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INFLUENCE OF MULTI-WALLED CARBON NANOTUBES (MWCNTs) ON VIABILITY OF *Paecilomyces fumosoroseus* (WISE) BROWN & SMITH (*Deuteromycotina*: *Hyphomycetes*) FUNGUS SPORE

WPŁYW WIELOŚCIENNYCH NANORUREK WĘGLOWYCH (MWCNTS) NA ŻYWOTNOŚĆ ZARODNIKÓW GRZYBA *Paecilomyces fumosoroseus* (WISE) BROWN & SMITH (*Deuteromycotina*: *Hyphomycetes*)

Abstract: The study aimed at testing the influence of pristine multi-walled carbon (MWCNTs) and carboxyl (MWCNT(COOH)) nanotubes on spores of entomopathogenic *Paecilomyces fumosoroseus* fungus. The effect of the nanotubes on the fungus linear growth, biomass increment and sporulation was determined. The character of linear growth of *P. fumosoroseus* mycelium obtained from the spores contacted with MWCNTs was different in comparison with the control. Pristine carbon nanotubes significantly stimulated *P. fumosoroseus* mycelium linear growth and limited its sporulation in comparison with the control. Carboxylation changed the influence of carbon nanotubes on the spores of the tested fungus. The activity of MWCNT(COOH) was definitely weaker and the obtained linear growth and sporulation did not differ significantly from the control. No apparent effect of MWCNTs on the increment of *P. fumosoroseus* biomass was found.

Keywords: multi-walled carbon nanotubes, carboxylated multi-walled carbon nanotubes, *Paecilomyces fumosoroseus*

Nanotechnology is a modern scientific discipline evoking wide interest. Nanomaterials and nanocompounds find numerous applications in various fields of economy. Carbon nanotubes (CNTs) are novel nanotechnological material researched by numerous scientists. Currently, as in the case of a majority of such materials, analyses focus primarily on their physical and chemical properties and potential practical applications [1–5].

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There have been reports pointing out the necessity to test carbon nanotube toxicity, especially for humans. Some of the obtained results may cause some anxiety [6–9]. Few of the studies conducted so far aim to test carbon nanotube toxicity for lower living organisms [10]. Researchers from the Yale University pointed out to the strong antibacterial effect of single-walled carbon nanotubes (SWNTs) on *Escherichia coli* [11]. Toxicity of this kind of nanomolecules for prokaryotic bacteria cells was demonstrated in the direct contact with the cell wall, which caused the death of the bacteria.

The studies aimed at determining the influence of multi-walled carbon nanotubes (MWCNTs) and carboxylated multi-walled carbon nanotubes (MWCNTs(COOH)) on the spores of *Paecilomyces fumosoroseus* fungus.

Material and methods

Carboxylated multi-walled nanotubes MWCNTs(COOH) were obtained by oxidation of pristine MWCNTs purchased in Echo-Nanobio Trading Co. Ltd., (Taipei, Taiwan). These are carbon nanotubes with the outer diameter of 40–60 nm, specific weight between 140 and 300 g/dm^3 and carbon content over 80 %.

Commercial MWCNTs were heated in nitric(V) acid for 2 days at boiling temperature. Subsequently they were drained and washed with water to obtain a neutral filtrate. They were dried at 120 °C for two days. TG analysis and acid-base titration of the carboxylated carbon nanotubes obtained in this way revealed the presence of carboxyl groups gravimetrically 3(± 0.5), purity > 99 %, pH = 4.5, outer diameter 10–40 nm (SME). The nanotubes prepared in this way were used for further analyses.

The experiment used a Polish strain of *P. fumosoroseus* entomopathogenic fungus, from the Department's own collection and was isolated from the soil for trap insects. The strain was multiplied to obtain sporulating mycelium. Fungus spore suspensions, in concentrations calculated in Bürker haemocytometer, were prepared in sterile distilled water. 200 cm3 of spore suspension in $5.5 \cdot 10^8$ pcs per cm^3 concentration was introduced into glass flasks. The control was provided by pure suspension without added nanotubes. The experimental objects contained multi-walled carbon nanotubes and carboxylated multi-walled carbon nanotubes, which were added to the spore suspensions in the volume of 0.1 cm^3 each. The control flask and flasks with added nanotubes were placed in a shaker and shaken for 1 hour. 10 mm^3 of the solution was collected with a micropipette from each of the shaken suspensions and inoculated on dishes with a standard Sabouraud solid medium to obtain mycelium linear growth. Six replications were used. When the observation of linear growth was completed (23rd day of fungi culturing) and sporulating mycelia were obtained, spore suspensions were prepared again by means of collecting 5 mycelium rings, 1 cm in diameter, from each experimental object and counting their sporulation. Mycelium biomass increment was also examined after 1 hour contact with the nanotubes by inoculating 3 cm^3 of the suspension to the flasks containing 100 cm^3 of liquid Sabouraud medium in 6 replications and incubating them at 20 °C for 40 days. After this period the post culture liquid with the mycelium was drained through filter paper. The mycelium was dried in sterile glass at 80 °C to constant weight. Dry mycelium mass was weighed on electronic analytical balance.

Analysing the obtained results, the curves of the linear growth trend were matched with the Gompertz curve according to the following formula:

$$Y = A \cdot \exp(-\exp(-k\,(x-x_c)))$$

where:
A – amplitude,
k – process speed rate,
x_c – curve inflection point.

Results and discussion

Figure 1 presents linear growth of *P. fumosoroseus* obtained from the fungi spores after one-hour contact with pristine multi-walled carbon and carboxylated nanotubes inoculated on solid media.

The trend of mycelium growth after the contact with multi-walled carbon nanotubes was different in character as compared with the control (spore suspension stirred for 1 h). Following the contact with pristine carbon nanotubes, at the five hundredth hour of growth, the mycelium reached a significantly larger size in comparison with the control. It was not observed for carboxylated carbon nanotubes. The analysis of growth according to Gompertz distribution revealed differences in the rate of the process (k) and the growth saturation time, which corresponds to the curve inflection point. The results of analysis were compiled in Table 1.

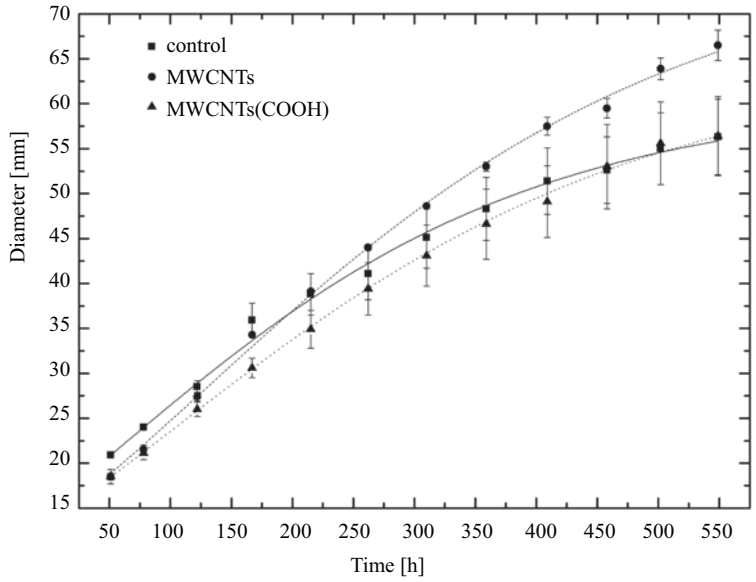

Fig. 1. Linear growth of *P. fumosoroseus* fungus obtained in culturing after 1 hour contact with carbon nanotubes

Table 1

Linear growth parameters obtained on the basis of the Gompertz distribution.

Specification	Control		MWCNTs		MWCNTs(COOH)	
	value	error	value	error	value	error
Amplitude – A	60.76	4.12	77.35	2.03	64.98	6.74
Growth rate – k	0.00510	0.00067	0.00435	0.00024	0.00439	0.00076
Time of fastest growth – x_c	63.76	13.28	130.21	6.62	103.43	24.89

In the case when both kinds of carbon nanotubes were used, the growth trend revealed a greater amplitude in comparison with the control, a lower process rate and a significantly longer time of growth saturation, which means that the control mycelium was growing faster, but also its growth rate was slowed earlier. In may be concluded that culturing the fungus from *P. fumosoroseus* spores following their contact with both kinds of MWCNTs initially only slightly limited the linear growth intensity in time, whereas its collapse was observed significantly later in comparison with the control and the mycelia reached greater sizes, particularly for non-carboxylated multi-walled carbon nanotubes.

Biomass obtained from the spores after their contact with carbon nanotubes did not change notably in comparison with the control. Differences were observed within the measuring error range, as has been shown in Figure 2.

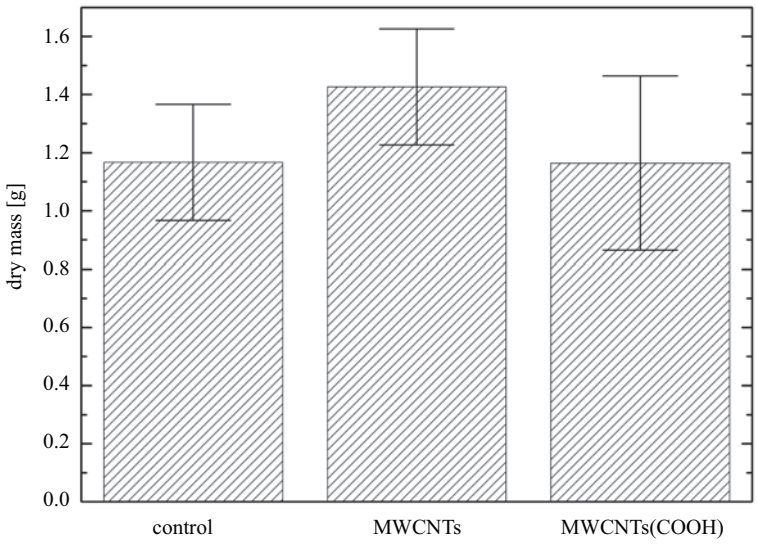

Fig. 2. Biomass of *P. fumosoroseus* obtained in the fungus culturing after 1 hour contact with carbon nanotubes

Results of fungus sporulation were diversified (Fig. 3). *P. fumosoroseus* sporulated significantly worse after the contact with non-carboxylated nanotubes, which was not

noted for carboxylated nanotubes, where the results obtained were very close to the control.

Kang et al [12], who observed a strong antibacterial effect of carbon nanotubes on *E. coli*, obtained different results. However, in their studies the above-mentioned authors were using single-walled carbon nanotubes (SWNTs) at the concentration on of 5 μg/cm^3 introduced to 0.9 % NaCl. Moreover, their test organism were bacteria and not a fungus spore.

Initial observations conducted on *P. fumosoroseus* fungus spores require more detailed research including, among others, testing if carbon nanotubes may contain free radicals. At present the *P. fumosoroseus* spore response to the contact with nanotubes may be explained by the fact that non-carboxylated carbon nanotubes, although hydrophobic, provide a source of carbon necessary for the growth of living organisms. However, the applied dose of nanotubes was not a considerable source of amorphous carbon. The more probable cause of the fungus growth stimulation may be the mutagenicity of non-carboxylated carbon nanotubes expressed as a faster cell division and therefore growth at simultaneous incapacitating sporulation.

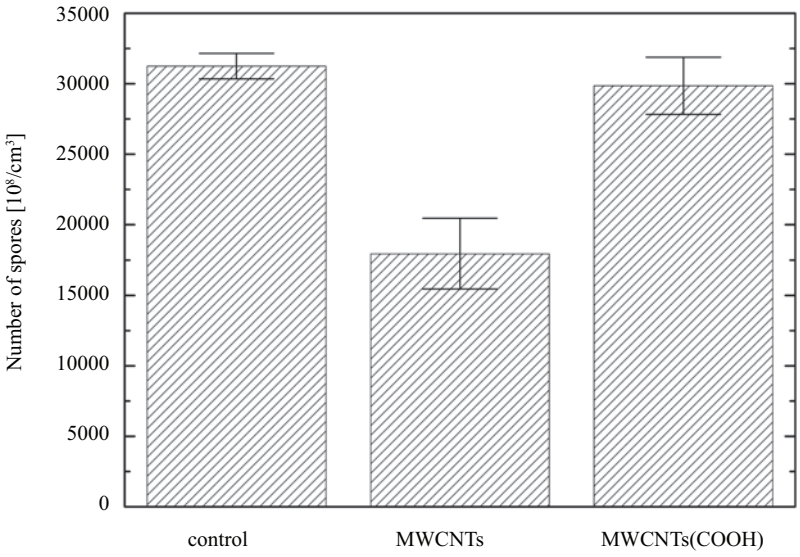

Fig. 3. Sporulation of *P. fumosoroseus* obtained in the fungus culturing after 1 hour contact with carbon nanotubes

Conclusions

1. A different character of *P. fumosoroseus* mycelium linear growth obtained from the spores in contact with multi-walled carbon nanotubes was observed in comparison with the control.

2. Pristine carbon nanotubes led to a significant stimulation of surface growth of *P. fumosoroseus* mycelium and reduction of its sporulation in comparison with the control.

3. Carboxylation changes the properties of multi-walled carbon nanotubes towards fungus spores.

4. The effect of carboxylated carbon nanotubes was definitely weaker and the obtained linear growth and sporulation did not differ significantly form the control.

5. No significant effect of multi-walled carbon nanotubes on *P. fumosoroseus* fungus biomass increment was observed.

References

[1] Iijima S.: *Helical microtubules of graphitic carbon*. Nature, 1991, **354**(6348), 56–58.

[2] Stafiej A. and Pyrzyńska K.: *Adsorption of heavy metal ions with carbon nanotubes*. Separ. Purific. Technol., 2007, **58**, 49–52.

[3] Wong D.H. and Lieber C.M.: *Probing electrical transport in nanomaterials: conductivity of individual carbon nanotubes*. Science, 1996, **272**(5261), 523–526.

[4] Ebbesen T.W., Lezec H.J., Hiura H., Bennet J.W., Ghaemi H.F. and Thio T.: *Electrical conductivity of individual carbon nanotubes*. Nature, 1996, **382**(6586), 54–55.

[5] Saito Y., Hamaguchi K., Hata K., Uchida K., Tasaka Y. and Ikazaki F.: *Conical beams from open nanotubes*. Nature, 1997, **389**(6651), 554.

[6] Hurt R.H., Monthioux M. and Kane A.: *Toxicology of carbon nanomaterials: Status, trends, and perspectives on the special issue*. Carbon 2006, **44**, 1028–1033.

[7] Lacerda L., Bianco A., Prato M. and Kostarelos K.: *Carbon nanotubes as nanomedicines: From toxicology to pharmacology*. Adv. Drug Delivery Rev. 2006, **58**, 1460–1470.

[8] Smart S.K., Cassady A.I., Lu G.Q. and Martin D.J.: *The biocompatibility of carbon nanotubes*. Carbon 2006, **44**, 1034–1047.

[9] Kalbacova M., Kalbac M., Dunsch L. and Hempel U.: *Influence of single-walled carbon nanotube films on metabolic activity and adherence of human osteoblasts*. Carbon 2007, **45**, 2266–2272.

[10] Tuzen M., Saygi K.O., Usta C. and Solak M.: *Pseudomonas aeruginosa immobilized multiwalled carbon nanotubes as biosorbent for heavy metal ions*. Bioresource Technol. 2008, **99**, 1563–1570.

[11] Reinhold C.: *Carbon nanotubes show germ-fighting promise*. Toxicol. Environ. Nanotoday., 2007, **2**(5), 10.

[12] Kang S., Pinault M., Pfefferle L.D. and Elimelech M.: *Single-Walled Carbon Nanotubes Exhibit Strong Antimicrobial Activity*. Langmuir., 2007, **23**, 8670–8673.

WPŁYW WIELOŚCIENNYCH NANORUREK WĘGLOWYCH (MWCNTS) NA ŻYWOTNOŚĆ ZARODNIKÓW GRZYBA *Paecilomyces fumosoroseus* (WISE) BROWN & SMITH (*Deuteromycotina*: *Hyphomycetes*)

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Abstrakt: Zbadano wpływ wielościennych nanorurek węglowych surowych (MWCNTs) i karboksylowanych (MWCNT(COOH)) na zarodniki owadobójczego grzyba *Paecilomyces fumosoroseus*. Określono wpływ nanorurek na wzrost liniowy grzyba, przyrost biomasy i zarodnikowanie. Charakter wzrostu liniowego grzybni *P. fumosoroseus* uzyskanego z zarodników kontaktowanych z MWCNTs w porównaniu do kontroli był inny. Nanorurki węglowe surowe powodowały znaczną stymulację wzrostu powierzchniowego grzybni *P. fumosoroseus* i ograniczenie jej zarodnikowania w porównaniu do kontroli. Karboksylacja zmieniła wpływ nanorurek węglowych na zarodniki badanego grzyba. Oddziaływanie MWCNT(COOH) było zdecydowanie słabsze, a uzyskany wzrost liniowy i zarodnikowanie nie różniły się istotnie od kontroli. Nie stwierdzono istotnego wpływu MWCNTs na przyrost biomasy grzyba *P. fumosoroseus*.

Słowa kluczowe: wielościenne nanorurki węglowe, karboksylowane wielościenne nanorurki węglowe, *Paecilomyces fumosoroseus*