

Influence of Yeast Strains on Microbiological Stability of Wheat Bread

E. Soboleva, E. Sergacheva, S. G. Davydenko, T. V. Meledina

Abstract—Problem of food preservation is extremely important for mankind. Viscous damage ("illness") of bread results from development of *Bacillus* spp. bacteria. High temperature resistant spores of this microorganism are steady against 120°C) and remain in bread during pastries, potentially causing spoilage of the final product. Scientists are interested in further characterization of bread spoiling *Bacillus* spp. species. Our aim was to find weather yeast *Saccharomyces cerevisiae* strains that are able to produce natural antimicrobial killer factor can preserve bread illness. By diffusion method, we showed yeast antagonistic activity against spore-forming bacteria. Experimental technological parameters were the same as for bakers' yeasts production on the industrial scale. Risograph test during dough fermentation demonstrated gas production. The major finding of the study was a clear indication of the presence of killer yeast strain antagonistic activity against rope in bread causing bacteria. After demonstrating antagonistic effect of *S. cerevisiae* on bacteria using solid nutrient medium, we tested baked bread under provocative conditions. We also measured formation of carbon dioxide in the dough, dough-making duration and quality of the final products, when using different strains of *S. cerevisiae*. It is determined that the use of yeast *S. cerevisiae* RCAM 01730 killer strain inhibits appearance of rope in bread. Thus, natural yeast antimicrobial killer toxin, produced by some *S. cerevisiae* strains is an anti-rope in bread protector.

Keywords—Bakers' yeasts, rope in bread, *Saccharomyces cerevisiae*.

I. INTRODUCTION

THE main infection sources during bread production are flour, bran and grain [1], [2]. Violation of processing procedures and poor sanitary conditions can be the cause of the final product disease. The vegetative cells of *Bacillus* bacteria die during baking in the oven, but their spores are heat resistant and can germinate during bread storage under favorable conditions [3].

At the first stage of spoilage, the bread loses its native flavor and then specific sweet odor appears similar to that of overripe melon or valerian [4]-[6]. During spoilage, the odor intensifies and takes on a putrefactive character. The crumb becomes sticky; when the loaf is torn, one can see slimy stretchy strings. Sometimes these thin silvery slimy web like strings stretch up to 50 sm. Hence the other name of this

disease, the rope. In German such phenomenon is called "Fadenziehen des Brotes", in English, "Rope in bread", in Russian, "Potato disease". Crumb color changes, blotches of yellow, brown and muddy pink appear. As the disease enters its final stage, bread turns into a dark slimy mass with a coarse odor and strong off-taste. The pores collapse, small holes and then larger ruptures appear in the crumb [7]-[10].

Bacterial cells have active amylolytic and proteolytic enzymes. The proteins are broken down and amides, amines, amino acids and peptides appear. Dextrin, mono- and disaccharides appear as a result of starch is hydrolyzes. The former contribute to crumb stickiness. Substances with a specific acrid odor appear due to interaction of mono- and disaccharides with amines and amides. The amount of aldehydes and other compounds rises sharply which contributes to the rotten smell of the diseased bread [5], [6]. Consumption of bakery products affected by rope can lead to accumulation of metabolites such as toxins and degradation products.

Previously the rope in bread appeared in regions with hot climate, mainly in summer. Now outbreaks of rope in bread arise in the northern regions of European Russia, the Urals and Siberia. In addition, bread has become prone to rope not only in summer but also in spring, autumn and even winter [11].

The most favorable conditions for *Bacillus* bacteria spores germination and growth are high relative humidity (over 80%), storage temperature (30 to 40°C/85 to 105° F) and low titratable acidity (under 3 degrees, while pH ranges from 5 to 10). Products made of rye flour are less prone to rope because of their higher titratable acidity (more than 5 degrees, pH less than 4.5) [12]. Therefore, this type of microbial spoilage is most typical for bakery products made of wheat flour.

The main factors that inhibit the development of rope in bread and corresponding pathogens are high acidity, low humidity, increased content of sugar and fat in the product (15–20% of the total weight of flour), the antibiotic activity of the medium. In this regard, bakeries use different methods, devices and techniques to fight rope in bread at all stages of manufacturing. There exist various methods for preventing the development of microbial spoilage of bakery products, chemical, physical and biological.

The most effective methods of rope control are biological methods, such that involve liquid yeast, sourdough and starter cultures. In recent years, there is significant demand for research of yeast strains capability to fight microbial spoilage of food substances of microbial origin [12]. Microorganisms of starter cultures used in baking are able to synthesize substances inhibiting exogenous microflora development in

E. Soboleva is with ITMO University, 191002, St. Petersburg, Lomonosova str., 9 Russia (phone: +7 921 923 88; e-mail: elenavsoboleva@mail.ru).

E. Sergacheva, T. V. Meledina are with ITMO University, 191002, St. Petersburg, Lomonosova str., 9 Russia (e-mail: essergacheva@mail.ru, tatiana.meledina@yandex.ru).

S. G. Davydenko is with Baltika Breweries Part of Carlsberg Group 194292 St. Petersburg, 6 Verkhny per., 3 Lomonosova str., 9 Russia (e-mail: davydenko@baltika.com).

final products. The antagonistic activity of these microorganisms is due to accumulation of their metabolic products in semi products. The main batch of active metabolites is built up by lactic acid bacteria, which produce an array of natural antibacterial substances, including organic acids, carbon dioxide, hydrogen peroxide, diacetyl, ethanol, bacteriocins, reuterin, reutericyclin [13]-[16]. As to the yeast, their antagonistic role is not normally considered. However, in 1909 F. Hayduck published data on yeast proteins (killer factors) being toxic to other strains [17].

Antagonistic activity of yeast is caused by competition for nutrients, medium pH changing due to the ion equilibrium shift or formation of organic acids, production of large quantities of ethanol, release of antibacterial and antimicrobial substances, such as killer toxins — mycocins [18], [19]. Mycocins are extracellular proteins, mainly glycoproteins that inhibit normal cell membrane functioning (integrity, osmotic permeability) in yeasts that have receptors with corresponding sensitivity [18]. Mycocin activity effects yeast cells genetically closely related to the killer producer strain, serving as a protective factor.

The yeast *S. cerevisiae* synthesizes several types of killer factors, some of which attach to β -1,3-glucan chains, opening channels in the cell membrane of sensitive strains, and others attach to mannoproteins of the cell wall and inhibit DNA synthesis [20].

Mycocin production occurs not only in *Saccharomyces* yeasts but also in *Candida*, *Cryptococcus*, *Debaryomyces*, *Kluveromyces*, *Pichia*, *Torulopsis*, *Williopsis* and *Zygosaccharomyces* [18], [19]. Effect of killer factors on bacterial microflora is a part for further investigations.

The development of spoilage microorganisms has a direct impact on final product quality. Therefore adding antagonistic yeast starter cultures is bound to enhance product safety by inhibiting pathogenic microorganisms growth during fermentation and final product sensory qualities and shelf life by inhibiting spoilage flora development [5], [6], [21]-[24].

Saccharomyces cerevisiae yeasts are the principal microorganisms in bread production, and as such, they show promise as an innate antagonist to bacteria *Bacillus* spp. that cause rope in bread.

II. MATERIALS AND METHODS

Yeast strains *Saccharomyces cerevisiae* RCAM 02150 (designated further as strain A) and *S. cerevisiae* RCAM 01730 (designated further as strain B), bacteria strains *Bacillus subtilis* KM and *B. licheniformis* 1, dough, wheat bread.

We showed yeast antagonistic activity against spore-forming bacteria by diffusion method. [24].

Bread production and storage tests. Yeast strains *Saccharomyces cerevisiae* RCAM 02150 and *S. cerevisiae* RCAM 01730 used in the experiment were grown in pilot plant conditions subject to all parameters and stages applied in pressed bakers' yeasts production on the industrial scale.

Kneading 1,000 g of wheat flour (labeled "premium" according to the Russian classification) and 560 g of tap water in a mixer (Kitchen Aid 5KPM5 Professional, St. Joseph,

Mich.) for 5 min at room temperature formed bread doughs. Yeast inoculum added at this stage was such that resulted in about 1.0×10^9 CFU g^{-1} (1%), 2.5×10^9 CFU g^{-1} (2.5%), 4.0×10^9 CFU g^{-1} (4.0%) viable yeast cells in the resulting doughs. After mixing, we shaped the doughs into roughly 400 g loaves, placed them in aluminum pans, and leavened at 35°C until the volume was twice the initial volume. We baked leavened doughs in an oven at 210°C for 25 min. After cooling, the samples were stored at 37°C, 90% humidity (provocative conditions). In 24 hours, we examined the loaves externally for the presence of the typical sweet fruity odor, which characterizes the first stage of rope disease. We cut the breads that gave off an intense smell cut into halves and inspected their crumb.

Risograph test fermentation showed the volume of gas produced by different strains during dough fermentation (National Manufacturing). We left the dough ferment for a maximum of 240 min at 30°C in the risograph test chamber and measured gas volume continuously at 1 min intervals [25].

III. RESULTS AND DISCUSSION

Based on antagonistic activity and gas production capacity we selected *S. cerevisiae* RCAM 01730 through the screening of yeast strains from different microorganism collections.

The purpose of the research was to investigate the impact of *S. cerevisiae* yeast microorganisms on development of bacteria causing rope in bread, following the steps listed:

- define antagonistic effect of *S. cerevisiae* on bacteria causing rope in bread using a solid nutrient medium, and then by putting test baked bread under provocative conditions;
- determine the effect of *S. cerevisiae* on carbon dioxide production the dough, the dough-making duration and the quality of the final products.

Control samples were prepared using *S. cerevisiae* RCAM 02150 yeasts widely used in the baking industry.

We showed yeast antagonistic activity against spore-forming *Bacillus subtilis* KM and *B. licheniformis* 1 by diffusion to meat-peptone agar method. See the results of yeast culture liquid supernatants influence on testing bacterial culture in Table I.

It is determined that culture liquid supernatant of the strain *S. cerevisiae* RCAM 01730 (strain B) inhibits the growth of bacteria of the genera *Bacillus* in the lunulas. On the other hand, culture liquid supernatant of the strain *S. cerevisiae* RCAM 02150 (strain A) intensifies bacterial growth. It allows an assumption about the presence of antagonistic activity of *S. cerevisiae* RCAM 01730 against bacteria of the genera *Bacillus*. This strain can prevent of rope spoilage of bread during storage.

The main yeasts processing behavior is gas-production property resulting from enzyme activity. The maltase and zymase activities, dough fermentation property and osmo sensitivity have been determined. The tested yeasts have the higher zymase and maltase activities and dough fermentation property than the control sample by 17%, 15% and 25% correspondingly.

TABLE I
 INFLUENCE OF YEAST STRAINS ON DEVELOPMENT OF BACTERIA CAUSING ROPE IN BREAD

Effects	Yeast strain <i>S. cerevisiae</i>			
	A		B	
	<i>B. subtilis</i> KM	<i>B. licheniformis</i> 1	<i>B. subtilis</i> KM	<i>B. licheniformis</i> 1
bacteria growth in the lunula	+	-	+	-
stimulating rings	+	-	+	-
inhibition zone, mm	10		12	

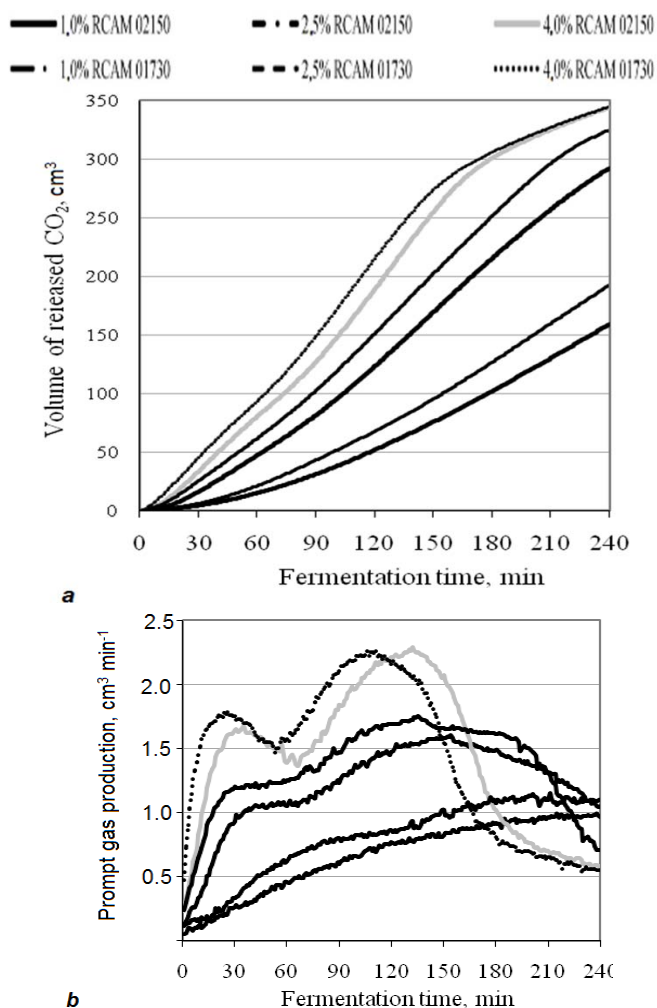


Fig. 1 Yeasts strains influence on total volume of released gas (a) and volume of prompt gas production (b)

Yeasts fermentation activity influenced on dough maturation time and finished product quality has been estimated by total volume of released gas, the instant gas-production, amount of residual sugars and free α -amine nitrogen FAN in the dough.

The carbon dioxide production intensity was determined in dough samples prepared with the using of yeasts in dosages 1.0%, 2.5% and 4.0%, which correspond to sponge and dough method, straight dough bulk fermentation and rapid processing. Yeasts strain *S. cerevisiae* RCAM 01730 (dosage 4.0%) increase volume of carbon dioxide by 15% during 120 min of fermentation, then 2.5% during 180 min gives more by

18%, 1.0% during 240 min- more by 21% in comparison with yeasts *S. cerevisiae* RCAM 02150 shown (Fig. 1 (a)).

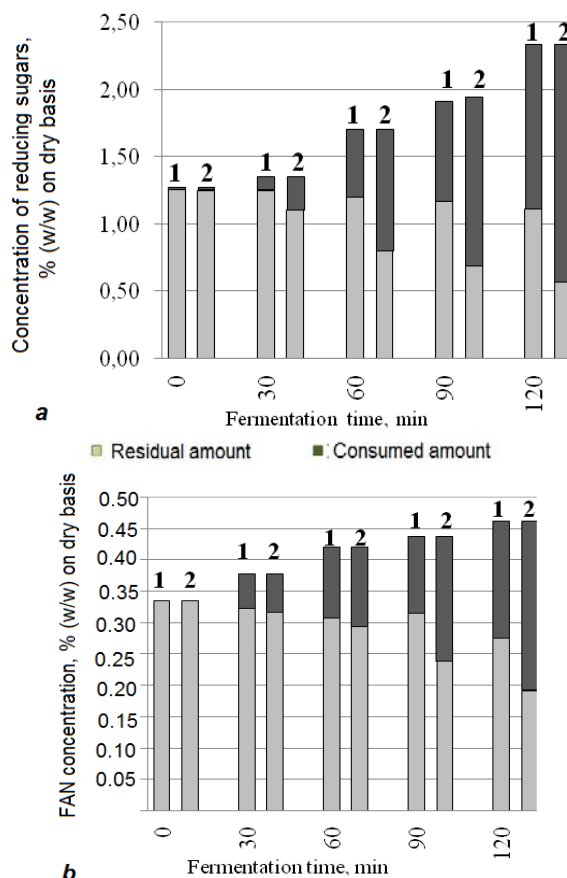


Fig. 2 Changes in the content of reducing sugars (a) and FAN (b) in the dough with *S. cerevisiae* RCAM 02150 (1) and *S. cerevisiae* RCAM 01730 (2).

The prompt gas-production permits to compare the activity of different yeasts and determine the end of the dough fermentation by means of maximum and reduction trend of this characteristic under straight dough method and the end of the dough piece proofing under rapid processing.

The investigation has shown that the maximum prompt gas-production when adding yeasts 4,0% of *S. cerevisiae* R CAM 01730 was at 98-105 min, for RCAM 02150 – 130-136 min. 2.5%: of *S. cerevisiae* RCAM 01730 gave maximum at 128-136 min, RCAM 02150 – 153-158 min.

Due to the action of amylolytic enzymes, the reduction of sugars and FAN utilization by microorganisms occurs during the fermentation.

Studying changes in the content of reducing sugars and the FAN during the dough fermentation (Fig. 2), showed samples prepared with the yeast *S. cerevisiae* RCAM 01730, had intensive decrease of reducing sugars and FAN. In two hours of fermentation, with RCAM 01730 number of fermented sugars was more than 21%, and consumed FAN - 17% compared with the control. Thus, the results of the study showed that the yeast strain *S. cerevisiae* RCAM 01730 are not inferior to strain RCAM 02150 in respect of

biotechnological properties, therefore, these yeasts are perspective for bread-production.

TABLE II
EFFECT OF STRAINS OF THE YEAST *S. CEREVISIAE* TO PROCESS PARAMETERS, QUALITY PARAMETERS OF SEMI-FINISHED AND FINAL PRODUCTS

Parameters and indicators	Methods of dough production	Pre-fermented			
		Firm		Liquid	
		A	B	A	B
Humidity, %	Pre-ferm.	41.5	41.5	68.9	68.8
	Dough	42.2	42.3	42.1	41.9
Acidity, deg	Pre-ferm.	3.2	3.5	2.8	3.0
	Dough	2.8	3.0	2.6	2.8
Fermentation, min	Pre-ferm.	210	210	240	240
	Dough	40	30	70	60
Proofing, min		80	65	90	60
Dough prod., min		330	305	400	360
Crumb humidity, %		41.5	41.5	41.5	41.5
Crumb acidity, deg		2.0	2.0	2.0	2.0
Porosity, %		73	78	74	80
Volume, sm ³ /g		3.35	3.60	3.45	3.65
Shape stability		0.52	0.55	0.50	0.54
		Non-PF		Accelerated	
Yeast strain			A	B	
Humidity, %		42.1	42.3	42.5	42.5
Acidity, deg		2.6	2.8	-	-
Fermentation, min		150	135	25	25
Proofing duration, min		41.5	41.5	41.5	
Total dough -making, min		1.8	1.6	1.8	
Crumb humidity, %		78	69	72	
Crumb acidity, deg		3.60	2.90	3.20	
Porosity, %		0.56	0.52	0.54	

The influence of yeasts strain *S. cerevisiae* RCAM 01730 on the maturation time, the characteristics of pre-fermented products under different technologies, the total time of dough production and the bread quality has been estimated. The dough was prepared from baker's top-grade wheat flour by rapid processing, straight dough fermentation and sponge and dough method (big tight and liquid sponges were used) with such dosages of yeasts as 4.0%, 2.5% and 1.0% correspondingly. The control was dough prepared with yeasts *S. cerevisiae* RCAM 02150.

Experimental yeast allows dough fermentation reduction and proofing the dough pieces (Table II). For example, by using the yeast *S. cerevisiae* RCAM 01730, the total duration of the dough development reduced by 8%, 10%, 14% and 22% by pre-fermented (firm and liquid pre-ferments), non-pre-fermented and accelerated methods respectively in comparison with using the yeast *S. cerevisiae* RCAM 02150. Thus, bread made by using strains of the yeast *S. cerevisiae* RCAM 01730 is not inferior to the control physical and chemical parameters (Table II).

The sensory analysis (Fig. 3) showed that experimental samples have developed a thin wall porosity and have a more pronounced pleasant smell and taste.

It is determined that the use of yeast *S. cerevisiae* RCAM 01730 inhibits appearance of signs rope in bread.

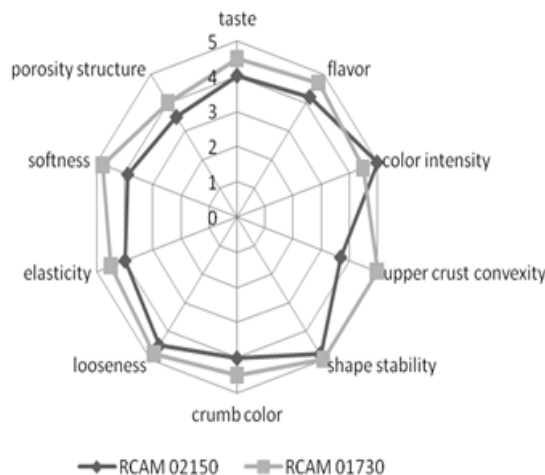


Fig. 3 Organoleptic quality indicators of bread made by non-pre-fermented method using different yeast strains

TABLE III
INFLUENCE OF YEAST STRAINS ON DEVELOPMENT OF MICROBIOLOGICAL SPOILAGE DURING THE BREAD STORAGE

Storage, hours	Dough-making method					
	sponge		straight		quick	
	A	B	A	B	A	B
	Development of rope in bread					
24	-	-	+	-	+	-
48	+	-	++	+	++	+
72	++	+	++	++	++	+
96	+++	++	+++	++	+++	++

"-" - no signs of microbiological spoilage;
"+" - initial signs of microbiological spoilage;
"++" - middle state of microbiological spoilage;
"+++" - intense microbiological spoilage

We performed test laboratory baking of wheat bread with these yeast. We prepared dough by sponge dough method, straight dough method and quick dough method with the yeast dosage yeast respectively 1.0, 2.5 and 4.0%. Bread was stored in provoking conditions - temperature $37 \pm 1^\circ\text{C}$ and relative humidity of 90%. We tested organoleptic quality and the appearance of signs rope in bread (Table III).

According to the research results it is possible to conclude that the strain *S. cerevisiae* RCAM 01730 has the antagonistic activity against the bacteria *B. subtilis*, *B. licheniformis*.

IV. CONCLUSIONS

During our research we have ascertained that yeast strain *S. cerevisiae* RCAM 01730 has a bacteriostatic influence on causative agencies of rope in bread. Usage of the strain *S. cerevisiae* RCAM 01730 instead of *S. cerevisiae* RCAM 02150 accelerates gas production in dough, intensifies process of dough maturation and improves organoleptic properties of bread quality.

ACKNOWLEDGMENT

This work was partially financed by the government of the Russian Federation, Grant 074-U01.

REFERENCES

- [1] Fangio, M. F., Roura, S. I., & Fritz, R. 2010. Isolation and identification of *Bacillus* spp. and related genera from different starchy foods. *Journal of food science*. 75 M218-M221.
- [2] Ybar, A., Cetinkaya, F., & Soyutemiz, G. E. 2012. Detection of rope-producing *Bacillus* in bread and identification of isolates to species level by Vitek 2 System. *Journal of Biological and Environmental Sciences*. 6(18), 243-248.
- [3] Sorokulova, I. B., Reva, O. N., Smirnov, V. V., Pinchuk, I. V., Lapa, S. V., & Urdaci, M. C. 2003. Genetic diversity and involvement in bread spoilage of *Bacillus* strains isolated from flour and rony bread. *Letters in applied microbiology*. 37(2), 169-173.
- [4] Puchkova, L.I.; Polandova, R.D.; Matveeva, I. 2005. *The technology of bread, pastry and pasta, vol. 1*. GIORD Publishing House, St Petersburg, 309 pp. (in Russian).
- [5] Von Holy, A. & Allan, C. 1990. Current perspectives on rope in bread. In Smith, M.F., Kort, M.J., Clarke, I.R. and Bush, P.B.: *Proceedings of the Second National Bakery Symposium*. Technikon Natal Printers, Durban, pp. 119-125
- [6] Voysey, P. A. 1989. Rope: a problem for bakers. *J. Appl. Bacteriol.* 67, xxv-xxvi.
- [7] Blackburn, C. (Ed.). 2006. *Food spoilage microorganisms*. Woodhead Publishing, 736 pp
- [8] Cauvain, S. P., & Young, L. S. (Eds.) 2007. *Technology of breadmaking*. Springer, New York, 420 pp.
- [9] Brandt, M. & Spicher, Gottfried. 2006. *Handbuch sauerteig: biologie, biochemie, technologie*. Behr, Stuttgart-Feuerbach, 428pp
- [10] Puchkova, L.I.; Polandova, R.D.; Matveeva, I. 2005. *The technology of bread, pastry and pasta, vol. 1*. GIORD Publishing House, St Petersburg, 309 pp. (in Russian).
- [11] Kosovañ, A.P. 2010. *Microbiological control production of food products from grains*. JSC Moscow Printing House 2, Moscow, 423 pp. (in Russian).
- [12] Hatoum R, Labrie S. and Fliss I. 2012 Antimicrobial and probiotic properties of yeasts: from fundamental to novel applications. *Front. Microbio.* 3:421. doi: 10.3389/fmicb.2012.00421.
- [13] Casas, I. A., & Dobrogosz, W. J. 2000. Validation of the probiotic concept: *Lactobacillus reuteri* confers broad-spectrum protection against disease in humans and animals. *Microbial ecology in health and disease*. 12(4), 247-285.
- [14] Gänzle, M. G., Höltzel, A., Walter, J., Jung, G., & Hammes, W. P. 2000. Characterization of Reutericyclin Produced by *Lactobacillus reuteri* LTH2584. *Applied and Environmental Microbiology*, 66(10), 4325-4333.
- [15] Ouwehand A. C., Vesterlund, S. 2004. *Antimicrobial Components from Lactic Acid Bacteria in Lactic Acid Bacteria Microbiological and Functional Aspects, Third Edition*. Editors Seppo Salminen, Atte von Wright, and Arthur Ouwehand, CRC Press, USA, <http://dx.doi.org/10.1201/9780824752033.ch11>
- [16] Talarico, T. L., & Dobrogosz, W. J. 1989. Chemical characterization of an antimicrobial substance produced by *Lactobacillus reuteri*. *Antimicrobial agents and chemotherapy*. 33(5), 674-679. doi:10.1128/aac.33.5.674.
- [17] Hayduck, F. 1909. Über einen Hefengiftstoff in Hefe. *Wochenschr. Brau.* 26, 677-679.
- [18] Golubev, W.I. 2006. *Antagonistic interactions among yeasts. Biodiversity and Ecophysiology of Yeasts*. Editors G.Péter and C.Rosa, Springer, p. 197-219.
- [19] Suzuki, C., Ando, Y., & Machida, S. 2001. Interaction of SMKT, a killer toxin produced by *Pichia farinosa*, with the yeast cell membranes. *Yeast*, 18(16), 1471-1478.
- [20] Priest, F. G. 2005. *Brewing Microbiology* / F. Priest, I. Campbell; translation from English under the editorship of Meledina, T.V., Soidla N. Professiya, St. Petersburg, 368 pp. (in Russian).
- [21] Blackburn, C. (Ed.). 2006. *Food spoilage microorganisms*. Woodhead Publishing, 736 pp
- [22] Puchkova, L.I.; Polandova, R.D.; Matveeva, I. 2005. *The technology of bread, pastry and pasta, vol. 1*. GIORD Publishing House, St Petersburg, 309 pp. (in Russian).
- [23] Salkinoja-Salonen, M. S., Vuorio, R., Andersson, M. A., Kämpfer, P., Andersson, M. C., Honkanen-Buzalski, T., & Scoging, A. C. 1999. Toxicogenic strains of *Bacillus licheniformis* related to food poisoning. *Applied and environmental microbiology*. 65 (10), 4637-4645.
- [24] Pepe, O., Blaiotta, G., Moschetti, G., Greco, T., & Villani, F. 2003. Rope-producing strains of *Bacillus* spp. from wheat bread and strategy for their control by lactic acid bacteria. *Applied and environmental microbiology*. 69(4), 2321-2329.
- [25] Rattin, G. E., Faubion, J. M., Walker, C. E., & Mense, A. L. 2009. Measuring yeast CO₂ production with the risograph. *Cereal foods world*. 54(6), 261-265.