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SCREENING OF EPIPHYTIC AND ENDOPHYTIC YEAST-LIKE FUNGI AS POTENTIAL BIOCONTROL AGENTS AGAINST GREY MOULD OF THE STRAWBERRY

Summary. The aim of this work was to identify endophytic and epiphytic isolates of yeast-like fungi with potential for biological control of *Botrytis cinerea*. A total of 7648 colonies of epiphytic and endophytic yeast-like fungi from the leaves and fruit were isolated, including 435 colonies isolated from fruit. Under *in vitro* conditions, 19% of the tested isolates significantly inhibited the growth of phytopathogen colonies, and 8.3% of them exhibited considerable antagonist activity against *B. cinerea* infection on strawberry leaves. The isolates obtained from the surface of winter wheat leaves were characterised by high antagonist potential against *B. cinerea*.

Key words: yeast-like fungi, strawberry, *Botrytis cinerea*, biocontrol

Introduction

The total area under strawberries in Poland is 53 500 ha, with the average yield reaching 37.1 dt/ha (STATISTICAL YEARBOOK... 2009). During the growing season, strawberries are exposed to the risk of infections caused by various pathogens, including the fungus *Botrytis cinerea* Pers., the causative agent of grey mould. The pathogen attacks strawberry flowers and causes severe yield losses estimated at 39% (MAZUR 2009). Chemical and biotechnological control reduces the incidence of grey mould on strawberry fruit, yet the applied methods are not always effective (SAS-PIOTROWSKA and PIOTROWSKI 2001, MAZUR 2009). Biological control agents may provide a viable alternative to chemicals in strawberry protection against *B. cinerea* (MELESHKO 2004). Yeast-like fungi could be used as biocontrol agents due to their fast growth rate, the ease of fruit and leaf colonisation, and high levels of fungicide tolerance. Citrus fruit is protected against pathogens with the use of commercial biocontrol products, Aspire (*Candida oleophila* isolate 182, Ecogen Inc., Langhorne, PA) and Yield Plus (*Crypto-*

coccus albidus, Anchor Yeast, Cape Town, South Africa) (TAQARORT et AL. 2008). The suitability of yeast-like fungi for strawberry protection against grey mould is currently being tested (HELBIG 2002, LONG and YUAN 2009, FAN et AL. 2009).

The objective of this study was to analyse the abundance of epiphytic and endophytic yeast-like fungi isolated from the fruit and leaves of plants, and to determine the inhibitory effect of selected isolates against *B. cinerea.*

Material and methods

Pathogen

The isolate of *B. cinerea* was obtained from ripe, naturally infected strawberry fruit (*Fragaria* × *ananassa* Duch.) cv. 'Senga Sengana'. This isolate, compared with the remaining ones in the collection, was characterised by high aggressiveness. The fungus was cultured on potato dextrose agar (PDA) medium at a temperature of 24°C, in darkness.

Isolation of antagonist yeast-like fungi

The isolates of yeast-like fungi showing potential antagonist activity against *B. cinerea* were obtained from the fruit and leaves of various plants. The fruit of apple (*Malus domestica* Borkh.), common pear (*Pyrus communis* L.), domestic plum (*Prunus domestica* L.), common grapevine (*Vitis vinifera* L.) and strawberry (*Fragaria* × *ananassa* Duch.) were harvested in north-eastern Poland, and orange fruit (*Citrus sinensis* L.) was imported from Spain. Depending on species, the fruit was stored for two to three months. Fresh leaves of winter wheat (*Triticum aestivum* L.), common stinging nettle (*Urtica dioica* L.), wild strawberry (*Fragaria vesca* L.) and strawberry (*Fragaria* × *ananassa*) were collected from plants grown in north-eastern Poland.

Samples of fruit surface tissue weighing 3 g were placed in 250 ml flasks filled with 50 ml sterile water. One-centimetre segments with known width were collected from winter wheat leaves. One-square centimetre fragments were collected from the leaves of the remaining plants. The samples were placed in 250 ml flasks filled with 15 ml sterile water, 15 fragments per flask. The flasks and their contents were shaken in a vortex shaker, type 358S, for 30 min. The isolates of endophytic fungi were obtained by grinding 3-gram fruit flesh samples or 15 leaf fragments with a surface area of 1 cm^2 in a mortar filled with 10 ml sterile water. Prior to grinding, the plant material was surface disinfected for 3 min in a 1% sodium hypochlorite solution. The resultant fungal cell suspensions or homogenates with a volume of 0.1 ml were transferred to three Petri dishes. Martin medium cooled to 40°C was poured into the dishes. Fungal colonies were counted after seven to ten days of incubation at 24°C. Individual, morphologically different colonies of yeast-like fungi were transferred onto agar slants and were stored at 4°C.

Screening of fungal antagonists against *Botrytis cinerea*

The inhibitory activity of 72 isolates of yeast-like fungi against *B. cinerea* was tested. Petri dishes were filled with potato dextrose agar (PDA) medium. Five-millimetre diameter disks were collected from the colonies of seven-day pathogen cultures. The disks were placed in the centre of Petri dishes. The suspensions of 48-hour cultures of fungal antagonists were transferred to Petri dishes with an inoculation loop, and were placed at a distance of 2 cm from the disks. The biological activity of yeast-like fungi was evaluated after four days of incubation at 24°C, in darkness. A measure of inhibitory activity was the elliptic coefficient of *B. cinerea* colonies, calculated by dividing the length of the short (minor) axis by the length of the long (major) axis of the ellipse circumscribed around the colony. The fungal isolates in whose presence the colonies of the tested phytopathogen assumed the shape of an ellipse with a coefficient below 0.69 were considered to be biologically active.

Screening of fungal antagonists against *Botrytis cinerea* infections of strawberry leaves

Fresh strawberry leaves were immersed in the suspension of yeast-like fungal isolates at a concentration of 2° on the McFarland scale. Strawberry leaves immersed in sterile water served as control. The leaves were placed on wet filer paper, in glass Petri dishes. After 24 h the spores of 14-day *B. cinerea* cultures were transferred to strawberry leaves with an inoculation loop. After three, four, five and six days of incubation at 21°C, the size of spots was estimated with the use of an image analysis system consisting of a Sony digital camera, a PC computer and a NEC monitor. The surface area of the spots was measured using the ImageJ program. The actual surface area of the spots was calculated based on the virtual and actual surface area of Petri dishes.

Statistical analysis

Quantitative data were subjected to transformation (log(cfu + 1)) and were processed statistically by an analysis of variance (ANOVA), using Statistica 9.0 software. The significance of differences between mean values in laboratory tests was estimated by Duncan's multiple range test ($p = 0.05$). The correlation between the elliptic coefficient of pathogen colonies and the surface area of grey mould lesions on strawberry leaves was also calculated.

Results

A total of 380 colonies of epiphytic yeast-like fungi and 55 colonies of endophytic yeast-like fungi were isolated from the fruit of the analysed plants (Fig. 1). The communities of epiphytic fungi isolated from fruit were much more abundant than the communities of endophytic fungi. The degree of fungal colonisation was highest on the surface of apples. Significantly more epiphytic colonies were isolated from orange fruit than from grapes and strawberries. The abundance of endophytic fungal communities in fruit flesh was low, with no significant differences between fruit species.

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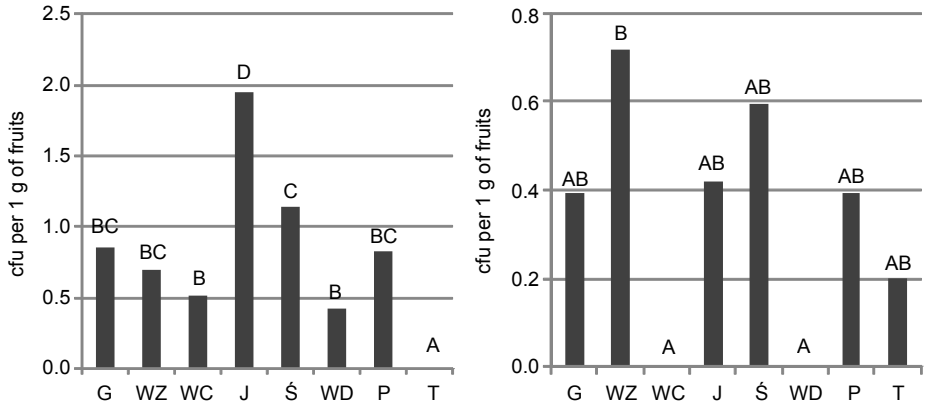

Fig. 1. The abundance of communities of epiphytic (on the left) and endophytic (on the right) yeast-like fungi isolated from the fruits of plants; G – pear, WZ – grapevine green, WC – grapevine dark, J – apple, Ś – plum, WD – grapevine large, P – orange, T – strawberry (mean values followed by the same letters are not significantly different according to SNK test: p = 0.05)

Rys. 1. Liczebność epifitycznych (z lewej) i endofitycznych (z prawej) zbiorowisk grzybów drożdżoidalnych uzyskanych z owoców roślin; G – gruszka, WZ – winogrona zielone, WC – winogrona ciemne, J – jabłko, Ś – śliwka, WD – winogrona duże, P – pomarańcza, T – truskawka (jednakowymi literami oznaczono wartości nie różniące się istotnie według testu Newmana-Keulsa: p = 0,05)

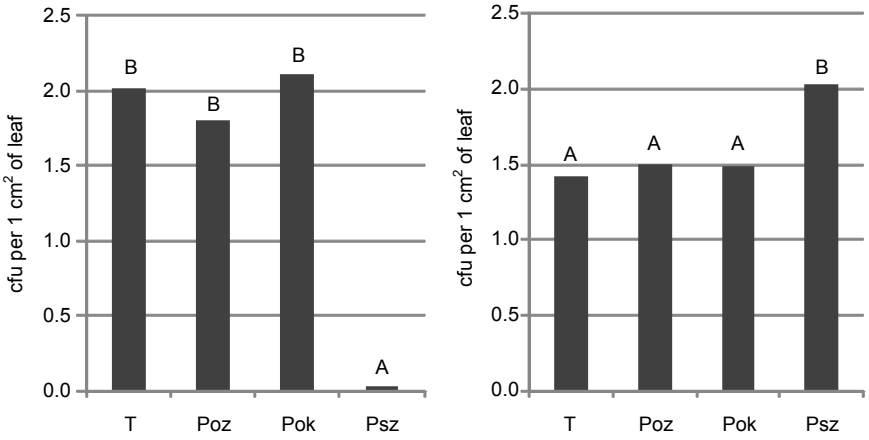

Fig. 2. The abundance of communities of epiphytic (on the left) and endophytic (on the right) yeast-like fungi isolated from the leaves of plants; T – strawberry, Poz – wild strawberry, Pok – common stinging nettle, Psz – winter wheat (mean values followed by the same letters are not significantly different according to SNK test: p = 0.05)

Rys. 2. Liczebność epifitycznych (z lewej) i endofitycznych (z prawej) zbiorowisk grzybów drożdżoidalnych uzyskanych z liści roślin; T – truskawka, Poz – poziomka, Pok – pokrzywa, Psz – pszenica ozima (jednakowymi literami oznaczono wartości nie różniące się istotnie według testu Newmana-Keulsa: p = 0,05)

A total of 1391 colonies of epiphytic yeast-like fungi and 5822 colonies of endophytic yeast-like fungi were isolated from the leaves of the analysed plants (Fig. 2). The communities of epiphytic fungi isolated from the leaves of common stinging nettle, wild strawberry and strawberry were much more abundant than the communities of endophytic fungi. The community of epiphytic fungi isolated from winter wheat leaves was much less abundant than the community of endophytic fungi.

Under *in vitro* conditions, 19% of the tested isolates significantly inhibited the growth of *B. cinerea* colonies (Table 1). Those isolates were obtained from the surface of oranges (three isolates: PEp1, PEp2, PEp3), plums (one isolate: ŚEp4) and apples (one isolate: JEp3) (Table 1). One isolate obtained from strawberry leaves (TEp6) and four isolates from winter wheat leaves (6Ep6 2.06., 4Ep3 2.06., 3Ep10, 5Ep4 2.06) exhibited high antagonist activity against *B. cinerea* infection. The isolates obtained from wheat, in particular isolate 6Ep6 2.06, were characterised by the greatest activity against *B. cinerea*.

Table 1. The effect of epiphytic and endophytic yeast-like fungi on the growth of *Botrytis cinerea* colonies
Tabela 1. Wpływ epifitycznych i endofitycznych grzybów drożdżoidalnych na wzrost kolonii *Botrytis cinerea*

Origin of isolates	Not antagonist effect	Antagonist effect	Elliptic coefficient of colonies of *Botrytis cinerea*
1	2	3	4
Orange (fruit)	–	PEp4	0.61
		PEp1	0.65
		PEp3	0.66
		PEp2	0.83
		PEn1	0.56
Plum (fruit)	ŚEn2, ŚEp1, ŚEp3	ŚEp4	0.66
		ŚEp2	0.69
		ŚEn1	0.68
Apple (fruit)	JEn1, JEp1, JEp2, JEp4, JEp5	JEp3	0.67
Pear (fruit)	GEp3, GEp4, GEp2, GEp1	–	–
Grapevine green (fruit)	WEp1, WEp2, WEn2, WEn1	–	–
Wild strawberry (leaf)	PozEn1, PozEn2, PozEn3, PozEn4, PozEp5, PozEp4, PozEp6, PozEp9	–	–

Table 1 – cont. / Tabela 1 – cd.

1	2	3	4
Strawberry (leaf)	TEn2, TEn3, TEn4, TEn5, TEp7, TEp8, TEp2, TEp3, TEp4	TEp6	0.50
Common stinging nettle (leaf)	PokEp2, PokEp3, PokEp4, PokEp5, PokEp6, PokEp7	–	–
Winter wheat (leaf)	Ep3 5.06, Ep1 5.06, 6Ep4 2.06,	6Ep6 2.06	0.34
	5Ep3 2.06, 6Ep5 2.06, 6Ep1 2.06,	4Ep3 2.06	0.55
	6Ep8 2.06, 6Ep9 2.06, 5Ep1 2.06,	3Ep10	0.45
	4Ep3 2.06, Ep6 5.03, 5Ep2,	5Ep4 2.06	0.47
	5Ep2 2.06, 6Ep1 2.06, 3Ep4,	5En1/III25	0.44
	Ep3 5.06, Ep5 5.06	5En1/III20	0.48

Among the isolates obtained from fruit flesh, isolate PEn1 from oranges was found to be biologically active under *in vitro* conditions (Table 1). Two isolates obtained from winter wheat leaves (5En1 25.03, 5En1 20.03) exerted a profound inhibitory effect on the growth of *B. cinerea* colonies.

The size of spots on strawberry leaves was evaluated three, four, five and six days after inoculation with *B. cinerea* spores (Tables 2, 3). The highest dynamics of spot development was observed on the fifth day after inoculation. Forty six isolates of epiphytic fungi had varied effects on lesion development on strawberry leaves (Table 2). The spots did not develop on strawberry leaves treated with the suspensions of fungal isolates obtained from winter wheat leaves (6Ep6 2.06, 3Ep10, 6Ep4 2.06, 4Ep3), the fruit of plum (ŚEp3) and pear (GEp4, GEp2). Eight isolates obtained from winter wheat leaves considerably inhibited lesion development on strawberry leaves. The spots that appeared on strawberry leaves treated with the cell suspensions of the above isolates had a surface area smaller than 50 mm^2. Three isolates of endophytic fungi, obtained from winter wheat leaves and the fruit of strawberry and apple, also inhibited the process of *B. cinerea* infection (Table 3).

There was no correlation (r = 0.21) between the inhibitory effect of isolates on the growth of *B. cinerea* colonies and the influence of those isolates on strawberry leaf infection (Tables 1, 2, 3). Only five isolates obtained from winter wheat (6Ep6 2.06, 4Ep3 2.06, 3Ep10, 5Ep4 2.06., 5En 20.03) exhibited high antagonist activity under both *in vitro* and *in vivo* conditions. One isolate from plums (ŚEp3), two isolates from pears (GEp4, GEp2), one isolate from wheat (4Ep3) and one endophyte from strawberry leaves (TEn2) did not inhibit the growth of *B. cinerea* colonies, but controlled the infection process. Spots did not develop on strawberry leaves treated with the suspensions of the above isolates.

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Table 2. The size of spots on strawberry leaves treated with the suspensions of epiphytic yeast--like fungi and inoculated with *Botrytis cinerea* (mm^2)
Tabela 2. Wielkość plam na liściach truskawki traktowanych zawiesiną epifitycznych grzybów drożdżoidalnych i inokulowanych patogenem *Botrytis cinerea* (mm^2)

Origin of isolates	Isolate	After three days	After four days	After five days	After six days	Mean
1	2	3	4	5	6	7
Orange	PEp4	29.3 ab	179.2 a-c	454.4 a-j	741.9 a-j	351.2 A-F
	PEp1	135.0 ab	375.8 a-h	671.9 a-j	998.9 a-l	545.4 A-G
	PEp3	101.2 ab	240.0 a-c	492.5 a-j	905.2 a-l	434.7 A-F
	PEp2	119.6 ab	272.5 a-e	595.8 a-j	1004.8 a-l	498.2 A-G
	PEp6	199.5 a-c	484.6 a-i	1 062.3 a-j	1 823.6 j-k	892.5 C-G
Plum	ŚEp4	81.1 ab	271.4 a-e	519.6 a-j	757.6 a-j	407.5 A-F
	ŚEp2	0.0 a	0.0 a	44.5 ab	146.1 ab	47.7 AB
	ŚEp1	68.5 ab	179.0 a-c	625.1 a-j	1 368.0 g-l	560.2 A-G
	ŚEp3	0.0 a	0.0 a	0.0 a	0.0 a	0.0 A
Apple	JEp3	25.7 ab	72.8 a-c	249.0 a-d	619.0 a-j	241.6 A-F
	JEp1	26.1 ab	173.2 a-c	534.3 a-j	1 070.7 a-l	451.1 A-F
	JEp2	9.4 a	46.5 ab	203.6 a-c	461.4 a-j	180.2 A-F
	JEp4	16.7 a	45.7 ab	226.0 a-c	772.8 a-j	265.3 A-F
	JEp5	25.5 ab	135.4 ab	377.7 a-h	723.7 a-j	315.6 A-F
Pear	GEp3	0.0 a	0.0 a	49.1 ab	114.1 ab	40.8 AB
	GEp4	0.0 a	0.0 a	0.0 a	0.0 a	0.0 A
	GEp2	0.0 a	0.0 a	0.0 a	0.0 a	0.0 A
Grapevine	WEp1	0.0 a	9.1 a	62.3 ab	222.7 a-c	73.5 AB
	WEp2	0.0 a	0.0 a	26.0 ab	28.2 ab	13.6 AB
Wild straw-berry	PozEp2	24.6 ab	44.3 a	103.9 ab	451.0 a-j	155.9 A-D
	PozEp5	9.5 a	31.2 ab	125.0 ab	277.5 a-e	110.8 A-C
	PozEp4	32.4 ab	27.8 ab	238.4 a-c	1 423.0 h-l	430.4 A-F
	PozEp6	10.2 a	50.2 ab	156.2 a-c	598.7 a-j	203.8 A-F
	PozEp9	12.3 a	55.0 ab	75.2 ab	142.4 a-c	71.2 A-F

Table 2 – cont. / Tabela 2 – cd.

1	2	3	4	5	6	7
Strawberry	TEp6	0.0 a	42.6 ab	113.6 ab	382.9 a-i	134.8 A-D
	TEp8	11.7 a	112.4 ab	236.1 a-c	635.1 a-j	248.8 A-F
	TEp2	20.3 a	111.2 ab	420.9 a-i	948.7 a-l	375.3 A-F
	TEp4	10.4 a	82.0 ab	278.7 a-e	570.8 a-j	235.5 A-F
Winter wheat	6Ep6 2.06	0.0 a	0.0 a	0.0 a	0.0 a	0.0 A
	Ep3 5.06	14.3 a	398.5 a-i	398.4 a-i	849.5 a-k	415.2 A-F
	4Ep3 2.06	19.9 a	25.8 ab	26.2 ab	67.6 ab	34.9 AB
	3Ep10	0.0 a	0.0 a	0.0 a	0.0 a	0.0 A
	6Ep4 2.06	0.0 a	0.0 a	0.0 a	0.0 a	0.0 A
	6Ep5 2.06	3.8 a	12.9 a	59.8 ab	160.5 a-c	59.2 AB
	Ep1 5.06	0.0 a	0.0 a	33.8 ab	494.0 a-j	131.9 A-D
	5Ep3 2.06	15.2 a	42.7 ab	171.1 a-c	484.7 a-j	178.4 A-F
	6Ep1 2.06	131.7 ab	468.4 a-j	852.1 a-k	629.3 a-j	520.4 A-G
	6Ep9 2.06	0.0 a	9.8 a	33.8 ab	1 312.5 d-l	339.0 A-F
	5Ep1 2.06	0.0 a	0.0 a	13.7 ab	138.1 a-c	38.0 AB
	4Ep3	0.0 a	0.0 a	0.0 a	0.0 a	0.0 A
	Ep6 5.03	233.4 a-c	514.3 a-j	885.0 a-k	1 312.5 c-l	736.3 B-G
	5Ep2	0.0 a	0.0 a	42.9 ab	236.6 a-c	69.9 AB
	5Ep2 2.06	0.0 a	0.0 a	43.0 a	236.6 a-c	69.9 AB
	3Ep4	0.0 a	0.0 a	176.0 a	nt	44.0 AB
	Ep3 5.06	427.2 a-i	nt	1 223.7 c-l	1 456.3 i-l	776.8 C-G
	Ep5 5.06	151.0 a-c	485.9 a-j	1 059.7 a-l	1 935.5 l	908.0 FG
Control		30.4 ab	105.4 ab	401.2 a-i	855.4 a-k	348.1 AG

Mean values followed by the same letters are not significantly different according to SNK test (p = 0.05).
nt – not tested.

Table 3. The size of spots on strawberry leaves treated with the suspensions of endophytic yeast--like fungi and inoculated with *Botrytis cinerea* (mm^2)
Tabela 3. Wielkość plam na liściach truskawki traktowanych zawiesiną endofitycznych grzybów drożdżoidalnych i inokulowanych patogenem *Botrytis cinerea* (mm^2)

Origin of isolates	Isolate	After three days	After four days	After five days	After six days	Mean
1	2	3	4	5	6	7
Orange (fruit)	PEn1	11.1 a	30.8 ab	120.7 ab	360.1 a-g	130.7 A-D
Plum (fruit)	ŚEn1	41.9 ab	246.4 a-c	534.1 a-j	1 031.6 a-l	463.5 A-G
	ŚEn2	43.5 ab	230.7 a-c	598.2 a-j	872.6 a-k	436.2 A-F
Apple (fruit)	JEn1	0.0 a	0.0 a	0.0 a	6.6 a	1.6 A
Grapevine (fruit)	WEn2	30.6 ab	137.2 ab	373.9 a-h	968.8 a-l	377.6 AB
	WEn1	8.2 a	72.9 ab	280.4 a-e	556.4 a-j	229.5 A-F
Wild strawberry (leaf)	PozEn1	94.0 ab	329.2 a-g	648.9 a-j	1 348.4 f-l	605.1 A-G
	PozEn2	186.4 a-c	119.1 ab	705.3 a-j	1 446.5 i-l	614.3 A-G
	PozEn3	44.1 ab	228.4 a-c	637.4 a-j	1 498.6 j-l	602.1 A-G
	PozEn4	56.7 ab	116.4 ab	302.4 a-f	nt	118.9 C-G
Strawberry (leaf)	TEn2	0.0 a	0.0 a	0.0 a	0.0 a	0.0 A
	TEn3	26.9 ab	150.7 a-c	298.3 a-f	563.5 a-j	259.8 A-F
	TEn4	31.5 ab	168.0 a-c	499.2 a-j	851.5 a-k	387.6 A-F
	TEn5	68.4 ab	68.4 ab	356.9 a-g	672.9 a-j	291.7 A-F
Winter wheat (leaf)	5En1 25.03	0.0 a	26.7 ab	100.9 ab	360.2 a-g	122.0 A-D
	5En 20.03	0.0 a	0.0 a	0.0 a	0.0 a	0.0 A
Control		30.4 ab	105.4 ab	401.2 a-i	855.4 a-k	348.1 A-F

Mean values followed by the same letters are not significantly different according to SNK test (p = 0.05).
nt – not tested.

Discussion

The abundance of yeast-like fungi colonizing the surface and the inner tissue of the fruit and leaves of various plants was analysed in the growing seasons of 2009 and 2010. The aim of screening tests was to identify environments abundantly colonised by yeast-like fungi with potential for biological control of *B. cinerea.* A total of 7648 colo-

nies of yeast-like fungi were isolated, including 435 colonies isolated from fruit. The applied method enabled to obtain an abundant fungal community from leaves. It was also found that the isolates from this environment showed potential for the biocontrol of *B. cinerea*. Most screening tests focus on isolates from fruit (TAQARORT et AL. 2008, ZHANG et AL. 2008, BLEVE et AL. 2006), and less frequently on those obtained from other environments, including olive brines, beverages and foods (SANTOS et AL. 2004). The leaves of crops, including wheat, are abundantly colonised by yeast-like fungi whose role in this environment has not been investigated in detail to date (DIK et AL. 1992). The present results indicate that isolates characterised by high antagonist activity against *B. cinerea* can be found in the above environment.

The majority of the studied isolates of yeast-like fungi had an inhibitory effect on the growth of *B. cinerea* colonies in *in vitro* tests. Pathogen colonies grown in the presence of antagonists had an elliptical shape, and inhibition zones were formed between the colonies of the tested pathogen and yeast-like fungi. Most probably, some isolates of yeast-like fungi released substances that inhibited the growth of pathogen colonies. Previous studies (SANTOS et AL. 2004, WALKER et AL. 1995) demonstrated that yeast--like fungi may produce killer toxins or hydrolytic enzymes. The secretion of enzymes by yeasts caused cell wall degeneration in phytopathogens. Killer toxins may vary in size from 6.3 to 110 kDa, and their mechanism of action is relatively well understood (MARQUINA et AL. 2002).

Among the 72 tested isolates only six (8.3%) exhibited considerable antagonist activity against *B. cinerea* infection on strawberry leaves. Five isolates were obtained from winter wheat leaves. In a study by TAQARORT et AL. (2008), only 8% of isolates of yeast-like fungi obtained from citrus fruit showed antagonist activity against *Penicillium digitatum*. According to the cited authors, the method that involves the selection and preliminary testing of the inhibitory effect of microbial suspensions on the fruit inoculated with the analysed pathogen is much more effective (TAQARORT et AL. 2008).

In the present study there was no correlation between the antagonist activity of isolates against *B. cinerea* colonies on PDA medium in Petri dishes and their effect on the infection process on strawberry leaves. It probably resulted from the varying ability of the tested isolates to colonise strawberry leaves. The isolates which actively colonised strawberry leaves exerted a more profound antagonist effect on *B. cinerea*. Research results (TAQARORT et AL. 2008) demonstrated that on the surface of citrus fruit the population size of *Debaryomyces hansenii* was increasing slowly over seven days, while the abundance of *Hanseniaspora guilliermondii* was increasing at a faster rate, and reached a maximum as soon as after 24 h of incubation. It seems that the rate of leaf colonisation on the first day of experiment could be of primary importance. The isolates characterised by a fast rate of leaf colonisation during the first hours after inoculation could have provided effective protection against *B. cinerea* infection.

Conclusions

1. The surface of winter wheat leaves may be a good source of isolates of yeast-like fungi with potential for biological control of *B. cinerea.*

2. The antagonist activity of yeast-like fungi against the causative agent of grey mould of the strawberry was dependent upon their inhibitory effect on the growth of pathogen colonies and the rate of colonisation of the protected crop.

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SELEKCJA EPIFITYCZNYCH I ENDOFITYCZNYCH GRZYBÓW DROŻDŻOIDALNYCH JAKO POTENCJALNYCH ANTAGONISTÓW SPRAWCY SZAREJ PLEŚNI TRUSKAWKI

Streszczenie. Celem badań skryningowych było wytypowanie środowisk licznie zasiedlanych przez grzyby drożdżoidalne o dużym potencjale antagonistycznego oddziaływania wobec *Botrytis*

cinerea. Łącznie z liści i owoców uzyskano 7648 kolonii epifitycznych i endofitycznych grzybów drożdżoidalnych, w tym z owoców – 435. W warunkach *in vitro* 19% ogółu testowanych izolatów znacznie ograniczało wzrost kolonii fitopatogenu, a 8,3% wykazywało znaczną antagonistyczną aktywność wobec procesu infekcyjnego *B. cinerea* na liściach truskawki. Izolaty pochodzące z powierzchni liści pszenicy ozimej wyróżniły się dużym potencjałem antagonistycznym w stosunku do *B. cinerea*.

Słowa kluczowe: grzyby drożdżoidalne, truskawka, *Botrytis cinerea*, ochrona biologiczna

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