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PROBIOTIC PROPERTIES OF *SACCHAROMYCES CEREVISIAE* ŁOCK 0119 YEAST

S u m m a r y

Probiotics including their combinations with prebiotics known as synbiotics have been extensively studied as an alternatives to antibiotic growth promoters commonly used in livestock breeding. Among many microorganisms that may be useful for their host, the *Saccharomyces cerevisiae* yeast exhibits probiotic potential.

The objective of the research study was to select a *S. cerevisiae* strain suitable for use as a probiotic in newly developed synbiotic preparations for monogastric animals (poultry, swine). The survivability of yeast strains in the presence of bile salts and under low-pH conditions varied and after 4 h of incubation it ranged from 66 to 94 % and from 68 to 97 %, respectively. The *S. cerevisiae* ŁOCK 0119 strain was characterised by the highest survival rate; the number of live cells of this strain was reduced not more than 9 % after 4 h of incubation under the fixed conditions. Also, this strain exhibited hydrophilic properties and a strong auto- and co-aggregation potential. The selected yeast strain aggregated with pathogenic bacteria (*Salmonella* spp., *Listeria monocytogenes*) to a varying degree ranging between 40 and 75 %. The *S. cerevisiae* ŁOCK 0119 strain inhibited the adhesion of pathogens to the Caco-2 cells, whereby the adhesion of morbific bacteria attachment was reduced to a varying degree (15 ÷ 37 %). Based on the results obtained, it was concluded that the selected *S. cerevisiae* yeast strain had beneficial properties since it was able to survive during gastrointestinal passage, to colonize the intestines and to reduce the growth of pathogenic microbiota.

Key words: probiotics, *S. cerevisiae* yeast, hydrophobicity, aggregation, adherence

Introduction

Probiotics are known as live microorganisms, which when administrated in the proper amount confer a health benefit on the host [10, 14]. Those beneficial microorganisms belong mostly to the bacterial species, namely *Lactobacillus*, *Bifidobacterium,*

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Bacillus or *Enterococcus*; nonetheless yeasts, such as *Saccharomyces cerevisiae*, are also known for their probiotic properties [23]. The positive effect of probiotics can be accomplished by modulating the intestinal microbiota and maintaining its balance, inhibiting the growth of pathogenic bacteria, increasing the resistance to infections, stimulating the immune system and what is more, supporting the digestion along with enhancing the bioavailability of nutrients [15].

The usefulness of probiotics in livestock breeding has been extensively studied since they improve the performance and health of animals; also because they are an alternative to antibiotic growth promoters (AGPs) [4, 16]. Since the 1940s AGPs have been extensively administrated to animals in order to reduce infections; in this way the growth of animals and the production efficiency have been enhanced. Nevertheless, the overuse of chemotherapeutics caused an issue to appear – the accumulation of drugs residues in animal food products, which could be also a transmission route of resistance genes to human pathogenic microbiota [26]. The documented negative consequences of using antibiotic growth promoters (AGPs) in livestock breeding led to taking legislative steps towards reducing the use of antibiotics in the European Union Member States as per 1st of January 2006 [9]. Furthermore, prebiotics and synbiotics were proposed as the substitutes for AGPs; the first are selectively fermented food ingredients, which contribute to health benefit of the host by stimulating the activity and growth of GIT microbiota while the synbiotics constitute a combination of pro- and prebiotics [12].

The objective of the research study was to select an *S. cerevisiae* yeast strain, which would possess probiotic features and would be included in the newly designed synbiotic preparations intended for use in monogastric animals, such as poultry and swine.

Materials and methods

Microorganisms, mediums and propagation

Of a group of 20 *S. cerevisiae* strains, 6 strains with amylolytic activity were screened for their probiotic properties. Their ability to degrade starch was analysed with the use of Waksman agar (distilled water 500 ml, potassium hydrogen phosphate 1.0 g/l, sodium chloride 1.0 g/l, ammonium sulphate 2.0 g/l, calcium carbonate 5.0 g/l, agar 20 g/l, 2 % w/v aqueous solution of potato starch 500 ml), on which the strains were inoculated by a streak technique and incubated (30 ºC, 24 h). A positive result was visible as a clear hydrolysis zone. The selected strains with amylolytic activity were taken from the Lodz Collection of Pure Cultures (ŁOCK 105) in the Institute of Fermentation Technology and Microbiology, the Lodz University of Technology (Tab. 1).

Table 1. *Saccharomyces cerevisiae* strains under analysis
Tabela 1. Analizowane szczepy *Saccharomyces cerevisiae*

Strain / Szczep	Source of isolation / Źródło izolacji
S. cerevisiae ŁOCK 0068	Forage / Pasza
S. cerevisiae ŁOCK 0113	Distiller's yeast / Drożdże gorzelnicze
S. cerevisiae ŁOCK 0119	Distiller's yeast / Drożdże gorzelnicze
S. cerevisiae ŁOCK 0137	Baker's yeast / Drożdże piekarskie
S. cerevisiae ŁOCK 0140	Baker's yeast / Drożdże piekarskie
S. cerevisiae ŁOCK 0142	Baker's yeast / Drożdże piekarskie

Moreover, the four pathogenic strains were used: *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 13311, *Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076, *Salmonella enterica* subsp. *enterica* serovar Choleraesuis PCM 2565 and *Listeria monocytogenes* ATCC 13932. Those strains were obtained from the American Type Culture Collection (ATCC, Manassas, USA) or from the Polish Collection of Microorganisms (PCM, Wroclaw, Poland).

Prior to the conducted analyses, the strains were stored at a temperature of -22 ºC with the use of Cryobanks™ (Copan Diagnostics Inc., Murrieta, USA). The microorganisms were activated before each and every analysis. Both the yeast and the pathogenic strains passed twice through a yeast-peptone-glucose broth (YPG, BTL, Łódź, Poland) at 30 ºC for 24 h and through a nutrient broth (Millipore, Darmstadt, Germany) at 37 ºC for 24 h, respectively, without oxygen limitation.

Resistance to bile salts and acidic conditions

The resistance to bile salts (Sigma-Aldrich, St. Louis, USA; 1 % and 2 % w/v concentration) and acidic conditions (pH 2 and pH 3) were analysed based on a method by Zielińska et al. with modifications [27].

The *S. cerevisiae* strains were incubated at 30 ºC for 24 h without oxygen limitation; then they were centrifuged at a relative centrifugal force (RCF) 3468 × g for 10 min (Centrifuge MPW-251, MPW, Warsaw, Poland) and the biomass was suspended in a physiological saline solution with bile salts added or having the specified pH levels. The samples were incubated at 30 ºC without oxygen limitation. The control sample, which was cultured in a 0.85 % (w/v) physiological saline solution with pH equalling 5.5, was also inoculated and incubated under the same conditions.

The serial dilutions of each bile salt solution, the physiological saline solutions with the defined pH levels, and the control sample were prepared at the beginning of incubation and then after 1, 2 and 4 h. The count of yeast cells was determined by a plate count method and using YPG agar. The plates were incubated at 30 ºC for 24 h

without oxygen limitation; thereafter the colonies were counted and the results were given in colony-forming units per ml (CFU/ml).

There were conducted three repetitions of the yeast monocultures in physiological saline solutions with a certain pH level or with the bile salts added and of the control samples. The plate method was also performed in three repetitions.

Hydrophobicity

The hydrophobic properties of the selected *S. cerevisiae* strain were assessed using a microbial adhesion to hydrocarbon (MATH) assay based on the methods first described by Rosenberg et al. with modifications [22].

The activated yeast cells were washed twice in a phosphate-buffered saline (PBS, Merck, Darmstadt, Germany) by centrifuging them with a relative centrifugal force (RCF) 3468 × g for 10 min and subsequently suspended in PBS to give a final optical density of 1.0 at 600 nm (A_0) as measured spectrophotometrically (Beckman DU 640, Beckman Instruments, Porterville, USA). The suspension of bacteria (5 ml) was mixed with 1 ml of hexadecane (apolar solvent; Sigma-Aldrich, St. Louis, USA), and vortexed for 2 min. The samples were incubated for 60 min at 20 ± 2 ºC and then the PBS fraction absorbance was measured (A).

The hydrophobicity (H) was analysed in three repetitions. The adhesion of yeast cells to hexadecane was calculated using a formula provided by Bellon-Fontaine et al. [1]:

$$\text{Hydrophobicity [\%]} = [(A_0 - (A/A_0)] \times 100$$

The yeast was classified as hydrophilic (H < 10 %), moderately hydrophilic (10 ÷ 34 %), medium hydrophobic (35 ÷ 70 %) or highly hydrophobic (71 ÷ 100 %) according to Chae et al. [5] with a modification consistent with Ben Taheur et al. [2].

Auto-aggregation and co-aggregation assay with pathogens

The activated yeast strain, the *Salmonella* spp. strains and *L. monocytogenes* ATCC 13932 were analysed for their aggregation properties by an assay as described by Kos et al. [18] and Janković et al. [17]. The microorganisms were centrifuged (3852 × g, 10 min), washed once and re-suspended in PBS with a final optical density of 1.0 at 600 nm. The cell suspensions of each strain were vortexed for 10 s and incubated at 20 ± 2 ºC. The upper suspension (100 µl) was transferred to another tube after 24 h and the absorbance was measured at 600 nm. The auto-aggregation percentage was determined using the equation:

$$\text{Auto-aggregation [\%]} = [1 - (A_t/A_0)] \times 100$$

where: A_t represents the absorbance after 24 h and A_0 the absorbance at the beginning of the incubation.

For the co-aggregation assay the cell suspensions were prepared similarly to the auto-aggregation test. Equal volumes of the yeast and each pathogen suspensions were mixed together by vortexing (10 s). As the reference tubes, there were used the samples from the auto-aggregation assay containing aliquots of a single bacterial suspension. The absorbance of suspensions was measured after 24 h as described above. The percentage of co-aggregation was calculated according to Handley et al. [13]:

$$\text{Co-aggregation [\%]} = [(A_x + A_y)/2 - (A_{(x+y)})]/[(A_x + A_y)/2] \times 100$$

where: x and y represent each of the two strains in the control tubes and (x + y) their mixture. Each experiment was performed in triplicate.

Adherence to Caco-2 cells in the competition assay with pathogens

The ability of selected *S. cerevisiae* strain to adhere to epithelial cells was determined using the Caco-2 cells, which at a concentration of 2.5×10^5 cells/well were cultured in 24-well tissue culture plates (Becton, Dickinson and Co., Franklin Lakes, USA) to obtain confluent monolayers.

The strains of yeast and pathogenic bacteria were grown at 30 ºC in YPG broth or at 37 ºC in a nutrient broth, respectively, then centrifuged (3852 × g, 10 min), washed with sterile PBS and re-suspended in fresh DMEM without antibiotics and supplements. Subsequently, the suspension of single strain (control) or combinations of *S. cerevisiae* with each pathogen (1:1, v/v) were deposited on the Caco-2 cells monolayers in an amount of 10^7 CFU/ml and incubated at 37 ºC in 5 % CO_2 for 2 h. Each strain or its combination was tested in triplicate. After incubation the unattached microorganisms were washed away, whereas the Caco-2 cells with bacteria adhered were detached from the bottom of the well with 1 % (w/v) trypsin-EDTA (Sigma-Aldrich, St. Louis, USA) and additionally scraped with a sterile cell scraper (Greiner Bio-One GmbH, Frickenhausen, Germany). Afterwards the Caco-2 cells were centrifuged (3852 × g, 10 min) and lysed with 0.1 % (v/v) Triton X-100 (Sigma-Aldrich, St. Louis, USA) for 5 min. The enumeration of the adhered microorganisms was performed by a plate method using YPG agar (yeast; incubation at 30 ºC, 48 h) or a nutrient agar (pathogens; incubation at 37 ºC, 48 h). The adherence inhibition rate [%] was expressed as a ratio of the count of adhered bacteria to the count of total bacteria added initially to each well according to the formula:

$$\text{Adherence inhibition [\%]} = \{(\log(\text{adherence of the tested sample}) \times 100)/[\log(\text{adherence of the control})]\} - 100$$

This represents a percentage reduction in the adhesion of pathogenic bacteria in the presence of the selected *S. cerevisiae* strains (as compared to the results for the pathogen alone).

The above adherence assay was also performed in a Nunc 8-well Lab-TekTM Chamber Slides system (Thermo Fisher Scientific, Waltham, USA) in order to visualise the yeast cells attached to Caco-2. After removing the non-adhered microorganisms the wells were washed with PBS and the Caco-2 cells were fixed with 80 % (v/v) methanol (15 min). After air-drying the preparations were stained with 0.1 % (w/v) crystal violet for 10 min. After staining the wells were washed with 70 % (v/v) ethanol until no colour remained and next dried overnight. The adhered *S. cerevisiae* strain was observed at 1000 × magnification under a phase-contrast microscope (Nikon, Tokyo, Japan) connected to a digital camera (Nikon Digital Sight DS-U3, Tokyo, Japan) using an imaging software (NIS-elements BR 3.0, Nikon, Tokyo, Japan).

Statistical analysis

The results presented in the research study constitute an arithmetic mean of the values from three repetitions. Before performing the ANOVA analysis of variances (at a significance level of $p < 0.05$), the Shapiro-Wilk's and Bartlett's tests were accomplished in order to confirm the normal distribution and homogeneity of variances of the variables, respectively. The multi-way ANOVA was carried out to statistically analyse the resistivity to acidic environment and the bile salts concentrations data. However, the one-way ANOVA was used to describe the auto- and co-aggregation data and single strains adhesion to the Caco-2 cells and the competition assay. Moreover, the Tukey's post hoc test was used after each ANOVA. Furthermore, the Pearson's correlation test was conducted to determine the association between the hydrophobicity and the aggregation abilities of the selected *S. cerevisiae* strain. The statistical analysis was performed with the use of XLSTAT software (Addinsoft, SARL, Paris, France).

Results and discussion

Resistance to bile salts and acidic conditions

The resistivity of the *S. cerevisiae* strains analysed in the presence of bile salts varied and ranged between 66.07 % and 98.97 % depending on the bile salts concentration and duration period of incubation (Tab. 2). The survivability of the strains was strain-specific and a significantly higher resistivity was found in the case of *S. cerevisiae* ŁOCK 0119, the endurance of which exceeded 90 % even up to 4 h of incubation. The above results correspond to the results as reported by Diosma et al. [8], who reported no significant changes in the abundance of live *S. cerevisiae* cells in the presence of bile salts for 6 out of 15 strains, which indicated a high bile salts resistance.

Moreover, the strains were able to survive in the acidic environment (pH 2, pH 3), for which the yeast population density was reduced by up to 33 % after 4 h of incubation (Tab. 3). The *S. cerevisiae* ŁOCK 0119 was the only strain where no substantial decrease in the survivability was reported; the count of their live cells was reduced no

more than 7 % after 4 h of incubation and the results were in line with those obtained by Srinivas et al. [24].

Furthermore, no significant variations in the survivability of yeast were observed between pH levels or the different bile salts concentrations.

Table 2. Survivability of *S. cerevisiae* strains in the presence of bile salts
Tabela 2. Przeżywalność szczepów drożdży *S. cerevisiae* w obecności soli żółci

Survivability / Przeżywalność [%]					
S. cerevisiae strain Szczep *S. cerevisiae*	Bile salt concentration Stężenie soli żółci [% w/v]	Time / Czas [h]			
		0	1	2	4
ŁOCK 0068	1	100 k	88.26 ± 2.58 f, g, h, i, j, k	79.29 ± 2.48 b, c, d, e, f, g, h	71.37 ± 5.33 a, b
	2		88.19 ± 2.54 e, f, g, h, i, j, k	76.74 ± 2.14 a, b, c, d, e, f	70.76 ± 5.25 a, b
ŁOCK 0113	1		88.33 ± 2.96 f, g, h, i, j, k	78.17 ± 2.08 a, b, c, d, e, f, g	70.29 ± 3.61 a, b
	2		87.06 ± 1.88 d, e, f, g, h, i, j	75.66 ± 3.17 a, b, c, d	69.69 ± 4.90 a, b
ŁOCK 0119	1		98.97 ± 3.58 j, k	96.30 ± 3.29 i, j, k	93.89 ± 2.47 i, j, k
	2		97.00 ± 3.80 i, j, k	93.03 ± 1.11 i, j, k	91.47 ± 1.73 h, i, j, k
ŁOCK 0137	1		88.77 ± 1.25 f, g, h, i, j, k	76.00 ± 5.73 a, b, c, d, e	68.55 ± 1.16 a, b
	2		88.39 ± 3.18 f, g, h, i, j, k	73.63 ± 4.72 a, b, c	66.90 ± 2.70 a
ŁOCK 0140	1		88.96 ± 4.10 f, g, h, i, j, k	75.82 ± 4.57 a, b, c, d	67.89 ± 2.69 a, b
	2		85.75 ± 4.04 c, d, e, f, g, h, i	74.07 ± 5.37 a, b, c	66.07 ± 2.94 a
ŁOCK 0142	1		89.07 ± 5.06 g, h, i, j, k	77.11 ± 1.29 a, b, c, d, e, f, g	71.80 ± 3.43 a, b
	2		86.59 ± 6.49 d, e, f, g, h, i	75.41 ± 4.77 a, b, c, d	68.85 ± 1.70 a, b

Explanatory notes / Objaśnienia:
Table shows mean values ± standard deviations / W tabeli przedstawiono wartości średnie ± odchylenia standardowe; n = 3; a-k – mean values denoted by different letters differ statistically significantly at $p < 0.05$ / wartości średnie oznaczone różnymi literami różnią się statystycznie istotnie przy $p < 0,05$.

Table 3. Survivability of *S. cerevisiae* strains in acidic environment
Tabela 3. Przeżywalność szczepów drożdży *S. cerevisiae* w kwaśnym środowisku

Survivability / Przeżywalność [%]					
S. cerevisiae strain Szczep *S. cerevisiae*	pH	Time / Czas [h]			
		0	1	2	4
ŁOCK 0068	2	100 k	83.19 ± 6.99 c, d, e, f, g, h, i, j	71.64 ± 3.74 a, b, c	67.54 ± 0.89 a
	3		88.40 ± 1.31 e, f, g, h, i, j, k	75.76 ± 6.00 a, b, c, d, e, f	69.66 ± 4.93 a, b
ŁOCK 0113	2		89.05 ± 2.72 g, h, i, j, k	74.89 ± 5.47 a, b, c, d	70.15 ± 5.04 a, b, c
	3		88.95 ± 2.83 f, g, h, i, j, k	78.4 5 ± 1.60 a, b, c, d, e, f, g, h, i	70.52 ± 4.34 a, b, c
ŁOCK 0119	2		96.63 ± 5.00 k	94.41 ± 3.11 j, k	93.22 ± 3.21 j, k
	3		98.83 ± 5.17 k	97.79 ± 4.79 k	96.90 ± 3.98 k
ŁOCK 0137	2		82.67 ± 3.37 b, c, d, e, f, g, h, i, j	75.74 ± 5.48 a, b, c, d, e	71.62 ± 5.10 a, b, c
	3		89.44 ± 2.63 h, i, j, k	78.29 ± 2.67 a, b, c, d, e, f, g, h, i	72.77 ± 3.83 a, b, c
ŁOCK 0140	2		88.66 ± 3.01 e, f, g, h, i, j, k	76.00 ± 3.14 a, b, c, d, e, f, g	70.54 ± 2.08 a, b, c
	3		90.48 ± 3.61 i, j, k	78.46 ± 2.42 a, b, c, d, e, f, g, h, i	74.29 ± 5.06 a, b, c
ŁOCK 0142	2		87.70 ± 4.78 d, e, f, g, h, i, j, k	73.58 ± 4.22 a, b, c	69.06 ± 5.78 a
	3		89.22 ± 2.06 h, i, j, k	76.41 ± 3.07 a, b, c, d, e, f, g, h	73.45 ± 2.42 a, b, c

Explanatory notes as in Tab. 2. / Objaśnienia jak pod tab. 2.

Based on the high survival rate and the detoxification properties of mycotoxins described by Chlebicz and Śliżewska [6], *S. cerevisiae* ŁOCK 0119 was selected in order to further probe other probiotic features of the yeast strain.

Hydrophobicity, auto-aggregation and co-aggregation assay with pathogens

The hydrophobicity of *S. cerevisiae* ŁOCK 0119 was at a level of 6.51 %, which classified this strain as hydrophilic according to Chae et al. [5] and Ben Taheur et al. [2]. The results opposite to those reported in the authors' own study were obtained by

De Lime et al. [11] and Fernandez-Pacheco et al. [7], who tested 28 and 10 *S. cerevisiae* strains, respectively, and those strains showed strong hydrophobicity.

Despite the hydrophilic characteristic of cell surface of *S. cerevisiae* ŁOCK 0119, this strain exhibited a strong ability to aggregate, which was in line with the data presented by Suvarna et al. [25]. A strong negative correlation was found between the hydrophobicity and the auto-aggregation capability of yeast (Pearson's correlation $r = -0.906$, $p < 0.05$). Moreover, a substantially higher yeast auto-aggregation (88.31 %) was observed in comparison to pathogenic bacteria (Tab. 4).

Table 4. Auto-aggregation of *S. cerevisiae* yeast and pathogenic bacteria
Tabela 4. Autoagregacja drożdży *S. cerevisiae* oraz bakterii patogennych

Strain / Szczep	Auto-aggregation / Autoagregacja [%]
S. cerevisiae ŁOCK 0119	$88.31^{c} \pm 2.44$
Pathogen / Patogen	
L. monocytogenes ATCC 13932	$70.02^{b} \pm 1.57$
S. Choleraesuis PCM 2565	$60.63^{a} \pm 0.93$
S. Enteritidis ATCC 13076	$73.68^{b} \pm 2{,}94$
S. Typhimurium ATCC 13311	$74.82^{b} \pm 1.09$

Table shows mean values ± standard deviations / W tabeli przedstawiono wartości średnie ± odchylenia standardowe; n = 3; a, b, c – mean values denoted by different letters differ statistically significantly at p < 0.05 / wartości średnie oznaczone różnymi literami różnią się statystycznie istotnie przy p < 0,05.

Table 5. Co-aggregation of *S. cerevisiae* ŁOCK 0119 with pathogenic bacteria from *Salmonella* genus and *L. monocytogenes*
Tabela 5. Koagregacja szczepu drożdży *S. cerevisiae* ŁOCK 0119 z patogennymi szczepami bakterii z rodzaju *Salmonella* oraz *L. monocytogenes*

Pathogen / Patogen	Co-aggregation / Koagregacja [%]
L. monocytogenes ATCC 13932	$70.25^{c} \pm 3.84$
S. Choleraesuis PCM 2565	$74.66^{d} \pm 0.88$
S. Enteritidis ATCC 13076	$46.87^{b} \pm 4.64$
S. Typhimurium ATCC 13311	$40.10^{a} \pm 5.32$

Table shows mean values ± standard deviations / W tabeli przedstawiono wartości średnie ± odchylenia standardowe; n = 3; a-d – mean values denoted by different letters differ statistically significantly at p < 0.05 / wartości średnie oznaczone różnymi literami różnią się statystycznie istotnie przy p < 0,05.

On the other hand, the co-aggregation of the selected yeast strain with pathogens was significantly diversified and differed from 40.10 % to 74.66 % (Tab. 5). *S. cerevisiae* ŁOCK 0119 co-aggregated with *S.* Choleraesuis PCM 2565 to the greatest extent, whereas the weakest aggregation of yeast was observed with *S.* Typhimurium ATCC 13311. Pizzolitto et al. [20] observed a substantially lower co-aggregation be-

tween the 4 *S. cerevisiae* strains tested and *Salmonella enterica* subsp. *enterica,* than that reported in the case of the authors' own strain. However, as for the co-aggregation of *S. cerevisiae* strains with *S.* Enteritidis, the data of the authors were in agreement with those of Binetti et al. [3]. Also, a strong co-aggregation feature of the yeast strain was also noted towards the *L. monocytogenes* ATCC 13932 (70.25 %).

Adherence to Caco-2 cells and the competition assay with pathogens

It was found that the adherence rate of *S. cerevisiae* ŁOCK 0119 monoculture (Tab. 6, Photo 1) was at a level of 77.56 %, which was considerably higher than the attachment rate of the yeast strains analysed by Menezes et al. [19], although it corresponded to the outcomes by Živković et al. [28].

Table 6. Adherence of monocultures of *S. cerevisiae* ŁOCK 0119 and pathogenic bacteria to human colon adenocarcinoma cell line (Caco-2)
Tabela 6. Adhezja monokultur szczepu drożdży *S. cerevisiae* ŁOCK oraz bakterii patogennych do linii komórkowej ludzkiego gruczolaka (Caco-2)

Strain / Szczep	Adherence rate / Stopień adherencji [%]
S. cerevisiae ŁOCK 0119	$77.56^{a} \pm 3.55$
Pathogen / Patogen	
L. monocytogenes ATCC 13932	$90.79^{b} \pm 4.65$
S. Choleraesuis PCM 2565	$96.31^{b} \pm 1.97$
S. Enteritidis ATCC 13076	$93.28^{b} \pm 3.59$
S. Typhimurium ATCC 13311	$91.68^{b} \pm 2.93$

Table shows mean values ± standard deviations / W tabeli przedstawiono wartości średnie ± odchylenia standardowe; n = 3; a, b – mean values denoted by different letters differ statistically significantly at $p < 0.05$ / wartości średnie oznaczone różnymi literami różnią się statystycznie istotnie przy p < 0,05.

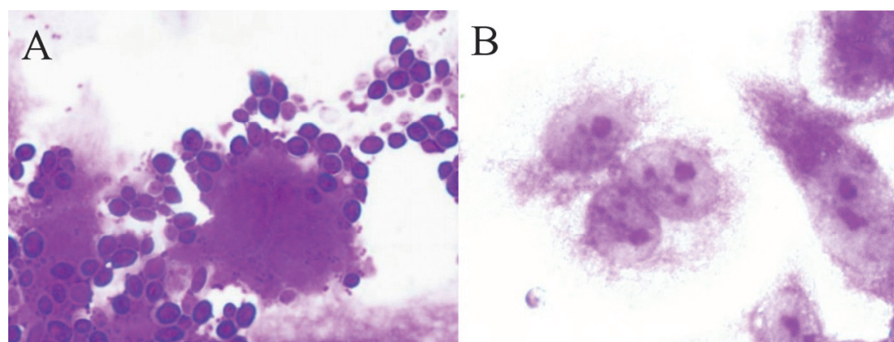

Photo 1. Adherence *S. cerevisiae* ŁOCK 0119 (A.) to human colon adenocarcinoma cell line (Caco-2) after 2 h incubation (1000 ×, Nikon – Eclipse, Tokyo, Japan) stained with crystal violet
Fot. 1. Adhezja komórek drożdży *S. cerevisiae* ŁOCK 0119 (A.) do linii komórkowej ludzkiego gruczolaka (Caco-2) po 2 h inkubacji (powiększenie 1000 ×, Nikon – Eclipse, Tokio, Japan) barwiona fioletem krystalicznym

Table 7. *S. cerevisiae* ŁOCK 0119-caused inhibition of pathogenic bacteria adherence to cell line of human colon adenocarcinoma cell line (Caco-2)

Tabela 7. Hamowanie przez drożdże *S. cerevisiae* ŁOCK 0119 adhezji bakterii patogennych do linii komórkowej gruczolaka ludzkiego (Caco-2)

Pathogen / Patogen	Inhibition rate / Stopień inhibicji [%]
L. monocytogenes ATCC 13932	$15.13^{a} \pm 3.76$
S. Choleraesuis PCM 2565	$36.70^{b} \pm 1.07$
S. Enteritidis ATCC 13076	$37.30^{b} \pm 1.06$
S. Typhimurium ATCC 13311	$24.62^{a,b} \pm 5.12$

Table shows mean values ± standard deviations / W tabeli przedstawiono wartości średnie ± odchylenia standardowe; n = 3; a, b – mean values denoted by different letters differ statistically significantly at $p < 0.05$ / wartości średnie oznaczone różnymi literami różnią się statystycznie istotnie przy $p < 0,05$.

S. cerevisiae ŁOCK 0119 was able to inhibit the attachment of those strains to the Caco-2 cells (Tab. 7) despite the weaker attachment capability of the analysed yeast strain in contrast to the one exhibited by each of the pathogenic bacteria, which varied between 90.85 ÷ 96.31 % (mean 92.81). The adhesion of *S.* Choleraesuis PCM 2565 and *S.* Enteritidis ATCC 13076 was reduced 36.70 % and 37.30 %, respectively, which was a significantly stronger effect than that as reported in the case of *L. monocytogenes* ATCC 13932 (15.13 %). The attachment of *S.* Typhimurium ATCC 13311 was also inhibited by *S. cerevisiae* ŁOCK 0119 at a level of 24.62 %.

Conclusions

1. The analysed yeast strain, i.e. *S. cerevisiae* ŁOCK 0119 can exert a beneficial impact on the health of monogastric animals since it has the potential to survive gastrointestinal passage as well as to colonize the intestines.
2. *S. cerevisiae* ŁOCK 0119 can decrease the number of pathogenic bacteria, through the co-aggregation and competition for adhesion site.

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WŁAŚCIWOŚCI PROBIOTYCZNE DROŻDŻY *SACCHAROMYCES CEREVISIAE* ŁOCK 0119

Streszczenie

Probiotyki, jak również ich połączenia z prebiotykami określane mianem synbiotyków, są szeroko badane jako alternatywa antybiotykowych stymulatorów wzrostu, które powszechnie stosowano w hodowli zwierząt. Spośród wielu mikroorganizmów, które mogą być pożyteczne dla gospodarza, drożdże *Saccharomyces cerevisiae* wykazują potencjał probiotyczny.

Celem pracy było wyselekcjonowanie szczepu *S. cerevisiae* odpowiedniego do stosowania jako probiotyk w nowo opracowywanych preparatach synbiotycznych dla zwierząt monogastrycznych (dla drobiu, trzody chlewnej). Przeżywalność szczepów drożdży w obecności soli żółciowych i niskiego pH była zróżnicowana, przy czym wahała się w zakresie odpowiednio: 66 ÷ 94 % i 68 ÷ 97 % po 4 h inkubacji. Najwyższą przeżywalnością charakteryzował się szczep *S. cerevisiae* ŁOCK 0119, którego liczba żywych komórek została obniżona o nie więcej niż o 9 % po 4 h inkubacji w ustalonych warunkach. Szczep ten wykazywał również właściwości hydrofilowe oraz silny potencjał auto- i koagregacji. Wyselekcjonowany szczep drożdży koagregował z bakteriami chorobotwórczymi (*Salmonella* spp., *Listeria monocytogenes*) w zróżnicowanym stopniu w zakresie 40 ÷ 75 %. *S. cerevisiae* ŁOCK 0119 hamował adhezję patogenów do komórek Caco-2, przy czym przyleganie komórek bakterii chorobotwórczych było ograniczane

w zróżnicowanym stopniu (15 ÷ 37 %). Na podstawie uzyskanych wyników stwierdzono, że wyselekcjonowany szczep *S. cerevisiae* ŁOCK 0119 wykazuje korzystne właściwości, takie jak możliwość przetrwania pasażu jelitowego i kolonizacji danego środowiska oraz ograniczanie wzrostu patogennej flory bakteryjnej.

Słowa kluczowe: probiotyki, drożdże *S. cerevisiae*, hydrofobowość, agregacja, adherencja

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