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EFFICACY OF *Candida melibiosica* FOR CONTROL OF POST-HARVEST FUNGAL DISEASES OF CARROT (*Daucus carota* L.)

Monika Kordowska-Wiater, Anna Wagner, Beata Hetman
University of Life Sciences in Lublin, Poland

Abstract. Biological control using antagonistic yeast has emerged as one of the most promising alternatives in postharvest protection of fruit and vegetables. The aim of the present studies was to investigate the potential of the yeast *Candida melibiosica* for biocontrol of *Botrytis cinerea*, *Alternaria alternata* and *A. radicina*, fungi pathogenic to carrot roots, and explain the possible mode of action of this antagonistic microorganism. The ability of the yeast to inhibit fungal growth *in vitro*, produce volatile compounds and lytic enzymes, and protect infected carrot roots was studied. *C. melibiosica* inhibited the growth of *B. cinerea* on potato-dextrose agar and malt agar by 25.9% and 33.3%, respectively, but only slightly restricted the growth of *A. radicina* and had no effect on *A. alternata*. The volatile compounds acting against *B. cinerea* and *A. radicina* were produced by *C. melibiosica* growing on malt agar. The yeast produced β-glucanase and chitinase with activities between 34.0–232.86 U · cm^{-3} and 20.74–43.7 U · cm^{-3}, respectively. Application of the yeast to carrot roots before inoculation with the fungi *B. cinerea* and *A. radicina* reduced the progress of the disease

Keywords: biocontrol, antagonistic yeast, phytopathogenic fungi

INTRODUCTION

In the post-harvest stages of fruits and vegetables, the application of fungicides is limited due to safety concerns. Biological control using yeast antagonists has emerged as one of the most promising alternatives to fungicides treatment, the common management strategy of combating plant diseases. These organisms deserve particular attention as their activity does not generally depend on the production of toxic metabolites. Also, they do not produce allergic spores or mycotoxins, are easily cultivated on simple media and can be produced on a large scale as commercial preparations [Zhang et al. 2010]. Intensive studies on screening and selection of appropriate yeast strains have

Corresponding author – Adres do korespondencji: Monika Kordowska-Wiater, Department of Biotechnology, Human Nutrition and Science of Food Commodities, University of Life Sciences in Lublin, Skromna 8, 20-704 Lublin, Poland, e-mail: monika.kordowska-wiater@up.lublin.pl

been continued for about twenty years now. Some yeasts have been identified as post-harvest biocontrol agents of different plant diseases caused by fungi, e.g., *Cryptococcus humicola* [Filonow et al. 1996], *C. laurentii, Rhodotorula glutinis* [Lima et al. 2003], *Debaryomyces hansenii, Pichia guilliermondii, Metschnikowia fructicola* [Ferrari et al. 2007], *M. pulcherrima* [Janisiewicz et al. 2001, Grebenisan et al. 2008] and the yeast-like fungus *Aureobasidium pullulans* [Leibinger et al. 1997, Lima et al. 2003, Zhang et al. 2010]. Yeasts of the genus *Candida* have also been the subject of interest to some researchers. Lima et al. [1997] reported the application of *C. oleophila* for the protection of strawberries against *Botrytis cinerea* and Schena et al. [2000] studied control of the growth of *B. cinerea, Penicillium digitatum, Aspergillus niger* and *Rhizopus stolonifer* by these yeasts on grapes, grapefruits and tomatoes. Teixidó et al. [1998] reported using *C. sake* to inhibit *P. expansum* on apples and Benbow and Sugar [1999] used *C. infirmominatus* before harvest for the control of *B. cinerea* and *P. expansum* on pears. There are some information about using *C. valida* against *Rhizoctonia solani* on sugar beets [El-Tarabily 2004], *C. glabrata, C. maltosa* and *C. sloofiae* against *Cephalosporium maydis* on corn [El-Mehalawy et al. 2004] and *C. steatolytica* against *Fusarium oxysporum* rot of bean [El-Mehalawy 2004]. *Candida melibiosica, C. butyri* and *C. parapsilosis* have been studied for their abilities to inhibit post-harvest grey mould of apples. *C. melibiosica* efficiently controlled *Botrytis* rot, inhibiting the development of necrosis by 75% 5 days after inoculation and by 25% two weeks after inoculation. *C. butyri* and *C. parapsilosis* reduced gray mould by 70% and 10–13% after the same inoculation periods [Wagner et al. 2006].

Understanding of the mode of action of antagonistic microorganisms is a prerequisite for the development of successful biocontrol strategies. Scientists suggest the following modes of yeast action: the ability to develop quickly in natural habitats and dominate the environment by inhibition of the growth of other microorganisms through competition for space and nutrients; the capacity to produce hydrolytic enzymes such as β-glucanases and chitinases, which destroy other fungal cell walls; the ability to produce siderophores or toxic volatile compounds that inhibit fungal growth; and in some yeast species, production of killer toxins which may be responsible for inhibition of phytopathogenic fungi [Saligkarias et al. 2002, Rosa et al. 2010].

The aim of the present studies was to investigate the potential of the yeast *C. melibiosica* for post-harvest protection of carrot roots against the pathogenic fungi *B. cinerea, A. alternata* and *A. radicina* and explain the mode of action of this antagonistic microorganism.

MATERIALS AND METHODS

Microorganisms. The yeast *Candida melibiosica* 2515 was obtained from the yeast collection of the Department of Biotechnology, Nutrition and Science of Food Commodities of the University of Life Sciences in Lublin, Poland. The microorganism was maintained on malt agar slants at 4°C. The phytopathogenic fungi *Botrytis cinerea* Pers. ex Nocca & Balb, *Alternaria alternata* (Fr.) Keissler and *Alternaria radicina* Meier, Drechsler & Eddy were isolated from carrot roots in the Department of Plant Protection

and Quarantine of the University of Life Sciences in Lublin, Poland. These fungi were maintained on potato dextrose agar slants at 4°C.

Evaluation of *in vitro* antagonism in solid medium. The studies were conducted on Petri dishes with malt agar and potato-dextrose agar (PDA). Petri dishes were inoculated at two sites with 40 µl of yeast suspension at a cell concentration of about 4×10^7 cfu · cm^{-3} in aqueous Tween 40 solution. After one hour, fungal mycelium discs, about 5 mm in diameter, were inoculated at the same sites as the yeast. The microorganisms were incubated at 28ºC for 5 days in darkness. Control fungal discs were incubated without the yeast under the same conditions. After incubation, the diameters of the mycelia were measured. The experiment was repeated twice. The fungal mycelia were microscopically observed to assess the possible hyphal damage caused by the yeast. Observations were done at 300× magnification and the hyphae were photographed.

Production of antifungal volatile compounds. Evaluation of the antifungal volatile compounds produced by the yeast was carried out on Petri dishes containing malt agar or PDA on the top and bottom of the dish. The bottom part of the dish was inoculated as a line with yeast from an agar slant, and the upper part was inoculated with a mycelial disc from an earlier prepared mycelial culture. After closing the Petri dish, the incubation was continued at 28ºC for 5 days in darkness. Fungal colonies incubated without the yeast were used as controls. After incubation, the diameters of the mycelia were measured and compared with the control. The experiment was repeated twice.

Production of hydrolytic enzymes (β-glucanase and chitinase). Yeast cells were transferred by loop to 100-ml Erlenmeyer flasks containing 40 cm^3 of a special medium composed of yeast extract (3 g · dm^{-3}), $(NH_4)_2SO_4$ (5 g · dm^{-3}), KH_2PO_4 (5 g · dm^{-3}), in which glucose, chitin or dried mycelium of *B. cinerea* or *A. radicina* or *A. alternata* (5 g · dm^{-3}) were used as carbon sources. Cultures were incubated at 28ºC under shaking at 200 rpm for 5 days. After that time, they were centrifuged at 10.000 g for 15 min at 4ºC. The supernatants were used for the analysis of β-glucanase, chitinase and protein contents.

β-glucanase activity was analyzed colorimetrically by the method of Miller [1959]. 0.1 cm^3 of sample was incubated with 0.9 cm^3 1% (w · v^{-1}) laminarin solution in acetate buffer (0.1 M, pH 4.8) at 50ºC for 50 min. Next, the amount of glucose released from laminarin was determined by adding 3 cm^3 of the dinitrosalicylic acid (DNS) reagent and boiling the samples for 5 min. After adding 11.5 cm^3 of distilled water, absorbance at 550 nm was measured on a Biorad Smartspec Plus spectrophotometer. The enzymatic activity was expressed in U · cm^{-3} and one unit of activity was defined as 1 µmol of reducing sugar released from laminarin per minute under the assay conditions.

Chitinase activity was evaluated by estimating the release of N-acetylglucosamine from 0.5 % (w/v) colloidal chitin solution in acetate buffer (0.1 M, pH 4.8). 0.5 cm^3 of sample was incubated with 0.5 cm^3 of the above solution at 50ºC for 60 min and then the Miller method [1959] using the DNS reagent was done. The enzymatic activity was expressed in U · cm^{-3} and one unit of activity was defined as 1 µmol of reducing sugar released from colloidal chitin per minute under the assay conditions.

Total protein concentration was determined by the Bradford method [1976] using Coomasie Brillant Blue. This concentration was used to evaluate the specific activity of hydrolytic enzymes, which was expressed in $U \cdot mg^{-1}$ of protein.

Evaluation of biological control of fungi by *C. melibiosica* on carrot roots. Roots of carrot cv. Elegance were washed in warm water, air dried, and externally decontaminated using 80% ethanol. Four wounds were made on each vegetable using a sterile needle, and 40 µl of a yeast suspension was applied onto each wound. The yeast suspension was prepared by introducing a loop of yeast biomass to sterile water with Tween 40 and gentle mixing to obtain a cell concentration of 4×10^7 cfu $\cdot$ cm^{-3}. The inoculated roots were placed on sterile trays, and after 24 hours the wounds were inoculated with 3 mm discs of mycelium, after which the vegetables were protected with food foil. Control roots were inoculated with discs of mycelium alone, and water with Tween 40 was used instead of the yeast cell suspension. The experiment was done in eight replicates (32 wounds). The samples were kept in phytotrone at room temperature, and the results were observed after 5, 10, and 15 days. The diameters of lesions on yeasts-inoculated and control vegetables were measured, and rot changes were observed. Additionally, after 10 days, half of the samples were treated with the yeast suspension for a second time to compare the protective effect of the yeasts *C. melibiosica*.

Data were analysed with Tukey's test using the SAS statistical system [SAS Ver. 9.1, SAS Inst., Cary, N.C., USA].

Fragments of mycelium from appropriate samples were collected to obtain microscopic preparations. Observations were done at 400× magnification to assess the possible hyphal damage caused by the yeast, and the hyphae were photographed.

RESULTS

Evaluation of *in vitro* antagonism in solid medium. The experiments showed that *C. melibiosica* inhibited *B. cinerea* growth on potato-dextrose agar and malt agar after 5 days of incubation by 25.9% and 33.3%, respectively. However, this species of yeast did not inhibit or minimally inhibited the growth of *Alternaria* genus on both media. Microscopic observations did not confirm hyphal deformities or other interactions between yeast cells and the mycelia of *B. cinerea* and *A. alternata*. On the other hand, cracked and partially destroyed spores were microscopically observed after co-culture of the studied yeast and *A. radicina* on a Petri dish, which is shown in fig. 1.

Production of antifungal volatile compounds. It was found that growth of fungi on potato-dextrose agar was not inhibited by the action of the yeast growing separately on the other part of the Petri dish. No volatile compounds were produced by *C. melibiosica* 2515 on this medium. However, a reduction in the growth of *A. radicina* (28.5%) and *B. cinerea* (60%) was observed during their culture in the presence of the yeast on malt agar, so it is possible that some volatile compounds were produced by *C. melibiosica* growing on this medium.

Production of hydrolytic enzymes. The enzymatic assays proved the ability of the investigated yeasts to produce the hydrolytic enzymes β-glucanase and chitinase, and this phenomenon depended on the source of carbon in the medium, which could be an

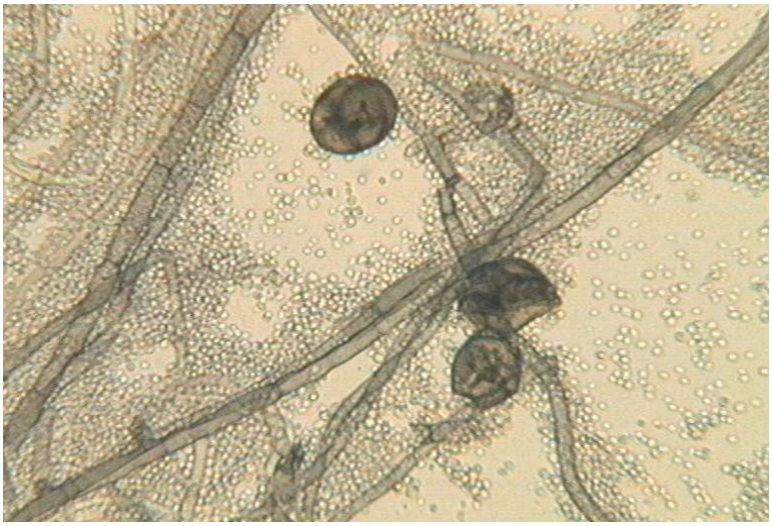

Fig. 1. *A. radicina* conidia and mycelium in co-culture with *C. melibiosica* in solid medium (magnification 300×)

Ryc. 1. Konidia i grzybnia *A. radicina* we wspólnej hodowli z *C. melibiosica* w podłożu stałym (powiększenie 300×)

Table 1. Average activity of β-glucanase and chitinase and specific activity of *C. melibiosica* depending on carbon source in the medium (inducer)

Tabela 1. Średnia aktywność β-glukanazy i chitynazy oraz aktywność specyficzna *C. melibiosica* w zależności od źródła węgla w podłożu (induktora)

Inducer in the medium Induktor w podłożu	Activity of β-glucanase Aktywność β-glukanazy U · cm^{-3}	Specific activity of β-glucanase U · protein mg^{-1} Specyficzna aktywność β-glukanazy U · mg białka^{-1}	Activity of chitinase Aktywność chitynazy U · cm^{-3}	Specific activity of chitinase U · protein mg^{-1} Specyficzna aktywność chitynazy U · mg białka^{-1}
Glucose – glukoza	198.63	1023.89	42.31	218.09
Chitin – chityna	232.86	5821.5	43.7	1092.5
Dried mycelium of *B. cinerea* Wysuszona grzybnia *B. cinerea*	92.2	1084.7	27.94	328.76
Dried mycelium of *A. radicina* Wysuszona grzybnia *A. radicina*	34.0	409.63	20.74	249.8
Dried mycelium of *A. alternata* Wysuszona grzybnia *A. alternata*	70.0	843.4	30.0	361.4

Table 2. Biocontrol efficacy of *C. melibiosica* in reducing lesions caused by *B. cinerea*, *A. radicina* and *A. alternata* on carrot roots

Tabela 2. Skuteczność biokontroli *C. melibiosica* w ograniczaniu zmian powodowanych przez *B. cinerea*, *A. radicina* i *A. alternata* na korzeniach marchwi

Fungus species Gatunek grzyba	Experimental combination Kombinacja doświadczenia	Mean diameter of necrosis, mm Średnia średnica nekrozy, mm			
		5 days 5 dni	10 days 10 dni	15 days 15 dni	15 days # 15 dni
Botrytis cinerea	Control – kontrola	13.5a*	41.2a*	45.3a*	45.3a*
	C. melibiosica	8.7b	28.1b	37.1b	27.4b
LSD (0.05) – NIR (0,05)		1.81	2.19	1.80	1.95
Alternaria radicina	Control – kontrola	10.3a*	22.1a*	46.7a*	46.7a*
	C. melibiosica	6.4b	17.7b	35.9b	23.8b
LSD (0.05) – NIR (0,05)		1.09	1.39	1.96	1.88
Alternaria alternata	Control – kontrola	3.6a*	3.6a*	3.7a*	3.7a*
	C. melibiosica	3.9a	4a	4.3a	3.2a
LSD (0.05) – NIR (0,05)		0.94	0.94	0.83	0.82

#Results after a second application of the yeast suspension after 10 days of incubation

#Wyniki po powtórnej aplikacji zawiesiny drożdży po 10 dniach inkubacji

*values designated with the same letters (a,b ..) within columns do not significantly differ at 5% error (Tukey's test)

* wartości oznaczone tą samą literą (a,b..) w kolumnach nie różnią się istotnie na poziomie istotności 5% (test Tukeya)

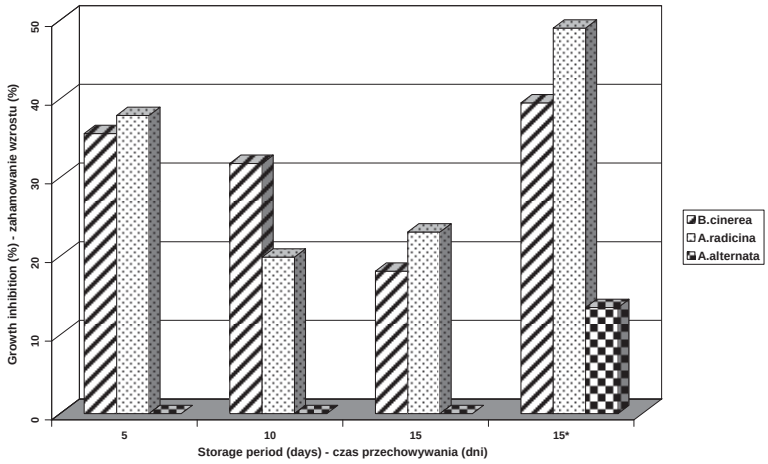

Fig. 2. Effect of *C. melibiosica* on the inhibition of diseases development caused by *B. cinerea*, *A.radicina* and *A. alternata* on carrot (%). *Results after a second application of the yeast suspension after 10 days of incubation

Ryc. 2. Wpływ *C. melibiosica* na hamowanie rozwoju chorób powodowanych przez *B. cinerea*, *A. radicina* i *A. alternata* na marchwi (%). *Wyniki po powtórnej aplikacji zawiesiny drożdży po 10 dniach inkubacji

inducer of enzyme production or not. In this experiment, glucose, chitin or dried mycelium of the test fungi were added separately to the medium, and the data on enzymatic activities and specific activities of β-glucanase and chitinase obtained after 5 days of yeast incubation are presented in Table 1. The activities of β-glucanase were higher than chitinase activities and ranged between 34.0–232.86 U · cm^{-3}, while chitinase activities were at a level of 20.74–43.7 U · cm^{-3}. Specific activities, dependent on protein concentration, were in the range of 409.63–5821.5 and 218.09–1092.5 U · mg^{-1} of protein. It was shown that the best inducer of production of hydrolytic enzymes by the investigated yeast strain was chitin.

Fig. 3. The lesions on carrot roots infected with *B. cinerea, A. alternata* and *A. radicina*. Top – roots infected with fungi, bottom – roots infected with fungi and yeast *C. melibiosica*

Ryc. 3. Zmiany na korzeniach marchwi zakażonej *B. cinerea, A. alternata* i *A. radicina*. Góra – korzenie zainfekowane grzybami, dół – korzenie zakażone grzybami i drożdżami *C. melibiosica*

Evaluation of biological control of fungi by *C. melibiosica* on carrot roots. The *in vivo* experiment was carried out to check the effect of the antagonistic yeast against the phytopatogenic fungi which cause diseases of plants, especially vegetables. Application of the yeast to carrot before inoculation with the investigated fungi reduced their growth and the progress of the disease. The effectiveness of the yeast depended on the fungus species, and the strongest lesion inhibition was observed during the growth of *B. cinerea* and *A. radicina*, whereas *A. alternata* was the most resistant to the antagonistic action of *C. melibiosica* (tab. 2, fig. 2). It was shown that better results were obtained when the yeasts suspension was applied for a second time after 10 days of incubation. Then, the reduction of pathogen growth was higher and ranged from 13.5% for *A. alternata* to 25.9% for *A. radicina*, which resulted in a 49% growth inhibition in comparison with the control combination (fig. 2). The lesions on carrot roots infected by the fungi and the antagonistic yeasts are shown in fig. 3.

DISCUSSION

The results obtained in the *in vitro* and *in vivo* experiments indicate that the yeast *C. melibiosica* has potential as an antagonist in biological control of *B. cinerea*, responsible for diseases of many fruits and vegetables, and *A. radicina*, causing black rot of carrot. Only *A. alternata* appeared to be resistant to the activity of this yeast in experiments on Petri dishes and on carrot roots. On the other hand, it only caused very small lesions on the roots. The studied yeast, when applied twice, reduced the development of the disease on carrot roots by as much as about 39–49% over two weeks. When one dose of the yeast suspension was applied, the reduction of lesions on carrot roots was at a level of about 20%. A study investigating the effectiveness of *C. melibiosica* in controlling *B. cinerea* on apples showed that, after two weeks of incubation, the yeast reduced necrosis by 25% when the yeast suspension was applied only once [Wagner et al. 2006]. Our experiment showed that volatile compounds could be responsible for the reduction of the rot caused by *B. cinerea* and *A. radicina* .

It was shown in this study that the yeast *C. melibiosica* is a producer of the lytic enzymes β-glucanase and chitinase and that the activities of these enzymes depend on the carbon source in the culture medium. Chitin was the best inducer of enzymes production. This mode of antagonistic action of yeasts has been investigated by several scientists. Segal et al. [2002] reported exo-glucanase activity of *C. oleophila* during cultivation on YEPD medium at a level of about 40 units/ml after 54 h of incubation. Masih and Paul [2002] investigated the biocontrol potential of the yeast *Pichia membranifaciens* against *B. cinerea*. They showed that this yeast produced very low activities of exo- and endo-β-1,3-glucanase when grown in YNB medium with glucose as a sole carbon source, but after medium supplementation with laminarin or a *B. cinerea* cell wall preparation, the level of the enzymes was higher and reached a maximum after 24 h; the specific activities of exoglucanase were 0.002 and 0.01 μg of glucose · mg^{-1} of protein, respectively. Similarly, in a study by Wisniewski et al. [1991] it was reported that *P. guilliermondii* and *P. anomala* produced high levels of lytic enzymes during cultivation on a medium supplemented with fungal cell walls. In turn, Saravanakumar et

al. [2009] investigated chitinase activity of the yeast *M. pulcherrima* and *Rhodotorula* sp., which restricted grey mould lesions caused by *B. cinerea* on apples. They observed that *M. pulcherrima* was a better producer of this enzyme than *Rhodotorula* sp., and the best results were obtained on YPD medium or apple juice extract with an addition of *B. cinerea* cell wall preparation. Maximum chitinase activity was recorded 6 days after inoculation. *M. pulcherrima* was also more efficient than *Rhodotorula* sp. in reducing fungal growth on apples in an *in vivo* experiment [Saravanakumar et al. 2009]. Moreover, Saligkarias et al. [2002] reported secretion of detectable amounts of β-1,3-exoglucanase and chitinase by *C. guilliermondii* and *C. oleophila* grown in different carbon sources. These reports suggest that secretion of lytic enzymes by antagonistic yeasts may be one of their essential modes of action against pathogenic fungi. The results obtained in this work confirm these findings, especially that some partially destroyed spores of *A. radicina* were microscopically observed after co-culture with yeasts *in vitro* and *in vivo*.

CONCLUSIONS

Candida melibiosica can be used as a biocontrol agent against postharvest diseases of carrot caused by *B. cinerea* and *A. radicina*. The probable mode of action of this yeast is the ability to produce the lytic enzymes that damage the cell wall of fungi and cause the damage of hyphae and spores and then restrict the growth of mycelia and occurrence of rot lesions on carrot roots.

REFERENCES

Benbow J.M., Sugar D., 1999. Fruit surface colonization and biological control of postharvest diseases of pear by preharvest yeast applications. Plant Dis., 83, 839–844.

Bradford M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72, 248–254.

El-Mehalawy A.A., Hassainen N.M., Khater H.M., Karam El-Din E.A., Youssef Y.A., 2004. Influence of maize root colonization by the rhizosphere actinomycetes and yeast fungi on plant growth and on the biological control of late wilt disease. Int. J. Agric. Biol., 6, 599–605.

El-Mehalawy A.A., 2004. The rizosphere yeast fungi as biocontrol agents for wilt disease of kidney bean caused by *Fusarium oxysporum*. Int. J. Agric. Biol., 6, 310–316.

El-Tarabily K.A., 2004. Suppression of *Rhizoctonia solani* diseases of sugar beet by antagonistic and plant growth-promoting yeasts. J. Appl. Microbiol., 96, 69–75.

Ferrari A., Sicher C., Prodorutti D., Pertot I., 2007. Potential new applications of Shemer, a *Metschnikowia fructicola* based product, in post-harvest soft fruit rots control. Bull. OILB/SROP, 30, 43–46.

Filonow A.B., Vishniac H.S., Andrson J.A., Janisiewicz W.J., 1996. Biological control of *Botrytis cinerea* in apple by yeasts from various habitats and their putative mechanisms of antagonism. Biol. Control, 7, 212–220.

Grebenisan I., Cornea P., Mateescu R., Cimpeanu C., Olteanu V., Campenu G.H., Stefan L.A., Oancea F., Lupu C., 2008, *Metschnikowia pulcherrima*, a new yeast with potential for biocontrol of postharvest fruit rots. Acta Hortic., 767, 355–360.

Janisiewicz W.J., Tworkoski T.J., Kurtzman C.P., 2001. Biocontrol potential of *Metchnikowia pulcherrima* strains against blue mold of apple. Biol. Control, 91, 1098–1108.

Leibinger W., Breuker B., Hahn M., Mendgen K., 1997. Control of postharvest pathogens and colonization of the apple surface by antagonistic microorganisms in the field. Phytopathol., 87, 1103–1110.

Lima G., De Curtis F., Castoria R., De Cicco V., 2003. Integrated control of apple postharvest pathogens and survival of biocontrol yeasts in semi-commercial conditions. Europ. J. Plant Pathol., 109, 341–349.

Lima G., Ippolito A., Nigro F., Salerno M., 1997. Effectiveness of *Aureobasidium pullulans* and *Candida oleophila* against postharvest strawberry rots. Postharvest Biol. Technol., 10, 169–178.

Masih E.I., Paul B., 2002. Secretion of β-1,3-glucanases by the yeast *Pichia membranifaciens* and its possible role in the biocontrol of *Botrytis cinerea* causing grey mold disease of the grapevine. Curr. Microbiol., 44, 391–395.

Miller G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal. Chem., 31, 426–428.

Rosa M.M., Tauk-Tornisielo S.M., Rampazzo P.E., Ceccato-Antonini S.R., 2010. Evaluation of the biological control by the yeast *Torulaspora globosa* against *Colletotrichum sublineolum* in sorghum. World J. Microbiol. Biotechnol., 26, 1491–1502.

Saligkarias I.D., Gravanis F.T., Epton H.A.S., 2002. Biological control of *Botrytis cinerea* on tomato plants by the use of epiphytic yeasts *Candida guilliermondii* strains 101 and US 7 and *Candida oleophila* strain I-182: II a study on mode of action. Biolog. Contr., 25, 151–161.

Saravanakumar D., Spadaro D., Garibaldi A., Gullino M.L., 2009. Detection of enzymatic activity and partial sequence of a chitinase gene in *Metschnikowia pulcherrima* strain MACH1 used as post-harvest biocontrol agent. Eur. J. Plant Pathol., 123, 183–193.

Schena I., Ippolito A., Zahavi T., Cohen I., Droby S., 2000. Molecular approaches to assist the screening and monitoring of postharvest biocontrol yeasts. Europ. J. Plant Pathol., 106, 681–691.

Segal E., Yehuda H., Droby S., Wisniewski M., Goldway M., 2002. Cloning and analysis of CoEXG1, a secreted 1,3-β-glucanase of the yeast biocontrol agent *Candida oleophila*. Yeast, 19, 1171–1182.

Teixidó N., Viñas I., Usall J., Magan N., 1998. Control of blue mould of apples by preharvest application of *Candida sake* grown in media with different water activity. Phytopatol., 88, 960–964.

Wagner A., Kordowska-Wiater M., Hetman B., 2006. Wpływ wybranych szczepów drożdży na rozwój szarej pleśni na owocach jabłoni. Prog. in Plant Prot., 46, 625–628.

Wisniewski M.E., Biles C., Droby S., Mclaughlin R., Wilson C.L., Chalutz E., 1991. Mode of action of the postharvest biocontrol yeast, *Pichia guilliermondii*. I. Characterization of attachment to *Botrytis cinerea*. Physiol. Mol. Plant Pathol., 39, 245–258.

Zhang D., Spadaro D., Garibaldi A., Gullino M.L., 2010. Selection and evaluation of new antagonists for their efficacy against postharvest brown rot of peaches. Postharv. Biol. Technol., 55, 174–181.

SKUTECZNOŚĆ *Candida melibiosica* W KONTROLI POZBIOROWYCH CHORÓB GRZYBOWYCH MARCHWI (*Daucus carota* L.)

Streszczenie Kontrola biologiczna z użyciem antagonistycznych drożdży jest jedną z najbardziej obiecujących metod ochrony pozbiorowej owoców i warzyw. Celem badań była ocena potencjału drożdży *Candida melibiosica* do biokontroli wzrostu *Botrytis cinerea*, *Alternaria alternata* i *A. radicina*, grzybów chorobotwórczych dla korzeni marchwi i wyjaśnienie możliwego sposobu działania tego antagonistycznego mikroorganizmu. Badano zdolność drożdży do hamowania wzrostu grzybów *in vitro*, produkcji związków lotnych i enzymów litycznych (β-glukanazy i chitynazy) oraz ochrony zakażonych korzeni marchwi. Gatunek *C. melibiosica* ograniczał wzrost *B. cinerea* na podłożu PDA o 25,9%, a na agarze brzeczkowym o 33,3%, natomiast słabo ograniczał wzrost *A. radicina* i w ogóle nie oddziaływał na *A. alternata*. Drożdże na agarze brzeczkowym produkowały związki lotne działające przeciwko *B. cinerea* i *A. radicina*. Wytwarzały β-glukanazę i chitynazę z aktywnościami wynoszącymi odpowiednio 34,0–232,86 U · cm^{-3} i 20,74–43,7 U · cm^{-3}. Zastosowanie drożdży na korzeniach marchwi przed inokulacją grzybami zredukowało na nich rozwój choroby podczas przechowywania.

Słowa kluczowe: biokontrola, drożdże antagonistyczne, grzyby fitopatogenne

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