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Effect of pH on Paramagnetic Centers in *Cladosporium cladosporioides* Melanin

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Paramagnetic centers in melanin existing in pigmented soil fungi *Cladosporium cladosporioides* cultured at acidic (4, 5, 6), neutral (7), and alkaline (8) pH were studied by EPR method. *o*-semiquinone free radicals (g: 2.0032–2.0040) concentration in melanin biopolymer increased for pH from 4 to 6, decreased at pH 7, and reached the maximum value at pH 8. It may be expected that melanin free radicals reactions with small molecules (metal ions, drugs) are the most effective at pH between 6 and 8. Slow spin–lattice relaxation processes exist in the all studied melanin samples.

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1. Introduction

Pigmented soil fungi *Cladosporium cladosporioides* cultured in medium of different pH were studied by the use of electron paramagnetic resonance spec-

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troscopy. Both eu- and pheomelanin were spectroscopically found in *Cladosporium cladosporioides* [1–4]. EPR spectrum of *Cladosporium cladosporioides* is a superposition of simple single line of eumelanin and complex EPR curve with unresolved hyperfine structure of pheomelanin [1–4]. EPR line of eumelanin is the main component of EPR spectrum of *Cladosporium cladosporioides*. EPR line of pheomelanin is clearly visible in *Cladosporium cladosporioides* spectra recorded at higher microwave powers.

Cladosporium cladosporioides play an important role in detoxification processes of the environment. Metal ions can accumulate in melanin biopolymer existing in these fungi. Changes of free radical properties of *Cl. cladosporioides* melanin after binding of metal ions were examined earlier [2–4]. Paramagnetic metal ions quench EPR line of fungal melanin, while diamagnetic metal ions increase *o*-semiquinone free radical concentration in this biopolymer.

Influence of pH on free radicals in *Cl. cladosporioides* has not been studied so far. The pH value of solution strongly affects dissociation of those functional groups in melanin biopolymer which are responsible for metal ions and drugs binding. Free radicals also play an important role in melanin reactions with small molecules [4, 5]. The aim of this work was to compare free radical properties and concentrations of melanin in *Cladosporium cladosporioides* fungi cultured at different pH of medium. Spin–spin and spin–lattice interactions in the natural melanin samples are discussed.

2. Experimental

Natural melanin biopolymers existing in *Cladosporium cladosporioides* pigmented soil fungi were tested.

Microscopic fungi were cultured in the standard media containing glucose (20 g), yeast extract (10 g), peptone (10 g) and bidistilled water (ad 1 dm^3). To examine the effect of pH on fungi growth and pigmentation the liquid media were adjusted to a suitable pH (4, 5, 6, 7, or 8) by adding hydrochloric acid or sodium hydroxide solution. After 14 days of culture the fungi were filtered, washed with bidistilled water and dried to a constant weight.

The ground samples of dry mycelium *Cladosporium cladosporioides* were measured using an X-band (9.3 GHz) EPR spectrometer with modulation of magnetic field 100 kHz. *g*-factor, line width ΔB_{pp}, amplitude of EPR line, and concentrations of paramagnetic centers in the samples were determined. Changes of EPR spectra with increase in microwave power were analyzed.

3. Results and discussion

Concentrations of paramagnetic centers, *g*-factors, and line widths ΔB_{pp} of the melanin EPR spectra are presented in Table. The broad EPR lines (ΔB_{pp},

TABLE

Free radical concentration N, g-factor, and line widths $\Delta B_{\rm pp}$ of EPR lines of melanin in *Cladosporium cladosporioides* fungi cultured at different pH of medium.

pH of medium	$N \times 10^{-17}$ [spin/g]	$\Delta B_{\rm pp}$ [±0.02 mT]	g [±0.0002]
4	0.7	0.41	2.0032
5	0.9	0.40	2.0039
6	2.2	0.40	2.0039
7	1.3	0.33	2.0039
8	4.3	0.39	2.0040

0.33–0.41 mT) were measured for all the studied *Cladosporium cladosporioides* samples, which indicates that strong dipolar interactions of unpaired spin magnetic moments occur in the natural melanins. Strong dependence of pH of medium on *o*-semiquinone radical concentration was observed. The concentration increased for pigmented fungi cultured at acidic pH increasing from 4 to 6. The highest free radical concentration was obtained for the sample cultured at alkaline 8 pH. Slow spin–lattice relaxation processes are characteristic of *Cl. cladosporioides* melanin. EPR lines of the all studied samples saturate at low microwave powers (Fig. 1a). The lowest spin–lattice relaxation time was obtained for the samples cultured at neutral 7 pH. Microwave saturation of EPR spectra of the samples cultured at 7 and 8 pH does not differ considerably. Power of microwave saturation of EPR spectra increases with increase in acidic pH from 4 to 6. Line widths of the analyzed

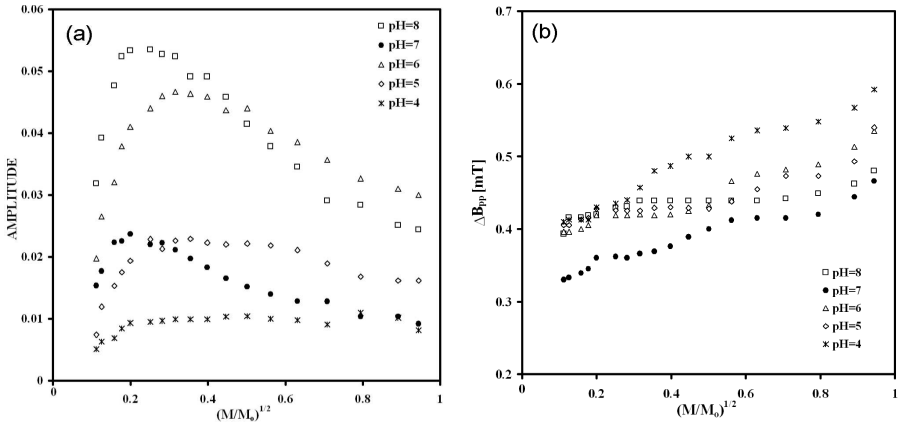

Fig. 1. Influence of microwave power on amplitudes (a) and line widths $\Delta B_{\rm pp}$ (b) of EPR lines of melanin in *Cladosporium cladosporioides* cultured at different pH. M — microwave power used during the measurement of the EPR line, M_0 — total microwave power produced by klystron (70 mW).

EPR spectra increase with increase in microwave power (Fig. 1b). EPR lines of the all samples obtained at different pH are homogeneously broadened (Fig. 1).

4. Conclusions

High amount of *o*-semiquinone free radicals ($\approx 10^{16}-10^{17}$ spin/g) are located in *Cladosporium cladosporioides* melanin biopolymer. pH of the culture strongly affects free radical concentration and spin–lattice relaxation processes in the examined natural melanins. The obtained results suggest that the described earlier [2–5] metal ions and other small molecules bound by *Cl. Cladosporioides* may be strongly affected by pH of the environment.

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