BENEFICIAL YEASTS WITH BIOCONTROL POTENTIAL AGAINST SWEET POTATO STORAGE PATHOGENS

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Abstract. The sweet potato is less known in Romania. The highly different storage conditions between sweet potatoes (*Ipomoea batatas*, *Convolvulaceae* family) and potatoes (*Solanum tuberosum*, *Solanaceae* family) make it difficult to maintain the quality of the tubers during storage. Sweet potatoes are sensitive to relatively low temperatures (<12°C), which can produce chilling injuries. The wounds created by the cold can lead to over-infection with storage pathogenic fungi. In order to reduce the potential losses caused by storage fungal infections, we recommend the use of beneficial yeasts, with biocontrol activity. These yeasts were selected from the natural epiphytic microbiota and do not present toxicity risks to consumers. The selected isolates have shown inhibitory activity against the main storage pathogenic fungi of sweet potato: *Botrytis cinerea*, *Fusarium oxysporum*, *Penicillium* sp., *Rhizopus stolonifer* and *Sclerotium bataticola*.

Keywords: beneficial yeasts, *Metschnikowia* sp., sweet potato, biocontrol of storage fungal pathogens.

Rezumat. Drojdii benefice cu potențial de biocontrol asupra patogenilor de depozit ai cartofului dulce. În România, cartoful dulce este mai puțin cunoscut, iar condițiile foarte diferite de stocare dintre cartoful dulce (*Ipomoea batatas*, fam. *Convolvulaceae*) și cartof (*Solanum tuberosum*, fam. *Solanaceae*) pun în dificultate menținerea calității tuberculilor pe parcursul depozitării. Cartofii dulci sunt sensibili la temperaturi relativ scăzute (sub 12° C). Rănile create de frig pot duce la suprainfecții cu fungi patogeni de depozit. Pentru a reduce potențialele pierderi cauzate de infecțiile patogene propunem utilizarea unor drojdii benefice, cu activitate de control biologic. Aceste microorganisme au fost selecționate din microbiota naturală epifită și nu prezintă riscuri de toxicitate pentru consumatori. Izolatele selecționate au dovedit activitate inhibitoare față de principalii fungi patogeni de depozit ai cartofului dulce: *Botrytis cinerea*, *Fusarium oxysporum*, *Penicillium* sp., *Rhizopus stolonifer* și *Sclerotium bataticola*.

Cuvinte cheie: drojdii benefice, *Metschnikowia* sp., cartof dulce, biocontrol al fungilor de depozit.

INTRODUCTION

Consumer interest for sweet potato is starting to increase in Romania. At the beginning, this legume was consumed in order to experience new tastes (CIOFU et al., 1987). But currently, due to its beneficial traits, it started to be appreciated, especially as baby food, and in different diets, including for diabetics. The availability of this food on the market is expanding, not only from imports, but also from local productions. Consumption availability throughout the year is closely related to storage possibilities. The storage conditions for sweet potato (*Ipomoea batatas* (L.) Lam., fam. Convolvulaceae), differ greatly from those of potato (*Solanum tuberosum* L., fam. Solanaceae). Tubers should be cured before storing. This stage ensures the healing of wounds and improves flavour. The curing process should take place under warm, moist conditions, at 26.5-32°C and 85-90% relative humidity, for 5 to 10 days. If these parameters are not achieved, the curing stage will take at least 2 weeks, at 15-23°C. During storage, sweet potatoes should be kept at 13-15°C, in high relative humidity, and proper ventilation. In order to prevent pathogenic infections, it is recommended to maintain the required storage conditions for sweet potato tubers and apply the general phytosanitary measurements used in deposits (AMES et al., 1996).

Sweet potatoes tubers are sensitive to chill and frost. Temperatures lower than 12°C can produce chilling injuries. The opened wounds can be easily infected with storage pathogens, which will rapidly decrease the quality and quantity of commercial tubers (BOIU-SICUIA et al., 2018). Therefore, an intact rhizoderm is important for maintaining tuber health during storage (AMES et al., 1996). Natural and added biological barriers can also contribute to tuber protection. In the case of some fruits and vegetables, different waxes and artificially added bio-layers are used to cover the skin or packaging of the stored products (PAWLIKOWSKA et al., 2019) in order to prolong the storage period, maintain their quality and prevent microbial contamination.

Studies have showed that many epiphyte yeasts found on fruits are natural enemies of phytopathogenic fungi (CSUTAK et al., 2013; PAWLIKOWSKA et al., 2019). Some of these epiphyte yeasts, like *Saccharomyces cerevisiae*, *Metschnokowia pulcherrima*, *M. fructicola*, are safe for humans and animal health, and sometime are used in the agro-alimentary industry. The status of *Generally Recognised as Safe* (GRAS) was first introduced by the United States Food and Drug Administration (FDA, 2019). The FDA listed the microorganisms and microbial derived ingredients used in food (FDA, 2001), which mentions many epiphyte and endophyte microorganisms. The European Food Safety Authority (EFSA) introduced in 2007 the status of *Qualified Presumption of Safety* (QPS) for those microorganisms used in food and feed production. Moreover, the QPS is also applied for some plant protection microorganisms (BOURDICHON et al., 2012).

In this paper we highlight the possibility of using yeast-based bioproducts to inhibit the development of pathogens in the warehouse. The ability of these yeasts to inhibit fungal growth is conferring biological protection against storage pathogens, such as *Botrytis cinerea*, *Fusarium oxysporum*, *Penicillium* spp., *Rhizopus stolonifer* and

Sclerotium bataticola. These yeasts are generally regarded as safe; they are not a menace for human and animal health if ingested, and have a good shelf live for long time periods.

MATERIAL AND METHODS

Epiphyte yeasts isolation. For the isolation of new epiphyte microorganisms, healthy grapes of Chasselas D'ore variety, organically produced in a family plantation, were used. The ripe grapes were immersed in sterile distilled water supplemented with 2-3 drops of Tween 80. The sample was kept under gentle shaking for 30 minutes. The suspension was collected and samples of 100µl were plated on sucrose-agar medium. After 48 hours of incubation at 28°C the colonies with characteristic yeast morphology were selected and purified on YPG medium, containing 1% yeast extract, 2% peptone, 2% glucose, and 2% agar.

Beneficial yeasts. Among the newly isolated yeasts we also used several other strains from the microbial collection of the Faculty of Biotechnology, from the UASVM of Bucharest (Table 1).

Table 1. Beneficial yeast strains.

No.	Strain	Source
1	*Metschnikowia pulcherrima* MUCL 29874	Microbial collection of the Faculty of Biotechnology University of Agronomical Sciences and Veterinary Medicine Bucharest, Romania (USAMV)
2	*Metschnikowia* sp. SG1	
3	*Metschnikowia* sp. SG2	
4	*Metschnikowia* sp. CPM1	
5	*Saccharomyces cerevisiae* SMR4	
6	*Saccharomyces cerevisiae* x208	
7	*Metschnikowia pulcherrima* NCAIM Y 01466	National Collection of Agricultural and Industrial Microorganisms Szent István University, Faculty of Food Science Budapest, Hungary
8	*Yeast strain* DjIBNA	National Research-Development Institute for Biology and Animal Nutrition, Bucharest, Romania
9	*Saccharomyces cerevisiae* Dr.Oetker	Marketed baker's yeast

Yeasts characterisation. The newly isolated yeasts were macroscopically characterized for colony morphology. All strains were microscopic analysed in Lugol solution.

Purified strains were subjected to different microbiologic tests in order to evaluate their potential for biological control. The production of lytic enzymes was analysed to detect chitinase, protease and lipase activity. Red pigment production was also tested in the presence of iron enriched media.

Chitinase activity was evaluated on specific culture media based on colloidal chitin. This medium contained 4.5 g/L colloidal chitin, 3.0 g/L $MgSO_4$, 3.0 g/L $(NH_4)_2SO_4$, 2.0 g/L K_2HPO_4, 1.0 g/L citric acid, 0.15 g/L purple bromocresol; 200 µl/L Tween 80, 20g/L agar, at pH = 4.7±0.2. Yeasts were spotted on the surface of the medium and incubated at 28°C. The positive reactions were those of purple colour, associated with the pH change of the growing substrate from acid pH (of yellow colour), to basic pH (purple), due to the enzymatic breakdown of chitin into N-acetyl glucosamine.

For protease activity assay, skim milk agar medium was used. The skim milk and water-agar solution were autoclaved separately and then aseptically mixed and plated. The yeasts were spotted on the solidified medium and incubated at 28°C. The positive reactions were revealed by a clear halo around colonies, due to the protease activity and casein hydrolysis.

Lipase activity was evaluated as mentioned by SICUIA et al., (2015). For these tests we used modified Schoofs medium, containing 10.0 g/L peptone, 5.0 g/L sodium chloride, 0.5 g/L calcium chloride, 1 ml/L Tween 80 and 18.0 g/L agar. Lipolytic enzymes production was correlated with Tween 80 hydrolysis and the appearance of white precipitated calcium soap around the colonies.

Pigment production was tested on PDA medium supplemented with 100µg/ml $FeCl_3$.

Storage pathogens.

Three of the tested pathogens were previously isolated from sweet potato produced in Romania. Tubers revealing storage rots were selected and brought into the plant protection laboratory. *Botrytis cinerea* and *Rhizopus stolonifer* were isolated from tubers of KSP1 cultivar, naturally contaminated with grey mould, or decayed by soft rot. *Penicillium* spp. were isolated from stored tubers of KSC1 cultivar contaminated with blue mould (BOIU-SICUIA et al., 2016). *Fusarium oxysporum* was isolated from Hayanmi variety of sweet potato (BOIU-SICUIA et al., 2017). *Sclerotium bataticola* (sin. *Macrophomina phaseolina*) was provided from the RDIPP collection.

Although *Aspergillus* species are not common pathogens of stored sweet potatoes, these fungi are commonly present on storage facilities (ACHAGLINKAME et al., 2017). Therefore, strains of *A. flavus* (GE2, T11, GIS 2 and GIS 9), *A. ochraceus* O1 and *A. niger* (AN1 and MA2) were also included in our tests.

All fungal pathogens were grown on PDA for 5 to 10 days, at 27°C, in order to refresh the cultures (Fig. 1).

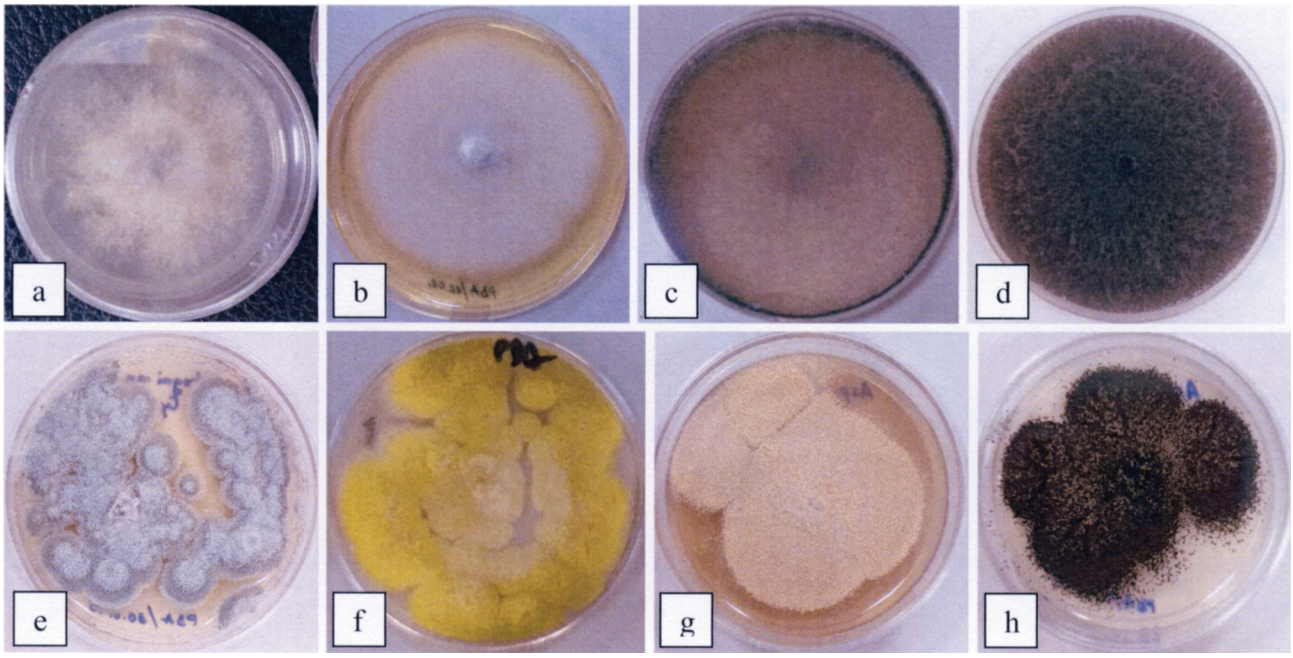

Figure 1. Storage fungal pathogens grown in pure cultures on PDA medium.
a) *Botrytis cinerea* b) *Fusarium oxysporum* c) *Rhizopus stolonifer* d) *Sclerotium bataticola*
e) *Penicillium* sp. f) *Aspergillus flavus* g) *A. ochraceus* h) *A. niger*.

***In vitro* biocontrol assays**. Two direct confrontation methods were used to evaluate the antagonistic potential of the selected yeasts. The double culture technique was the first to be used. This method is appropriate for fast colonizing pathogens, such as *Botrytis cinerea*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Sclerotium bataticola*. In the case of *Penicillium* spp. and *Aspergillus* spp. we used the double layer technique.

The double culture technique was performed as in Fig. 2a on BDA (Batata-Dextrose-Agar) medium. This medium was similarly prepared as PDA, but sweet potato infusion was used instead of potato. The yeasts were the first ones inoculated on the substrate, four strains per plate. The fungi were calibrated at 5 mm diameter and then placed in the center of the plates. Controls were also prepared, for each of the tested fungi. The test was repeated twice each time in triplicate. Plates were incubated at 28°C and periodically analysed up to 14 days.

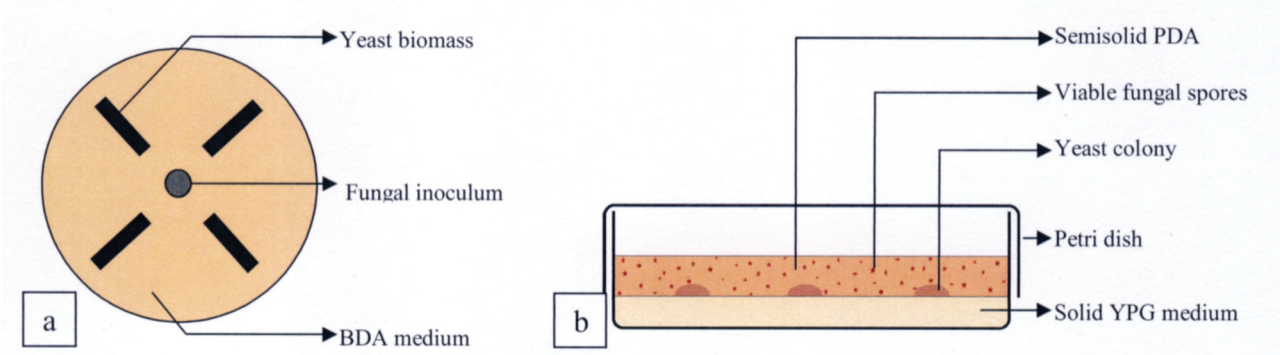

Figure 2. Co-inoculation model for antagonism assay.
a) double culture technique b) double layer technique.

The double layer technique was performed in two steps. At first, one layer of YPG medium was inoculated in spots with fresh yeast cultures. After two days of incubation the plates were covered with a second layer of semi-solid PDA containing fungal spores of *Penicillium* spp. and *Aspergillus* spp., respectively (Fig. 2b).

RESULTS AND DISCUSSION

A number of eight new epiphytes were isolated from the Chasselas D'ore grapes (Fig. 3a). But only five were proven to be yeasts. The newly isolated strains were named sa1, sa5, sa6, sa7 and sa8. Their selection was made based on two criteria: typical yeast morphology and normal-fast growth under *in vitro* conditions. The selected yeasts showed rapid, abundant growth on YPG medium. The pure cultures revealed circular, creamy colonies (Fig. 3b). All strains had ellipsoidal to globular shape cells, depending on their age. In the mature cells, a drop with high refractive index could be observed (Fig. 3c).

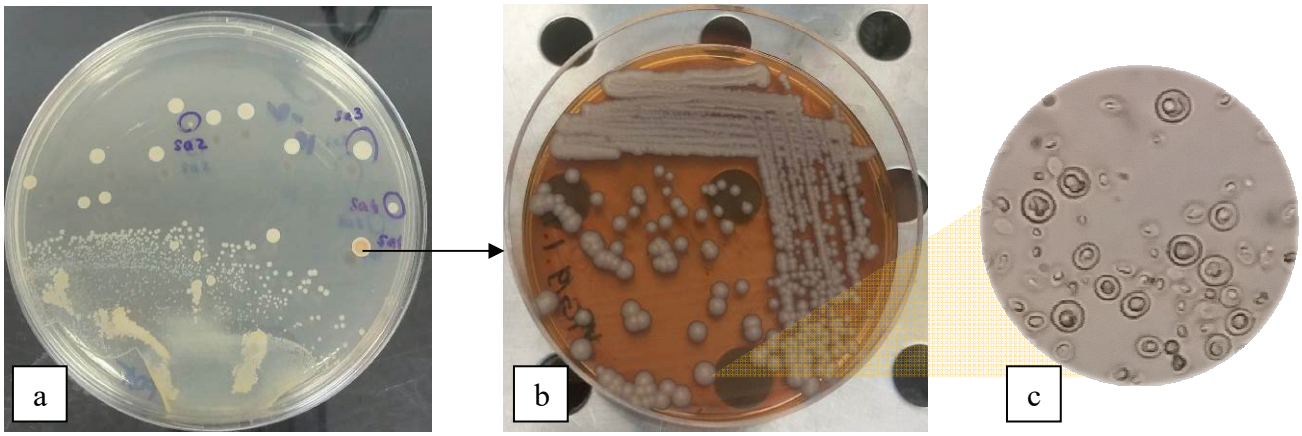

Figure 3. Newly isolated yeasts: a) Freshly isolated epiphyte microorganisms, on glucose-agar medium; b) Pure yeast culture of sa1 strain, on YPG; c) yeasts cells under 100X magnification.

Due to the similarities between cells morphology of the newly isolated strains and those of the *M. pulcherrima* references, the presumption that our newly isolated yeasts could belong to *Metschnikowia* taxon is not excluded.

The *M. pulcherrima* MUCL 29874 reference strain, *Metschnikowia* sp. SG2 and *Metschnikowia* sp. CPM1 produced red-brownish pigmented colonies, associated to pulcherrimin synthesis (Fig 4a). Pulcherrimin synthesis is mentioned not only in *M. pulcherrima*, but also in other related yeast species such as: *M. andauensis*, *M. fructicola*, *M. shanxiensis*, *M. sinensis*, and *M. ziziphicola* (SIPICZKI et al., 2018). Moreover, when sa1, sa5, sa6, sa7, and sa8 isolates were grown on PDA supplemented with $FeCl_3$, they also produced red-brownish pigmented colonies, as for pulcherrimin synthesis (Fig. 4b).

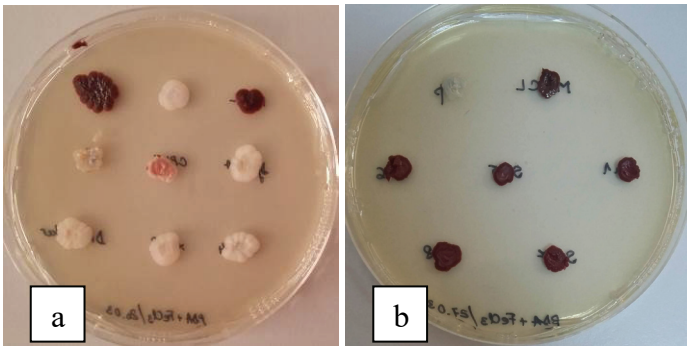

Figure 4. Pulcherrimin production by yeasts cultures grown on iron supplemented medium
- positive strains reveal a pink to red-brownish pigmentation -
a. Pigmented growth of *Metschnikowia* sp. MUCL29874, SG2 and CPM1 producing pulcherrimin, compared to non-producing strains;
b. Pulcherrimin producing strains, MUCL29874, sa1, sa5, sa6, sa7, and sa8, with red-brownish pigmented colonies.

Pulcherrimin production is stimulated by the presence of $FeCl_3$ in the culture medium (SIPICZKI, 2006). Sipiczki (2006) consider that pulcherrimin producing *Metschnikowia* inhibit the growth of other microbes due to their capacity to immobilize the iron from the growing substrate. As iron is essential for microbial growth, Fe sequestration by non-pathogenic microorganisms can be exploited in biological control for postharvest protection against damaging storage fungi. This iron depletion mechanism has similar advantages as siderophores (ferric ion-specific chelators) that reduce the iron availability in the environment for competitive microorganisms, including phytopatogenic fungi (SARAVANAKUMAR et al., 2008; FERRAZ et al., 2016).

Lytic enzymes, such as chitinase, proteases, and lipases can contribute to the biological control of deleterious fungal pathogens. Such enzymes can digest specific constituents of the cell wall and cell membrane of phytopathogenic fungi. Therefore, beneficial microorganisms releasing these enzymes can be used to control phytopathogenic growth. Such enzymatic activity is also described in yeasts, as additional mechanisms involved in their biological control activity (LIU et al., 2013; BANANI et al., 2015; PRETSCHER et al., 2018).

The enzymatic activity of the studied yeast strains was evaluated visually and biometrically on plate assays. The measurements were also used to calculate the hydrolysis index, using the following formula:

$$HI = \frac{CD + HD}{CD}$$

where: HI = hydrolysis index; CD = microbial colony diameter; HD = halo diameter.

One of the mechanisms involved in biological control is chitinase production. As chitin is the main polysaccharide present in the structure of the fungal cell wall, beneficial microorganisms expressing chitinase enzymatic activity contribute to

chitin degradation, fungal cell lysis and biocontrol of pathogenic fungi. Among the studied yeasts only 6 strains revealed chitinolytic activity. *Metschnikowia* spp. SG1 and SG2 were able to degrade colloidal chitin very fast, and after the first 3 days of growth, they revealed a hydrolysis index of 2.75 and 3 (Table 2). The other positive strains (MUCL 29874, CPM1, sa5, sa7) activated their chitinolytic activity much more slowly, expressing a lower enzymatic action. Chitin degradation was revealed by the colour change of the medium, from yellow to purple, as a result of substrate pH modification, from acidic to alkaline (Fig. 5).

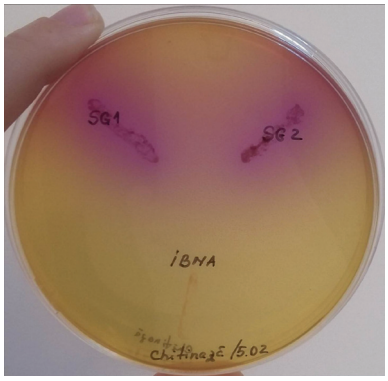

Figure 5. *Metschnikowia* spp. SG1 and SG2 degrading colloidal chitin.

Table 2. The enzymatic activity of yeasts.

Yeast strains	Chitinase				Lipase		Protease	
	Reaction	HI	Reaction	HI	Reaction & Halo	HI	Reaction & Halo	HI
	after 3 days		after 7 days		after 7 days		after 7 days	
MUCL 29874	—	0	+	1.1	—	0	+ 2 mm	1.3
NCAIM Y 01466	—	0	—	0	+ 8 mm	3,57	N.A.	N.A.
SG1	+	2.75	+ +	4.29	+ 4.5 mm	2,43	+ 2 mm	1.43
SG2	+	3	+ +	5	—	0	+ 2 mm	1.38
CPM1	—	0	+	1.1	—	0	N.A.	N.A.
IBNA	—	0	—	0	+ 2 mm	1,25	N.A.	N.A.
SMR4	—	0	—	0	+ 1 mm	1,1	N.A.	N.A.
x208	—	0	—	0	—	0	N.A.	N.A.
Dr.Oetker	—	0	—	0	—	0	N.A.	N.A.
sa1	—	0	—	0	N.A.	N.A.	+ 2 mm	1.43
sa5	—	0	±	1	N.A.	N.A.	+ 2 mm	1,5
sa6	—	0	—	0	N.A.	N.A.	+ 2 mm	1.38
sa7	—	0	±	1	N.A.	N.A.	+ 2 mm	1.62
sa8	—	0	—	0	N.A.	N.A.	+ 2 mm	1.46
Legend: HI = hydrolysis index; N.A. = not available								

Chitin degrading activity has been studied in many biocontrol yeasts, such as: *Candida*, *Debaryomyces*, *Metschnikowia*, *Pichia*, *Saccharomyces*, *Tilletiopsis* and different other genera (URQUHART & PUNJA, 2002; BAR-SHIMON et al., 2004; SARAVANAKUMAR et al., 2009; ZHANG et al., 2011; BANANI et al., 2015; LOPES et al., 2015; PRETSCHER et al., 2018; ZAJC et al., 2019), with various results among the analysed strains.

Screening lipase activity among some of the analysed yeasts revealed only four positive strains: NCAIM Y 01466, SG1, CPM1, SMR4 (Table 2). Due to lipase activity, the triglycerides from Tween 80 were hydrolysed in glycerol and fatty acids, which formed a white precipitate in the presence of calcium salts (Fig. 6). Lipase activity is not that common in *M. pulcherrima* strains; however they could produce esterase-lipases (PAWLIKOWSKA & KRĘGIEL, 2017).

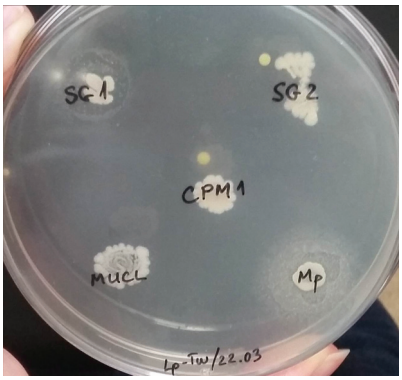

Figure 6. Lipase activity on Tween 80.

FREIMOSER et al. (2019) listed several studies highlighting the role of lipolytic enzymes in plant diseases and pest biocontrol. Therefore, the lipolytic activity of antagonistic yeasts may also represent a promising trait in warehouses biocontrol applications.

The proteolytic activity was evaluated on slim-milk-agar. After the first 5 days of incubation, all tested strains degraded casein from the substrate, revealing a translucent halo around the colonies, due to the hydrolysis (Fig. 7).

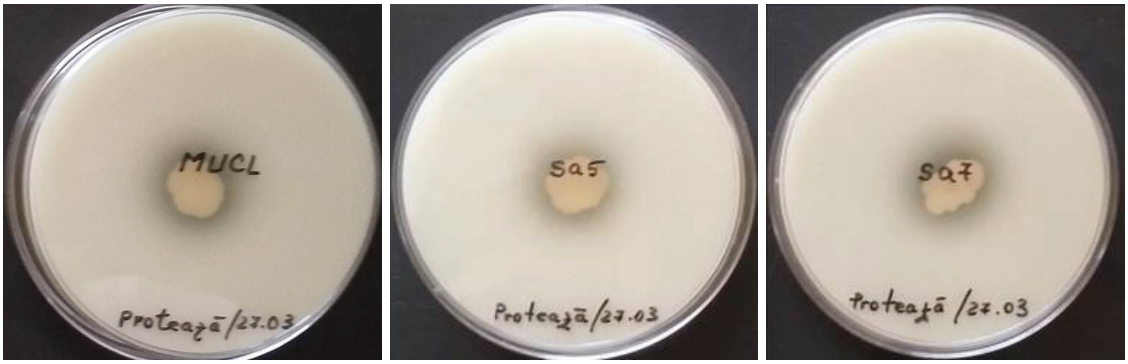

Figure 7. Milk casein hydrolysis by yeasts expressing proteolytic activity.

Protease activity has been reported in different yeasts genera, such as: *Metschnikowia*, *Pichia*, *Saccharomycopsis* and *Wickerhamomyces* (PRETSCHER et al., 2018). The protease activity of yeasts is usually detected in later growth stages (after one week of growth in rich nutrient media), therefore it is supposed to have a minor role in yeast biocontrol activity (BAR-SHIMON et al., 2004). However, alkaline serine protease expressed in biocontrol yeasts reduced spore germination and germ tube length of different storage pathogens such as: *Penicillium expansum*, *Botrytis cinerea*, *Monilia fructicola* and *Alternaria alternata*. Moreover, the inhibitory effect of the biocontrol yeast was proven to be dependent on the Alp5 gene expression and protease concentration (BANANI et al., 2014).

Enzymes degrading cellular components are a common feature in microbial interactions and biocontrol features. Enzymes such as chitinases, glucanases, proteases and lipases are regularly reported and highlighted in antagonistic interaction, fungal growth inhibition and mycoparasitism and biocontrol against pathogenic fungi (FREIMOSER et al., 2019).

The antifungal activity of the studied yeasts was evaluated by means of dual culture and double layer techniques. The first method was used to evaluate the antagonistic effect against: *Botrytis cinerea, Fusarium oxysporum, Rhizopus stolonifer* and *Sclerotium bataticola*. The antifungal activity was visually evaluated, and clear inhibition zones were measured (Table 3).

The newly isolated yeasts were the ones expressing the highest antifungal potential against grey mould. Best results in inhibiting *Botrytis cinerea* were obtained with sa5 and sa7 strains (Fig. 8b).

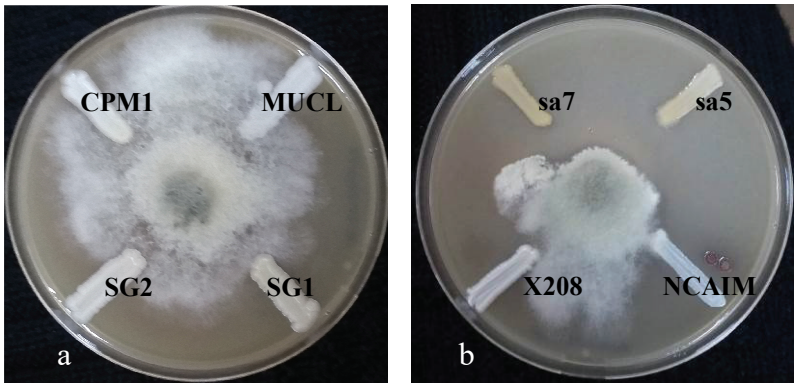

Figure 8. The antagonistic activity of some beneficial yeasts against *Botrytis cinerea*.
- after 7 days on Batata-Dextrose-Agar -
a. low inhibitory activity, b. high antifungal action of sa5 and sa7 yeast strains against grey mould

Table 3. Yeast biocontrol potential revealed by means of the dual culture technique.

Storage fungi →	***Botrytis cinerea***			***Fusarium oxysporum***		***Rhizopus stolonifer***	***Sclerotium bataticola***	
	7 days	10 days	14 days	3 days	7 days	3 days	3 days	7 days
Fungal control	5cm Ø	9cm Ø	9cm Ø	4,8cm Ø	9cm Ø	9cm Ø	9cm Ø grey	9cm Ø black
Yeasts strains	Antifungal activity and clear inhibition zones							
SG1	4 mm	±	±	±	−	−	±	−
SG2	5 mm	±	±	−	−	−	+	±
MUCL 29874	3 mm	−	−	−	−	−	+	±
CPM1	8 mm	+	+	−	−	−	+	±
sa1	8 mm	6.8 mm	6.8 mm	5 mm	3.2 mm	2 mm	1 mm	+
sa5	14 mm	12 mm	12 mm	6 mm	5.8 mm	8 mm	9 mm	5 mm
sa6	10 mm	9.2 mm	9.2 mm	5.6 mm	3.6 mm	2 mm	2 mm	+
sa7	11 mm	9 mm	9 mm	6 mm	5.4 mm	3 mm	2 mm	1 mm
sa8	8.6 mm	8.2 mm	8.2 mm	4.8 mm	2.8 mm	2 mm	1 mm	+
NCAIM Y 01466	5 mm	±	±	−	−	−	−	−
x208	8 mm	1 mm	1 mm	−	−	−	−	−

The newly isolated yeasts inhibit the development of grey rot without requiring direct contact with the pathogen. Clear inhibition zones suggest that yeasts are releasing antifungal compounds that are inhibiting the mycelia to approach them. This aspect is beneficial in terms of biocontrol potential. Yeast strains sa7 and sa5, revealed an inhibition efficacy of 70 to 82.5% against grey mould in the 7th day of interaction, and maintained their efficiency from 75.9 to 79.3% after 2 weeks.

The newly isolated yeasts inhibit also *Fusarium oxysporum*, maintaining clear inhibition zones around their colonies. Best results were obtained with sa5 and sa7 strains (Table 3, Fig. 9).

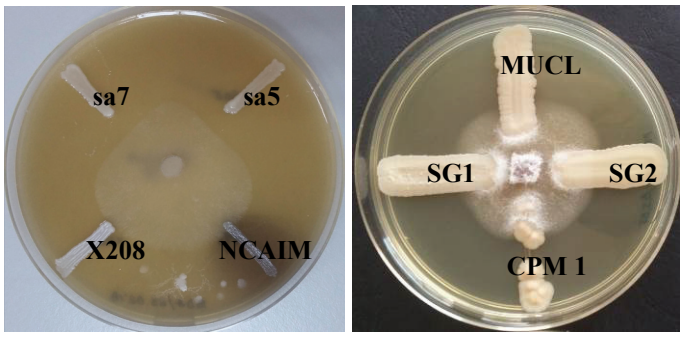

Figure 9. Antifungal activity of some yeast strains against *Fusarium oxysporum*, after 7 days on Batata-Dextrose-Agar (left) and Potato-Dextrose-Agar (right).

Rhizopus stolonifer is a fast growing and aggressive pathogen. Among the tested yeasts, only the newly isolated strains were able to inhibit its growth (Table 3). Best results were obtained with sa5 and sa7 strains (Fig. 10), which maintained their antagonistic activity for more than one week.

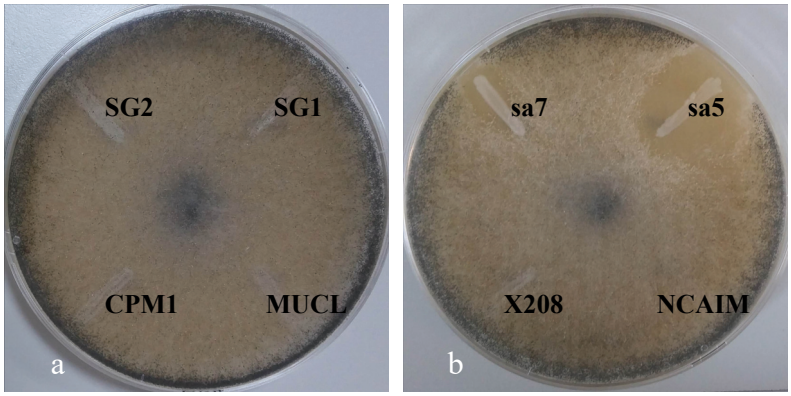

Figure 10. Antifungal evaluation of some beneficial yeasts against *Rhizopus stolonifer*.
a. No antifungal action, b. high antagonistic activity of sa5 and sa7 yeast strains against black mold

The growth of *Sclerotium bataticola* was inhibited by most of the tested yeast strains. The antagonistic effect was higher when using the newly isolated yeasts (Table 3). Best results were obtained with the sa5 strain, followed by sa7 (Fig. 11). These strains maintained their biocontrol activity for more than 14 days of incubation.

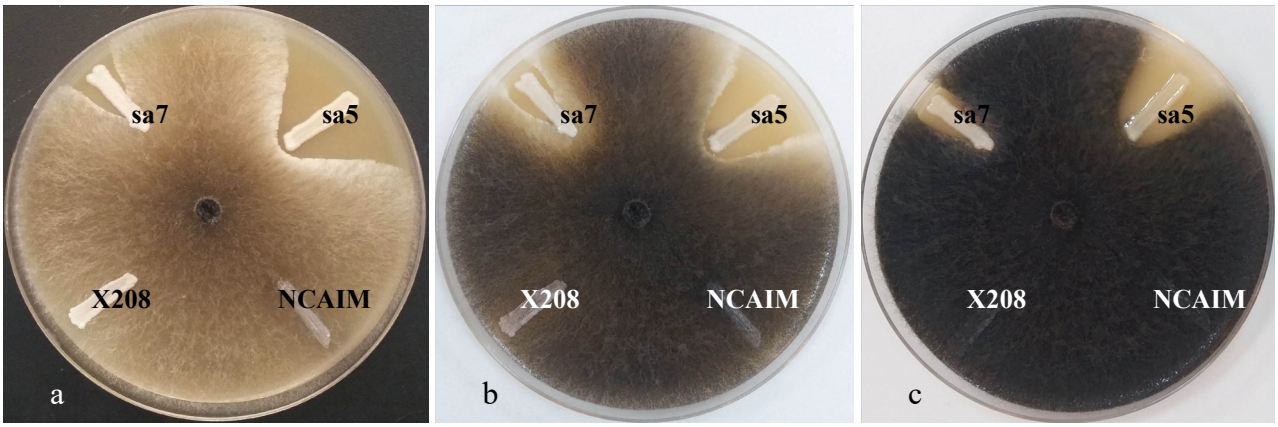

Figure 11. Antifungal activity of sa5 and sa7 yeast strains against *Sclerotium bataticola* after 3 (a), 7 (b) and 14 days (c) of incubation on Batata-Dextrose-Agar.

The antagonistic activity against *Penicillium* spp. and *Aspergillus* spp. storage pathogens was evaluated by means of the double layer technique. Only seven yeast strains were studied against these storage pathogens. The antifungal activity was visually evaluated and distinctive symbols were attributed according to the inhibitory action, as follows: "–" = no inhibition activity; "+" = slightly inhibition of the fungal growth, "+ +" = moderate inhibition; "+ + +" = high inhibition activity.

The yeasts' interaction with *A. ochraceus* O1 revealed MUCL 29874, SG1 and Dj IBNA as capable to inhibit the fungal growth (Table 4). The SG2 strain expressed antagonistic activity only in the first five days of co-cultivation.

Table 4. Yeasts' inhibitory activity against *Aspergillus* storage pathogens.

Mycotoxigenic storage fungi	**Yeast antifungal potential**						
	MUCL 29874	**SG1**	**SG2**	**sa5**	**sa7**	**Dj IBNA**	**CPM 1**
Aspergillus ochraceus O1	+ +	+ +	+	+	N.A.	+ +	+ +
Aspergillus flavus GE2	+ +	+ +	–	+	N.A.	+ +	–
Aspergillus flavus T11	+ +	+	+	+	+	+ +	+
Aspergillus flavus GIS2	+ + +	+ + +	+ +	–	N.A.	+ + +	+ + +
Aspergillus flavus GIS9	+ + +	+ +	–	+ +	N.A.	+ + +	+
Aspergillus niger AN1	+ + +	+ +	–	+ + +	N.A.	+ + +	N.A.
Aspergillus niger MA2	+ + +	+ +	–	+ + +	N.A.	+ + +	N.A.
where: N.A. = not available							

The interactions studies between the analysed yeasts and the four tested *A. flavus* pathogens revealed that GE2 and T11 strains are more virulent and harder to be controlled (Fig. 12). Fungal growth inhibition was much more efficient against GIS 2 and GIS 9 mycotoxigenic fungi. The best results were obtained with the MUCL and DjIBNA yeast strains, followed by the SG1 strain (Table 4).

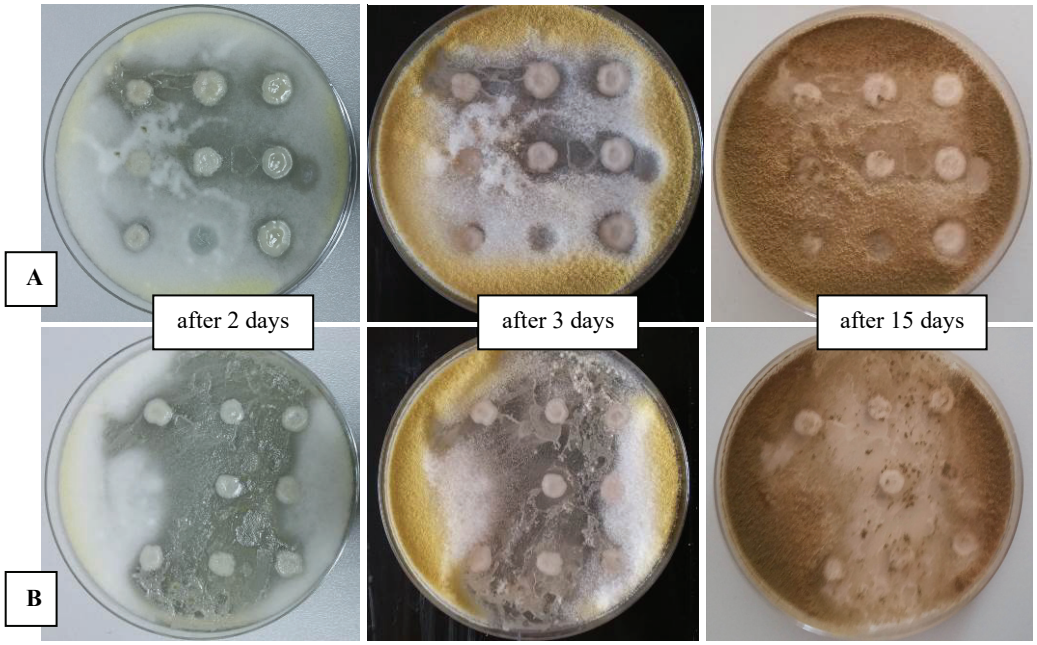

Figure 12. Double layer co-cultivation and yeasts antagonism activity against *A. flavus* GE2 (A) and T11 (B)at different incubation times.

Regarding yeast interaction with *Aspergillus niger*, similar results were obtained. The yeast strains MUCL, Dj IBNA, sa5, followed by SG1 revealed a high to moderate antifungal activity (Table 4). The antagonistic activity was maintained during the 15 days of incubation. No notable differences were seen among AN1 (Fig. 13) and MA2 biocontrol.

Figure 13. Antifungal activity of MUCL, Dj IBNA, sa5 and SG1 against *Aspergillus niger* AN1
- back (a) and front (b) view images of the interaction plate -

The antagonistic activity of yeasts was also tested against two *Penicillium* spp. isolates B1 and B2 collected from improper stored sweet potato tubers revealing blue mould infection. In vitro tests, performed by means of the dual layer technique (Fig. 14), revealed that *Metschnikowia* spp. strains are having a higher inhibitory activity than *S. cerevisie* strains against *Penicillium* spp fungi (Table 5).

Table 5. Yeasts inhibitory activity against *Penicillium spp.* blue mold.

Microbial strains		***Metschnikowia* spp.**				***Saccharomyces cerevisiae***			**Newly isolated *Metschnikowia* spp.**				
		MUCL	**CPM1**	**SG1**	**SG2**	**SMR4**	**x208**	**Dr.Oetker**	**sa1**	**sa5**	**sa6**	**sa7**	**sa8**
Penicillium spp.	B1 strain	+	+ +	+	+ +	+	+	+	+	+ +	+ + +	+ +	+
	B2 strain	+ +	+ +	+	+ + +	+	+	+	+	+ + +	+ + +	+ +	+ +

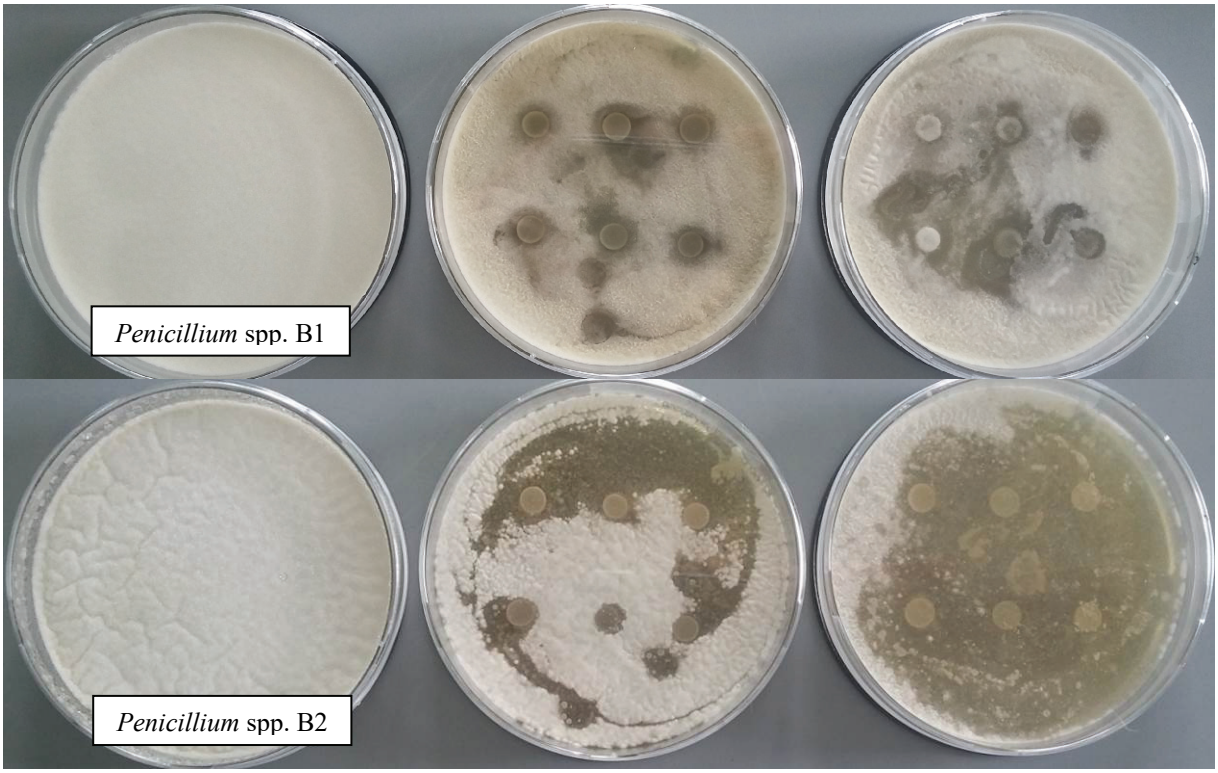

Figure 14. Yeasts antagonistic activity against *Penicillium* spp. B1 and B2 strains.

The biocontrol of storage pathogens was also studied for sweet potato (RAY & NEDUNCHEZHIYAN, 2012). Several studies revealed *Debaryomyces hansenii*, *Pichia anomala* and *Saccharomyces cerevisiae* strains as efficient against the *Botryodiplodia* rot and the *Rhizopus* soft rot of sweet potato (RAY & DAS, 1998; STEVENS et al., 1997).

Chemical control is generally avoided to prevent spoilage during storage, mostly because postharvest application of fungicides may cause residue problem. However, some fungicides are mentioned for disinfecting planting materials (RAY & NEDUNCHEZHIYAN, 2012). Therefore, to reduce disease incidence during storage, it is important to apply correct prevention methods and storage conditions. Among the biocontrol measurements tested so far, antagonistic epiphyte yeasts are to be used primarily (GREBENISAN et al., 2008). GRAS and QRS are also considered, which have a long shelf-life on fruit and vegetable skin (BOURDICHON et al., 2012; FDA, 2019; PAWLIKOWSKA et al., 2019).

CONCLUSIONS

Sweet potato is vulnerable to various fungal diseases during storage. To prevent spoilage losses, specific technical preventive measures and storage conditions are recommended. The chemical control of storage pathogens is to be avoided. However, physical and microbiological measurement could be applied. Biocontrol yeasts are primarily preferred to bacteria, mostly due to their long shelf-life as epiphytes on vegetable surface. The current study is describing several yeast strains with antifungal activity against storage spoilage fungi, such as: *Aspergillus* spp., *Botrytis cinerea, Fusarium oxysporum, Penicillium* spp., *Rhizopus stolonifer and Sclerotium bataticola*. Among the 11 yeast stains analysed, the newly isolated *Metschnikowia* spp. sa5 and sa7 strains were the ones revealing the widest antifungal range and inhibitory activity against tested plant pathogens.

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