

EXPERIMENTAL PAPER

Plant endophytic fungi as a source of paclitaxel

ALICJA MICHALCZYK*, ANNA CIENIECKA-ROSŁONKIEWICZ, MAŁGORZATA CHOLEWIŃSKA

Institute of Industrial Organic Chemistry
Annopol 6
03-236 Warszawa, Poland

*corresponding author: e-mail: michalczyk@ipo.waw.pl

Summary

Endophytic fungi were isolated from different parts of the plants *Corylus avellana* (Corylaceae) and *Ocimum basilicum* (Lamiaceae) and then identified to the genus level based on the morphology of the fungal culture and the characteristics of the spores. The fungicidal and antitumor activity of isolates and extracts obtained from them was determined. We found that a few isolates from *Corylus avellana* and *Ocimum basilicum* produced metabolites that inhibited the growth of *Oomyces* fungi to a highly significant extent. In the potato disc bioassay only an extract from the isolate C-9 showed an inhibitory action in tumor development. Paclitaxel presence in extract from the isolate C-9 was confirmed using the thin layer chromatography method (TLC) and UV-VIS spectroscopic analysis. The above results indicated that the tested samples revealed an antitumor activity. The detection of paclitaxel in the isolate C-9 suggests that the antitumor activity resulted probably from the presence of this taxane in the tested material.

Key words: *endopytic fungi, paclitaxel, Oomyces, Crown Gall Tumor Disc Method*

INTRODUCTION

Paclitaxel (taxol) is the best known taxane used for the treatment of human tumors, including ovarian, breast, lung, prostate and head and neck cancers [1]. Paclitaxel can be obtained from yew (*Taxus* sp.), but such obtaining requires destruction

of trees. Alternative methods for paclitaxel production such as chemical synthesis, tissue and cell cultures of the *Taxus* sp. are expensive and give low yields [2]. Recently, paclitaxel as well as other taxanes have been detected in shells and leaves of *Corylus avellana* and also in leaves of *Ocimum basilicum* [3, 4]. It is well known that in the tissues of living plants reside the endophytic fungi which possess analogical ability to perform a biosynthesis of secondary metabolites as the host plant. The endophytic fungi are therefore expected to be a potential source of many natural bioactive products including also paclitaxel and other taxanes. The endophytes can be isolated from a host plant and cultivated *in vitro* on artificial media. In view of this, taxanes can be produced industrially from endophytic culture in the fermentation process. The mode of action of paclitaxel is to preclude tubulin molecules from depolymerizing during the processes of cell division. Paclitaxel reveals also a fungicidal activity. Fungal plant pathogens from *Oomycetes* class such as *Pythium* spp. and *Phytophthora* spp. are the most sensitive to it [5, 6]. Their sensitivity to paclitaxel is based on their interaction with tubulin in a manner identical to that in a rapidly dividing human cancer cell. This is why the finding that the tested extracts from plant endophytes reveals the high activity against *Oomycetes* fungi and indicates that the extract has also an antitumor activity. Thus, *Oomycetes* fungi can be used as a screening tool in searching for anticancer compounds such as paclitaxel. Among the different bioassays offered for screening, the antitumor properties of plant extracts of the crown gall tumor inhibition (potato disc) is a safe, rapid and inexpensive assay. This test is based on *Agrobacterium tumefaciens* infection on a potato disc. This gram-negative bacterial strain induces the neoplastic disease of plants named crown gall. The bacteria possesses large Ti (tumor inducing) plasmids which carry genetic information (T-DNA) that transforms regular plant cells into autonomous tumor cells. Galsky [7, 8] demonstrated that an inhibition of crown gall tumor distribution on potato disc showed the agreement with compounds and plant extracts known to be active in the 3PS (*in vivo*, mouse leukemia) antitumor assay. The inhibition of the growth of the tumors and inhibition of the tumor initiation agreed well with 3PS activity [8]. The rationale for the use of this bioassay is the tumorigenic mechanism indicated in plant tissues by *Agrobacterium tumefaciens* which is in many ways similar to that of animals [9]. The aim of the present work was to isolate the endophytic fungi from different parts of the plants *Corylus avellana* and *Ocimum basilicum* and to verify whether they produced paclitaxel. The antitumor properties of endophytic samples were checked using the potato disc assay and the determination of their activity against *Oomycetes* fungi.

MATERIALS AND METHODS

Source of plant material

In this study, we used the hazel (*Coryllus avelana*) and basil (*Ocimum basilicum*) growing in the Botanical Garden, Polish Academy of Science, Powsin. The aerial parts of these plants were collected between May and October 2011.

Isolation and culture of endophytic fungi

Endophytic fungi were isolated from different parts of *Corylus avellana* (twigs, shells, leaves) and *Ocimum basilicum* (young and mature leaves). The samples were cut into small pieces (approximately 5 mm x 5 mm). To minimize the risk of isolating epiphytic microorganisms during tissue sampling, the plant organs were surface sterilized by dipping them in 70% ethanol (v/v) for 3 min and 0.01% mercuric chloride (HgCl₂) for 1 min and rinsed two times in sterilized water. Small pieces of samples were placed on the surface of water agar supplemented with streptomycin (10 µg/ml) and tetracycline (3 µg/ml) and incubated at 25°C in darkness [10]. After several days, fungal mycelia emerged from the cut surface of the samples. The hyphal tips of the various fungi were cut and placed in Petri dishes containing potato dextrose agar (PDA) and incubated at 25°C for at least 10–12 days. Each fungal culture was checked for purity and transferred to agar slants. The isolated endophytic fungi were identified to genus level using standard monographs [11].

Antifungal bioassay:

The antifungal assay was performed on a PDA agar medium in Petri dishes using the dual culture method [12, 13].

Endophytic fungi and two plant pathogenic fungi: *Phytophthora cactorum* PH0281 and *Phytophthora capsici* PH0010 (Main Inspectorate of Plant Health and Seed Inspection of Poland) were cultured separately on PDA medium at 25°C for 5–7 days. The mycelial plugs (5 mm in diameter) of pathogens and endophytic fungi were placed on the same Petri dish 6 cm one from another. Dishes inoculated only with test pathogens were used as controls. Plates were incubated at 25°C for 5-7 days. The experiment was performed in triplicate. The percent growth inhibition of fungal pathogens was calculated by the following formula:

$$\text{Percent inhibition} = \frac{\text{Radius of the pathogen in the control plate} - \text{Radius of pathogen in the dual culture plate}}{\text{Radius of the pathogen in the control plate}} \times 100$$

The percentage of inhibition was categorized on a growth inhibition category scale from 0 to 4 modified from Živković [13], where

1. 0–30% growth inhibition means low antifungal activity
2. 31–50% growth inhibition means moderate antifungal activity
3. 51–70% growth inhibition means high antifungal activity
4. 71–100% growth inhibition means very high antifungal activity

The endophytic fungi with a percentage of inhibition of more than 30% were kept for further study.

Endophytes showing a good or moderate antifungal activity were selected for a secondary screening step, which was performed using the crown gall tumor disc method.

Fermentation and extraction procedure

Microorganisms were cultured in 500 ml Erlenmeyer flasks containing 250 ml of MID liquid medium supplemented with 1 g/l of soytone [14]. The cultures were incubated for 3 weeks at 25°C in darkness under the stationary conditions. The extraction process was performed according to the method proposed by Strobel [15, 16]. After incubation, the cultures were filtered through several layers of gauze to remove mycelium. Then 0.25 g of Na₂CO₃ was added to the filtrate and frequently shaken to reduce the amount of fatty acids. The culture filtrate was extracted with two equal volumes of dichloromethane. The organic phase was collected and then the solvent was then removed by evaporation under reduced pressure at 35°C. The dry extracts from endophytic fungi were dissolved in methanol for analytical tests and in DMSO for biological assays, respectively.

Chromatographic analysis

Thin layer chromatography (TLC) analysis for the endophytic extracts were carried out on Merck 0.25 mm silica gel plates developed in solvents:

- A. chloroform:methanol (7:1, v/v)
- B. chloroform:acetonitrile (7:3, v/v)
- C. ethyl acetate:2-propanol (95:5, v/v)
- D. methylenechloride:tetrahydrofuran (6:2)
- E. methylenechloride:methanol:dimethylformamide (90:9:1, v/v/v) [16].

The presence of paclitaxel was detected with vanillin/sulphuric acid reagent (1% w/v) after gentle heating [17]. The appearance of a bluish spot of fading to dark gray after 24 h indicated the presence of paclitaxel. The R_f values of the samples were calculated and compared with authentic paclitaxel.

Ultraviolet–visible (UV-VIS) spectroscopic analysis

The presence of paclitaxel in the fungal extracts was further confirmed by UV spectroscopy. After the TLC chromatography, the area of the plate containing potential paclitaxel was carefully removed by scrapping off the silica gel at the appropriate R_f and exhaustively eluting it with methanol. After the elution, the crude paclitaxel was performed for the qualitative analyses. The taxol samples were analyzed by UV absorption (Shimadzu UV 1800 Spectrophotometer), dissolved in 100% methanol and compared with authentic paclitaxel (Sigma-Aldrich).

Antitumor potato disc bioassay

The first step of the assay was to check the sensitivity of the test *Agrobacterium tumefaciens* B6 strain to the standard antibiotics and the tested extracts from

endophytes. The sensitivity of test *A. tumefaciens* strain was performed using the disc diffusion method (Kirby-Bauer method) [18]. Dimethylsulfoxide (DMSO) was used as a negative control, tetracycline (30 µg/ml), and ampicillin (10 µg/ml) were used as positive controls. Sterilized paper discs (6 mm diameter) were impregnated with 10 µl of extracts (10 µg/ml, 100 µg/ml, 1000 µg/ml), antibiotics and DMSO allowed to dry in a laminar flow cabinet and placed on to inoculated Petri dishes containing Luria Bertani (LB) agar medium [19]. Petri dishes were placed at 4°C for 2 h to allow the extracts to diffuse into the medium and then incubated at 28–30°C for 24 hours. The sensitivity test was evaluated by the measurement of inhibition zone's diameter (mm) against *A. tumefaciens* strain. The experimental studies were replicated three times.

An antitumor assay of endophytic extracts was performed according to the potato disc bioassay [7, 20-22] *Agrobacterium tumefaciens* B6 strain (PCM 2354) (Polish Collection of Microorganisms, Wrocław) was cultured on LB broth and incubated for 48 hours at 28°C. Suspensions of *A. tumefaciens* in phosphate-buffered saline (PBS) were standardized to 1.0×10^9 Colony Forming Units (CFU) as determined by an absorbance value of 0.96–0.02 at 600 nm [20]. The assay was performed using the red potatoes (Pontiac red or red Russet varieties). The potatoes were washed under running water for 10 min and surface sterilized by immersion in 0.1% mercuric chloride solution for 10 min. The sterilized potatoes were cut into pieces 15 mm × 5 mm in size from the centre of potato tissue by a sterilized cork borer.

The discs were placed on Petri plates containing water agar medium (1.5%). The following solution containing 600 µl of extracts or control solution, 150 µl of sterile distilled water and 750 µl of standardized of *A. tumefaciens* in PBS were prepared. The above solutions were used in 50 µl aliquots to inoculate the surface of each potato disc. Camptothecin and paclitaxel (obtained from Sigma-Aldrich) dissolved in DMSO were used as a positive control and DMSO was used as a negative control.

The plates were incubated at 28°C in darkness. After 3 weeks, the discs were treated with Lugol's solution (5% I₂ + 10% KI in distilled water) and tumors on each disc were counted. The experiments were performed three times. The percent inhibition of tumors was calculated using the formula [23]

$$\text{percent inhibition} = 100 - \frac{\text{Number of tumors with sample}}{\text{Number of tumors with control}} \times 100$$

More than 20% tumor inhibition is considered significant [21, 24]. Data were analyzed using Microsoft Excel-2010 software.

RESULTS AND DISCUSSION

A total of 120 isolates were obtained from the samples of *Ocimum basilicum* and *Corylus avellana* (tab. 1).

Table 1.

Fungal isolates from *Corylus avellana* and *Ocimum basilicum*

Genus	Number of fungal isolates					Total
	Samples of <i>Ocimum basilicum</i>		Samples of <i>Corylus avellana</i>			
	Young leaves	Mature leaves	Twigs	Shells	Leaves	
<i>Fusarium</i>	ND	ND	5	1	5	11
<i>Nigrospora</i>	2	2	ND	ND	ND	4
<i>Botrytis</i>	ND	ND	1	1	ND	2
<i>Phoma</i>	3	1	12	2	5	23
<i>Pestalotiopsis</i>	1	ND	1	ND	ND	2
<i>Cladosporium</i>	ND	ND	1	1	1	3
<i>Aspergillus</i>	ND	ND	1	ND	1	2
<i>Curvularia</i>	5	2	ND	ND	ND	7
<i>Colletotrichum</i>	ND	2	1	2	ND	5
<i>Alternaria</i>	ND	ND	6	1	5	12
<i>Penicillium</i>	3	3	ND	ND	ND	6
<i>Phyllosticta</i>	1	5	ND	ND	ND	6
<i>Phomopsis</i>	3	4	ND	ND	ND	7
<i>Trichoderma</i>	ND	1	2	ND	ND	3
Unidentified	2	3	10	5	7	27
Total	19	22	37	20	19	120

ND-not detected

The number of fungal isolates received from twigs, shells and leaves of *Corylus avellana* was greater than those obtained from young and mature leaves of *Ocimum basilicum*. The endophytic fungal isolates were identified and belonged to 14 commonly isolated endophytic genera such as *Alternaria*, *Fusarium*, *Phoma*, *Pestalotiopsis*, which have also been reported as producers of the anticancer compounds: taxol, cytochalasins, podophyllotoxin [25-29]. Among all the isolates, *Phoma* with about 19% isolates was the dominant genus.

The antitumor activity of endophytic fungal isolates and extracts obtained from them were studied using the dual culture method and the potato disc bioassay technique.

Among 120 of endophytic fungal isolates tested for antifungal activity, 15 isolates inhibited the growth of at least one of the fungal pathogens at the level of more than 30% (tab. 2).

An endophytic fungus of the genus *Alternaria* (C-9) isolated from hazelnut shells showed the best results. This fungus inhibited the growth of both indicator fungal pathogens in the field of 60–70%. The isolates C-45, C-96, C-100, O-42 exhibited a good activity against *Phytophthora capsici* and a moderate activity against *Phytophthora cactorum*. Other isolated endophytes inhibited the growth of both

pathogens within the range of less than 30%. The present study clearly indicates that endophytic fungi isolated from *Corylus avellana* and *Ocimum basilicum* produced secondary metabolites that inhibited the growth of phytopatogenes belonging to the *Oomycetes* class. However, further studies were necessary to screen compounds responsible for the antitumoregenic activity.

Table 2.

Inhibition of *Oomycetes* fungi growth by endophytic isolates (%)

Isolates	Host plants	Plant organ	Genus	Inhibition of test fungi growth (%)	
				<i>Phytophthora cactorum</i>	<i>Phytophthora capsici</i>
C-5	<i>Corylus avellana</i>	Twigs	<i>Alternaria</i>	19	31
C-9	<i>Corylus avellana</i>	Shells	<i>Alternaria</i>	60	70
C-11	<i>Corylus avellana</i>	Shells	<i>Alternaria</i>	5	33
C-45	<i>Corylus avellana</i>	Leaves	<i>Fusarium</i>	37	58
C-48	<i>Corylus avellana</i>	Twigs	<i>Botrytis</i>	13	32
C-78	<i>Corylus avellana</i>	Twigs	<i>Pestalotiopsis</i>	38	14
C-82	<i>Corylus avellana</i>	Leaves	<i>Aspergillus</i>	25	28
C-96	<i>Corylus avellana</i>	Shells	<i>Fusarium</i>	32	58
C-98	<i>Corylus avellana</i>	Twigs	<i>Trichoderma</i>	16	32
C-100	<i>Corylus avellana</i>	Leaves	<i>Cladosporium</i>	38	55
O-12	<i>Ocimum basilicum</i>	Young leaves	<i>Phomopsis</i>	18	38
O-42	<i>Ocimum basilicum</i>	Mature leaves	<i>Phyllosticta</i>	34	52
O-55	<i>Ocimum basilicum</i>	Mature leaves	<i>Curvularia</i>	24	40
O-79	<i>Ocimum basilicum</i>	Mature leaves	<i>Penicillium</i>	21	38
O-101	<i>Ocimum basilicum</i>	Mature leaves	<i>Colleotrichum</i>	34	25

The extracts from the endophytic culture were examined for the presence of paclitaxel by TLC chromatographic and spectroscopic analyses. The compound demonstrating chromatographic properties comparable to paclitaxel in solvent system and giving a color reaction with the vanilin/sulfuric acid reagent was consistently obtained from the endophytic extract C-9. The presence of paclitaxel in the fungal extract was confirmed by TLC showing the color blue to grey with the vanilin sulfuric acid reagent. The UV absorption spectrum of fungal paclitaxel is similar to that of authentic paclitaxel with a maximum absorption at 273 nm (fig. 1).

The checking of the sensitivity of *A. tumefaciens* B6 strain against endophytic extracts showed that five of the tested extracts revealed no effect on the viability of *A. tumefaciens*. The minimum inhibitory concentration (MIC) of all endophytic extracts against *A. tumefaciens* B6 strain was found to be higher than 1000 µg/ml. *A. tumefaciens* B6 was sensitive to kanamycin but was resistant to tetracycline and ampicillin. A comparatively low concentration (10, 100, 1000 µg/ml) of extracts obtained from the fungus isolated from *Corylus avellana* and *Ocimum basilicum* were used for antitumor activity.

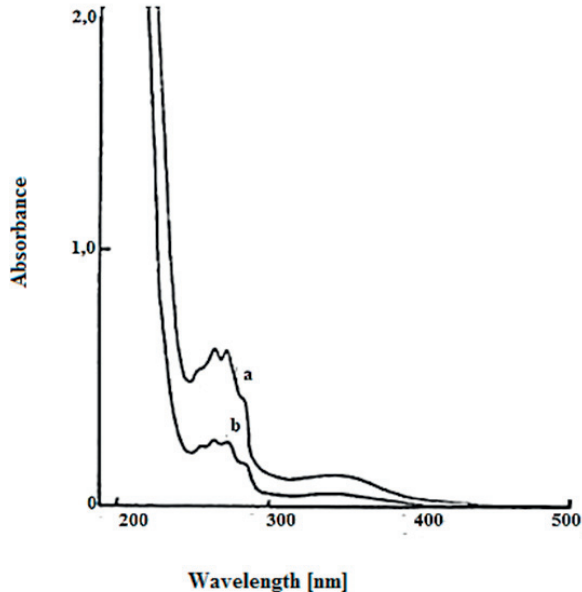


Figure 1.

The UV absorption spectrum of (a) authentic paclitaxel, (b) fungal extracted paclitaxel

The results showed that the five endophytic extracts inhibited tumor growth to a highly significant extent (tab. 3).

A significant tumor inhibition was observed at 100 $\mu\text{g/ml}$ and 1000 $\mu\text{g/ml}$ concentrations, but not at 10 $\mu\text{g/ml}$. Among the studied endophytic extracts, the highest percentage of tumor inhibition showed the extracts from the isolate C-9. Other extracts showed a slightly lower activity camptothecin and paclitaxel at 10 $\mu\text{g/ml}$ concentrations were employed as positive controls and caused 100% inhibitions.

Endophytic fungi are a rich and reliable source of bioactive and chemically novel compounds with a huge medicinal and agricultural potential [30-32]. In the recent years, the quest for isolation of new compounds from medicinal plants has become a fascinating area of research. Plants with ethno-pharmaceutical importance are being exploited because of their healing properties. However, a large scale harvesting of medical plants has already become a major threat to biodiversity. As an alternative, microbes that live inside the plants (endophytes) may be a potential source of therapeutic compounds. Hence, this study was investigated for the isolation and identification of paclitaxel from endophytic fungi. Most of the earlier reported paclitaxel producers were isolated as endophytic forms, especially from yews (*Taxus* sp.) rather than other plant groups [33]. Whereas, in the present study, *Alternaria* sp. isolated from shells of *Corylus avellana* exhibited an extracellular paclitaxel production in the MID medium for the first time.

Table 3.

The effect of five endophytic fungal extracts on crown gall tumor formation by *Agrobacterium tumefaciens* strain B6 on potato discs

Isolates	Concentration [$\mu\text{g/ml}$]	Mean number of tumor \pm SE	Tumor inhibition (%)
C-9	10	12.8 \pm 0.89	15.8
	100	9.7 \pm 1.48	36.2
	1000	6.3 \pm 0.36	58.6
C-45	10	14.2 \pm 0.87	6.6
	100	11.3 \pm 1.42	25.7
	1000	9.8 \pm 0.31	35.5
C-96	10	14.7 \pm 1.28	3.3
	100	11.8 \pm 0.15	22.4
	1000	10.0 \pm 0.21	34.3
C-100	10	12.2 \pm 1.17	19.74
	100	11.6 \pm 1.22	23.7
	1000	10.9 \pm 0.26	28.3
O-42	10	14.3 \pm 0.24	5.93
	100	11.4 \pm 0.18	25.0
	1000	10.7 \pm 0.29	29.6
<i>Agrobacterium tumefaciens</i> strain B6 +DMSO	–	15.2 \pm 0.89	–
DMSO	–	0	–
Camptothecin	10	0	100
Paclitaxel	10	0	100

In future studies, the cytotoxic activity of paclitaxel produced by the isolate C-9 (*Alternaria* sp.) will be determined against human tumor cell lines. Furthermore, the following research directions are predicted during the realization of these studies: optimization of culture conditions for the isolate C-9 through the addition of paclitaxel biosynthesis precursors and stimulators to culture media and also testing the paclitaxel production in a large scale.

CONCLUSION

As the plant producing paclitaxel has become endangered, the endophytes can be used as an alternative source for paclitaxel production. The endophytic fungus of the genus *Alternaria* (C-9) isolated from hazelnut shells produces paclitaxel. Its presence is confirmed in biological research as well as in a chromatographic and spectrophotometric analysis. The isolated fungi can be a good source of paclitaxel for large scale production by pharmaceutical industries in near future.

ACKNOWLEDGEMENTS

This research project was supported by the Ministry of Science and Higher Education, grant No. NN 209133440.

REFERENCES

1. Mekhail TM, Markman M. Paclitaxel in cancer therapy. *Expert Opin Pharmacother* 2002; 3:755-766, doi: 10.1517/14656566.3.6.755.
2. Guenard D, Gueritte-Voegelein F, Potier P. Taxol and taxotere: discovery, chemistry and structure activity relationship. *Acc Chem Res* 1993; 26:160-167.
3. Ottaggio L, Bestoso F, Armirotti A, Balbi A, Damonte G, Mazzei M, Sancandi M, Miele M. Taxanes from Shells and Leaves of *Corylus avellana*. *J Nat Prod* 2008; 71(1):58-60.
4. Gangadevi V, Muthumary J. Endophytic fungal diversity from young, mature and senescent leaves of *Ocimum basilicum* L. with special reference to taxol production. *Indian J Sci Technol* 2007; 1(1):1-15.
5. Bollon AP, Mu J-H, Sidhu RS. Fungal beta-tubulin genes. *EP 1163345 A1* 2000.
6. Young DH, Michelotti EL, Swindell CS, Krauss NE. Antifungal properties of taxol and various analogues. *Experientia* 1992; 48(9):882-885.
7. Galsky AG, Wilsey JP, Powell RG. Crown-gall tumor disc bioassay; a possible aid in the detection of compounds aid in antitumor activity. *Plant Physiol* 1980; 65:184-185.
8. Galsky AG, Kozimor R, Piotrowski D, Powell RG. The crown-gall potato disk bioassay as a primary screen for compounds with antitumor activity. *J Natl Cancer Inst* 1981; 67(3):689-92.
9. Srirama R, Ramesha BT, Ravikanth G, Shaanker RU, Ganeshiah. Are plants with anti-cancer activity resistant to crown gall? A test of hypothesis. *Curr Sci* 2008; 10:1407-1408.
10. Kim SU, Strobel G, Ford E. Screening of taxol-producing endophytic fungi from *Ginkgo biloba* and *Taxus cuspidata* in Korea. *Agric Chem Biotechnol* 1999; 42(2):97-99.
11. Barnett HL, Hunter BB. *Illustrated genera of imperfect fungi* (4th ed.). APS Press, St. Paul, Minnesota, USA 1998.
12. Fokkema NJ. Fungal antagonism in the phyllosphere. *Ann Appl Biol* 1978; 89:115-117.
13. Živković S, Stojanović S, Ivanović Ž, Gavrilović V, Popović T, Balaž J. Screening of antagonistic activity of microorganisms against *Colletotrichum acutatum* and *Colletotrichum gloeosporioides*. *Arch. Biol. Sci* 2010; 62:611-623.
14. Pinkerton R, Strobel G. Serinol as an activator of toxin production in attenuated cultures of *H. sacchari*. *Proc Natl Acad Sci USA* 1976; 73:4007-4011.
15. Strobel GA, Hess WM, Ford E, Sidhu RS, Yang X. Taxol from fungal endophytes and the issue of biodiversity. *J Ind Microbiol* 1996; 17:417-423.
16. Strobel GA, Yang X, Sears J, Kramer R, Sidhu R, Hess WM. Taxol from *Pestalotiopsis microspora*, an endophytic fungus of *Taxus walllichiana*. *Microbiology* 1996; 142:435-440.
17. Cardellina JH. HPLC separation of taxol and cephalomannine. *J Chromatogr* 1991; 14:659-665.
18. Andrews JM. BSAC Standardized disc susceptibility testing method. *J. Antimicrob Chemother* 2004; 53:713-728.
19. Anand VK, Heberlein GT. Crown Gall Tumorigenesis in potato tuber tissue. *Amer J Bot* 1977; 64 (2):153-158.
20. Coker PS, Radecke J, Guy C, Camper ND. Potato disc tumor induction assay; A multiple mode of drug action assay. *Phytomedicine* 2003; 10:133-138.
21. Ferrigini NR, Putnam JE, Anderson B, Jacobsen LB, Nichols DE, Moore DS, et al. Modification and evaluation of the potato disc assay and antitumor screening of *Euphorbiaceae* seeds. *J. Nat Prod* 1982; 45:679-686.
22. McLaughlin JL. Crown gall tumours on potato discs and brine shrimp lethality: Two simple bioassays for higher plant screening. In: Hostettmann K (ed.). *Methods Plant Biochem*. London: Academic Press 1991:1-31.

23. Hussain A, Zia M, Mirza B. Cytotoxic and antitumor potential of *Fegonia cretica* L. Turk J Biol 2007; 31:19-24.
24. Hadizadeh F, Moradi A, Naghibi G, Vojdani M, Behravan J, Ramezani M. Synthesis and antitumor activity of substituted succinamides using a potato disc tumor induction assay. Int J Biomed Sci 2007; 3(1):60–6.
25. Tian R, Yang Q, Zhou G, Tan J, Zhang L, Fang C. Taxonomic study on a taxol producing fungus isolated from bark of *Taxus chinensis* var. *mairei*. J Wuhan Botan Res 2006; 24:541-545.
26. Chakravarthi BVSK, Das P, Surendranath K, Karande AA, Jayabaskaran C. Production of paclitaxel by *Fusarium solani* isolated from *Taxus celebica*. Journal of Biosciences 2008; 33:259-26
27. Strobel GA, Hess WM, Li JY, Ford E, Sears J, Sidhu RS, Summerell B. *Pestalotiopsis guepinii*, a taxol-producing endophyte of the Wollemi pine, *Wollemia nobilis*. Austral J Bot 1997; 45:1073-1082.
28. Kim EL, Li JL, Dang HT, Hong J, Lee CO, Kim DK, Yoon WD, Kim E, Liu Y, Jung JH. Cytotoxic cytochalasins from the endozoic fungus *Phoma* sp. of the giant jellyfish *Nemopilema nomurai*. Bioorg Med Chem Lett 2012; 1:22(17):5752.
29. Yang X, Guo S, Zhang L, Shao H. Selection of producing podophyllotoxin endophytic fungi from podophyllin plant. Nat Prod Res Develop 2003; 15:419-422.
30. Tan RX, Zou WX. Endophytes: a rich source of functional metabolites. Nat Prod Rep 2001; 18:448–459. doi:10.1039/b100918o
31. Li HY, Qing C, Zhang YL, Zhao ZW. Screening for endophytic fungi with anti-tumour and antifungal activities from Chinese medicinal plants. World J Microbiol Biotechnol 2005; 21:1515–1519. doi:10.1007/s11274-005-7381-4
32. Pandey PK, Singh S, Yadav RNS, Singh AK, Singh MChK. Fungal Endophytes: Promising Tools for Pharmaceutical Science. Int J Pharm Sci Rev Res 2014; 25(2):128-138.
33. Zhu X, Zhu H, Liu L, Lin J, Tang K. A review: recent advances and future prospects of taxol - producing endophytic fungi. Appl. Microbiol. Biotechnol 2010; 86:1707-1717.

ROŚLINNE GRZYBY ENDOFITYCZNE JAKO ŹRÓDŁO PAKLITAKSELU

ALICJA MICHALCZYK*, ANNA CIENIECKA-ROSŁONKIEWICZ, MAŁGORZATA CHOLEWIŃSKA

Instytut Przemysłu Organicznego
Annopol 6
03-236 Warszawa

Streszczenie

Endofity grzybowe izolowano z różnych części tkanek *Corylus avellana* (*Corylaceae*) i *Ocimum basilicum* (*Lamiaceae*). Następnie przypisywano do rodzaju w oparciu o cechy morfologiczne i budowę struktur zarodnikujących. Sprawdzano aktywność grzybobójczą oraz przeciwnowotworową izolatów oraz uzyskanych z nich ekstraktów. Stwierdzono, że niektóre izolaty z *Corylus avellana* i *Ocimum basilicum* wytwarzają metabolity hamujące w znacznym stopniu wzrost grzybów z klasy *Oomycetes*. W badaniu na krążkach ziemniaków, tylko eks-

trakt z izolatu C-9 wykazywał działanie hamujące rozwój tumorów. Obecność paklitakselu w ekstrakcie z izolatu C-9 potwierdzono, stosując metodę chromatografii TLC oraz analizy spektroskopowej UV-VIS. Powyższe wyniki sugerują, że grzyby endofityczne mogą wytwarzać paklitaksel, który jest odpowiedzialny za aktywność przeciwnowotworową badanych próbek i że grzyby endofityczne mogą znaleźć zastosowanie do produkcji paklitakselu w procesie fermentacji.

Słowa kluczowe: *endofity grzybowe, paklitaksel, oomycetes, Crown Gall Tumor Disc*