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Fluorescence in the *Badhamia utricularis* plasmodium

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Abstract: A plasmodium of *Badhamia utricularis* was collected in the Bio Bio Region in Chile. Fluorescence was documented in the UV region and a qualitative characterization of the plasmodial extract was carried out in seven solvents with different dielectric constants. Also, a reverse phase chromatographic analysis coupled with a detector of fluorescence was used to identify the amount of fluorescent compounds. In the studied species, three or four fluorophores were identified, and two low polarity compounds that exhibited fluorescence were registered. This investigation has the potential to expand the information on the vegetative stage of myxomycetes and lead to new research on these organisms.

Keywords: biotechnology, Chile, ecology, vegetative stage.

Introduction

Myxomycetes (also called slime molds) are cosmopolitan, amoeboid organisms of variable shapes and colors (Stephenson and Stempen 1994). At present they are part of the Kingdom Protista but were formerly classified within the Kingdom Fungi and more than a thousand species are known worldwide. One of the vegetative stages, known as a plasmodium, is a multinucleated cell without cell walls, capable of actively moving over various substrates, even in extreme environments such as desert or snow (Keller et al. 2022). Myxomycetes are an excellent study group for their abundant production of secondary metabolites with a number of antifungal, antibacterial, and anticancer applications.

The present study focused on autofluorescence, a phenomenon understudied in myxomycetes that can potentially be useful for applied research. Such singularity has been previously reported for both sporocarp extracts (Steglitch 1989) and their surfaces (Carmarán et al. 2013). It is also known in cellular slime molds (Uchiyama et al. 1994), but in both groups of mycetozoans, there is no clear explanation supporting the mechanism of its existence. In that way, a number of potential explanations have been offered, but given the poor record of study of this phenomenon, most of those arguments are speculative.

The objective of this study was not to provide a potential explanation of the phenomenon but to document the presence of autofluorescence in the plasmodium of *Badhamia utricularis* collected in a

temperate Chilean forest. This documentation is made with the purpose of communicating the phenomenon and generating discussion around a topic that is both interesting and can derive possible applications in the future.

Materials and methods

A plasmodium of *B. utricularis* was collected in a temperate forest of the Bio Bio Region of Chile on 12 July 2020 during the winter season. The species was identified based on sporocarps (Fig. 1) growing on the plasmodium by using the book of Neubert et al. (1995) and asking Dr. Carlos Lado, who had previously worked in that area.

From the collected plasmodium, a culturing process was initiated in Petri dishes lined with filter paper (Fig. 2). For this, a section of the plasmodium was carefully set on the filter paper and oat flour was provided as a food source along with small quantities of bottled water. The plasmodium was re-cultured every 3 or 4 days using the same protocol during the length of the study in order to have fresh material for documentation.

Figure 1. Plasmodium (A) and sporocarps (B) of *B. utricularis* originally collected in the Bio Bio region of Chile for culturing the plasmodia for the experimentation explained in this study.

A completely dark study chamber with an installed UV light emitting at 395-400 nm was used to document the fluorescence. For this, both an examination of cultures until the plasmodia dried out completely and after carefully tearing the outer layer of the plasmodium was carried out. Also, sections of the plasmodia at different stages of development were placed on glass slides for documenting the fluorescence under the microscope.

Two excitation wavelengths to determine the peaks of fluorescence intensity were revealed the from the absorbance spectrum of plasmodial dilutions using a Jasco V-650 spectrophotometer, and one was selected for being close to the maximum of excitation of pteridines, the molecules observed in other mycetozoans. The intensity of fluorescence was determined by making plasmodial extracts (100 mg of

plasmodial mass and diluting it in 4 ml of solvent) using seven different solvents (water, acetone, ethanol, ethyl acetate, chloroform, methanol, and acetonitrile). 1.5 ml of each solution was centrifugated for ten minutes at 3400 rpm and used to obtain the emission spectra in a Shimadzu RF-5301PC spectrofluorophotometer by using the three excitation wavelengths determined earlier. Finally, to identify the potential fluorescent compounds, a reversed-phase chromatography was carried out in a Shimadzu Nexera UHPLC with 0.1% formic acid as the mobile phase A and 100% acetonitrile as phase B (10 µl dilution in methanol at 0.5 ml/min).

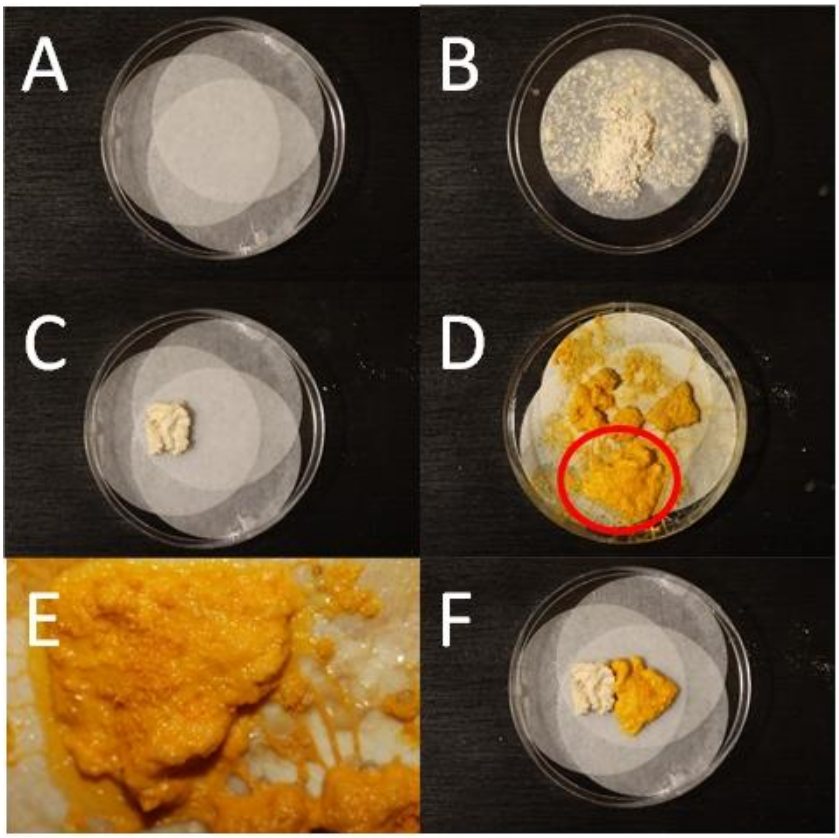

Figure 2. Culturing process of the studied plasmodium. A, initial setup. B and C, wet oat flour and oat flour mixture with a doughy texture. D and E, growing plasmodium and oat flour pile covered by it used to generate the new culture. F, new culture.

Results

Fluorescence, in the UV region, was detected in the plasmodium of *B. utricularis*, and any damage to the outer layer of the plasmodium increased the intensity of the phenomenon (Fig. 3). Plasmodia in the process of drying out completely showed the highest intensity. Fluorescence observed under the microscope was more intense in both the plasmodium in vegetative mode and at initial stages of sporocarp formation (Fig. 4). Upon damage of about 1 cm^2, the maximum intensity of fluorescense was observed after 3-4 minutes (Fig. 5) and the plasmodium healed in about three hours.

The three excitation wavelenghts used were 290, 360 and 390 nm. In general, the peaks of fluorescense intensity showed a redshift at higher wavelengths (Fig. 6). The chromatogram showed two fluorescent peaks at 29.8 and 30.1 minutes, when the concentration of the Phase B was 100%. The first signal had two absorption bands at 320 and 361 nm, whereas the second signal absorbed at 311 nm (Fig. 7).

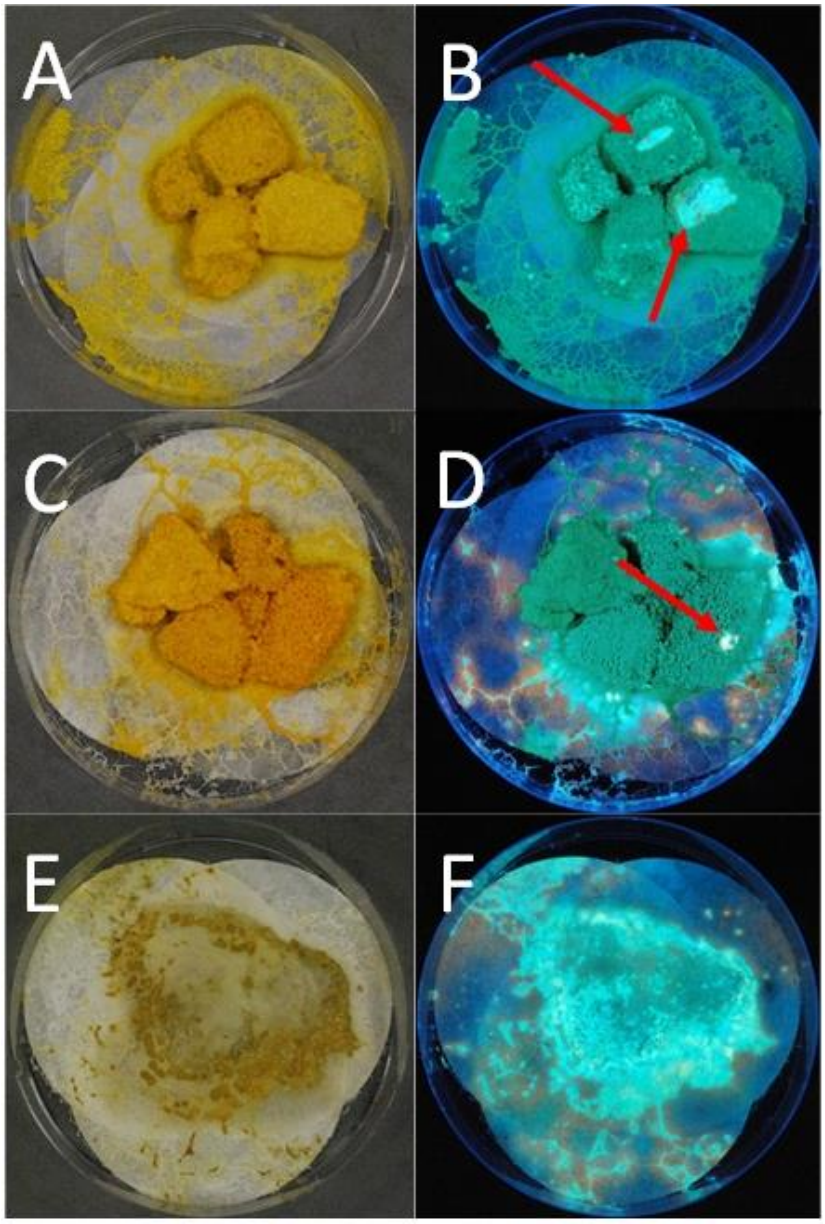

Figure 3. Cultured plasmodium seen under natural (left) and UV light at 366 nm (right) where the autofluorescence can be observed at different intensities (bluish green). From A to C the yellow color of the plasmodium intensified but there is no effect on the emitted fluorescence (B and D). In E, when the plasmodium is at a further stage of drying out, the fluorescence intensified to a maximum (F). All arrows show the effect of mechanical damage on the outer layer of the plasmodium, which promoted fluorescence.

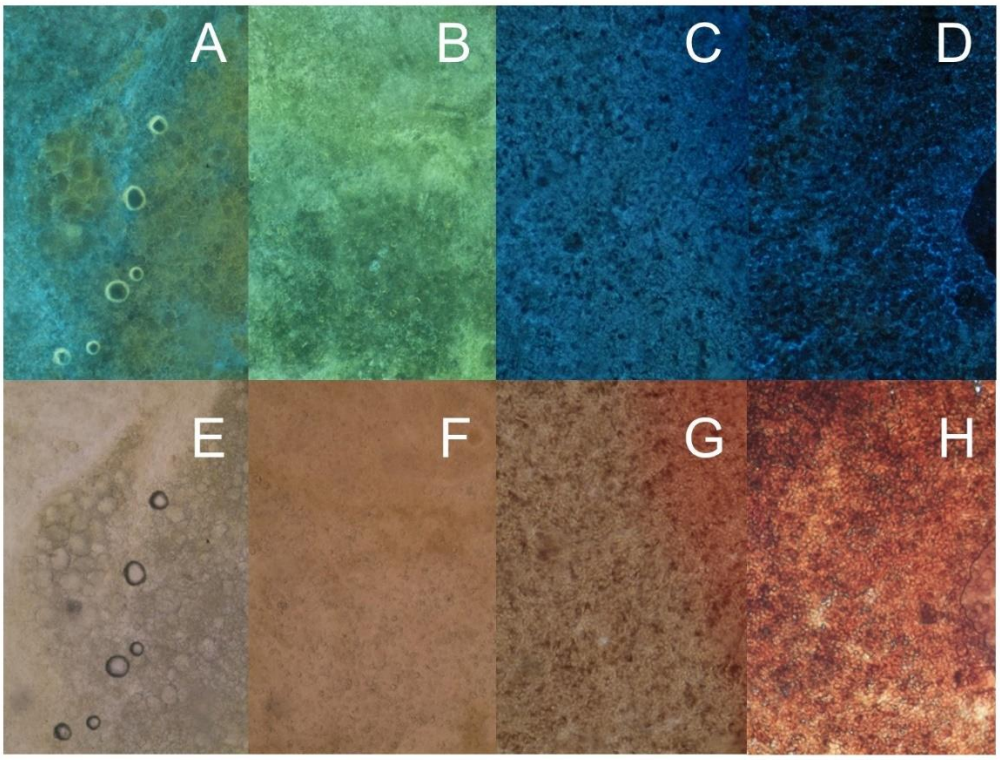

Figure 4. Fluorescence (top) during the process of sporocarp formation as observed under the microscope. View with normal transmitted light at the bottom. A and E correspond to the normal plasmodium. B and F to a plasmodium in the process of sporocarp formation (highest fluorescence in B). C and G to a plasmodium that initiated sporocarp formation. D and H show the plasmodium at a late stage of sporocarp formation, with lowest fluorescence and tighter structure.

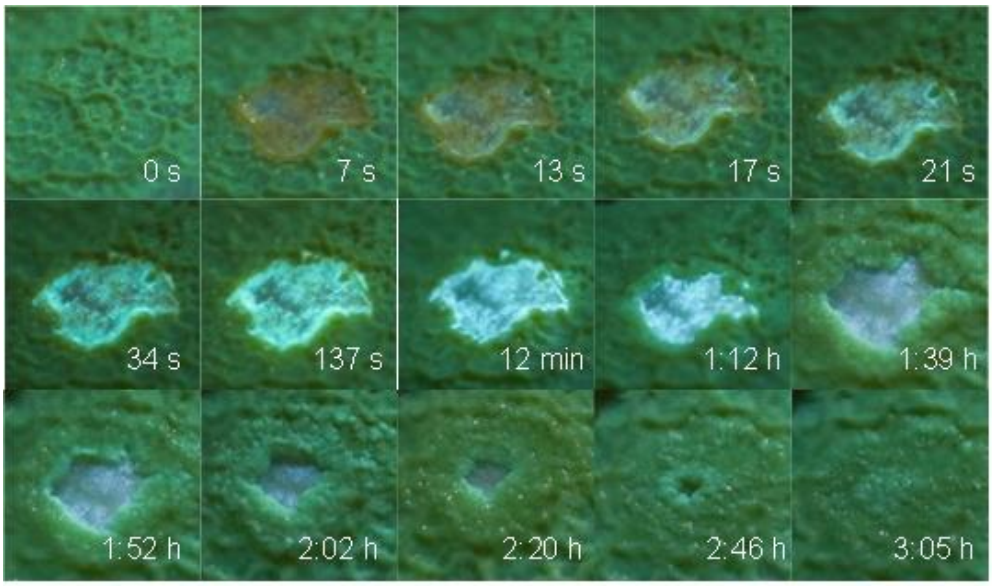

Figure 5. Plasmodium showing a mechanical damage to its outer layer and the subsequent process of healing over a period of about three hours. Fluorescence was observed during the healing process.

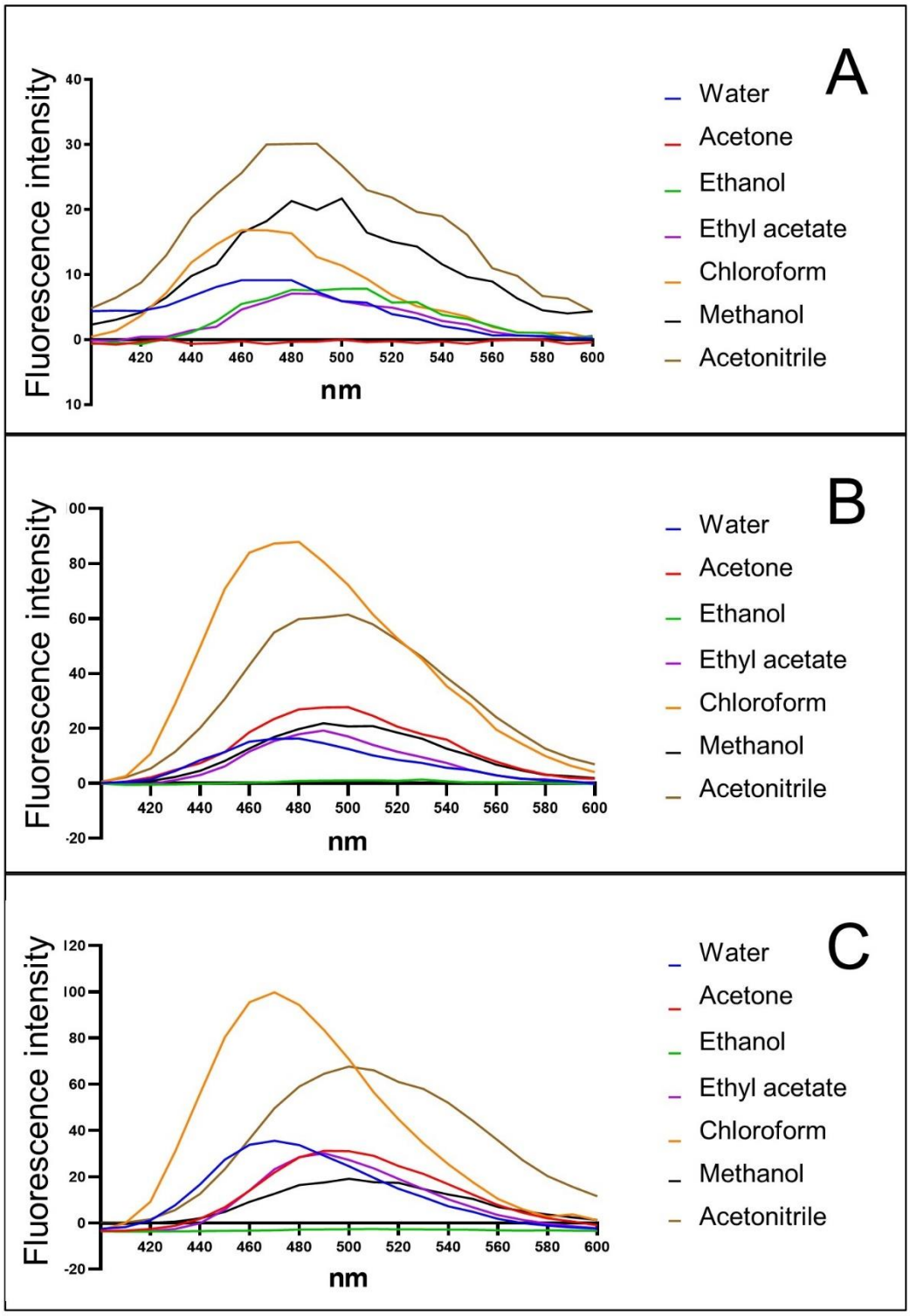

Figure 6. Comparison of the emitted fluorescence in the plasmodial extracts at three excitation wavelengths (A=290 nm, B=360 nm, C=390 nm) using different solvents. A redshift trend is observed at higher wavelengths (from A to C).

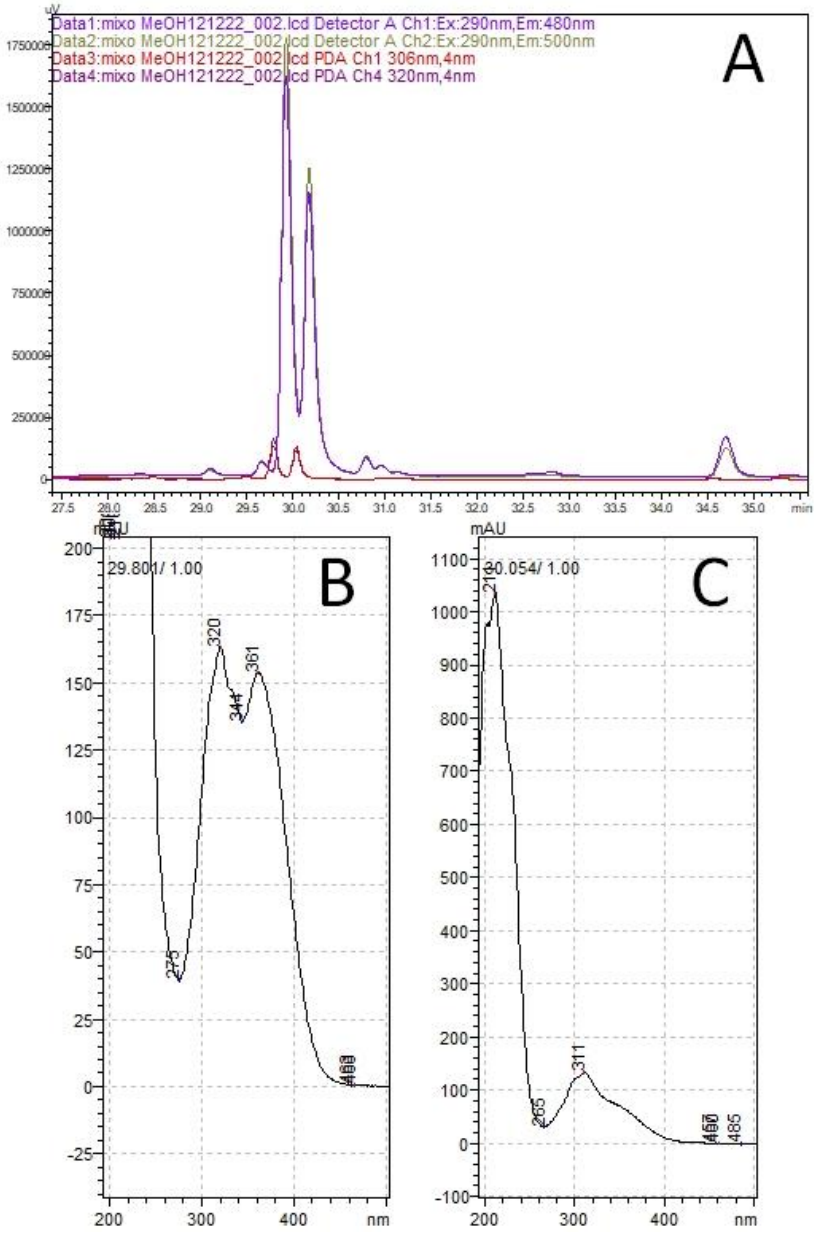

Figure 7. Chromatographic representation of the qualitative analysis of a sample of plasmodial extract excited at 290 nm showing emissions at 480 and 500 nm (A) and absorption spectra of two obtained compounds using UHPLC (B and C).

Discussion

The fluorescence in the *B. utricularis* plasmodium was documented succesfully in the UV region of the spectrum. Based on the literature, similar species can contain a number of photoreceptors such as pteridines, carotenoids, phenolic compounds, peptides, flavones, and polyenes that contain nitrogen (Nowak and Steffan 1997). From all of those, the most similar to the ones observed in this study are the

pteridines that have yellow color and have two peaks of absorption between 220-290 nm and 300-400 nm (Lorente 2003; Serrano 2014).

Uchiyama et al. (1997) reported pteridines in the spore mass of *Dictyostelium discoideum* (at 466 nm) and suggested that these molecules would work as photoprotectors, absorbing damaging UV-C light and emitting inofensive fluorescence. Such observations would explain the increase in intensity in metabolically active stages (i.e., plasmodium to sclerotium switch, healing after mechanical damage) and would suggest that fluorescence in myxomycete plasmodia is a strategy for radiation protection.

Based on the observations presented in this study, it seems that there are three or four fluorophores, but the UHPLC only elucidated two. However, these only appeared when the phase B was at 100% suggesting apolarity. Since these procedures were only carried out once in the present work, it is possible that other replicas would bring extra information (more compounds) not observed herein. In this manner, the results presented in this study could be complemented with more analyses in the future.

If the UV protection idea could be supported with more data and further analysis, the fluorescence observed in *B. utricularis*, that presumably can occur in other plasmodia as well, could serve as a starting point for various practical applications. As explained by Stoyneva-Gärtner et al. (2023), biotechnological approaches for the cosmetic industry or bioengineering applications could be based on this phenomenon.

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