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Original Article

Probiotic yeast *Saccharomyces cerevisiae* Az-12 isolated from pomegranate juice presented inhibitory effects against pathogenic bacteria

Levedura probiótica *Saccharomyces cerevisiae* Az-12 isolada de suco de romã apresentando efeitos inibitórios contra bactérias patogênicas

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Abstract

The potential probiotic yeast was isolated from the Kyzyl Anor pomegranate variety growing in the Turkestan region (Kazakhstan). The yeast strain was identified as *Saccharomyces cerevisiae* Az-12. Molecular genetic identification was carried out using the Sanger sequencing method. The degree of homology of the *S. cerevisiae* Az-12 strain with the strain MH608341.1 Saccharomyces cerevisiae isolate extr03 was 99.65%. Antagonistic effect of the yeast against pathogenic bacteria was confirmed according inhibition zones for *Staphylococcus aureus* 13.5 ± 0.05 mm; the inhibition zones for *Escherichia coli* 12.8 ± 0.05 mm; and 10.7 ± 0.05 mm for *Pseudomonas aeruginosa*. Scanning microscopy of *S. cerevisiae Az-12* and *S. aureus* confirmed the adhesive ability of the yeast cell surface to *S. aureus. S. cerevisiae* Az-12 were chosen as the most promising, as they are able to quickly ferment juices. Functional drinks containing pomegranate juice and yeast with a probiotic effect can be considered as a useful synbiotic product formulation.

Keywords: pomegranate juice, probiotics, *Saccharomyces cerevisiae* Az-12, inhibitory effect, bacteria.

Resumo

A levedura probiótica potencial foi compreendida da variedade de romã Kyzyl Anor que cresce na região do Turquestão (Cazaquistão). A cepa de levedura foi identificada como *Saccharomyces cerevisiae* Az-12. A identificação genética molecular foi realizada pelo método de sequenciamento de Sanger. O grau de homologia da cepa *S. cerevisiae* Az-12 com a cepa MH608341.1 *Saccharomyces cerevisiae* isolado extr-03 foi de 99,65%. O efeito antagônico da levedura contra bactérias patogênicas foi confirmado de acordo com as zonas de inibição para *Staphylococcus aureus* 13,5 ± 0,05 mm; as zonas de inibição para *Escherichia coli* 12,8 ± 0,05 mm; e 10,7 ± 0,05 mm para *Pseudomonas aeruginosa.* A microscopia de varredura de *S. cerevisiae Az-12* e *S. aureus* confirmou a capacidade de adesão da superfície celular de levedura a *S. aureus. S. cerevisiae Az-12* foram escolhidas como as mais promissoras, pois são capazes de fermentar sucos rapidamente. Bebidas funcionais contendo suco de romã e fermento com efeito probiótico podem ser consideradas como uma formulação de produto simbiótico útil.

Palavras-chave: suco de romã, probióticos, *Saccharomyces cerevisiae* Az-12, efeito inibitório, bactérias.

1. Introduction

An analysis of the health status of the Kazakhstan population shows that many residents have health problems. This is connected both with the rhythm of life and with the consumption of fast food, the environment also has a great influence (Abilkayir, 2019). Pomegranate fruits, belonging to the Punicaceae family, are an important source of sugars, vitamins, minerals, antioxidant polyphenols, especially tannins, anthocyanins and ellagic acid derivatives, which are beneficial for health. Therefore, recently there has been a massive increase in the popularity of the use of pomegranate (Stockton and Al-Dujaili, 2022; Rinaldi et al., 2013; Kaplan et al., 2001; Gil et al., 2000; Danesi and Ferguson, 2017; Calín-Sánchez et al., 2013; Cano-Lamadrid et al., 2017). Pomegranate has been clinically and experimentally proven to play a protective role against atherosclerosis, hypertension, cancer, type II diabetes, and obesity (McFarlin et al., 2009; Al-Muammar and Khan, 2012).

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A number of scientists suggest that fruit juices can serve as a suitable medium for the cultivation of probiotic microorganisms. At the same time, fruit juices already have an established market sector as a functional drink. Such juices are regularly consumed, which is extremely important when creating a new drink through the use of probiotics (Siti et al., 2016; Sheehan et al., 2007).

Modern methods of fermentation of plant products make it possible to use the metabolism of microorganisms to obtain valuable products (Wenli et al., 2022; Erkmen and Bozoglu, 2016a; Erkmen and Bozoglu, 2016b; Christensen et al., 2022; Feng et al., 2018, Pessoa et al., 2023). *Saccharomyces cerevisiae* is a widely used organism in both genetic engineering, electroporation manipulation (Alqosaibi et al., 2022) and fermentation industry (Willaert, 2017), scientists have identified the probiotic and beneficial potential of yeast for the human body. (Chigaeva and Dudikova, 2017; Guslandi et al., 2000, 2003).

Saccharomyces cerevisiae var. *boulardii* (Sb) is a yeast used in preparations with proven probiotic efficacy (McFarland, 2006; Beaugerie and Petit, 2004; Czerucka et al., 2007; Agarbati et al., 2020). Potentially probiotic yeast can be used to produce various fermented foods, improving their nutritional and organoleptic properties (Staniszewski and Kordowska-Wiater, 2021). There is information about the genome of Sb is a strain in an attempt to trace the genomic causes of this yeast's probiotic behavior. It has been found that proteins that appear to be specifically present in Sb are also present in some *Saccharomyces cerevisiae* (Sc) strains (Khatri et al., 2013). Sb was found not to form spores on sporulation medium even after one week of incubation (Edwards-Ingram et al., 2007). One of the hypotheses proposed to explain the probiotic activity of Sb against enteropathogenic microorganisms is antagonism by the formation of inhibitory compounds (Rajkowska et al., 2012). However, there are only a few studies that have shown that this yeast inhibits the growth of various bacteria. This effect has been demonstrated for *Proteus mirabilis, Proteus vulgaris, Salmonella typhi, Salmonella typhimurium, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* (Rajkowska et al., 2012; Pontier-Bres et al., 2014). *In vivo* studies show that the protective reflex against *S. typhimurium*, *Shigella flexneri* and *Clostridium difficile* found in mice fed yeast-treated food was not associated with a decrease in the overall bacterial population in the gut (Rodrigues et al., 2000). Other properties that could explain the protective effect against enteropathogenic bacteria are immunomodulation, modulation of the production of substances with antitoxic action (Martins et al., 2005).

Thus, the aim of this study was the evaluate antagonistic effect of *S. cerevisiae-Az-12* isolated from pomegranate on the pathogenic bacteria.

2. Materials and Methods

2.1. Isolation and identification of S. cerevisiae-Az-12

Pure culture of the yeast *S. Cerevisiae* Az-12 was isolated from pomegranate juice, cultural and morphological properties and physiological and biochemical properties were determined in accordance with standard methods accepted in microbiology according to researches (Saparbekova et al., 2019).

Wort agar, Sabur agar (glucose-peptone media), and YPD medium (Yeast Extract-Peptone-Dextrose) were used as nutrient media for the cultivation and study of yeast.

Molecular genetics identification of microorganisms was performed by Sanger sequencing. Genomic DNA from 1 day-old cultures was isolated using the Plant/Fungi DNA Isolation Kit from Norgen Biotek Corp (Canada) according to the manufacturer's protocol. The concentration of DNA in the samples was determined using a Qubit fluorometer (Invitrogen, USA) on the scale for dsDNAHS.

Universal primers of the ITS-region yeast such as ITS1 (5·-TCCGTAGGTGAACCTGCGG-3·) and ITS 2(5·-TCCTCCGCTTATTGATATGC-3·) were used in this work.

The reaction mixture for amplification consisted of: 12.5 µLQ5® Hot Start High-Fidelity 2X MasterMix, 1.25 µl Forward primer (10 µm), 1.25 µl Reverse primer (10 µm), 1.5 µl DNA, and 8.5 water. The total volume of the PCR mixture was 25 µl. PCR with universal primers was performed on an amplifier Eppendorf in the amplification mode: 94 °C-30 sec; 55 °C-1 min; 72 °C-40 sec-a total of 30 cycles; 72 °C-10 min. PCR products were stained and added to 1.2% agarose gel. They were visualized in a UV transilluminator.

2.2. Study of antagonistic abilities of S. cerevisiae Az-12

The antagonistic abilities of *S. cerevisiae* Az-12 were investigated with the following opportunistic or pathogenic bacteria: *Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus.*

Antagonistic effect of the yeast was studied on the YPD medium using the agar plate method. The method is based on the observation of parallel growth of strains: indicator and antagonistic. Agar recesses with a diameter of 14 mm were aseptically cut from agar with YPD, overgrown with lawn *S. cerevisiae* Az-12 incubated for 48 hours at 37 °C. For the co-cultivation of yeast and opportunistic and pathogenic microorganisms, a modified medium was used, similar in content and consistency to the intestinal medium.

The modified medium consisted of the following components (in g•L^{-1} of distilled water): starch 5.0, pectin 2.0, guar gum 1.0, xylan 2.0, arabinogalactan 2.0, inulin 1.0, casein 3.0, peptone 3.33, trypton 5.0, raffinose 10.0, bile acid salts - 0.4, yeast extract - 4.5, $FeSO_4 \cdot 7H_2O$ - 0.005, NaCl - 6.16, KCl - 4.5, KH_2PO_4 - 0.5, $MgSO_4 \cdot 7H_2O$ -1.25, $CaCl_2 \cdot 6H_2O$ - 0.15, $NaHCO_3$ - 1.5, cysteine - 0.8, chemine - 0.05; pH was adjusted to 6.2.

The analysis of yeast and bacterial growth was carried out with an initial inoculation of CFU (Colony-Forming Unit) 10^5 CFU • mL^{-1}, incubation was carried out at 37°C under anaerobic conditions. The number of microorganisms was estimated by counting every 2 hours on the first day, then after 4 hours of incubation. For yeast, CFU• mL^{-1} values were determined by applying appropriate dilutions to the YPD agar medium with the addition of gentamicin (40 mg in 100mL) and for bacteria by applying to the nutrient agar. Pathogenic or opportunistic human bacteria: *Escherichia*

coli, Salmonella Typhimurium, Enterococcus faecalis, Pseudomonas aeruginosa and *Staphylococcus aureus* were used as indicator microorganisms.

To study the adhesion of *S. aureus* to *S. cerevisiae* Az-12 yeast cells during their joint cultivation, a JSM-6490LM scanning electron microscope with an Energy INCA 350 energy dispersive X-ray microanalysis system and an HKL Basic system was used.

3. Results and Discussion

From fresh juices (grapes, apples, pomegranates, plums, pears, apricots, etc., growing in the South Kazakhstan region), 180 species of yeast have been isolated. 71 pure cultures were identified, most of yeast belong to Saccharomyces.

Two strains of yeast *Saccharomyces cerevisiae* Gul -8 (isolated from grapes) and *Saccharomyces cerevisiae* Az - 12 (isolated from pomegranate) were chosen as the most promising, since they are able to ferment fruit juices relatively quickly (Saparbekova et al., 2019). The leading factors were also organoleptic indicators: fruity smell, pleasant natural sweet-sour taste and the absence of visible turbidity. Consumption of beverages prepared with probiotics containing active microbial cells and their metabolites improves the functional properties of beverages. Fermentation of pomegranate juice was carried out at room temperature 25°C for 24 hours using 5 g of yeast per 100 ml of freshly squeezed juice.

A one-day old culture of the yeast *S. cerevisiae* Gul -8 and *S.cerevisiae* Az-12 were used for DNA isolation. The separation of the nutrient medium is carried out by centrifuging the culture for 5 minutes at 1000 ×g. Removal of the supernatant made it possible to get rid of the remnants of the nutrient medium. DNA isolation was carried out sequentially by first lysing the yeast cell and then removing bioorganic compounds, including carbohydrates, lipids, proteins, RNA, etc.

For separation of RNA, 30 µl of RNaseA solution was used; after addition of the enzyme, the mixture was incubated for 5 minutes at 37°C. For complete purification from fermentation and denaturation products, 10 µl of 4 M ammonium acetate solution and 1 ml of 100% ethanol were used. After intensive mixing, the tube was centrifuged for 3 minutes at maximum speed (14500 rpm) at room temperature. After removing the supernatant at room temperature, the precipitate with DNA was dried and dissolved in 100 µl of TE buffer.

Molecular genetic identification is carried out using the Sanger sequencing method.

Genomic DNA from 1 day old cultures of the yeast *S. cerevisiae* Gul -8 and *S. cerevisiae* Az - 12 was isolated using «Plant/Fungi DNA Isolation Kit» companies Norgen Biotek Corp. (Canada) according to the manufacturer's protocol. The DNA concentration in the samples was determined using a fluorometer Qubit (Invitrogen, USA) according to the scale for dsDNA HS.

The universal primers of the yeast ITS region were used: ITS1 (5·-TCCGTAGGTGAACCTGCGG-3·) and ITS 2 (5·-TCCTCCGCTTATTGATATGC-3·). The amplification reaction mixture consisted of: 12.5 µl Q5® Hot Start High-Fidelity 2X Master Mix, 1,25 µl Forward primer (10 µM), 1,25 µl Reverse primer (10 µM), 1,5 µl DNA and 8,5 µl water. The total volume of the PCR mixture was 25 µl.

PCR with universal primers was carried out on an Eppendorf amplifier at the amplification mode: 94ºC – 30 sec; 55ºC - 1 min; 72ºC - 40 sec - 30 cycles in total; 72ºC - 10 min. The PCR products were stained and loaded onto a 1.2% agarose gel. Visualized in a UV transilluminator.

The sequencing reaction was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applide Biosystems) according to the manufacturer's instructions [BigDye® Terminator v3.1 Cycle Sequencing Kit Protocol Applied Biosystems USA], followed by separation of the fragments on a 3500 DNA Analyzer (Applied Biosystems). The sequencing results were processed using the SeqA program (Applied Biosystems). The obtained nucleotide sequences of the ITS region of fungal DNA were compared with the data from the Gene Bank database (www.ncbi.nih.gov) using the BLAST program. Phylogenetic analysis was performed using MEGA6 software. Nucleotide sequence alignment was performed using the ClustalW algorithm. The Neiighbor-Joining (NJ) method was used to build phylogenetic trees.

The DNA concentration according to the readings of the Quibit fluorometer was -60,8 ng/µl. As a result of amplification with ITS primers, a PCR product of about 550 bp was obtained (Figure 1).

The results of the analysis of the ITS gene sequences in the studied strains are presented in the form of phylogenetic trees built in the MEGA 6 program using the Neiighbor-Joining cluster method for calculating genetic distances.

The nucleotide sequence of the PCR product *Saccharomyces cerevisiae* Gl-8:

GGATTTTTTTGTTTTGGCAAGAGCATGAGAGCTTTTACTGG
GCAAGAAGACAAGAGATGGAGAGTCCAGCCGGGCCTGCGCT
TAAGTGCGCGGTCTTGCTAGGCTTGTAAGTTTCTTTCTTGCTA
TTCCAAACGGTGAGAGATTTCTGTGCTTTTGTTATAGGACAA
TTAAAACCGTTTCAATACAACACACTGTGGAGTTTTCATATCT
TTGCAACTTTTTCTTTGGGCATTCGAGCAATCGGGGCCCAGA
GGTAACAAACACAAACAATTTTATCTATTCATTAAATTT

The degree of homology of the strain *S. cerevisiae* Gl-8 with the strain MH608341.1 *Saccharomyces cerevisiae* isolate extr03, (Figure 2).

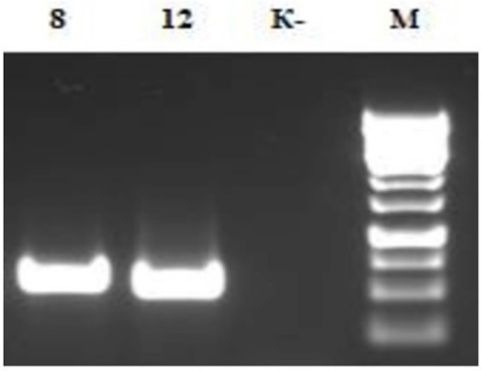


Figure 1. PCR product of *Saccharomyces cerevisiae* Gl-8 and *Saccharomyces cerevisiae* Az-12 obtained with ITS primers Note: M - O'GeneRuler 1 kb DNA Ladder length marker.

The degree of homology of the strain *S. cerevisiae* Gl-8 with the strain MH608341.1 *Saccharomyces cerevisiae* isolate extr03 составила 100%.

The nucleotide sequence of the strain Saccharomyces cerevisiae Az-12:

TTTTTTGTTTTGGCAAGAGCATGAGAGCTTTTACTGGGCA
AGAAGACAAGAGATGGAGAGTCCAGCCGGGCCTGCGCTTA
AGTGCGCGGTCTTGCTAGGCTTTGTTAAGTTTCTTTCTTGCT
ATTCCAAACGGTGAGAGATTTCTGTGCTTTTGTTATAGGACA
ATTAAAACCGTTTCAATACAACACACTGTGGAGTTTTCATAT
CTTTGCAACTTTTTCTTTGGGCATTCGAGCAATCGGGGCCCA
GAGGTAACAAACACAAACAATTTTATCTATTCATTAAATTTTT
GTCAAAAACAAGAATTTTCGTAACTGGAAATTTTAAAATATT
AAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGA
AGAACGCAGCGAAATGCGATACGTAATGTGAATTGCAGAAT
TCCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTT
GGTATTCCAGGGGGCATGCCTGTTTGAGCGTCATTTCCTTC
TCAAACATTCTGTTTGGTAGTGAGTGATACTCTTTGGAGTTA
ACTTGAAATTGCTGGCCTTTTCATTGGATGTTTTTTTT

The degree of homology of the *S. cerevisiae* Az-12 strain with the *S. cerevisiae* isolate extr03 strain MH608341.1 was 99.65%, (Figure 3).

The genome of *S. boulardii* is so similar to *Saccharomyces cerevisiae* that they should be classified as conspecific (Edwards-Ingram et al., 2007). However, a single genome sample may not be enough to study the probiotic properties of the studied yeasts. Understanding the evolution and quantitative variation among Sb strains would require an entire sequencing, including complete genomes.

One of the most valuable properties of probiotic yeast is its antibacterial activity against pathogenic and opportunistic human microorganisms: *E. coli*, *E. faecalis*, *P.aeruginosa*, *S. typhimurium* and *S. aureus*. The inhibitory mechanisms of the yeast *S. cerevisiae* in relation to the above-listed bacteria are shown in a number of studies (Hatoum et al., 2012).

S.cerevisiae Az-12 strain isolated from pomegranate was a facultative aerobic, easily ferments glucose, fructose, sucrose, maltose, maltotriose, did not use galactose, consumes a small amount of pentose-arabinose, xylose and ribose, and used many simple compounds glycerin as a carbon source, as a result of the fermentation of sugars, it formed CO_2 and ethyl alcohol. The optimal temperature range is 37±1°C, which was close to the temperature of the human body. The cells grow in the range from 5°C to 45°C. The optimal pH value of the medium was 3.5-5.5. It retains viability in the pH range from 1.2 to 10. It grows when the bile content in the medium is up to 3.0%.

S. cerevisiae Az-12 colonies on malt agar wort was small, smooth, and convex, with even edges. The average cell size was 5.0 × 6.4 microns. The shape of the cells was mostly rounded, (Figure 4). It was propagated by budding. It did not form a spore.

Studies conducted to determine the action of the yeast *S.cerevisiae* Az-12 on pathogenic or opportunistic bacteria showed that this strain exhibits features of the probiotic culture of *S.cerevisiae* var. *boulardi.* A clear antagonism of yeast against bacteria was confirmed only for *E.coli, P. aeruginosa*, and *S.s aureus*. Consistent results were obtained for the *S. cerevisiae* Az-12 strain isolated from pomegranate growing in the Turkestan region. The inhibition zones are equal (12.8 ± 0.05) mm for *E.coli*; (13.5 ± 0.05) mm for *S. aureus* and (10.7 ± 0.05) mm for *P. aeruginosa*.

For the joint cultivation of yeast and pathogenic and opportunistic microorganisms, a modified medium was used, similar in content and consistency to the intestinal medium.

The study of the antibacterial activity of *S.cerevisiae* Az-12 was carried out in two variants. In the first variant, the growth of a monoculture in a modified medium was studied (Figure 5A).

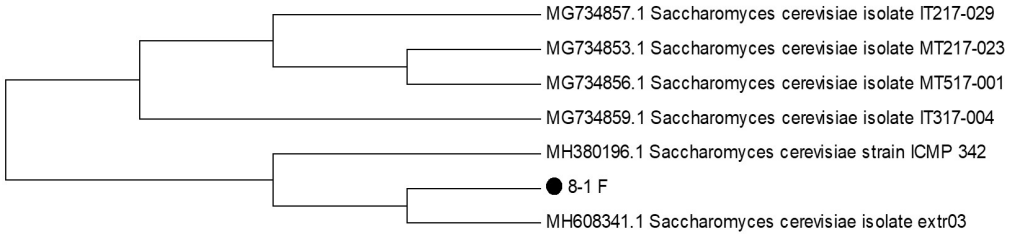


Figure 2. The degree of homology of the strain Saccharomyces cerevisiae Gl-8 with the strain MH608341.1 *Saccharomyces cerevisiae* isolate extr03.

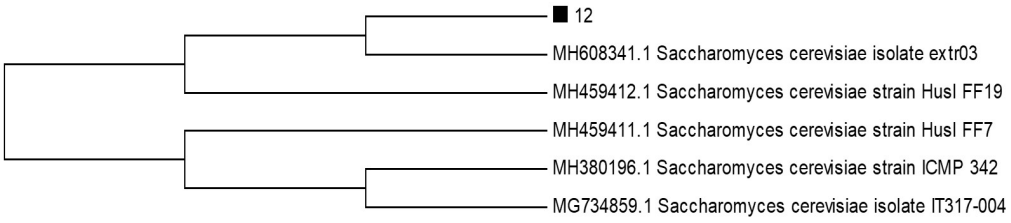


Figure 3. The degree of homology of the Saccharomyces cerevisiae Az-12 strain with the Saccharomyces cerevisiae isolate extr03 strain MH608341.1.

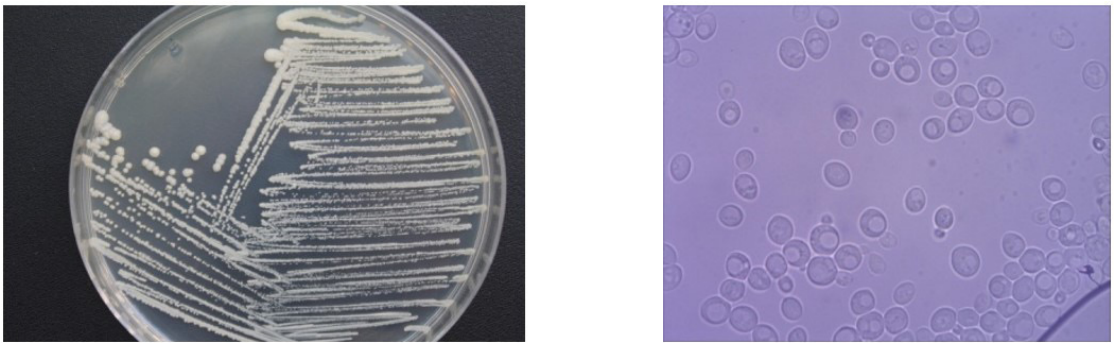
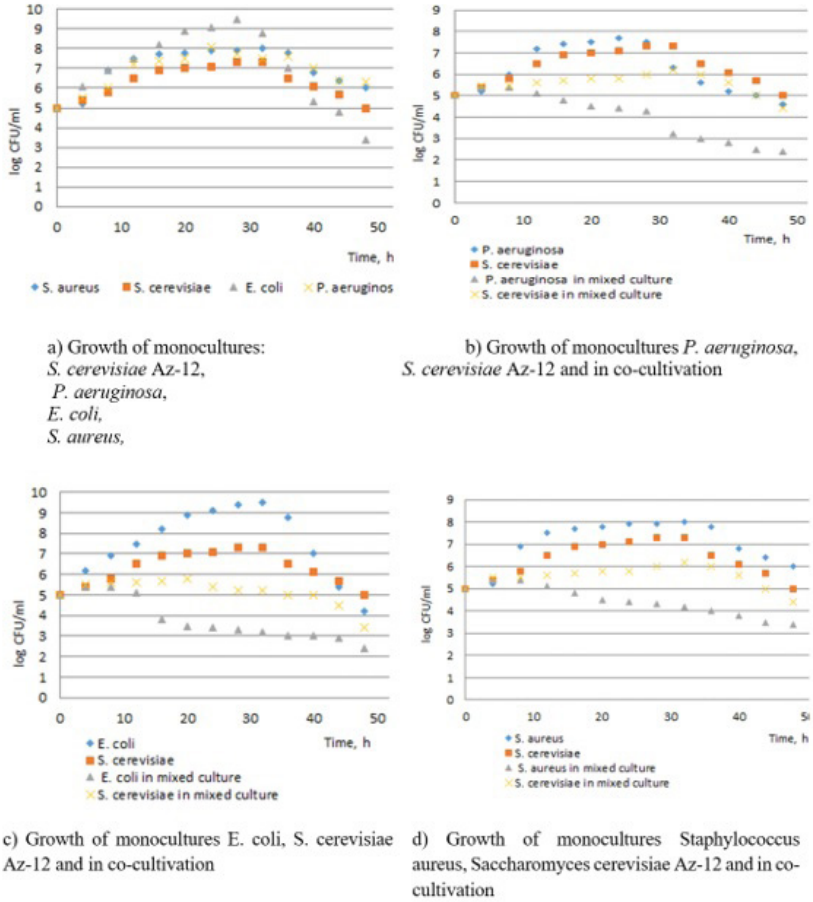


Figure 4. Yeast culture Saccharomyces cerevisiae Az-12 (from pomegranate juice).



a) Growth of monocultures: *S. cerevisiae* Az-12, *P. aeruginosa*, *E. coli*, *S. aureus*,

b) Growth of monocultures *P. aeruginosa*, *S. cerevisiae* Az-12 and in co-cultivation

c) Growth of monocultures E. coli, S. cerevisiae Az-12 and in co-cultivation

d) Growth of monocultures Staphylococcus aureus, Saccharomyces cerevisiae Az-12 and in co-cultivation

Figure 5. Joint and separate cultivation of yeast and bacterial strains of human pathogens.

In the second variant, the growth of microorganisms was studied during the co-cultivation of yeast and a bacterial strain of human pathogens.

P. aeruginosa is one of the most common infectious agents, especially in immunocompromised individuals. The pathogenicity of *P. aeruginosa* is expressed by the presence of sufficient mobility and the ability to form toxins.

In the case of co-cultivation of *P. aeruginosa* strains with the yeast *S. cerevisiae* Az-12 in mixed cultures, a decrease in the number of bacterial cells was observed. A monoculture of *P. aeruginosa* in exponential phase has a maximum level of 7.7 log CFU • mL^{-1} after 24 hours. Subsequently, on the second day, there was a decrease in viable cells by 1.5 times. In a mixed culture with yeast, we found a 43% decrease

in bacterial cells on the first day and almost 50% after 48 hours. This is evidence of the negative effects of yeast on *P. aeruginosa*. The development and growth of yeast *S. cerevisiae* Az-12 during co-cultivation with *P.aeruginosa* also decreases, but not so dramatically (Figure 5B).

Escherichia coli is a bacteria that occurs naturally in the human intestine. In most cases, *E. coli* strains are harmless, but some strains can cause food poisoning, especially if they are heavily contaminated.

In the modified medium, a stable growth of *E. coli* culture is observed on the first day, reaching a maximum 9.5 log units. Subsequently, due to a decrease in nutrients, they are reduced to 4.1 log units. When *E.coli* is co-cultured with the yeast Saccharomyces cerevisiae Az-12, there is a significant decrease in the number of viable *E. coli* cells to 3.4 log units on the second day. This suggests a significant suppressive effect of probiotic yeast on *E. coli.* It is necessary to note the negative impact of *E. coli* on the development of *S.cerevisiae* Az-12, as compared with a monoculture, their number decreases on the second day in a mixed culture by more than 1.8 times. The results of experiments with *E.coli* and *S.cerevisiae* Az-12 with separate and joint cultivation (Figure 5C).

The number of viable cells of *S. aureus, S. cerevisiae* Az-12 in separate and joint cultivation (Figure 5D). *S.aureus* is one of the most common microorganisms that cause diseases of the skin and mucous membranes of the upper respiratory tract. Up to 40% of the population are temporary or permanent carriers of this bacterium. At the same time, *S. aureus* can cause many diseases, from mild skin infections to deadly diseases, among which pneumonia ranks first.

The monoculture of *S. aureus* in a modified medium develops rapidly. The maximum growth of this culture reaches 8.1 log CFU mL $^{-1}$. The stationary growth phase lasts 28 hours.

The probiotic yeast *S. cerevisiae* Az-12 strongly and negatively influenced the growth of *S. aureus*. Co-cultivation of *S. cerevisiae* Az-12 and *S. aureus* leads to a decrease in the causative agent of infections almost from the first hours of cultivation. At 32 hours of co-cultivation, the number of viable *S. aureus* cells was almost halved. The growth of the yeast *S.cerevisiae* Az-12 during co-cultivation in the first hours also decreased in comparison with the control. However, at 32 hours, there is a slight increase in the amount of the studied culture.

Despite the fact that there is some evidence of the antagonistic effect of yeast on human pathogens, the mechanism of its action is not clearly defined. In our study, we used scanning microscopy techniques to evaluate the adhesion of bacterial cells to the yeast probiotic cell wall. After co-cultivation, a 1 ml sample of the culture suspension containing *S. cerevisiae* Az-12 and *S. aureus* was taken. The probiotic strain *S. cerevisiae* Az-12 (Figure 6) and *S. aureus* were microscopically examined using a scanning electron microscope JSM-6490LM with Energy INCA 350 energy dispersive X-ray microanalysis sustem and HKL Basic system. Using the method of scanning microscopy, based on a subjective assessment of the relative position of the yeast cell and *S. aureus,* positive results were obtained confirming the adhesive ability of *S. aureus* to the surface of the yeast cell (Figure 7).

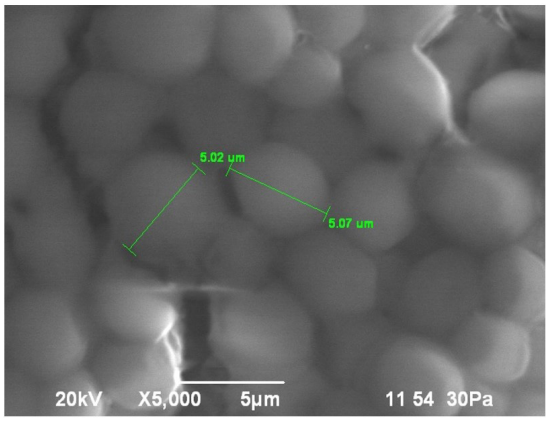


Figure 6. yeast *Saccharomyces cerevisiae* Az-12 (Magnification 5000 times).

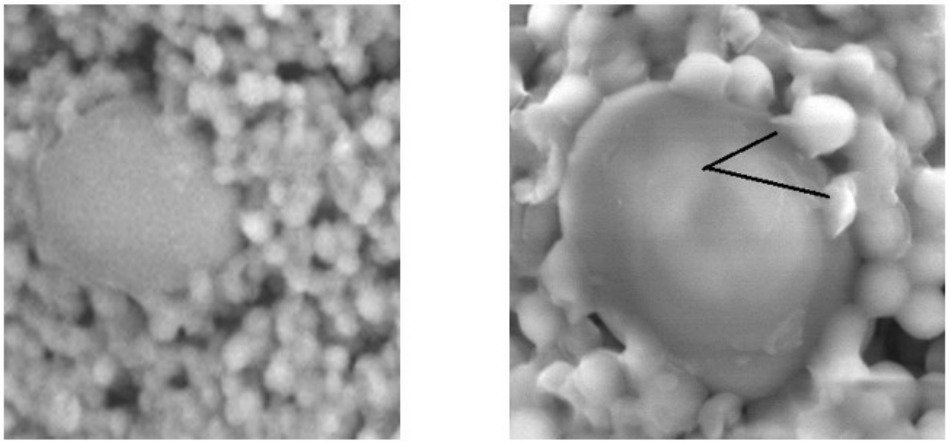


Figure 7. Adhesion of *Staphylococcus aureus* to wall of the yeast *Saccharomyces cerevisiae* Az-12 (Magnification 5000 times).

Thus, an important initial event in bacterial pathogenesis is the adherence of bacteria through their surface organelles to the host intestinal cells. The use of *S. cerevisiae* Az-12 as a probiotic for humans, can counteract the adhesion of pathogens to host tissues by providing alternative sites of adhesion to enterobacteria and thus prevent infections. They can also eliminate pathogens from the gastrointestinal tract of infected patients by removing them from the body in a yeast-cell-bound state.

4. Conclusions

Currently, in the Republic of Kazakhstan, the epidemiological picture for the class and types of diseases of the digestive system remains disappointing (Kausova et al., 2019; Suleimenova and Kuatbayeva, 2014). Consumption of pomegranate juice improves food digestion (Tsang et al., 2012) and pomegranate extract containing volatile substances, anthocyanins (Jurga et al., 2021) has also been shown to reduce blood pressure, insulin resistance, and stress hormone levels (Stockton et al., 2015).

Non-dairy probiotic products can be used by consumers of all food groups, including vegans and vegetarians, as well as consumers with dairy allergies. In this regard, the use of means of correction of the intestinal microbiota (probiotics, prebiotics, synbiotics) in the treatment of inflammatory bowel diseases is attractive and can serve as a worthy alternative to antibiotics (Avalueva et al., 2010). If the drink is prepared using materials of plant origin, then their oligosaccharides and fiber act as prebiotics. Both components (probiotic strain(s) and prebiotic substrate) exist in a synergistic relationship in the product and contribute to several nutritional and gut health benefits (Divakar and Poonam, 2022).

The strain of *S. cerevisiae* Az-12 isolated from pomegranate. The degree of homology of the Saccharomyces cerevisiae Az-12 strain with the *S. cerevisiae* isolate extr03 strain MH608341.1 was 99.65%. *S. cerevisiae* Az-12 caused a statistically significant reduction of pathogenic bacteria. The inhibition zones are (12.8 ± 0.05) for *E. coli*; (13.5 ± 0.05) mm for *S.aureus* and (10.7 ± 0.05) for *P. aeruginosa*. The use of *S. cerevisiae* Az-12 can limit bacterial invasiveness and infections caused by human pathogens due to the adhesive surface of the yeast, the ability to excrete from the gastrointestinal tract in a bound state. *S. cerevisiae Az-12* is registered as a non-pathogenic microorganism in the Republican Collection of Microorganisms, Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan.

Summarizing the results of our study showed that Saccharomyces cerevisiae-Az-12 has the properties of probiotic yeast, in particular, antibacterial activity against pathogenic and opportunistic human microorganisms and shows features similar to other registered probiotic yeasts.

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