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THE APPLICATION OF ELECTRON PARAMAGNETIC RESONANCE (EPR) SPECTROSCOPY FOR STUDY OF ACCUMULATION OF METAL IONS BY *CLADOSPORIUM CLADOSPORIOIDES*

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ABSTRACT: Melanin exists in *Cladosporium Cladosporioides* and electron paramagnetic resonance (EPR) spectroscopy can be used for its study. These fungi have ability of accumulation of metal ions from environment. Metal ions bonded with melanin change the parameters of EPR spectra (linewidth, intensity). For EPR study were prepared *Cl. Cladosporioides* (control samples) and its complexes with diamagnetic ions: Cd(II) and Zn(II). The spectroscopic splitting factor was calculated and the influence of microwave power on EPR parameters was discussed.

KEY WORDS: EPR, melain, *cladosporium cladosporioides*.

Introduction

Melanin (eumelanin and/or pheomelanin) is the polymer existing in different organisms (Williamson et al. 1998; Sarna et al. 2003; Buszman et al. 2003a; Płonka 2009). Melanin gives colour to skin, hair, and eyes. Eumelanin gives black color, and pheomelanin brownisch-red. Pheomelanin contains sulfur, which is responsible for this effect. The high concentration of paramagnetic centers in melanin makes the use of electron paramagnetic resonance spectroscopy EPR possible (Froncisz et al. 1980; Buszman et al.

2003a; Płonka 2009). The unpaired electrons localized on the oxygen or nitrogen atom of 5,6-indol semiquinone groups, are the paramagnetic centers in melanin. Melanin has the possibility to binding of metal ions (Mars and Larson 1999, Froncisz et al. 1980, Szpoganicz et al. 2002, Buszman et al. 2003a, 2003b, Senesi et al. 1987, Buszman et al. 2006, Birdelli 2008, Najder-Kozdrowska et al. 2009) and the EPR method is a useful for analyzing melanin-metal interaction. Diamagnetic metal ions causes increase, and paramagnetic metal ions decrease of the intensity of EPR signal connected with melanin radicals (Buszman et al. 2003a, Buszman et al. 2006, Najder-Kozdrowska et al. 2009).

Soil fungi have ability to absorption of metal ions and play role in protection of natural environment (Senesi et al. 1987, Buszman et al. 2003a, Buszman et al. 2006). Influence of paramagnetic and diamagnetic ions on EPR parameters of *Cladosporium cladosporioides* is described by Buszman et al. (2006). It was obvious that ions with dissimilar magnetic properties (dia- and paramagentism) were affected in differently way on paramagnetic centers in melanin. The influence of pH on paramagnetic properties of *Cl. Cladosporioides* was also analyzed (Pilawa et al. 2005).

In this work, the influence of two different diamagnetic ions: Cd(II) and Zn(II) on parameters of EPR spectra of melanin from *Cl. Cladosporioides* is compared.

Materials and method

Samples

The fallowing samples were prepared for EPR study: *Cl. Cladosporioides* (control sample), complex *Cl. Cladosporioides* with Cd(II) ions and complex *Cl. Cladosporioides* with Zn(II) ions. The concentration of added metal ions solution to *Cl. Cladosporioides* was $5x10^{-5}$ M.

Method

The EPR spectra were recorded using the spectrometer RADIOPAN Poznań at X-band (microwave frequency was 9.3 GHz). Modulation of magnetic field was 100 kHz. All spectra were recorded as the first derivative of absorption energy. The measurements were done in the range of microwave power: 0,7–70 mW. Spectroscopic splitting factor *g* was calculated from the equation:

$$g = \frac{h\nu}{\beta B_r}$$

where: h - Planck's constant, β - Bohr's magneton, ν - frequency of microwave light, B_r – magnetic resonance induction.

The linewidth peak-to-peak of EPR spectra was determined. The intensity of EPR signal (which proportional to concentration of paramagnetic centers) was calculated from the simply formula:

$$I = A(\Delta B_{pp})^2$$

where: A – amplitude of EPR signal, ΔB_{pp} – peak-to-peak linewidth.
The theoretical EPR signal and its parameters are presented on figure 1.

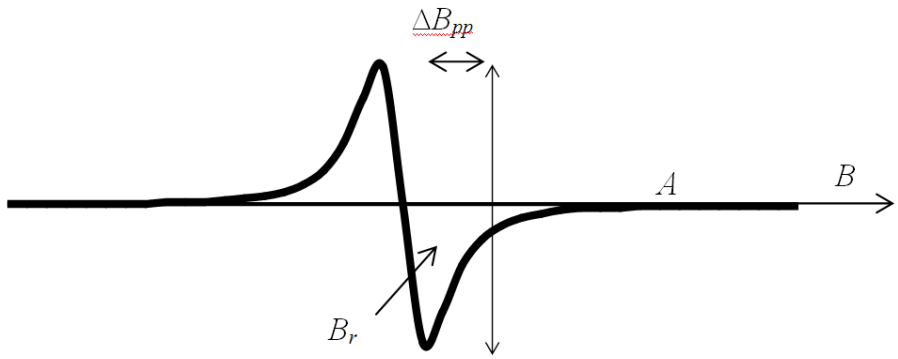

Fig. 1 The EPR parameters of the first derivative of the absorption line (*A*– amplitude, ΔB_{pp}– peak-to-peak linewidth, B_r – induction of magnetic resonance filed)

Results and discussion

The obtained EPR parameters allow to discus the interactions existing in *Cl. Cladosporioides* and its complexes with metal ions (Buszman et al. 1998, Buszman et al. 2003a, 2006). Metal ions change concentration of radicals in melanin and linewidth of EPR signal. Values of EPR linewidth for studied samples *versus* microwave power used during measurements are presented on figure 2. The control sample and complex with Zn(II) ions characterize by very closely value of linewidth (about 0,3 mT) (Fig. 2). The increase of linewidth (about 0,4 mT) was noticed for *Cl. Cladosporioides* -Cd(II) complex (Fig. 2).

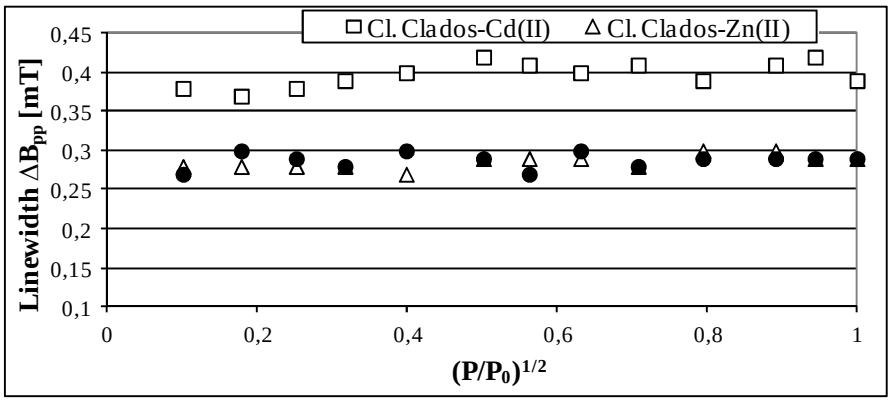

Fig. 2. The influence of microwave power on the linewidth of EPR signals of melanin from *Cl. Cladospiroides. P* – microwave power used during EPR measurements, P_0 – total microwave power produced by klystron

These values of linewidth, which characterize all studied samples, indicate for strong dipolar interactions between paramagnetic centers in melanin existing in *Cl. Cladosporioides*. Cd(II) ions are responsible for intensification of these interactions.

The values of g-factor (2,0030) indicate for localization of unpaired electrons on oxygen atoms. The dependence of the intensity of EPR signal *versus* microwave power for all samples is shown on figure 3. The intensity of EPR signal of *Cl. Cladosporioides* changes after adding metal ions (Fig. 3). The dependencies of intensity of EPR signal indicate for slow spin-lattice relaxation processes (Fig. 3). But the EPR signal of *Cl. Cladosporioides* and its complex with Zn(II) ions saturate at lower microwave power than complex *Cl. Cladosporioides* with Cd(II) ions (Fig. 3). Zn(II) ions caused fractional, and Cd(II) ions considerable increment of the EPR intensity in relation to control sample (Fig. 3).

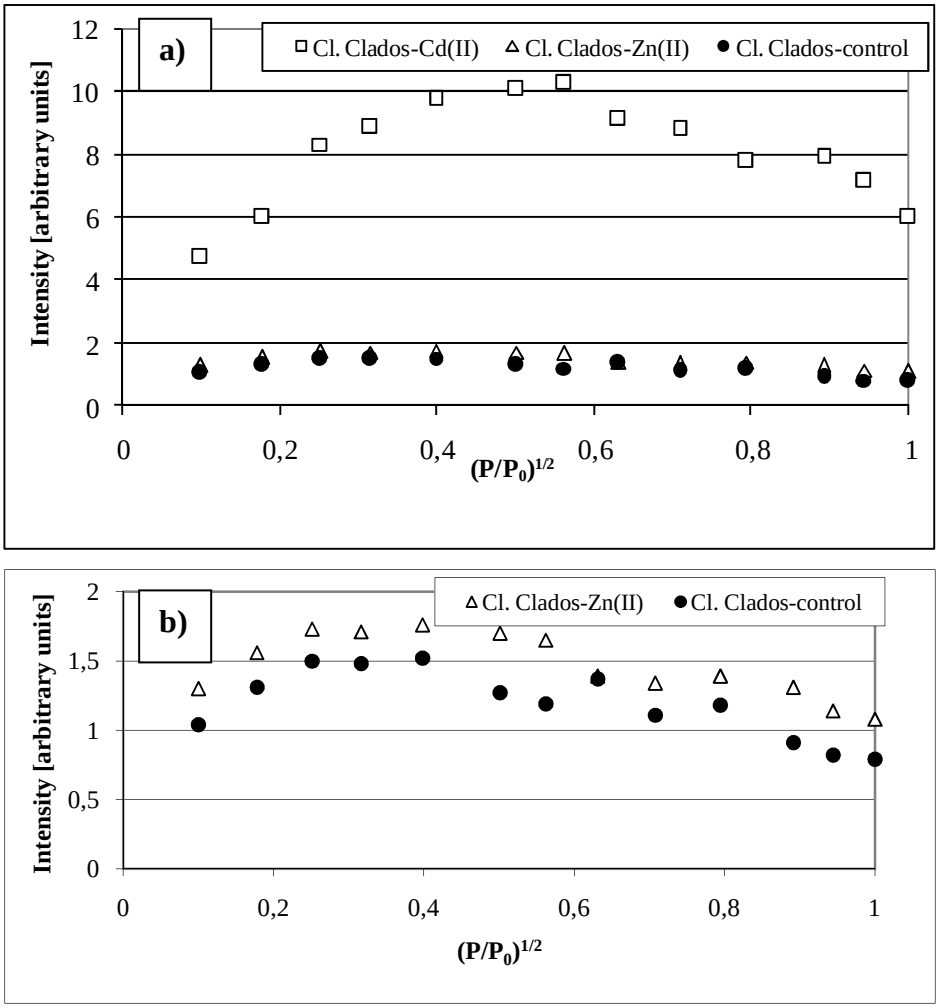

Fig. 3. The influence of microwave power on the intensity of EPR signals of melanin from *Cl. Cladospiroides. P* – microwave power used during EPR measurements, P_0 – total microwave power produced by klystron. a) all studied samples, b) enlargement for control sample and for complex with Zn(II) ions

Conclusion

The EPR method could be used for investigation of amount of metal ions in natural environment by using of EPR method and the phenomenon of binding these ions to melanin existing in fungi. The adding of diamagnetic metal ions: Cd(II) and Zn(II) influence on the intensity and linewidth of melanin EPR signal.

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