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Autofluorescence of ascospores and conidial spores in selected lichen species of the *Opegrapha* genus

ANETTA WIECZOREK

Department of Ecology, University of Szczecin
Wąska 13, PL-71-415 Szczecin, anettaw@univ.szczecin.pl

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The paper presents findings of the research work on autofluorescence phenomenon in ascospores and conidial spores that was carried out on 12 selected species of the *Opegrapha* genus occurring in Poland.

Key words: lichens, *Opegrapha*, autofluorescence

INTRODUCTION

Autofluorescence is one of luminescence types and consists in emitting the own light, which is induced by photon capture by a body. Own luminescence of cells depends on the presence of endogenous chromophores in tissue. The phenomenon of autofluorescence is frequent not only in plants but also in fungi (Rost 1992). It has been observed, among others, in the cytoplasm of ascospore and konidial spore hyphae (Žižka, Gabriel 2006). Studies on the usefulness of autofluorescence methods in identification of fungi species are used in examination of mycorrhiza phenomenon (Séjalon-Delmas et al. 1998) and in laboratory diagnostics, in particular of pathogenic fungi, and consist among other in determination of tissue colour differences in healthy plants and animals and those pathologically changed (Margo, Bombardier 1985; Elston 2001). In lichens, autofluorescence phenomenon has been used, among others, in evaluation of the viability of hyphae and algal cells according to the method of their storage (Honegger 2003) as well as in evaluation of damages in the chlorophyll apparatus of algal cells induced by high pollutant concentration (Favali et al. 1991; Silberstein et al. 1996). Sometimes, this phenomenon can be used for determining germination capacity (Cathy, Warren 1984). This study aimed at taking up an attempt to use the autofluorescence phenomenon in identification of lichen species of the *Opegrapha* genus.

MATERIAL AND METHODS

In the study were used selected epiphytic and epilithic species of the *Opegrapha* genus occurring in Poland (*O. atra* Pers., *O. calcarea* Sm., *O. culmigena* Lib., *O. dolomitica* (Arnold) Clauzade & CL. Roux, *O. gyrocarpa* Flot., *O. niveoatra* (Borrer) J. R. Laundon, *O. rufescens* Pers., *O. rupestris* Pers., *O. varia* Pers., *O. vermicellifera* (Kunze) J. R. Laundon, *O. viridis* (Ach.) Behlen & Desberger, and *O. vulgata* (Ach.) Ach. Species terminology was adopted after Santesson et al. (2004) and Dietrich et al. (2008). In order to determine the intensity of autofluorescence, a series of microscope slides was prepared, 10 samples per each species, using the fresh material collected by the author from the bark of different tree species within the area of the western Poland in 2004-2006 as well as the herbarium material. The samples collected from the herbarium material were from 1940-2001. In order to examine in detail the subtle structures, such as ascospores and conidial spores, squashed microscope slides were prepared. In the study, a LSM 510 laser scanning microscope (Zeiss), operating on an Axiovert 200M motorised inverted microscope with fluorescence (Zeiss), was used. The used laser was an argon-ion laser with 11% power output, emitting 488 nm light waves, equipped with a 63x objective for ascospores and a 100x objective for conidial spores and two red filters (between 505 nm–650 nm wavelength) and one green filter (to 505 nm wavelength); when observing ascospores and conidial spores, a Nomarski filter was used.

RESULTS AND DISCUSSION

In all examined species, the emission of light within the wavelength range from 488 nm to 505 nm, and sometimes over 650 nm, was found (Tab. 1). The examined taxa were separated into three groups with different fluorescence intensity. The ascospores of the following species were in the first group: *O. atra* (Fig. 1), *O. calcarea*, *O. dolomitica* (Fig. 2) and *O. rupestris* (Fig. 3), with the fluorescence intensity within both wavelength ranges being clearly observable. In the second group, the spores of *O. gyrocarpa*, *O. vermicellifera*, *O. vulgata* (Fig. 4), *O. viridis* and *O. culmigena* were found, the autofluorescence intensity of which was much weaker or almost completely inconspicuous. In case of the species of the second group, no expected effects were obtained despite intensification of autofluorescence with ultraviolet radiation. On the other hand, a more rapid burning of autofluorescence was observed after application of ultraviolet radiation.

Into the third group, the ascospores of such species as *O. niveoatra*, *O. varia* (Fig. 5) and *O. rufescens* (Fig. 6) were included, in which a variable intensity of fluorescence was found. Different level of autofluorescence intensity in these species can be induced on the one hand by a different depth of spore location in microscope slide but on the other hand by a different degree of ascospore maturity.

It should be emphasised that observation of spores at the stage of transversal wall formation (Figs 7-9) in the taxa with weak autofluorescence showed the occurrence of such phenomenon.

In the species with intense autofluorescence, a fading of red light emission was observed as deeper spore layers were scanned due to autofluorescence burning.

Table 1

Autofluorescence intensity of ascospores in selected lichen species of the *Opegrapha* genus

Species	Light emission intensity within wavelength range		
	488-659nm	488-505 nm	
	High	Weak	Very weak
<i>O. atra</i> Pers.	+		
<i>O. calcarea</i> Sm.	+		
<i>O. dolomitica</i> (Arnold) Clauzade & CL. Roux	+		
<i>O. rupestris</i> Pers.	+		
<i>O. varia</i> Pers.	+	+	
<i>O. rufescens</i> Pers.	+	+	+
<i>O. niveoatra</i> (Borrer) J. R. Laundon		+	+
<i>O. gyrocarpa</i> Flot.		+	
<i>O. vermicellifera</i> (Kunze) J. R. Laundon		+	
<i>O. vulgata</i> (Ach.) Ach.		+	
<i>O. viridis</i> (Ach.) Behlen & Desberger			+
<i>O. culmigena</i> Lib.			+

No fundamental differences were observed in the intensity of spore fluorescence in microscope slides prepared both from the fresh and herbarium materials.

The conidial spores of all examined species showed similar autofluorescence, irrespective of the origin of the analysed material and their size (Figs 10-12).

The taken up research is the first attempt of using autofluorescence phenomenon for identification of lichen species of the *Opegrapha* genus. The carried out analyses showed the presence of endogenous chromophores in the cytoplasm of ascospores and conidial spores in all examined taxa. In the literature a correlation was showed between the fluorescence intensity and the viability, maturity degree and storage conditions of ascospores and conidial spores (Cathy, Warren 1984; Honegger 2003). It was also proved that the fluorescence intensity of algal cells changed depending on the degree of environmental pollution (Di Toppi et al. 2005) and other factors. In the work by Favali et al. 1991, not only changes in autofluorescence intensity connected with the harmful effect of such chemical elements as aluminium (Al), silicon (Si), phosphorus (P), sulphur (S), chlorine (Cl), and calcium (Ca) were demonstrated but also differences were showed in the concentration of respective chemical elements in different elements of the thallus, including a considerable concentration of aluminium in the apothecia. It is not excluded that these stress factors could also affect the intensity of light emission in ascospores and conidial spores. Autofluorescence depends also on the type and the amount of chromophore occurring in the cytoplasm. The carried out analyses of images show a variable autofluorescent response of ascospores in *O. niveoatra*, *O. rufescens* and *O. varia*. The spectrum of luminescence in the examined taxa may undergo changes and is not stable. Thus, it is difficult to determine simple criteria that would describe a given group of species, while autofluorescence intensity can not be a trait of diagnostic importance since it may be affected by many external factors. It appears thus that it is not a good method for identification of taxa, at least in that group of species. Nevertheless, it is worth expanding the research work on the usefulness of that method in taxonomic studies of other lichen and fungi genera.

CONCLUSIONS

No fundamental taxonomic differences were found at the level of light emission intensity. The autofluorescent response of particular species results most probably from the size of spores and the same from the amount of cytoplasm inside them as well as from the degree of their maturity. As is showed by examinations, dead spores devoid of the cytoplasm as well as those being dormant do not show autofluorescence (Cathy, Warren 1984). The intensity of fluorescence in ascospores and conidial spores in the genus *Opegrapha* does not seem to be a good method in taxonomic identification of particular species.

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Autofluorescencja zarodników workowych i konidialnych wybranych gatunków porostów z rodzaju *Opegrapha*

Streszczenie

W pracy przedstawiono wyniki badań nad zjawiskiem autofluorescencji zarodników workowych i konidialnych wybranych gatunków porostów z rodzaju *Opegrapha*. Zaobserwowano i scharakteryzowano zjawisko autofluorescencji u 12 gatunków występujących w Polsce (*O. atra*, *O. calcarea*, *O. culmigena*, *O. dolomitica*, *O. gyrocarpa*, *O. niveoatra*, *O. rufescens*, *O. rupestris*, *O. varia*, *O. vermicellifera*, *O. viridis*, *O. vulgata*).

W badaniach wykorzystano laserowy mikroskop skaningowy LSM 510 (Zeiss) pracujący na odwróconym mikroskopie Axiovert 200M (Zeiss) z fluorescencją. Zastosowano laser argonowy o mocy 11% wysyłający fale o długości 488 nm, obiektyw 63x dla zarodników workowych i 100x dla konidialnych oraz dwa filtry, czerwony o długości fal od 505 do 650 nm i zielony o długości, do 505 nm. Przy obserwacji zarodników wykorzystano filtr nomarskiego. Nie stwierdzono zasadniczych różnic taksonomicznych w poziomie intensywności emisji światła. Zaobserwowane różnice w intensywności świecenia poszczególnych gatunków wynikają prawdopodobnie z wielkości, a tym samym ilości cytoplazmy wewnątrz zarodników oraz stopnia ich dojrzałości.

Po raz pierwszy opisano wykorzystanie zjawiska autofluorescencji w badaniach taksonomicznych porostów z rodzaju *Opegrapha*. Zaprezentowano pierwsze zdjęcia tego zjawiska, które uzupełniają oryginalny opis rodzaju.