

## A fast and cost-effective methodology for *Fonsecaea pedrosoi* ATCC46428 staining using ESIPT fluorescent dyes

Valeriano Antonio Corbellini<sup>c</sup>, Maria Lúcia Scroferneker<sup>b,\*</sup>, Mariana Carissimi<sup>b</sup>,  
Fabiano Severo Rodembusch<sup>a</sup>, Valter Stefani<sup>a,\*\*</sup>

<sup>a</sup> Instituto de Química, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, CP 15003, 91501-970 Porto Alegre-RS, Brazil

<sup>b</sup> Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Rua Sarmento Leite, 500, 90050-170 Porto Alegre-RS, Brazil

<sup>c</sup> Departamento de Química e Física, Universidade de Santa Cruz do Sul (UNISC), Av. Independência 2293, 96815-900 Santa Cruz do Sul-RS, Brazil

### ARTICLE INFO

#### Article history:

Received 27 November 2008

Received in revised form 18 March 2010

Accepted 19 March 2010

Available online 21 March 2010

#### Keywords:

Chromoblastomycosis

Benzazole

Cell staining

Proton transfer

Epifluorescence microscopy

Black fungus

### ABSTRACT

The microscopic morphology of *Fonsecaea pedrosoi* ATCC46428 was observed using two benzazole derivatives, 2-(2'-hydroxyphenyl)benzoxazole and 2-(5'-amino-2'-hydroxyphenyl)benzoxazole, which emit intense fluorescence by a proton transfer mechanism in the electronically excited state (ESIPT). The cell surface could be successfully stained with fluorescent dye solutions of 10  $\mu$ M–10 mM using two different fast and cost-effective procedures. At these concentrations, any structure or dye crystallization could be observed. Concerning the external microstructural details, only the amino derivative allowed the differentiation between hyphae and conidia. These dyes presented some advantages comparing to commercial dyes, since the stained cells showed high chemical, thermal and photochemical stability during the experiments and also after several months of storage at room temperature and normal light exposition. Procedure 1 presented the advantage to be used when heating can change the chemical or biochemical cell composition. On the other hand Procedure 2 showed to be useful as a routine methodology for cells staining.

The results allowed to propose a simple and highly sensitive assay to study the *F. pedrosoi* micromorphology by epifluorescence microscopy. This methodology can probably be extended for other fungi of clinical interest.

© 2010 Elsevier B.V. All rights reserved.

### 1. Introduction

Fluorescence techniques have been used as important tools in microscopy to study cell structure morphologies [1]. The development of new fluorochromes with more specific properties regarding tissue staining and light, air and temperature stability presents great interest. The epifluorescence microscopy (EFM) has been used in mycology to assess the cell viability of yeasts and spores [2,3], the growth of fungi [4,5], and their morphology in soil and clinical specimens [6–8]. For this purpose several dyes have been used, including Ethidium bromide, Propidium iodide [2,3], Fluorescein diacetate [9,10], Carboxyfluorescein diacetate [2,3], Sulfofluorescein diacetate derivatives [11] and Rhodamine B [12]. Another dye, Calcofluor White®, have been used since 1984 to evaluate the histoarchitecture of cell elements [13–15], and in diagnostic mycology to quantify and identify the fungal cells in soils [16], plants [17] and clinical specimens [18–21].

\* Corresponding author. Tel.: +55 51 3308 3934; fax: +55 51 3308 3135.

\*\* Corresponding author. Tel.: +55 51 3308 7206; fax: +55 51 3308 7304.

E-mail addresses: [scrofern@ufrgs.br](mailto:scrofern@ufrgs.br) (M. Lúcia Scroferneker), [vstefani@iq.ufrgs.br](mailto:vstefani@iq.ufrgs.br) (V. Stefani).

Compounds emitting fluorescence through an excited state intramolecular proton transfer (ESIPT) [22,23] mechanism (Fig. 1) have become a very attractive field of research by virtue of the widespread applications that can be envisaged for these dyes [24–30]. Particular interest has been presented in some benzoxazole derivatives, since these dyes have been used as probe to investigate hydrophobic–hydrophilic environments in systems that use polymers for drugs delivery [31]. Usually the normal enol form (Fig. 1) is the most stable conformer of these dyes in the ground state. This conformer on excitation undergoes ESIPT to form the keto tautomer, which gives rise to an emission with large Stokes shift. Additional conformers are described and do not undergo ESIPT [32]. These species are responsible for short wavelength emission bands, which can compete with the ESIPT-exhibiting dyes. Evidence of these conformational equilibria in solution in the ground state could already be observed experimentally through a solvatochromic effect in the UV–Vis absorption spectra or by dual fluorescence emission [32].

*Fonsecaea pedrosoi* is a black fungus responsible for the development of chromoblastomycosis in humans [33,34]. The fungus has several patterns of conidiogenesis, which requires an accurate morphological analysis for diagnostic and therapeutic purposes

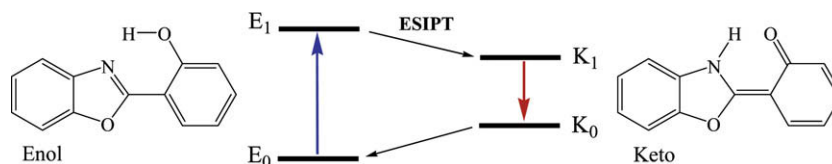
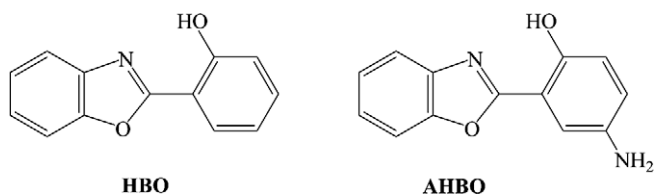


Fig. 1. ESIPT mechanism of the HBO.



Scheme 1. Structure of 2-(2'-hydroxyphenyl)benzoxazole (HBO) and 2-(5'-amino-2'-hydroxyphenyl)benzoxazole (AHBO).

[35]. Many studies in respect to differentiation [36,37] and cell surface components [38–41] of the different fungal forms have been performed, which can be related to the fungus adaptation to sustain the cellular viability [42–44]. Additionally, several methodologies have been used to follow those morphological changes to better understand the virulence and pathogenic mechanisms [45,46]. This paper presents a fast and cost-effective methodology of *F. pedrosoi* cell staining based on benzoxazole dyes comparatively to Fluorescein and Calcofluor White®.

## 2. Materials and methods

### 2.1. Biological and chemical materials

Reagents grade 2-aminophenol and 5-aminosalicylic acid, polyphosphoric acid, purchased from Aldrich (Saint Louis, United States), and salicylic acid from Synth (Diadema, Brazil) were used as received to the fluorescent dyes synthesis. Fluorescein Sodium

Salt was purchased from Carlo Erba (Rodano, Italy), Calcofluor White® and Rhodamine B from Aldrich (Saint Louis, United States) and used without any further purification. Hexane, dichloromethane, chloroform and absolute ethanol were purchased from Synth (Diadema, Brazil) and 1,4-dioxane and *n*-octanol from Riedel de Hën (Hanover, Germany) or Nuclear (Diadema, Brazil). All solvents were used as received. Silicagel 60 Merck (Darmstadt, Germany) was used for chromatographic column separations. Potato dextrose agar (PDA), Sabouraud dextrose broth (SDB) and Sabouraud dextrose agar (SDA) were purchased from DIFCO (Detroit, United States). Tween 80 was purchased from Synth (Diadema, Brazil). The *F. pedrosoi* ATCC46428 samples was obtained from the Laboratory of Fungal Immunology of the Institute of Basic Health Sciences of Universidade Federal do Rio Grande do Sul (Porto Alegre, Brazil) and was kept in SDA at 36.5 °C.

Table 1

Relevant photophysical data of the benzoxazole derivatives.

	Solvent	$\lambda_{\text{max}}^{\text{abs}}$ (nm)	$\lambda_{\text{max}}^{\text{em}}$ (nm)	$\Delta\lambda_{\text{ST}}$ (nm)
HBO	Dichloromethane	321	495	174
	1,4-Dioxane	321	500	179
	Octanol	322	493	171
AHBO	Dichloromethane	369	565	196
	1,4-Dioxane	395	460	65
	Octanol	383	507	124

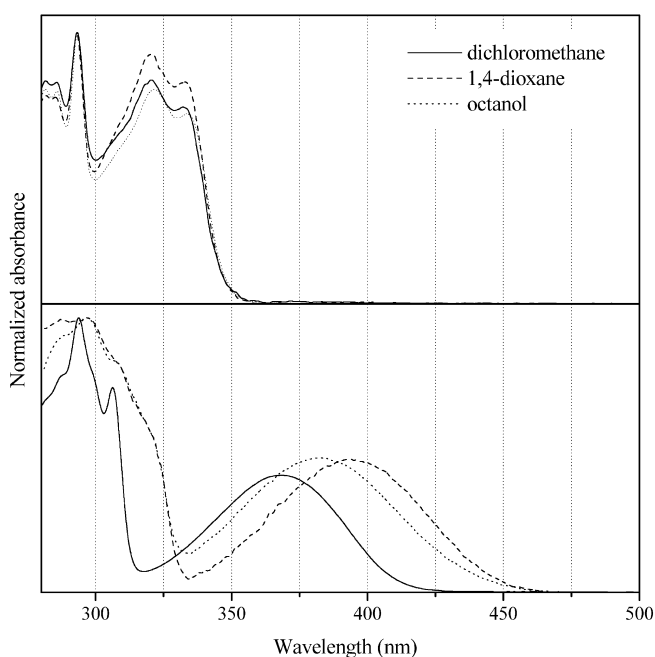


Fig. 2. Normalized UV-Vis absorption spectra of the HBO (top) and AHBO (bottom).

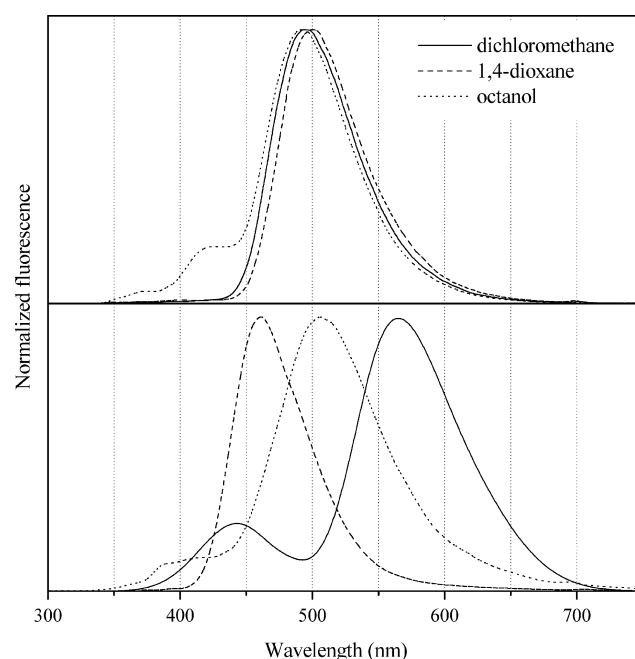


Fig. 3. Normalized fluorescence emission spectra of the HBO (top) and AHBO (bottom).

Download English Version:

<https://daneshyari.com/en/article/29768>

Download Persian Version:

<https://daneshyari.com/article/29768>

[Daneshyari.com](https://daneshyari.com)