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ANTI-MOLD PROPERTIES OF YEAST STRAINS AS A BIOLOGICAL AGENT FOR PROTECTION OF GRAIN DURING STORAGE

Summary

Contamination of cereal grains by mold is a serious threat to human and animal health and leads to economic losses. The antagonistic activity of yeast strains isolated from varied habitats against mold was assessed. Based on the in vitro experiments, three strains of yeasts, showing the antagonistic activity towards the growth of 13 mold species isolated from cereals, have been selected. The ability to inhibit the growth of mold was tested in wheat, rye, barley, and oat grains, the positive effect was demonstrated in wheat and rye grain only.
***Key words**: contamination by molds, yeast activity against molds*

WYKORZYSTANIE ANTYPLEŚNIOWYCH WŁAŚCIWOŚCI SZCZEPÓW DROŻDŻY DO BIOLOGICZNEJ OCHRONY ZIARNA PODCZAS PRZECHOWYWANIA

Streszczenie

Zanieczyszczenie ziarna zbóż pleśnią stanowi poważne zagrożenie dla zdrowia ludzi i zwierząt oraz prowadzi do strat ekonomicznych. Oceniono antagonistyczną aktywność szczepów drożdży izolowanych z różnorodnych siedlisk skierowaną wobec pleśń. Na podstawie doświadczeń in vitro wybrano trzy szczepy drożdży, wykazujące działanie antagonistyczne wobec wzrostu 13 gatunków pleśni, izolowanych ze zbóż. Zdolność do hamowania wzrostu pleśni badano w ziarnach pszenicy, żyta, jęczmienia i owsa, pozytywny efekt wykazano tylko w przypadku pszenicy i żyta.
***Słowa kluczowe**: skażenie pleśniami, aktywność antypleśniowa drożdży*

1. Introduction

Contamination of cereal grains by mold is a serious threat to human and animal health due to the toxic effects of secondary metabolites of mold fungi - mycotoxins. It is estimated that on a global scale even up to 25% of harvested food crops can be infected by spoilage fungi what, except health hazard, causes considerable economic losses [3]. Growth mycotoxigenic species of mold is influenced by abiotic and biotic factors including unfavorable atmospheric conditions during growth and harvesting as well as inadequate temperature and humidity conditions during storage and presence of natural microbiota on harvested plants.

The preservation of harvested grain by drying in high temperature requires a significant amount of energy Several species of *Aspergillus*, *Penicillium* and *Fusarium* are capable of growing in different climates and thus contamination of food crops with mycotoxins (ochratoxin A, fumonisins deoxsynivalenol, zaralenone) can occur worldwide [17, 20, 22]. To eliminate the threat of mold spoilage chemical fungicides are used commonly. However the trend to eliminate chemicals from food and feed, especially in the ecological system, induces/ encourages use of antimicrobial properties of microorganisms to protect crops and food from spoilage caused by the growth of pathogens, such as mold, as an alternative, non-hazardous method of postharvest preservation.

Antimicrobial activity of yeast was investigated, inter alia, for use it in biocontrol of undesirable microorganisms during technological processes in food production (wine fermentation, bread production) and in agriculture (fruit protection after harvesting, grain and silage storage) [3, 4, 8, 21]. Pathogen antagonistic activity is a valuable feature of probiotic microorgaisms, which have been used up to now with regard to the antimicrobial properties of bacteria [1, 10, 23, 32].

Yeast are capable of producing killer toxins - antimycotic compounds to which they themselves are immune. This ability, which was first found in *Saccharomyces cerevisiae*, occurs in yeast belonging to such genera as *Pichia*, *Torulopsis*, *Williopsis*, *Candida*, *Cryptococcus*, *Debaromyces*, *Hanseniaspora*, *Kluyveromyces* [18, 20]. The biocontrol ability is a species specific feature/characteristic [6]. Killer toxins have proteinaceous character (proteins or glycoproteins) and differ in terms of biochemical properties, mode and spectrum of action on microorganisms as well as molecular characteristic [7, 9, 13, 27, 28, 31]. Cell wall receptors of sensitive yeast constitute a yeast's killer toxins target, for example one of toxins produced by *Pichia anomala* (Pikt) interacts with β-glucan of cell wall [12, 28].

It has been shown that, beside closely related species of yeast, toxins secreted by yeast are active against sensitive bacteria and mold [11, 21, 24, 27]. Yeast *Meyerozyme guliermondii* which shows antimicrobial activity was found to be capable of inhibiting mycelial growth and sporulation of mold *Colletotrichum gloeosporioides*, responsible for significant losses in tropical crops, cocoa and papaya, among others [14]. *Aureobasidium pullulans* and *Rhodotorula mucilaginosa* isolated from the fruits are active against *Penicillium expansum* which cause spoilage of pears, during low temperature storage. Use of these yeasts as a biocontrol/ bioconserving agent was positively evaluated by Robiglio et al. [25]. The application of killer yeast in the production of wines may limit the need to add sulfur dioxide thanks to antagonistic properties towards wild yeast [26]. Killer toxin of *Pichia membranifaciens* exerted a fungicidal effect on *Botritis cinerea* (which is a cause grey mold disease) [29]. *Pichia anomala* is capable of producing killer toxin, that is active against *Dekkera*/*Brettanomyces* sp. yeasts, which are undesirable in wine production [19, 26, 30]. Characteriza-

tion of toxin secreted by *Pichia anomala* DBVPG 3003 (known as Pikt) revealed its similarity to ubiquitin, in terms of mode of action and biochemical properties. Yeast toxin of *Williopsis mrakii*, produced by transformed *Aspergillus niger*, was active at storage temperature against spoilage yeast in yogurt and silage [16]. Purified killer toxin of *Williopsis saturnus* var. *mrakii* exhibited a wide spectrum of antimicrobial activity [11, 15]. *W. saturnus* var. *mrakii* MUCL 41968 has been found to secrete a killer toxin exhibiting a lethal effect against a broad range of microorganisms pathogenic for human, including *Pneumocystis carinii*, *Candida albicans Mycobacterium tuberculosis*. *Pichia anomala* and *S. cerevisiae* [11].

Parallel to research on toxin production by yeasts and their purification, attempts are made to use yeast strains with proven antimicrobial activity in processes of food technology and production of animal feeds. Some studies reported greater inhibition of mold when active grooving cells of yeast were used, compared to growth performed with use of purified toxin [27]. In the paper on the use of yeast for biocontrol of mold growth during storage of high moisture wheat, it has been shown that *Pichia anomala* (synonymous with *Hansenula anomala*) limits the growth of *Penicillium roqueforii* and other molds both in vitro and in malfunctioning airtight silos [5, 6]. Depending on the strain (species) and number of yeast introduced, total mold growth inhibition or reduction of at least several levels were observed [5, 6, 24].

Despite the fact that some work on use of yeast as biocontrol agent has already been performed with promising results there is a few preparations for regular use in biopreservation [5]. Therefore, taking public concern about environmental and health issues into account, it is reasonable to search for new strains of yeast with antimicrobial activity against mold and bacteria because the possibility of their application in food and feed technology can be potentially widespread.

Consequently, the purpose of this study was to investigate the antimicrobial/ antimycotic activity of yeast isolated from natural environments and potential application of selected yeast strains/species as biocontroling agent during storage of grain.

2. Materials and methods

Yeast strains used in the study were isolated from different natural environmental niches (various plant materials and food). The isolates were identified to genus/species level based on morphology and physiology. The species identity was confirmed by sequence analysis of the ITS1 and ITS4 regions of 18S rDNA. The yeast strains used in this work are listed in Table 1.

Preliminary screening for the anti-mold activeness was done against molds of the genus *Pennicillium*, *Aspergillus*, *Fusarium* isolated from grass silage, belonging to the culture collection of IBPRS of Fermentation Department.

Based on the results of initial screening an anti-mold activity of selected strains, showing the best activity, was evaluated against *Fusarium sporotrichioides*, *Fusarium culmorum*, *Fusarium poae*, *F. langsethiae*, and *Penicilium* sp. isolated from cereals and kindly provided by the Department of Phytopathology and Plant Seed Science of Poznan University of Life Sciences. From cereal grains, which were used in this work six molds strains representing genus *Rhizopus* spp,. *Mucor* spp and species *Aspergillus flavus*, *Penicillium roqueforti* Z1, *Penicillium roqueforti* Z2, *Penicillium expansum* were isolated. Evaluation of anti-molds activity of yeast against those molds was done as well.

Table 1. Strains of yeast used in work
Tab. 1. Szczepy drożdży wykorzystane w pracy

Symbol in the collection	Source of isolation/ origin	Species
KKP 1780	milk	*Saccharomyces cerevisiae*
KKP 1781	milk	*Hanseniaspora uvarum*
KKP 1783	chees	*Saccharomyces cerevisiae*
KKP 1782	sour cream	*Saccharomyces cerevisiae*
KKP 1774	currants fruits	*Saccharomyces cerevisiae*
KKP 1775	currants fruits	*Hanseniaspora uvarum*
ZF7 1779	grass silage	*Pichia fermentans*
KKP 1771	apples	*Issatchenkia orientalis*
KKP 1772	apples	*Issatchenkia orientalis*
KKP 1773	plums fruit	*Hanseniaspora uvarum*
KKP 1776	manure	*Issatchenkia orientalis*
KKP 1777	manure	*Issatchenkia orientalis*
KKP 1778	manure	*Hanseniaspora uvarum*
KKP 1616	cherries fruits	*Saccharomyces cerevisiae*
KKP 1615	cherries fruits	*Saccharomyces cerevisiae*
KKP 1619	milk	*Candida kefir*
Kz 1	goat's digestive tract	*Candida tropicalis*
Kz 3	goat's milk	*Candida zeylanoides*
Sw 3	manure	*Candida tropicalis*
Regent	grapes	*Saccharomyces cerevisiae*
Solaris	grapes	*Saccharomyces cerevisiae*
KKP 1623	strawberries	*Issatchenkia orientalis*
KKP 1621	strawberries	*Candida qulliermondi*
ZFD 03	rye soudough	*Candida krusei/iconspicua*
MJ D_2	barley flour	*Candida krusei*
Jr	apples	*Candida utilis*
Jc	apples	*Candida sphaerica*
KKP 1617	cherries fruits	*Saccharomyces cerevisiae*
KKP 1622	strawberries	*Candida qulliermondi*
ZFD 04	rye flour	*Candida zeylanoides*
ZFD 03	rye soudough	*Candida krusei*
ZFD 10	rye soudough	*Candida krusei*
ZFD 16	rye soudough	*Candida pelicullosa*
Champion	apples	*Kloeckera sp.*
KKP 512	comercial bakery yeast	*Saccharomyces cerevisiae*
Ws F3	cherries fruits	*Saccharomyces cerevisiae*
Pr 4	currants fruits	*Saccharomyces cerevisiae*
KKP 905	NCAIM	*Kluyweromyces lactis*

Source: own work / Źródło: opracowanie własna

All microorganisms were stored in an atmosphere of liquid nitrogen (at -195,8°C).

Cereal grains

Cereal grains harvested in 2016: wheat, rye, barley, oat characterized by moistures respectively: 12,36%, 11,16%, 13,25%, 13,67% were used for the study, as well as the same grains after increasing their humidity by about 25%.

Media

The media used for the study were YEPD (1% yeast extract, 2% glucose, 2% peptone and 1,5-2% agar) and YEPD with 100mM citrate phosphate buffer.

Yeast and mold cultures were restored to activity after taking out liquid nitrogen in liquid YEPD or YEPD with citrate. Routinely yeast cultures were grown in YEPD at 30°C for 24 hours.

The spores from mold colonies were collected after 5 days of growth on YGC solid medium (Merck).

Number of viable yeast and mold were counted onto YGC agar and expressed as colony-forming units (CFU ml^{-1} or CFU g^{-1}).

Inocula

Yeast suspensions were prepared by inoculating 100 ml of a YEPG broth with 1 ml of yeast from a culture stored in YEPG at 4°C (at least a second step of culture after taking yeast out liquid nitrogen stored culture). After incubation at rotary shaker (100rpm) at 30°C for 24 h, the number of cells was enumerated in Thoma camera.

The suspension of mold spores was prepared by collecting spores from 5-day old colonies (grown on YGC at 25°C) in pepton water with 0,015% Tween 80 added to assist in the dispersal of conidia. The spore concentration was counted in Thoma camera.

Determination of antimicrobial activity of yeast against mold

The assay was performed in YEPG with citrate agar. Agar yeast plates were prepared by mixing the yeast suspension in adequate/right amount with medium to receive yeast cell number in level 10^3, 10^4, 10^6, 10^7 CFU ml^{-1} (each plates contained 10 ml of medium). Non inoculated yeast plates with mold colonies served as control.

On the surface of a seeded agar were poured 10µl of molds spores suspended in pepton water, counting $1x10^4$ CFU ml^{-1} spores as spots on Petri dishes (three on plate).

The degree of inhibition was determined /designated as the diameter of the mold colony compared to diameter of colony in control, or as "no growth".

Determination of mold number in cereals

The presence of mold was assessed in samples: before hydrating (1), after hydrating (with tap water to 25% moisture, at 2°C, 48h) (2), after moistening/hydrating and incubated at 25°C for 14 days (3), after moistening/hydrating and inoculated by yeast and incubated at 25°C for 14 days (4). The number of mold (in CFU) was determined using YGC broth, sample were treated according to standardized methods.

Inhibition in grain

The assessment of mold growth inhibition by yeast in grain was conducted according to procedure described by Druvefors and Schnurer [6]. Dry and wet grain, inoculated by yeast to 10^6 CFU g^{-1} , were put into 45 ml tubes and incubated at 25°C for 14 days. The tubes were sealed with parafilm in which small holes were made with a needle.
All experiments were performed in three replications.

3. Results and Discussion

Among 38 strains of yeast, screened for mold inhibition merely three strains: *Candida tropicalis* Kz1, *Issatchenkia orientalis* KKP 1623 and *Candida krusei* ZFD 03, exhibited anti- mold activity, therefore the results of that preliminary investigation are not shown in the table.

These (3) strains were evaluated in terms of antimycotic activity against molds isolated from cereal grains: *Fusarium sporotrichioides*, *Fusarium culmorum*, *Fusarium poae*, *F. langsethiae*, and *Penicilium*, results are presented in Table 2.

Experiments in vitro (on the plates) with three yeast strains showed the mold inhibitory activity of all tested strains. The highest anti-mold ability was observed for *C. krusei*/*iconspicua* ZFD03 beginning from the level of 10^4 CFU g^{-1} the growth three out of seven investigated molds was completely inhibited.

Because of the high degree of contamination of cereal grains (from the 2016 harvest) by mold – on the level 10^3 – 10^4 CFU g^{-1} (Table 4), the evaluation of the antimicrobial activity of selected yeast strains against endogenous molds, isolated from these cereals, was conducted. The results are presented in Table 3.

Table 2. Inhibition of mold by yeast, evaluation of antimicrobial activity against mold in vitro
Tab. 2. Hamowanie pleśni przez drożdże, ocena aktywności antymikrobiologicznej skierowanej wobec pleśni in vitro

Number of yeast CFU ml^{-1}	Yeast	Growth of mold, colony diameter, mm ±SD						
		Fl	Fc1	Fc2	Fp	Fs	P1	P2
Control	without yeast	90±14	60±7	50±4	35±2	40±4	30±4	30±2
10^3	*C. tropicalis* Kz1	6±2	10±4	10±2	9±3	10±2	-	15±3
	C. zeylanoides KKP1623	10±1	10±1	20±4	8±2	15±2	-	13±4
	C. krusei/ iconspicua ZFD03	6±1	20±4	15±2	5±1	15±1	-	10±2
10^4	*C. tropicalis* Kz1	4±1	10±7	5±1	5±0	6±0	-	5±1
	C. zeylanoides KKP1623	4±1	10±1	15±2	5±1	-	-	10±2
	C. krusei/ iconspicua ZFD03	6±1	10±4	-	4±7	-	-	6±1
10^6	*C. tropicalis* Kz1	-	5±1	-	-	-	-	-
	C. zeylanoides KKP1623	-	5±2	5±1	3±0	-	-	10±1
	C. krusei/ iconspicua ZFD03	-	5±1	-	-	-	-	8±1
10^7	*C. tropicalis* Kz1	-	-	-	-	-	-	-
	C. zeylanoides KKP1623	-	5±0	-	-	-	-	10±2
	C. krusei/ iconspicua ZFD03	-	-	-	-	-	-	8±1

Molds: Fl - *Fusarium langsethiae;* Fc - *Fusarium culmorum* 1; Fc2 - *Fusarium culmorum* 2; Fp - *Fusarium poae*; Fs - *Fusarium sporotrichioides*; P1 - *Penicillium roqueforti* S1; P2 - *Penicillim roqueforti* 2

Source: own work / Źródło: opracowanie własne

Table 3. Inhibition of mold by yeast, evaluation of antimicrobial activity against mold in vitro
Tab. 3. Hamowanie pleśni przez drożdże, ocena aktywności antymikrobiologicznej skierowanej wobec pleśni in vitro

Numer of yeast, CFU ml^{-1}	Yeast	Growth of mold, colony diameter, mm ±SD					
		Af	PZ1	Pe	Pz2	R	M
control	without yeast	25 ± 2	30 ± 1	35 ± 3	15 ± 2	90 ± 7	50 ± 4
10^3	*C. tropicalis* Kz1	8 ± 1	10 ± 1	8 ± 1	6 ± 1	15 ± 3	7 ± 1
	C. zeylanoides KKP1623	10 ± 3	12 ± 1	9 ± 1	10 ± 4	10 ± 1	7 ± 3
	C. krusei/ iconspicua ZFD03	9 ± 1	11 ± 1	7 ± 2	9 ± 1	14 ± 1	6 ± 2
10^4	*C. tropicalis* Kz1	5 ± 1	6 ± 2	5 ± 1	4 ± 1	10 ± 3	5 ± 1
	C. zeylanoides KKP1623	6 ± 1	10 ± 2	6 ± 1	6 ± 1	7 ± 2	4± 0
	C. krusei/ iconspicua ZFD03	5 ± 2	7 ± 1	5 ± 1	6 ± 1	10 ± 1	3 ± 1
10^6	*C. tropicalis* Kz1	-	3 ± 0	-	-	-	-
	C. zeylanoides KKP1623	2 ± 0	3 ± 1	-	5 ± 1	-	-
	C. krusei/ iconspicua ZFD03	3 ± 1	4 ± 1	-	4 ± 1	2 ± 0	-
10^7	*C. tropicalis* Kz1	-	-	-	-	-	-
	C. zeylanoides KKP1623	-	-	-	3 ± 1	-	-
	C. krusei/ iconspicua ZFD03	-	2 ± 1	-	4 ± 1	-	-

Molds: Af - Aspergillus flavus; PZ1 - Penicillium roquforti Z1; Pe - Penicillium expansum; PZ2 - Penicillium roquforti Z2; R- Rhizopus spp.; M - Mucor spp.

Source: own work / Źródło: opracowanie własne

Table 4. Effect of yeast applying on mold presence in dry grain and grain after moistening
Tab. 4. Wpływ zastosowania drożdży na obecności pleśni w ziarnie suchym i po nawilżeniu

Grain cereals	Number of mold, CFU g^{-1} ± SD				
	Dry grain	Dry grain with yeast a,b,c	Wet grain after 48h at 2°C	Wet grain after 14 days at 25°C	Wet grain with yeast (a,b,c) at 25°C
wheat	$1{,}0x10^3 \pm 0{,}2x10^2$	a $1{,}0x10^2 \pm 0$	$2{,}0x10^3 \pm 0{,}3x10^3$	$2{,}4x10^8 \pm 0{,}1x10^8$	a $1{,}9x10^8 \pm 0{,}1x10^8$
		b nd			b $1{,}0x10^6 \pm 0{,}2x10^6$
		c nd			c $8{,}0x10^5 \pm 0{,}4x10^5$
rye	$1{,}0x10^4 \pm 0{,}1x10^3$	a nd	$2{,}0x10^4 \pm 0{,}4\ x10^4$	$1{,}8x\ 10^8 \pm 0{,}4x10^8$	a $1{,}8x10^8 \pm 0{,}3x10^8$
		b nd			b $1{,}0x10^6 \pm 0{,}7x10^6$
		c nd			c $2{,}0x10^5 \pm 0{,}4x10^5$
barley	$8{,}0x10^3 \pm 0{,}3x10^2$	a $1{,}0x10^4 \pm 0{,}3x10^4$	$1{,}0x10^4 \pm 0{,}1x10^4$	$1{,}7x10^8 \pm 0{,}2x10^8$	a $2{,}0x10^8 \pm 0{,}1x10^8$
		b $3{,}0x10^3 \pm 0{,}2x10^3$			b $1{,}9x10^8 \pm 0{,}6x10^8$
		c $3{,}5x10^3 \pm 0{,}2x10^3$			c $1{,}8x10^8 \pm 0{,}2x10^8$
oat	$1{,}0x10^4 \pm 0{,}1x10^3$	a $2{,}0x10^5 \pm 0{,}4x10^5$	$2{,}0x10^4 \pm 0{,}3x10^4$	$1{,}0x10^7 \pm 0{,}2x10^7$	a $1{,}0x10^7 \pm 0{,}1x10^7$
		b $2{,}0x10^4 \pm 0{,}1x10^4$			b $1{,}3x10^8 \pm 0{,}3x10^8$
		c $1{,}0x10^4 \pm 0{,}1x10^4$			c $1{,}2x10^8 \pm 0$

a - *C. tropicalis* Kz1; b - *C. zeylanoides* KKP1623; c - *C. krusei/ iconspicua* ZFD03; nd - not detected

Source: own work / Źródło: opracowanie własne

All three strains of yeast showed the ability to inhibit the growth of molds isolated from the examined cereal grain. The anti-mold activity varied depending on the yeast strain and the mold species. The mold inhibitory effect was observed for *C. tropicalis* Kz1 strain, which showed strong inhibition against all tested molds at the level 10^7 $CFUg^{-1}$ and at the level 10^6 $CFUg^{-1}$ against 5 of 6 tested molds. The other tested yeast strains showed weaker activity, growth of molds *Penicillium roqueforti* (2 strains) was the most difficult to inhibit.

Taking into account the results of all experiments *in vitro* on the mold inhibitory activity of the tested yeast, against a total of 17 molds (4 strains in preliminary screening trials), it was found that the three yeast strains have the ability to inhibit the growth of various mold species. On this basis, laboratory-scale studies were carried out using four most common cereals. In experiments, both dry and moist grains were used, the mold inhibitory activity with high–moisture grain was conducted according to the method applied by Druvefors and Schnűrer [6]. The number of molds was evaluated in dry grain, dry grain with yeast, wet grain after 48 hours of moistening at 2°C, wet grain after humidification incubated at 25°C, wet grain after humidification incubated at 25°C inoculated by yeast. The results were presented in Table 4.

All examined cereal grains showed a relatively high mold presence with CFU on level of 10^3-10^4 g^{-1}. The inoculation of dry grains of wheat and rye with yeast inhibited the growth of mold. This effect was evident in case of *C. zeylanoides* KKP1623 and *C. krusei* ZFD03, whereas in case of *C. tropicalis* Kz1 this effect was less visible. In barley and oat grains the reduction in mold number was not observed.

Increasing the wetness of the grain to 25% caused that during the 14 days of incubation at 25°C, the number of endogenous molds increased to 10^7-10^8 CFU g^{-1}, it means

3-4 levels. With such a high mold abundance, the use of yeast did not have, in most cases a significant effect on the number of molds. Inhibition of mold growth was found in wheat and rye inoculated with yeast *Candida zeylanoides* KKP 1623 and *Candida krusei/iconspicua* ZFD03, which reduced mold number to levels of 10^6 CFU g^{-1} and 10^5 CFU g^{-1} respectively, in comparison to control samples with a number of mold at level of 10^8 CFU g^{-1}. Druvefors and Schnurer proved the efficacy of mold inhibition by Pichia anomala in moist feed grain during storage. In those investigations, distinguishing from current work, the number of yeast two times exceeded the number of molds (10^4 to 10^2 CFU g^{-2}) [5].

Contrary to expectations, in the experiments conducted on cereals, higher anti-mold activity was shown by yeast strains, which in vitro had weaker activity while the *C. tropicalis* Kz1 strain, which was the most active in vitro, showed no ability to inhibit the growth of mold in grain.

These results indicate the need to conduct mold growth inhibition tests in conditions as close as possible to the intended use in practice. Antagonistic (against mold) activity of yeast depends on the cereal to which they will be introduced. This is needed because both the production of compounds active against mold by yeast (like killer toxins or volatile organic compounds) and growth of mold are strongly affected by the culture conditions and environmental factors, such as water activity, pH and temperature, aeration of stored grain during grain storage [24]. Druvefors et al. [5] suppose that in the case of *Pichia anomala* the effect of mold inhibition is related to the synergistic effect of competition in the environment and the synthesis of other compounds outside the killer toxins and ethyl acetate.

The preparation containing the particular strain of yeast should be developed for a particular cereal grains.

Similar conclusions / suggestions for confirming the inhibition of mold growth by yeasts in natural ecosystems were presented by other authors. Considering this, a number of attempts have been made to mimic the storage conditions of food and feed matrices [4, 8, 24]. Yeast ability to decontaminate mycotoxins is another issue related to inhibition of mold growth.

The results show that the antagonistic effect should be tested/investigated under the conditions most likely to imitate the intended use in the environment, as in the studies conducted with respect to anti-mold activity [6, 15, 21, 24].

4. Conclusions

Out of a pool of 38 strains of yeast belonging to several species isolated from different natural habitats, only three strains can potentially be used for the biological protection of cereal grains against mold during storage. The antagonistic activity of yeast strains was assessed in vitro and in conditions similar to the natural in tubes (microsilos), in the case of molds associated with cereal grains. The results obtained are similar to the results of the studies conducted by Druveforst and Schűrer, who also observed variation among yeast strains in these properties [6]. The inhibition of mold growth by yeast is specific between specific yeasts and molds within the species. In addition, the importance of the environmental impact - taking into account physical factors - pH, temperature, humidity, chemical - cereal grain, biological - enzyme activity and presence of other microorganisms.

The mode of action for anti-mold effects of the tested strains was not determined, but it was found that they have the ability to inhibit the growth of mold in wheat and rye grain, especially if its moisture content is not very high.

Currently in progress are studies on the biochemical properties of this yeast strains. This provides the basis for further research into the development of biological preparations for the protection of cereal grains during storage.

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