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Detoxification of phenanthrene by *C. elegans* evaluated by calorimetry

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Abstract

The filamentous fungus *Cunninghamella elegans* IM 1785/21Gp has an ability to remove phenanthrene to hydroxylated products with high efficiency. The sensitivity of *C. elegans* spores to phenanthrene derived metabolic products, 9-phenanthrols, as a model example of hydroxyderivatives produced by this fungus, was determined in this work. Calorimetric measurements showed that 9-phenanthrol disturbed the metabolic activity and spore germination of *C. elegans* less than phenanthrene did. However, at concentrations exceeding 60–70 mg L^{-1}, phenanthrol strongly affected the intracellular processes of this fungus.

Keywords: *Cunninghamella elegans*; Microcalorimetry; 9-Phenanthrol; Detoxification

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large group of xenobiotics commonly found as pollutants in soils, estuarine waters and sediments. There is widespread contamination of the environment with PAHs from incomplete combustion of fossil fuels and organic wastes. Many PAHs are known to be mutagenic or carcinogenic [1]. Phenanthrene is a PAH but is noncarcinogenic [2] and nonmutagenic [3,4], and thus is a useful model for mammalian [5] and fungal [6–8] metabolism studies.

From the many PAH-degrading strains, the zygomycete *Cunninghamella elegans*, exhibits significant potential to metabolize PAHs [9]. *C. elegans* IM 1785/21Gp has an ability to remove phenanthrene with a high efficiency [10,11]. Previously, we observed the toxic effect of phenanthrene on *C. elegans* IM 1785/21Gp growth, by calorimetry [12]. The results showed that calorimetry could be a valuable supplement in the study on PAH toxicity on fungi, because it allows measurement of small changes in metabolic activity.

Fungi do not use phenanthrene as the sole source of carbon and energy but, instead, cometabolize the PAH to hydroxylated products [13]. Phenanthrene is metabolized by cytochrome P-450 monooxygenases in the initial attack. The product, arene oxide is further metabolized to dihydrodiols with the *trans*-configuration, phenanthrols or sulfate and glucoside conjugates [1,6,14,15]. Our previous results [11] showed that in the initial stage of phenanthrene metabolism by *C. elegans* IM 1785/21Gp, cytochrome P-450 is also involved. Our studies (unpublished) confirmed that *C. elegans* IM 1785/21Gp strain metabolizes phenanthrene to hydroxylated products, mainly phenanthrols, *trans*-dihydrodiols and glucoside conjugates, similar to metabolism of phenanthrene by fungi described by Casillas et al. [6] and Cerniglia and Yang [15]. Most of the metabolites produced from PAHs by fungi are less toxic to other organisms than the parent compounds [16]. Cerniglia et al. [13] described that conversion of PAHs to more polar oxygenated metabolites aided in the detoxification of these compounds. Nevertheless, our observation of *C. elegans* growth indicated that despite the intensive transformation of the toxic substrate into less toxic derivatives, the fungal growth was strongly limited as compared with the control culture (without PAH addition). The growth was much more inhibited than expected, which could

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indicate that the product formed also inhibited *C. elegans* IM 1785/21Gp growth within the time of its accumulation. In order to elucidate how this product limits fungal growth rate, calorimetric measurements of *C. elegans* IM 1785/21Gp germinating spores were done in this study. Germinating spores are a good research model, because they are more sensitive to PAHs [12] and metals [17] than mycelium. Because metabolization of phenanthrene at 9,10 position (K-region) is a principal way of phenanthrene oxidation by mammals [18], 9-phenanthrol, as a model example of hydroxyderivatives produced by *C. elegans* IM 1785/21Gp, was used in the present work.

2. Experimental

2.1. Chemicals

9-Phenanthrol was obtained from Aldrich [Steinheim, Germany]. Dimethylformamide was purchased from Serva [Heidelberg, Germany]. All chemicals and solvents were high purity grade reagents.

2.2. Microbial strain and cultural conditions

C. elegans IM 1785/21Gp from the Department of Industrial Microbiology and Biotechnology fungal strains collection was used. This strain was described in our earlier paper [10]. All tests were carried out at 28 °C using a Sabouraud liquid medium [Difco Laboratories, USA], inoculated with 10-day-old cultures of *C. elegans* IM 1785/21Gp on Sabouraud agar slants.

2.3. Sample preparation

C. elegans IM 1785/21Gp was inoculated in the Sabouraud medium, initially containing 5×10^7 spores mL^{-1}, then 9-phenanthrol dissolved in dimethylformamide (10 mg mL^{-1}) was added into the cell suspension (25–100 mg L^{-1}).

2.4. Microscopic observation

Fungal culture was done with Bioscreen C Analyzer System (Labsystems, Finland) as described earlier [12] with and without different amounts of 9-phenanthrol. For microscopic observation, an Axiovert 200M microscope with confocal scanning module LSM5 Pascal (Zeiss, Germany) with Nomarski differential interference contrast was used.

2.5. Calorimetry

Calorimetric measurements of the fungal growth were performed as described earlier [12] with a Micro DSC III (Setaram, France). The measurements were carried out in 1 mL stainless steel "closed, batch" vessels. The sample and reference vessels were sterilized. The sample ampoule contained 300 μL of *C. elegans* IM 1785/21Gp culture with the appropriate amount of 9-phenanthrol. The reference ampoule contained 300 μL of Sabouraud medium. Power–time curves for all measurements were performed at 28 °C.

3. Results

The heat rate versus time curves are presented in Fig. 1. The results show that 9-phenanthrol at 25 mg L^{-1} diminishes the maximum heat rate by about 8% as compared with the control. At 50 mg L^{-1} of 9-phenanthrol, the maximum heat production was decreased by 16%. Increasing the 9-phenanthrol concentration from 60 to 70 mg L^{-1}, caused a larger than predicted decrease in the maximum heat rate. Increasing the hydroxyderivative further to 100 mg L^{-1}, did not cause a further increase in the rate of decrease in the heat rate. The results suggest that 9-phenanthrol at ≥60 mg L^{-1} causes important changes in the intracellular processes of the fungus. Microscopic observations were performed to confirm this thesis.

The results of microscopic inspection of 9-phenanthrol supplemented cultures are presented in Fig. 2. Spores incubated in Sabouraud medium with 25 mg L^{-1} of 9-phenanthrol (Fig. 2B) formed long and branched filaments, about 40 μm, similar to the ones observed in the control culture without the xenobiotic (Fig. 2A). At a phenanthrol concentration of 50 mg L^{-1}, spores formed shorter germ tubes, about 20 μm (Fig. 2C). A phenanthrol concentration of 60 mg L^{-1} (Fig. 2D) stimulated the formation of conidiophore from the germinating spores. The production of spores from germinating spores occurs in some fungi when grown in nutrient-poor conditions [19]. Our microscopic observations indicated that germ tube formation was gradually limited at phenanthrol concentrations of 25–60 mg L^{-1}, nevertheless the granular structures inside the filaments and conidiophores were still

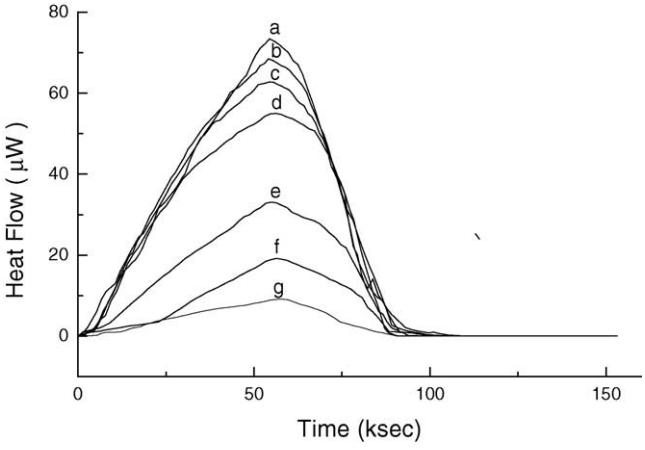

Fig. 1. Heat production rate by *C. elegans* spores incubated in growth medium without phenanthrol (a) and with 25 mg L^{-1} (b), 50 mg L^{-1} (c), 60 mg L^{-1} (d), 70 mg L^{-1} (e), 90 mg L^{-1} (f), and 100 mg L^{-1} (g) of 9-phenanthrol.

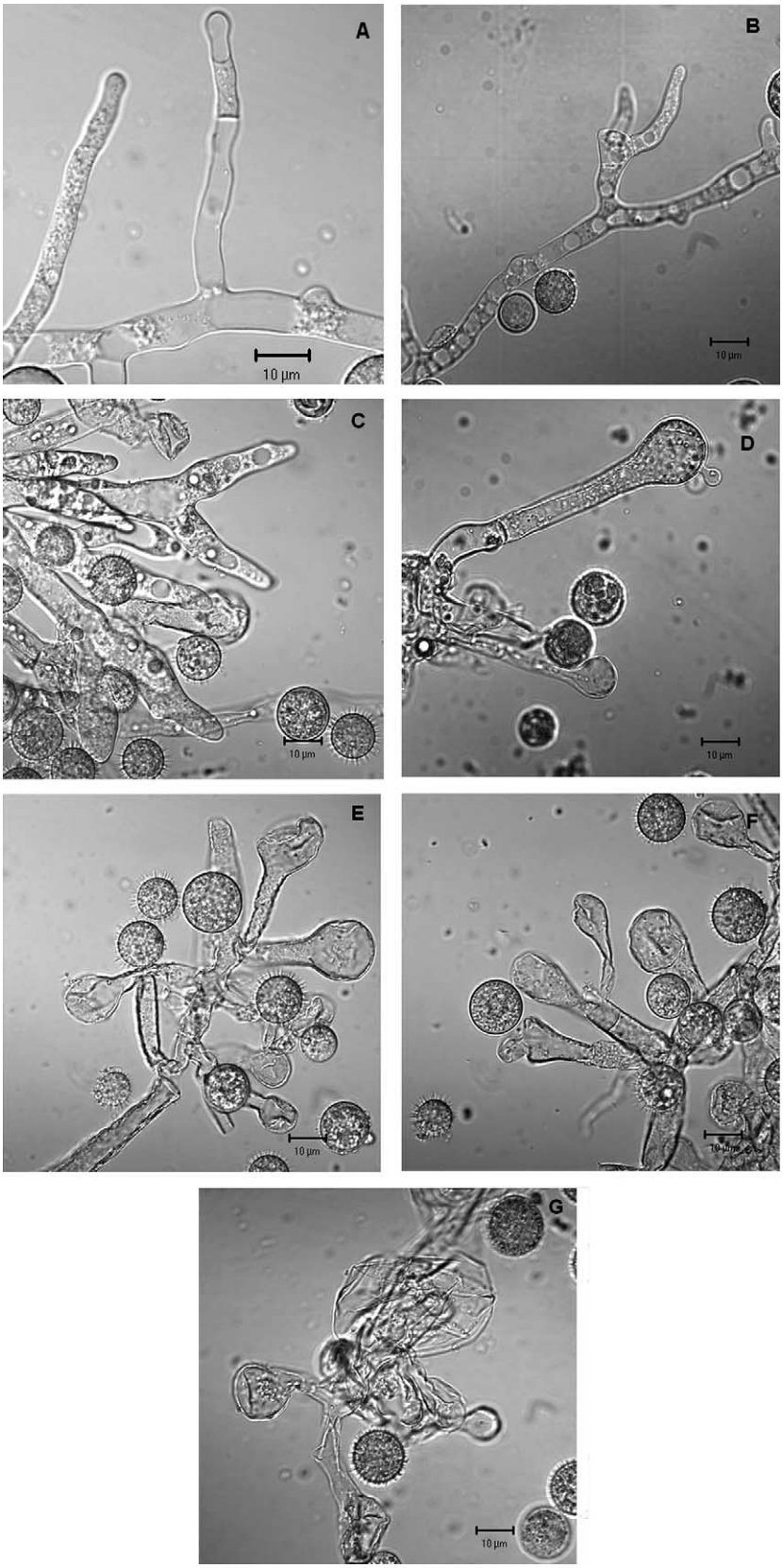

Fig. 2. *C. elegans* photomicrographs; germinating spores after 24 h of incubation in Sabouraud medium without 9-phenanthrol (A) and with 25 mg L^{-1} (B), 50 mg L^{-1} (C), 60 mg L^{-1} (D), 70 mg L^{-1} (E), 90 mg L^{-1} (F) and 100 mg L^{-1} (G) of 9-phenanthrol.

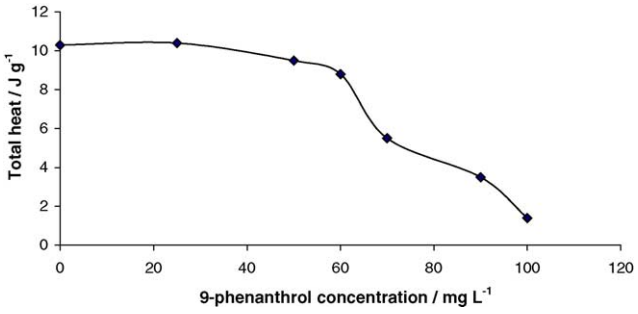

Fig. 3. Relation of total amount of heat production (enthalpy) by *C. elegans* spores vs. 9-phenanthrol content in the growth medium.

observed. Further increase in phenanthrol up to 70 mg L^{-1} resulted in the presence of empty and collapsed filaments (Fig. 2E). Spores incubated with 90–100 mg L^{-1} (Fig. 2F and G) did not differ significantly from the spores cultivated at 70 mg L^{-1}.

The total amount of heat produced during the *C. elegans* spore growth with 9-phenanthrol concentration of 25–50 mg L^{-1} was 10.2 and 9.8 J g^{-1} of culture, respectively, in comparison with 10.6 J g^{-1} in the control (Fig. 3). The total amount of heat produced by spores cultivated with phenanthrene at the same concentrations was significantly lower, 9.2 and 6.4 J g^{-1}, respectively [12]. A higher concentration of phenanthrene (60–90 mg L^{-1}) produced less than 2 J g^{-1} [12], while after the addition of 9-phenanthrol, the total heat was 8.8–4.2 J g^{-1} at the same concentrations.

There is no proportionality between the increase in phenanthrol concentrations and the decrease in the total metabolic heat. The significant decrease in total heat with a slight increase in phenanthrol concentration at 60–70 mg L^{-1} could indicate saturation of the system. The same dependence was observed in our earlier experiments with phenanthrene, but at different concentrations (50–60 mg L^{-1}) [12].

4. Discussion

The results of calorimetric experiments showed that phenanthrol disturbed the metabolic activity and spore germination of *C. elegans* IM 1785/21Gp less than phenanthrene did. But the product formed gradually accumulates, and when phenanthrol exceeds 60–70 mg L^{-1} an increased toxic effect on *C. elegans* IM 1785/21Gp growth was observed. After exceeding this concentration very slightly, phenanthrol strongly affected the intracellular processes of this fungus, which is typical of xenobiotics accumulated in mammalian lipids. Thus, the decrease in fungal activity is a result of the very toxic substrate amount and a growing concentration of less toxic product.

This thesis was confirmed by microscopic observation. Phenanthrol addition up to 70 mg L^{-1} resulted in the presence of empty and collapsed filaments. The cytoskeleton plays an important role in shaping fungal cells [20]. Some factors, like dehydration, eliminated the hydroskeleton, therefore conidia and germ tubes collapsed [21].

The results showed that during phenanthrene bioconversion by *C. elegans* IM 1785/21Gp, mutual completing of PAHs toxic effect was observed. The decrease in phenanthrene amount under critical value caused only small inhibition of fungal physiological processes. On the other hand, the increase in the product amount under critical value caused a strong inhibition of *C. elegans* IM 1785/21Gp metabolism.

References

[1] S. Harayama, Curr. Opin. Biotechnol. 8 (1997) 268–273.
[2] G. Grimmer, H. Brune, G. Dettbarn, J. Jacob, E. Mohtashamipur, K. Norpoth, R. Wenzel-Hartung, Polycycl. Aromat. Compd. 2 (1991) 39–47.
[3] J. Mc Cann, E. Choi, E. Yamasaki, B.N. Ames, Proc. Natl. Acad. Sci. U.S.A. 72 (1975) 5135–5139.
[4] C.E. Cerniglia, G.L. White, R.H. Heflich, Arch. Microbiol. 47 (1985) 105–110.
[5] J. Jacob, G. Raab, V. Soballa, W.A. Schmalix, G. Grimmer, H. Greim, J. Doehmer, A. Seidel, Environ. Toxicol. Pharmacol. 1 (1996) 1–11.
[6] R.P. Casillas, S.A. Crow, T.M. Heinze, J. Deck, C.E. Cerniglia, J. Ind. Microbiol. 16 (1996) 205–215.
[7] L. Bezalel, Y. Hadar, C.E. Cerniglia, Appl. Environ. Microbiol. 63 (1997) 2495–2501.
[8] S. Boehmer, K. Messner, E. Srebotnik, Biochem. Biophys. Res. Commun. 244 (1998) 233–238.
[9] C.E. Cerniglia, J. Ind. Microbiol. Biotechnol. 19 (1997) 324–333.
[10] K. Lisowska, J. Długoński, J. Basic Microbiol. 39 (1999) 117–125.
[11] K. Lisowska, J. Długoński, J. Steroid Biochem. Mol. Biol. 85 (2003) 63–69.
[12] K. Lisowska, B. Pałecz, J. Długoński, Thermochim. Acta 411 (2004) 181–186.
[13] C.E. Cerniglia, J.B. Sutherland, S.A. Crow, in: G. Winkelman (Ed.), Microbial Degradation of Natural Products, VCH, Weinheim, 1992, pp. 193–217.
[14] C.E. Cerniglia, W.E. Campbell, J.P. Freeman, F.P. Evans, Appl. Environ. Microbiol. 55 (1989) 2275–2279.
[15] C.E. Cerniglia, S.A. Yang, Appl. Environ. Microbiol. 47 (1984) 119–124.
[16] J.B. Sutherland, J. Ind. Microbiol. 9 (1992) 53–62.
[17] H. Amir, R. Pineau, Soil Biol. Biochem. 14 (1998) 2043–2054.
[18] J.B. Sutherland, P.J. Freeman, P.J. Selby, P.P. Fu, W.D. Miller, C.E. Cerniglia, Arch. Microbiol. 154 (1990) 260–266.
[19] J.W. Deacon, Modern Mycology, 3rd ed., Blackwell Science Ltd., Osney Mead, Oxford, 1997, pp. 53–54.
[20] I.B. Heath, Int. Rev. Cytol. 123 (1990) 95–127.
[21] N.P. Money, in: K. Esser, P.A. Lemke (Eds.), The Mycota: Growth, Differentiation and Sexuality, Springer-Verlag, 1994, p. 79.