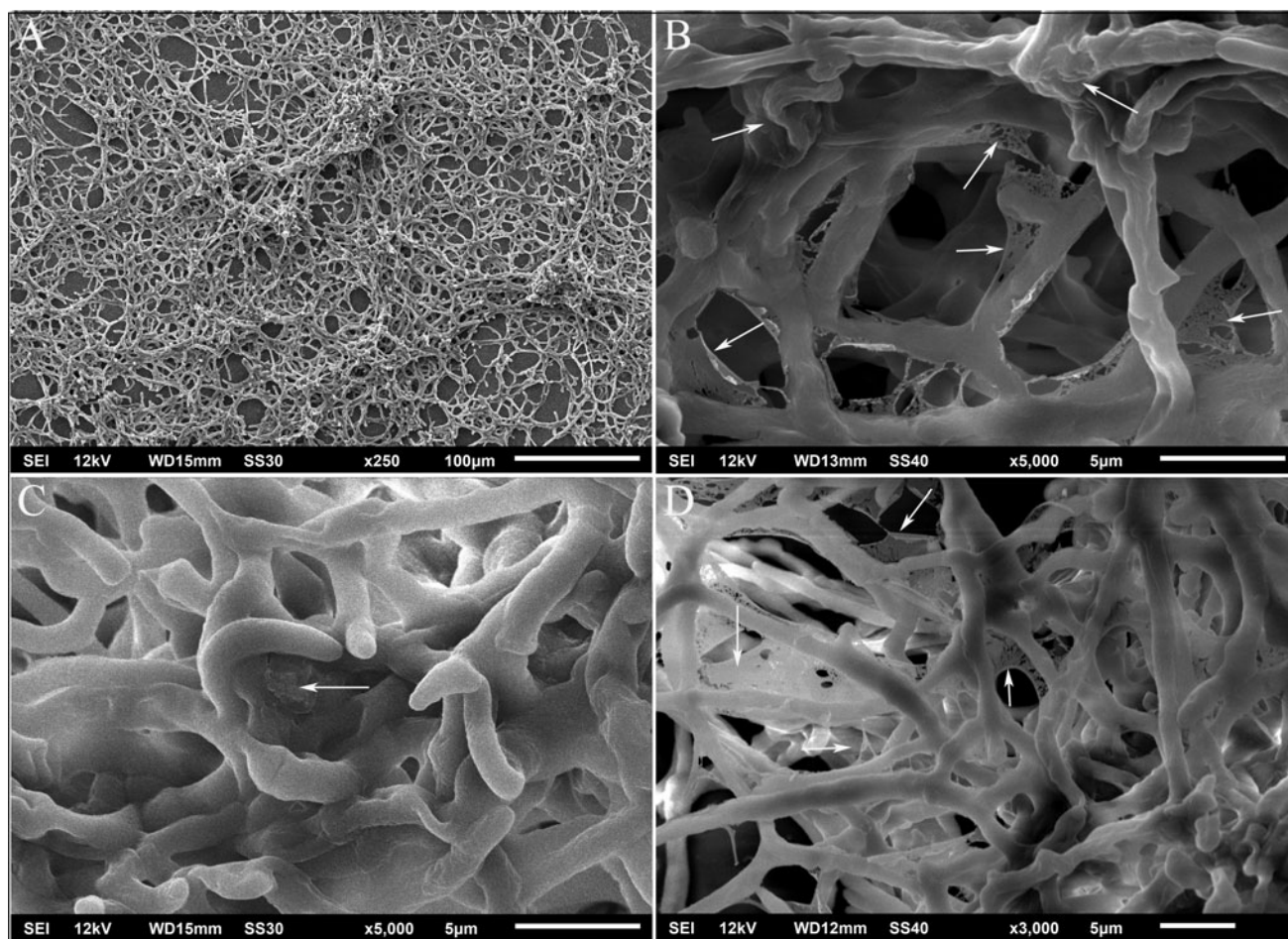


Figure 4. SEM images of mature *T. mentagrophytes* ATCC 11481 biofilm (A). Arrows denote the exopolymeric matrix (B, C and D).

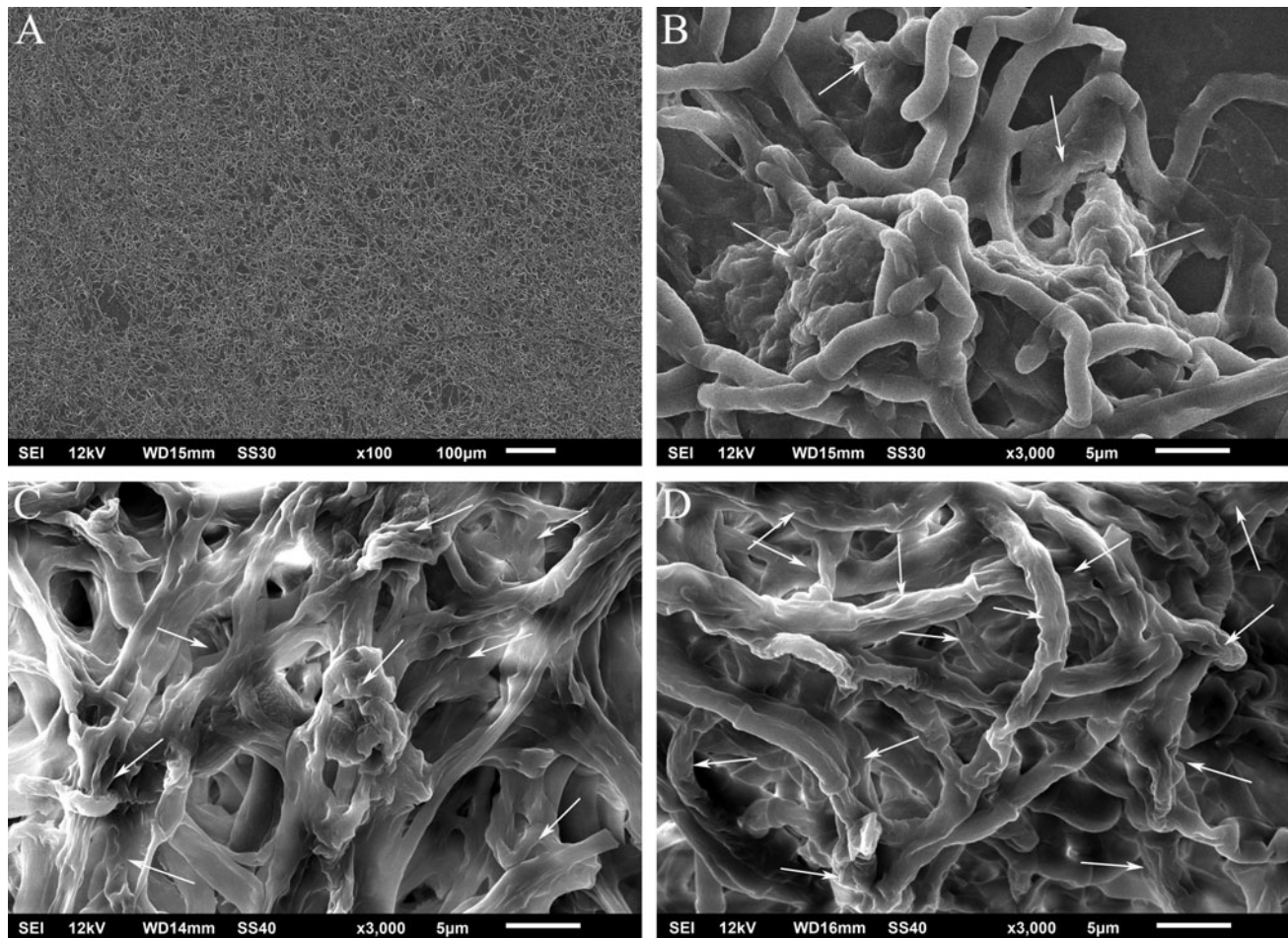


Figure 5. SEM images of mature *T. rubrum* ATCC 28189 biofilm (A). Arrows denote the exopolymeric matrix produced by *T. rubrum* covering the hyphae (B, C and D).

to fungal species and host status. The factors responsible for adhesion of dermatophytes to various surfaces are poorly understood, but it is known that the ability of *T. rubrum* to adhere to epithelial cells is due to the action of carbohydrate-specific adhesins that are expressed on the microconidial surface (Vermout et al. 2008).

Biofilms can be defined as extensive communities of organisms growing on surfaces, surrounded by a polysaccharide-rich extracellular matrix, with a pronounced capacity for resistance to antimicrobial agents and host defenses (Chandra et al. 2005; Ramage et al. 2009). Moreover, biofilm cells exhibit an altered phenotype with respect to growth rate and gene transcription (Harding et al. 2009; Percival et al. 2012). Burkhart et al. (2002) proposed the hypothesis that biofilm formation in dermatophytes could explain dermatophytomas, in which circumscribed dense white fungal masses live within and under the nail plate, but this has not previously been characterized. Dermatophytomas are more resistant to traditional therapies, host more than one microorganism species and have living fungal elements strongly adherent

to the nail plate that survive in histological studies. In this paper, the characteristics of the biofilms formed *in vitro* by *Trichophyton rubrum* and *T. mentagrophytes* are described for the first time. The ability to adhere and form biofilms was assessed first by light microscopy, using the IN Cell Analyzer to investigate the development of the biofilms on polystyrene surfaces. Two reference strains, *T. rubrum* ATCC 28189 and *T. mentagrophytes* ATCC 11481, were evaluated. The time of adherence corroborated with previous work by Zurita and Hay (1987), who described a time of 3 to 4 h for maximum adherence of arthroconidia of *Trichophyton* spp. in keratinocytes (Vermout et al. 2008). RPMI 1640 was used as the culture medium as it has been shown to be optimal for biofilm development. This medium is commonly used in *in vitro* biofilm formation for several species of fungi (Seidler, Salvenmoser & Muller 2008; Pires, Santos et al. 2011; Sengupta et al. 2012; Peiqian et al. 2013).

Colorimetric assays are important tools for studying viability in eukaryotic cells (Kuhn et al. 2003). XTT is a yellow tetrazolium salt which, in the presence of

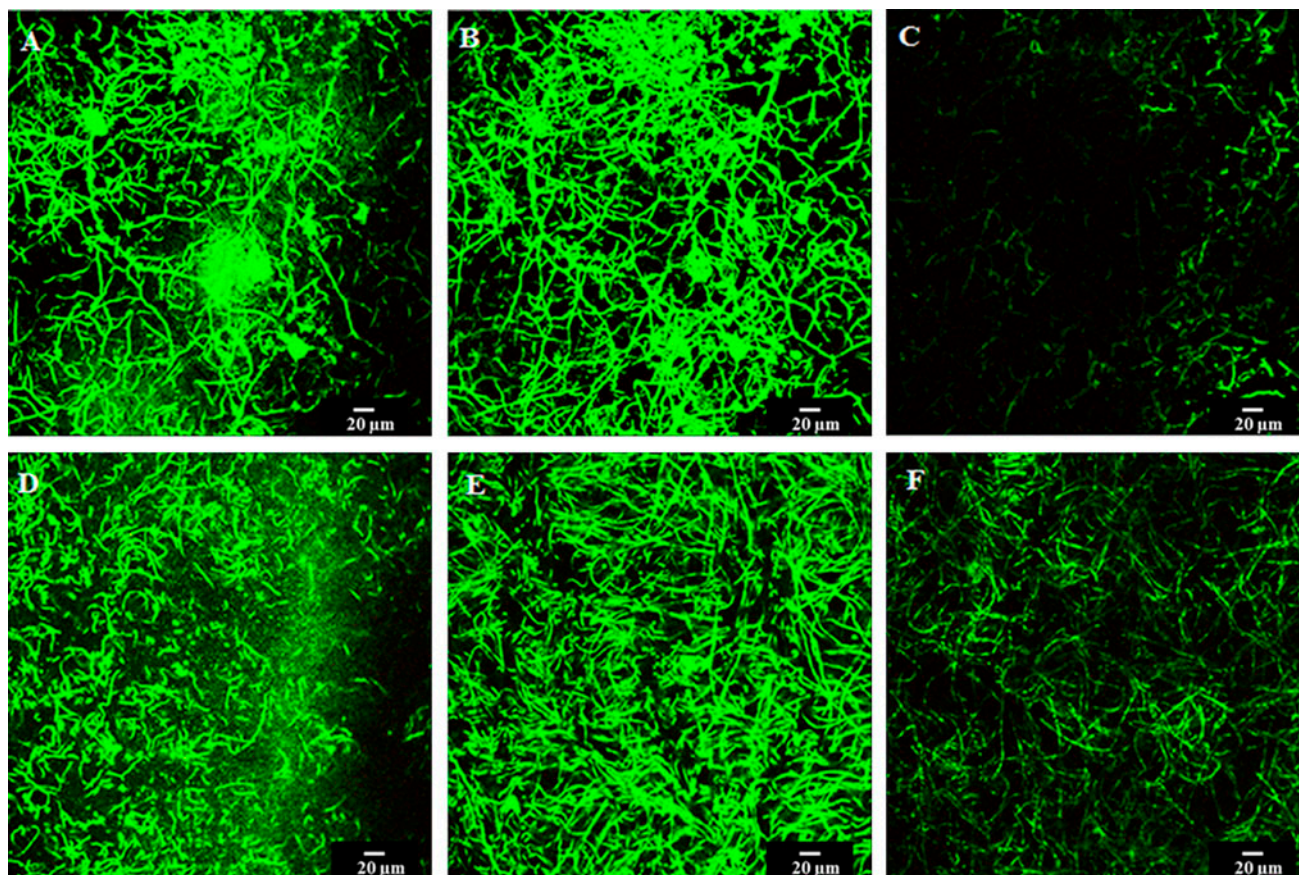


Figure 6. CLSM images of the mature biofilms of *T. mentagrophytes* ATCC 11481 and *T. rubrum* ATCC 28189. The images represent the bases (A, D), the middle sections (B, E) and the tops of the biofilms (C, F).

metabolic activity, is converted to orange-colored formazan salts, which are soluble in water and easily measured in cell supernatants. This makes them important in the study of biofilms, without the involvement of community structures. The overall result of the XTT reduction assays in the present study demonstrated that both species of dermatophyte showed similar results with regard to growth kinetics and 72 h was the peak of metabolic activity. Thus, 72 h was the ideal time for the development of mature *T. rubrum* and *T. mentagrophytes* biofilms.

Seidler et al. (2006, 2008) reported the ability of safranin to stain fungal polysaccharide structures and the extracellular matrix. The absorbance results showed that the *T. rubrum* ATCC 28189 biofilm produces more polysaccharide structures and/or extracellular matrix than the *T. mentagrophytes* ATCC 11481 biofilm. This fact was confirmed by SEM in which a larger amount of extracellular matrix was observed surrounding the hyphal network in *T. rubrum* biofilm. The same occurred when both fungi were stained with crystal violet. The *T. rubrum* ATCC 28189 biofilm showed a greater biomass when compared with the *T. mentagrophytes* ATCC 11481 biofilm.

SEM results demonstrated that *T. rubrum* and *T. mentagrophytes* form biofilms on polystyrene surfaces. After biofilm formation for 72 h, a coordinated network of hyphae was observed, growing in all directions, crossing each other and embedded in some areas within an extracellular polysaccharide matrix (EPS), and comprising the ultrastructure of the mature biofilm. The *T. rubrum* ATCC 28189 biofilm appeared more compact than that of *T. mentagrophytes* ATCC 11481 and, throughout its length, it appeared to have more areas containing EPS. In some places within the biofilm, the extracellular matrix seemed to be thick and compact, giving the hyphae a wrinkled appearance. The analysis of confocal imaging sections taken across the biofilm showed the fungal mass and its active cells. The thickness of the mature biofilms was different: that of *T. rubrum* ATCC 28189 had a thickness of 249.67 μm and that of *T. mentagrophytes* ATCC 11481 a thickness of 134.82 μm .

It is known that several fungi that form biofilms are more resistant to antifungal agents (Fanning & Mitchell 2012), so this may explain persistent infection, resistance and the long-term treatment required for dermatophytosis, especially in onychomycosis. Thus, the characterization

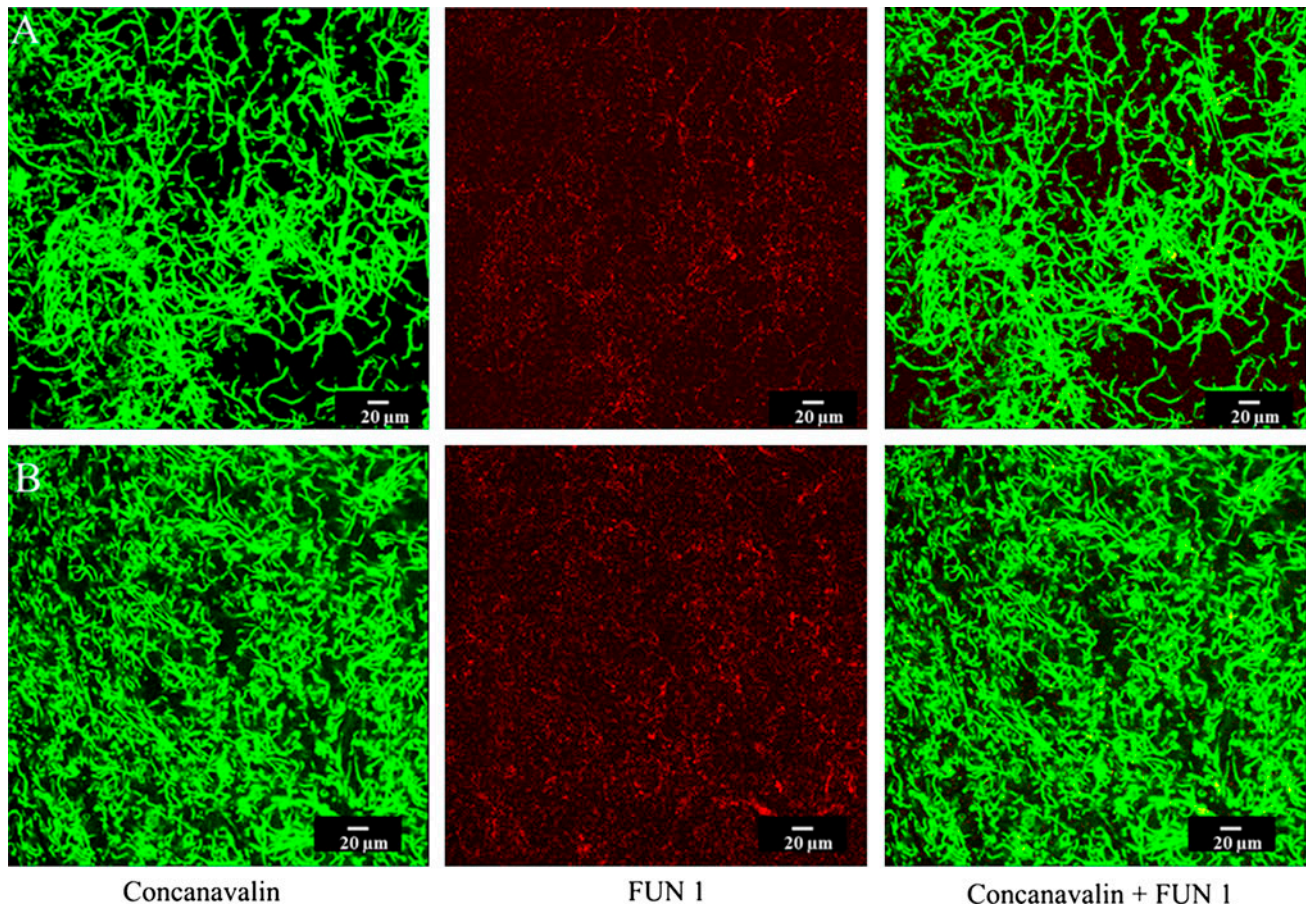


Figure 7. CLSM images of the mature biofilms *T. mentagrophytes* ATCC 11481 (A) and *T. rubrum* ATCC 28189 (B). ConA stains the fungal cell wall and the extracellular matrix green, whereas FUN 1 stains the active cells red.

and confirmation of biofilm formation by these pathogens is an extremely important tool for possible revision of antifungal doses and time of treatment, as well as for the discovery of new targets for new antifungal drugs.

Acknowledgements

This work was supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and PADC FCFAR UNESP (Programa de Desenvolvimento Científico – School of Pharmaceutical Sciences-UNESP. C.B. Costa-Orlandi has a fellowship from CNPq [process number: 141073/2012-8].

References

- Achterman RR, White TC. 2012. A foot in the door for dermatophyte research. *PLoS Pathog.* 8:e1002564.
- Ajesh K, Sreejith K. 2012. *Cryptococcus laurentii* biofilms: structure, development and antifungal drug resistance. *Mycopathologia.* 174:409–419.
- Aquino VR, Constante CC, Bakos L. 2007. Frequency of dermatophytosis in mycological examinations at a general hospital in Porto Alegre, Brazil. *An Bras Dermatol.* 82:239–244.
- Baldo A, Monod M, Mathy A, Cambier L, Bagut ET, Defaweux V, Symoens F, Antoine N, Mignon B. 2012. Mechanisms of skin adherence and invasion by dermatophytes. *Mycoses.* 55:218–223.
- Bojsen RK, Andersen KS, Regenber B. 2012. *Saccharomyces cerevisiae* – a model to uncover molecular mechanisms for yeast biofilm biology. *FEMS Immunol Med Microbiol.* 65:169–182.
- Burkhart CN, Burkhart CG, Gupta AK. 2002. Dermatophytoma: recalcitrance to treatment because of existence of fungal biofilm. *J Am Acad Dermatol.* 47:629–631.
- Chandra J, Patel JD, Li J, Zhou G, Mukherjee PK, McCormick TS, Anderson JM, Ghannoum MA. 2005. Modification of surface properties of biomaterials influences the ability of *Candida albicans* to form biofilms. *Appl Environ Microbiol.* 71:8795–8801.
- Colombo AL, Padovan AC, Chaves GM. 2011. Current knowledge of *Trichosporon* spp. and trichosporonosis. *Clin Microbiol Rev.* 24:682–700.
- Costa-Orlandi CB, Magalhães GM, Oliveira MB, Taylor EL, Marques CR, de Resende-Stoianoff MA. 2012. Prevalence of dermatomycosis in a Brazilian tertiary care hospital. *Mycopathologia.* 174:489–497.
- Ells TC, Truelstrup Hansen L. 2006. Strain and growth temperature influence *Listeria* spp. attachment to intact and cut cabbage. *Int J Food Microbiol.* 111:34–42.

- Fanning S, Mitchell AP. 2012. Fungal biofilms. *PLoS Pathog.* 8:e1002585.
- Figueredo LA, Cafarchia C, Desantis S, Otranto D. 2012. Biofilm formation of *Malassezia pachydermatis* from dogs. *Vet Microbiol.* 160:126–131.
- Harding MW, Marques LL, Howard RJ, Olson ME. 2009. Can filamentous fungi form biofilms? *Trends Microbiol.* 17:475–480.
- Kuhn DM, Chandra J, Mukherjee PK, Ghannoum MA. 2002. Comparison of biofilms formed by *Candida albicans* and *Candida parapsilosis* on bioprosthetic surfaces. *Infect Immun.* 70:878–888.
- Kuhn DM, Balkis M, Chandra J, Mukherjee PK, Ghannoum MA. 2003. Uses and limitations of the XTT assay in studies of *Candida* growth and metabolism. *J Clin Microbiol.* 41:506–508.
- Macura AB, Krzysciak P, Skora M, Gniadek A. 2010. Dermatophyte species in superficial mycoses in the Krakow district, Poland in the years 1972–2007. *Mycoses.* 53:148–152.
- Maranhao FC, Silveira HC, Rossi A, Martinez-Rossi NM. 2011. Isolation of transcripts overexpressed in the human pathogen *Trichophyton rubrum* grown in lipid as carbon source. *Can J Microbiol.* 57:333–338.
- Martins M, Henriques M, Lopez-Ribot JL, Oliveira R. 2012. Addition of DNase improves the *in vitro* activity of antifungal drugs against *Candida albicans* biofilms. *Mycoses.* 55:80–85.
- Monod M. 2008. Secreted proteases from dermatophytes. *Mycopathologia.* 166:285–294.
- Moraes MAP, Machado AAL, Medeiros Filho P, Reis CMS. 2001. Pseudomycetoma dermatofítico: relato de um caso devido a *Trichophyton tonsurans* [Dermatophytic pseudomycetoma: report of a case caused by *Trichophyton tonsurans*]. *Rev Soc Bras Med Trop.* 34: 291–294.
- Morris CE, Monier J, Jacques M. 1997. Methods for observing microbial biofilms directly on leaf surfaces and recovering them for isolation of culturable microorganisms. *Appl Environ Microbiol.* 63:1570–1576.
- Mowat E, Butcher J, Lang S, Williams C, Ramage G. 2007. Development of a simple model for studying the effects of antifungal agents on multicellular communities of *Aspergillus fumigatus*. *J Med Microbiol.* 56:1205–1212.
- Muszkietka L, Beauvais A, Pahtz V, Gibbons JG, Anton Leberre V, Beau R, Shibuya K, Rokas A, Francois JM, Kniemeyer O, et al. 2013. Investigation of *Aspergillus fumigatus* biofilm formation by various ‘omics’ approaches. *Front Microbiol.* 4:13.
- Peiqian L, Xiaoming P, Huifang S, Jingxin Z, Ning H, Birun L. 2013. Biofilm formation by *Fusarium oxysporum* f. sp. cucumerinum and susceptibility to environmental stress. *FEMS Microbiol Lett.* Oct 24. Epub 2013/10/30.
- Percival SL, Emanuel C, Cutting KF, Williams DW. 2012. Microbiology of the skin and the role of biofilms in infection. *Int Wound J.* 9:14–32.
- Peres NT, Sanches PR, Falcao JP, Silveira HC, Paiao FG, Maranhao FC, Gras DE, Segato F, Cazzaniga RA, Mazucato M, et al. 2010. Transcriptional profiling reveals the expression of novel genes in response to various stimuli in the human dermatophyte *Trichophyton rubrum*. *BMC Microbiol.* 10:39.
- Pinheiro AdQ, Moreira JLB, Sidrim JJC. 1997. Dermatofitoses no meio urbano e a coexistência do homem com cães e gatos [Dermatophytosis in the urban environment and the coexistence of man with dogs and cats]. *Rev Soc Bras Med Trop.* 30:287–294.
- Pires RH, Montanari LB, Martins CH, Zaia JE, Almeida AM, Matsumoto MT, Mendes-Giannini MJ. 2011. Anticandidal efficacy of cinnamon oil against planktonic and biofilm cultures of *Candida parapsilosis* and *Candida orthopsilosis*. *Mycopathologia.* 172:453–464.
- Pires RH, Santos JM, Zaia JE, Martins CH, Mendes-Giannini MJ. 2011. *Candida parapsilosis* complex water isolates from a haemodialysis unit: biofilm production and *in vitro* evaluation of the use of clinical antifungals. *Mem Inst Oswaldo Cruz.* 106:646–654.
- Pitangui NS, Sardi JC, Silva JF, Benaducci T, Moraes da Silva RA, Rodriguez-Arellanes G, Taylor ML, Mendes-Giannini MJ, Fusco-Almeida AM. 2012. Adhesion of *Histoplasma capsulatum* to pneumocytes and biofilm formation on an abiotic surface. *Biofouling.* 28:711–718.
- Ramage G, Vandewalle K, Wickes BL, Lopez-Ribot JL. 2001. Characteristics of biofilm formation by *Candida albicans*. *Rev Iberoam Micol.* 18:163–170.
- Ramage G, Mowat E, Jones B, Williams C, Lopez-Ribot J. 2009. Our current understanding of fungal biofilms. *Crit Rev Microbiol.* 35:340–355.
- Saenz FJ. 2001. Identificación de hongos dermatofitos [Identification of dermatophytes]. In: *Rev Iberoam Micol.* p. 1–11.
- Sardi JC, Scorzoni L, Bernardi T, Fusco-Almeida AM, Mendes Giannini MJ. 2013. *Candida* species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. *J Med Microbiol.* 62:10–24.
- Seidler M, Salvenmoser S, Muller FM. 2006. *In vitro* effects of micafungin against *Candida* biofilms on polystyrene and central venous catheter sections. *Int J Antimicrob Agents.* 28:568–573.
- Seidler MJ, Salvenmoser S, Muller FM. 2008. *Aspergillus fumigatus* forms biofilms with reduced antifungal drug susceptibility on bronchial epithelial cells. *Antimicrob Agents Chemother.* 52:4130–4136.
- Sengupta J, Saha S, Khetan A, Sarkar SK, Mandal SM. 2012. Effects of lactoferricin B against keratitis-associated fungal biofilms. *J Infect Chemother.* 18:698–703.
- Silveira HC, Gras DE, Cazzaniga RA, Sanches PR, Rossi A, Martinez-Rossi NM. 2010. Transcriptional profiling reveals genes in the human pathogen *Trichophyton rubrum* that are expressed in response to pH signaling. *Microb Pathog.* 48:91–96.
- Soares LA, Sardi JCO, Gullo FP, Pitangui NS, Scorzoni L, Sangalli-Leite F, Mendes-Giannini MJS, Fusco-Almeida AM. 2013. Anti-dermatophytic therapy – prospects for the discovery of new drugs from natural products. *Braz J Microbiol.* 4: 1035–1041.
- Vermont S, Tabart J, Baldo A, Mathy A, Losson B, Mignon B. 2008. Pathogenesis of dermatophytosis. *Mycopathologia.* 166:267–275.
- Vlassova N, Han A, Zenilman JM, James G, Lazarus GS. 2011. New horizons for cutaneous microbiology: the role of biofilms in dermatological disease. *Br J Dermatol.* 165:751–759.
- Weitzman I, Summerbell RC. 1995. The dermatophytes. *Clin Microbiol Rev.* 8:240–259.
- Zhang X, Wang Y, Chi W, Shi Y, Chen S, Lin D, Jin Y. 2013. Metalloprotease genes of *Trichophyton mentagrophytes* are important for pathogenicity. *Med Mycol.* 16. Epub 2013/07/19.
- Zurita J, Hay RJ. 1987. Adherence of dermatophyte microconidia and arthroconidia to human keratinocytes *in vitro*. *J Invest Dermatol.* 89:529–534.