

Morpho-Cultural and Quantitative Analysis of Microbial Strains Isolated from Spontaneous Yeast Based Yogurts Used in Aghdam Region

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The presented article is devoted to study the morpho – cultural and quantitative analysis of lactic acid bacteria and yeast fungi isolated from the spontaneously prepared yogurts of Uchoglan, Tazakend, Guzanli and Imamgulubayli villages of Aghdam region. 5 isolates of lactic acid bacteria and 4 isolates of yeast fungi strains were obtained from yoghurt samples. The highest amount of isolated lactic acid bacteria and yeast fungi was determined in Tazakend village yogurt. The amount of yeast cells in 1 ml of Guzanli village yogurt was 3.2 times more than the yeast cells in Uchoglan village yogurt, 5.5 times less than the yeast cells in Tazakend village yogurt, and 2.1 times less than the yeast cells in Imamgulubayli village yogurt. The amount of lactic acid bacteria in 1 ml of Tazakend village yogurt was 2.5 times more than Uchoglan village yogurt; 1.3 times more than Guzanli village yogurt and it was 1.8 times more than Imamgulubayli village yogurt. The amount of yeast cells contained in Uchoglan, Guzanli and Imamgulubayli village yogurts was less than lactic acid bacteria which were contained in those yogurts. The amount of lactic acid bacteria was 4,7 times more than the yeast cells in Uchoglan village yogurt, 2.9 times more than in Guzanli village yogurt and it was 1.1 times more in Imamgulubayli village yogurt. Only the number of yeast cells isolated from Tazakend village yogurt was 1.5 times more than the number of bacteria. The number of yeast cells was 1.5 times more than the number of bacteria only in Tazakend village yogurt. According to the morpho-cultural characteristic features of the investigated lactic acid bacteria strains, the size of the colonies was small, medium and they were in white, whitish, yellowish-white colours, their shape was round or coke-shaped, rod-shaped, their surface was slightly smooth, sometimes slightly convex forms. It was determined that, isolated lactic acid bacteria strains belonged to 2 genera *Streptococcus* spp. (BSU – UN1, BSU – QI4) and *Lactobacillus* spp. (BSU – UN2, BSU – TD6, BSU – II8). The size of the colonies which we obtained from isolated yeast strains was medium, relatively large, their shape was mostly round with paste – like consistency, their edges were wavy, flat with wrinkled and convex surface. The shape of the cells was mainly elongated – lemon – shaped or egg – shaped. It was determined that, all 4 isolated yeast strains (BSU – UN3, BSU – QI5, BSU – TD7, BSU – II9) belonged to the genus of *Saccharomyces*.

Keywords: Colony; Fermentation; Lactic Acid Bacteria; Morpho-Cultural Features; Spontaneous Yogurt; Yeast.

Yogurt is a fermented sour milk product that has been made by humans for centuries and is famous for its unique taste and health benefits^{2,6,7,23}.

The origin of yogurt can be traced back to ancient civilizations such as the Sumerians and Babylonians, who discovered the transformative

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effects of natural fermentation on milk. Today, yogurt is produced traditionally and used by the entire population of the world spontaneously at home and for commercial purposes on the basis of lactic starter culture. The main role in yeast based yogurt fermentation is played by lactic acid bacteria and yeast fungi. At this time, lactic acid bacteria absorb lactose sugar and produce lactic acid, and yeast fungi produce alcoholic fermentation. Lactic acid bacteria strains are known to produce metabolites with high antimicrobial properties and in our research work we have worked with the spontaneously prepared yogurts of Uchoglan, Tazakend, Guzanli and Imamgulubayli villages of Aghdam region^{1,8,10,11-13,15-18}.

Currently, yeast-based yogurt is being prepared and used in different areas of the Azerbaijan Republic. It is one of the important issues to study which types of microbial cultures are participate in this process^{5,21,22}. This point – the main purpose of presented work in the review was to bring out the microbial strains of spontaneous yeast base yogurt into pure cultures which prepared by the population in some regions of Aghdam district and to study their morpho – cultural and quantitative characteristics.

MATERIALS AND METHODS

Spontaneous yogurt samples taken from different areas of Aghdam region: Uchoglan, Tazakend, Guzanli, Imamgulubayli villages were used as the research objects. For the cultivation of yeast fungi, nutrient medium with malt agar was used. Meat – peptone agar medium was used for the cultivation of lactic acid bacteria¹⁴.

The collected samples were delivered to the microbiology laboratory through special cooling chambers within 6 hours. From the delivered yogurt samples to the laboratory, 1 ml of spontaneous yogurt was taken with a sterile pipette and added into the test bottles containing 9 ml of sterile water, and it diluted up to 5 times. The suspension obtained from the fourth and fifth dilution was taken with a sterile pipette, 0.1 ml from the end suspension was added to the meat-peptone agar and malt agar nutrient media in the petri dishes, and spread with a sterile spatula. Cultivation was carried out in four to five replicates. The cultured petri dishes were kept for 3 days in a

thermostat at a temperature of 30°C for incubation. Colonies were observed in Petri dishes after the incubation period. Colonies observed in Petri dishes differed in number and morpho-cultural characteristics. The number of microorganisms was determined according to the number of colonies observed in Petri dishes. Then, the color of the colonies, the shape of their edges and surface were determined. In order to determine the shape of the cells, preparations were prepared and looked at under a light microscope, and the strains were removed into pure culture.

It was investigated by Gram staining method whether the isolated bacterial strains were gram positive or gram negative or whether they produce or do not produce spores and pigments. We also tested lactic acid bacteria by using 3% hydrogen peroxide solution. For yeast strains, the forms of asci which formed from vegetative cells, the number and structure of ascospores in asci were determined.

By determining these characteristic features, bacterial strains were assigned according to Bergey's designation (Bergeys Manual, 1997), and yeast strains were assigned according to Lodder (1870), Babyeva and Golubeva (1979) designation for identify the genus of isolated species^{4,19}.

RESULTS AND DISCUSSIONS

Morpho-cultural and quantitative characteristics of lactic acid bacteria and yeast fungi isolated from each sample were determined during the research work.

The color of lactic acid bacteria strain BDU-UN1 isolated from Uchoglan village yogurt was in white color, in small size, the edges of them were round and their shape was in coke-shaped (Tab.1, Fig. 1)

The colony size of BDU – UN2 lactic acid bacteria was large, its color was whitish, the surface of it was wrinkled, the cell shape of it was rod – shaped (Tab.1, Fig. 2).

The colony surface of BSU – QI4 strain isolated from Guzanli village yogurt was wrinkled, it was in whitish colour and the shape of their cells was in coke – shaped (Tab.1, Fig. 3).

The colony size of BDU – TD6 lactic acid bacteria strain isolated from Tazakend yogurt was medium, its color was white with slightly smooth

and round surface, and the cell shape was rod – shaped (Tab.1, Fig. 4).

The colony size of BDU – II8 lactic acid bacteria strain isolated from Imamgulubayli village yogurt was small with round shape and its surface was slightly convex in white colour, it had a coke – shaped cellular form (Tab.1, Fig. 5).

Summarizing the above, it can be concluded that the color of the colony of the lactic

acid bacteria strains we studied was white, whitish, size – small, medium, large, surface – smooth, slightly convex, cellular shape was straw and coke – shaped.

Morpho-cultural features of isolated yeast strains

Morpho-cultural and quantitative characteristics of yeast strains isolated from yogurt

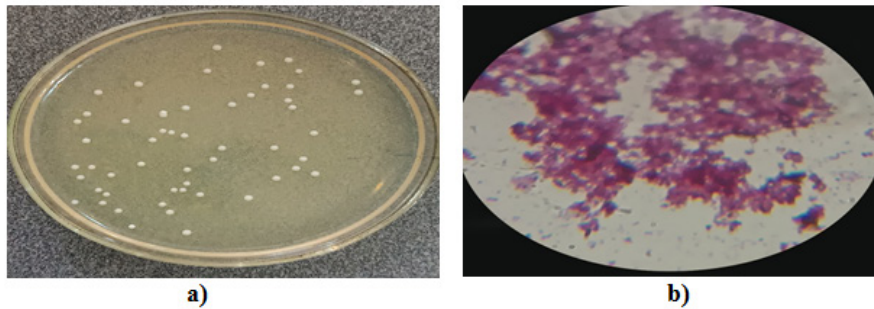


Fig. 1. BSU – UN1 lactic acid bacteria strain: a) growing colonies on solid nutrient agar medium, b) cells appearance under a microscope

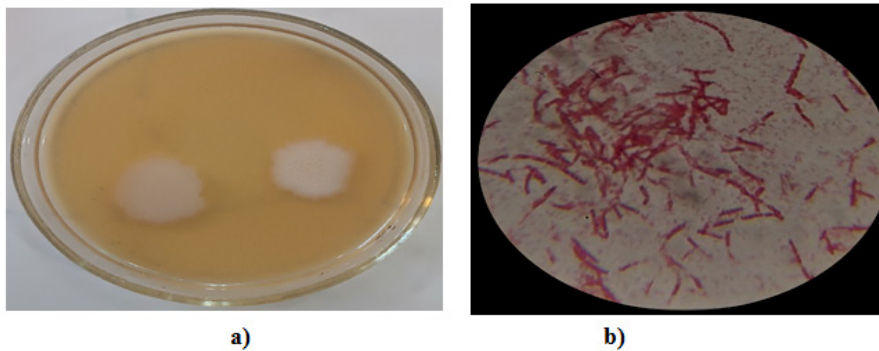


Fig. 2. BSU – UN2 lactic acid bacteria strain: a)growing colonies on solid nutrient agar medium, b) cells appearance under a microscope

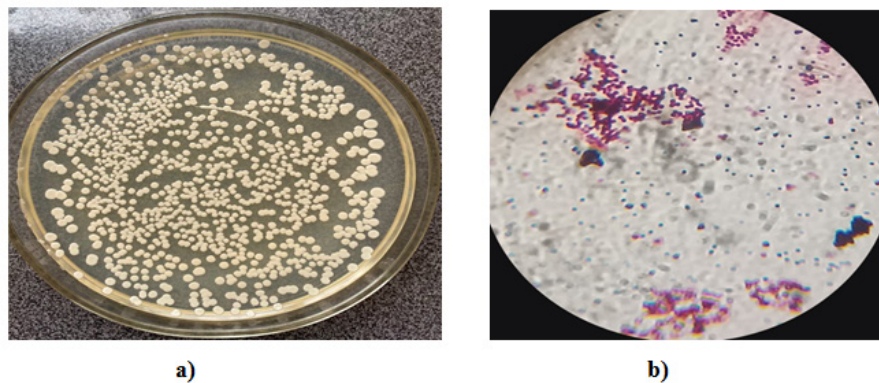


Fig. 3. BSU – QI4 lactic acid bacteria strain: a) growing colonies on solid nutrient agar medium, b) cells appearance under a microscope

samples taken from different settlements of Agdam region were studied.

It was determined that, the colony surface of BSU – UN3 yeast strain isolated from Uchoglan village yogurt had a slightly convex surface,

paste-like consistency, light yellow color, and their shapes were mostly in round cell shapes (Tab. 2, Fig. 6).

The colony color of BSU – QI5 yeast strain isolated from Guzanli village yogurt was

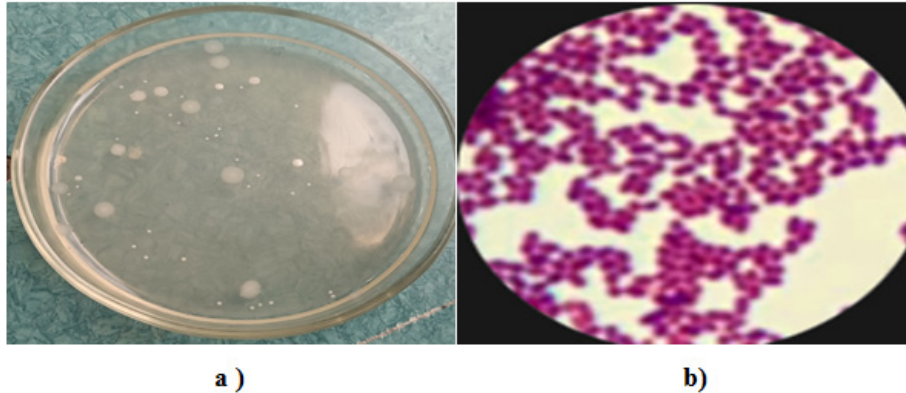


Fig. 4. BSU – TD6 lactic acid strain: a) growing colonies on solid nutrient agar medium, b) cells appearance under a microscope

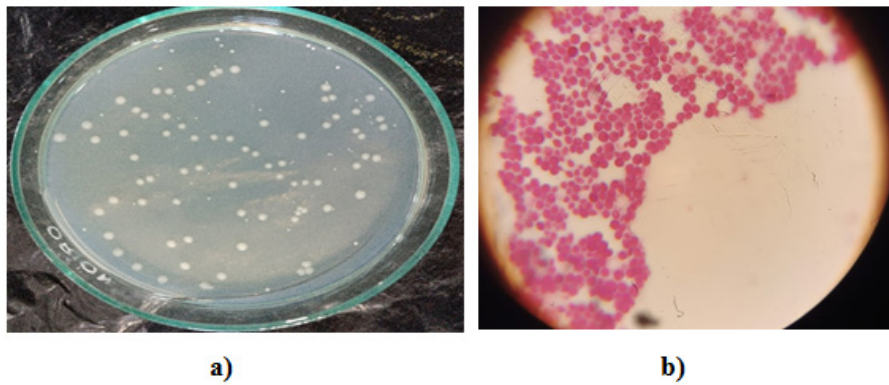


Fig. 5. BSU – ÝÝ8 lactic acid strain: a) growing colonies on solid nutrient agar medium, b) cells appearance under a microscope

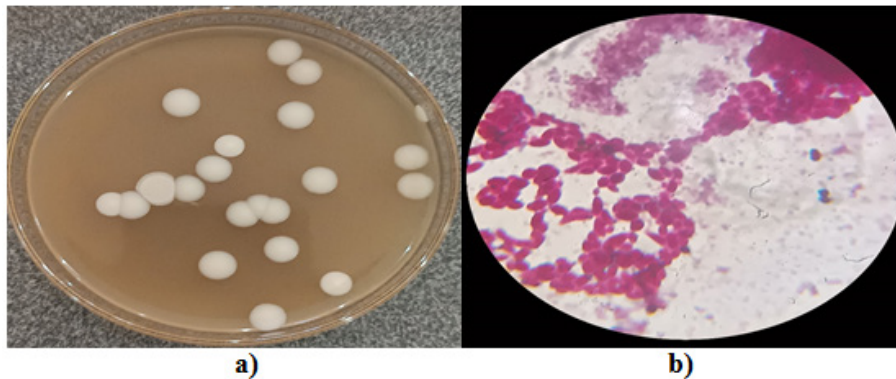


Fig. 6. BSU – UN3 yeast strain: a) growing colonies on solid nutrient agar medium, b) cells appearance under a microscope

light yellow, their edges were smooth with paste – like consistency and they had a slightly raised surface. Their cell shape was elongated lemon – shaped (Tab.2, Fig.7)

The consistency of BDU – TD7 yeast strain colony isolated from Tazakend village yogurt was paste – like, colony colour was white with

smooth surface and edges and their cells were like as lemon – shaped elongated cells (Tab. 2, Fig. 8).

Based on Figure 9, we can say that the colony shape of BSU – II9 yeast strains isolated from Imamangulubeyli village yogurt was round, their colonies were in yellowish – white colour with paste – like consistency, colonies were slightly

Table 1. Morpho-cultural features of lactic acid bacteria strains

Lactic acid bacteria strains	Cultural and morphological description of colonies		
	Colour	Surface form	The shape of the cells
BSU-UN1	white	smooth	coccus shape
BSU-UN2	whitish	wrinkled	straw shaped
BSU-QI4	whitish	wrinkled	coccus shape
BSU-TD6	white	a little smooth	straw shaped
BSU-II8	white	a little convex	coccus shape

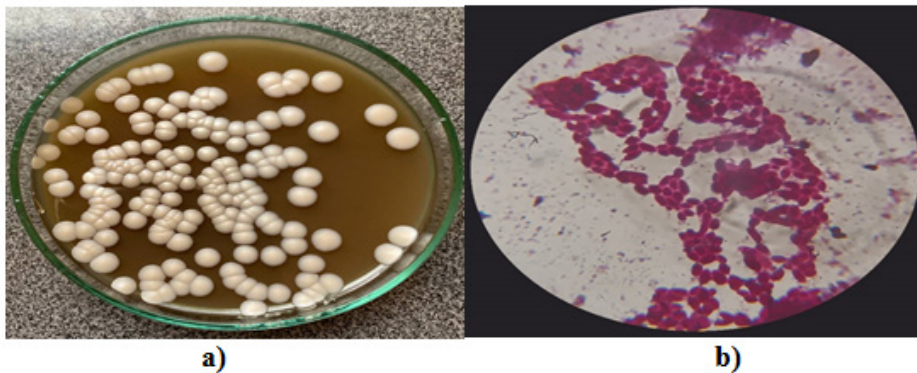


Fig. 7. BSU – Q15 yeast strain: a) growing colonies on solid nutrient agar medium, b) cells appearance under a microscope

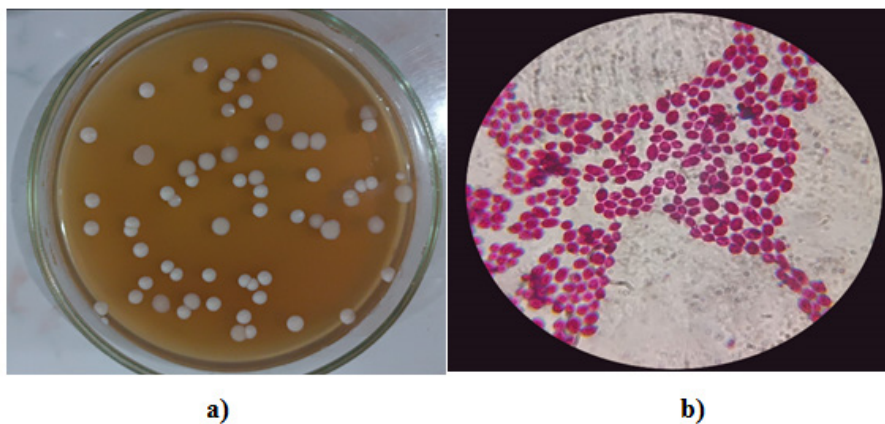


Fig. 8. BSU – TD7 yeast strain: a) growing colonies on solid nutrient agar medium, b) cells appearance under a microscope

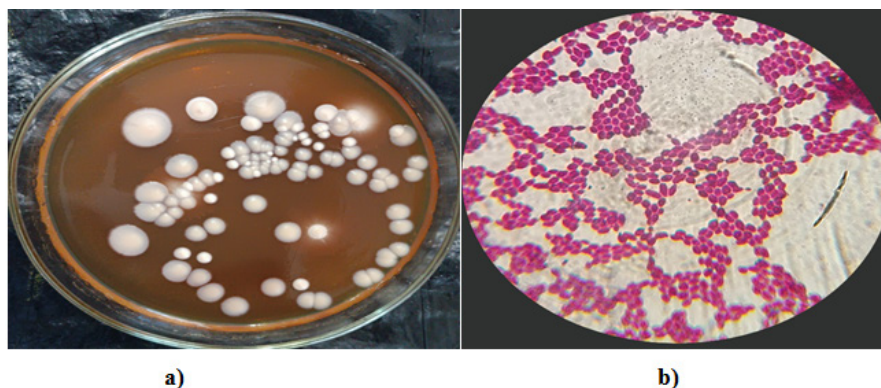


Fig. 9. BSU – II9 yeast strain: a) growing colonies on solid nutrient agar medium, b) cells appearance under a microscope

Table 2. Morpho – cultural features of yeast strains

Yeast strains	Colour	Cultural and morphological description of colonies		
		Surface form	Consistency	The shape of the cells
BSU-UN3	light -yellow	smooth	paste- like	balloon-shaped
BSU-Q15	light yellow	slightly convex	paste-like	lemon shaped
BSU-TD-7	white	smooth	paste-like	elongated
BSU-II9	light -yellow	slightly convex	paste-like	balloon-shaped

Table 3. The amount of microorganisms (per 1 ml) in spontaneous yogurts used in the territory of Aghdam region

Residential areas	The number of Lactic acid bacteria	The number of yeasts
Uchoglan	71x10 ⁴	15x10 ⁴
Guzanli	142x10 ⁴	48x10 ⁴
Tazakand	181x10 ⁴	268x10 ⁴
Imamgulubayli	102x10 ⁴	95x10 ⁴

convex on the surface, and slightly larger in size. Cells were elongated and balloon – shaped (Tab. 2, Fig. 9).

The morpho – cultural colony signs of the yeast strains isolated from spontaneous yogurt samples that we used as our study object were tabulated and their cellular structure is also reflected in the following table (Tab 2).

As a result of the research, the amount of lactic acid bacteria and yeast fungi obtained from spontaneous yogurt samples taken from the villages of Uchoglan, Imamgulubayli, Tazakend, Guzanli of Aghdam region was determined (Tab 3).

The amount of yeast cells in 1 ml of spontaneous yogurt samples taken from Uchoglan village was 15x10⁴, 48x10⁴ in Guzanli village yogurt, 268x10⁴ in Tazakend yogurt, and 95x10⁴ in Imamgulubeyli village yogurt.

The most yeast cells were observed in Tazakend yogurt, and a relatively small amount was observed in spontaneous yogurt brought from the village of Imamgulubayli.

The amount of yeast cells in 1 ml of Guzanli village yogurt was 3.2 times more than the yeast cells in Uchoglan village yogurt, 5.5 times less than the yeast cells in Tazakend yogurt, and 2.1 times less than the yeast cells in Imamgulubeyli village yogurt.

The amount of lactic acid bacteria was 71x10⁴ in 1 ml of yogurt sample taken from Uchoglan village, 142x10⁴ in Guzanli village yogurt sample, 181x10⁴ in Tazakend yogurt sample, and 102x10⁴ in Imamgulubeyli village yogurt sample. The most number of lactic acid bacteria cells were observed in Tazakend village yogurt sample, and the least amount of lactic acid bacteria was observed in Uchoglan village yogurt compared to other settlements.

It was determined that, the amount of lactic acid bacteria in 1 ml of Tazakend village yogurt was 2.5 times more than Uchoglan village yogurt, 1.3 times more than Guzanli village yogurt and it was 1.8 times more than Imamgulubayli village yogurt.

The amount of yeast cells contained in Uchoglan, Guzanli and Imamgulubayli village yogurts was less than lactic acid bacteria contained in those yogurts. The amount of lactic acid bacteria was 4,7 times more than yeast cells in Uçođlan village yogurt, 2,9 times more in Guzanli village yogurt, and it was 1,1 times more in Imamgulubayli village yogurt. The number of yeast cells was 1.5 times more than the number of bacteria only in Tazakend village yogurt.

Certain tests were carried on for bacterial strains isolated from spontaneously prepared yogurt samples in 4 settlements of Agdam region and it was determined that they do not form spores and pigments, they are gram positive and cannot produce catalase enzyme.

In yeast strains, diploid cells undergo vegetative reproduction. Asci are mainly formed from vegetative diploid cells. In them, the asci are round or oval. Up to 2 – 4 round colorless and smooth ascospores are located in the ascus. The establishment of these characteristic signs and tests has helped us to determine bacterial and fungal strains in a genus level.

CONCLUSION

Thus, it was determined that 5 lactic acid bacteria and 4 yeast strains were isolated from the studied yogurt samples. The highest amount of isolated lactic acid bacteria and yeast fungi was determined in Tazakend village yogurt.

According to the morpho – cultural characteristic features of the investigated lactic acid bacteria strains, their colony size was small, medium, colonies were white, whitish, yellowish – white with round or coke – shaped, rod – shaped, the colony surface was slightly smooth, sometimes slightly convex. Lactic acid bacteria strains were belonged to 2 genera; *Streptococcus spp.* (BSU – UN1, BSU – QI4) and *Lactobacillus spp.* (BSU – UN2, BSU – TD6, BSU – II8) genera.

The colony sizes of the isolated yeast strains were medium, relatively large, their shapes

were mostly round, their consistency was paste-like, the edges were wavy, flat, their surface was wrinkled, convex. The shape of the cells was mainly elongated – lemon – shaped or egg – shaped. In these strains, asci are mainly formed from vegetative diploid cells. Asci are round or oval. There are 1 – 4 round, colorless and smooth ascospores in the asket. By taking account these signs, all 4 yeast strains (BSU – UN3, BSU – QY5, BSU – TD7, BSU – II9) were belonged to the genus *Saccharomyces*.

In future studies, because we have no conditions for genetic analysis (PCR), the isolated strains will be identified at species level by studying their physiological and biochemical characteristics. After studying the antimicrobial activity of strains, their active ones will be applied for certain purposes in industry.

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Conflict of interest

There are no conflict of interest.

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Authors' Contribution

Contribute all author equally

Data Availability Statement

None.

Ethics Approval Statement

None.

REFERENCES

1. Anita Menconi., Gopala Kallapura et al. Identification and characterization of lactic acid bacteria in a commercial probiotic culture // Pub Med, *Bioscience of microbiota, food and health*, 2014; 33(1), 25-30.
2. Angelova T.T., Hristova Y., Pavlov A., Beshkova D. Lactic acid bacteria-from nature through food to health // *Advances in Biotechnology for food industry*, 2018; 4(1), 91-133.
3. Ááúááá È.Á., Áïéóááá È.È. Ìàòìàù áúááéáíëý è èääíòèòèèääöèè äðíææáé - Ì: - 1979; - c. 120.
4. Bergeys manual of determination bacteriology. The Williams Wilkins Company: Baltimore, 1997; 1225p.
5. Chandan R.C. An Overview of Yogurt Production and Composition // *Yogurt in Health and Disease Prevention*, 2017; 31-47.

6. Filippis F.D., Pasolli E., Ercolini D. The food – gut axis: lactic acid bacteria and their link to food, the gut microbiome and human health // *FEMS Microbiology Reviews*, 2020; 454-489.
7. Fiore G., Profio E., Sculati M. Health effects of yogurt consumption during paediatric age: a narrative review // *International Journal of Food Sciences and Nutrition*, 2022; 73(1), 738-759.
8. Ganbarov Kh.G., Jafarov M.M. Microbiology of home-made (spontaneous) yogurts in Azerbaijan. Monograph – 2013; 346 p.
9. Gumushtash A. Isolation and characterization of lactic acid bacteria and bacteriophages from various sources, Ankara-2015; 76p.
10. Haritha Meruvu., Sebnem Tellioglu. Lactic acid bacteria: isolation–characterization approaches and industrial applications // *Critical Reviews in Food Science and Nutrition*, 2022; <https://doi.org/10.1080/10408398.2022.2054936>, DOI:10.1080/10408398.2022; 2054936.
11. Huang H.C., Lee I., Huang C., Chang T. Lactic acid bacteria and lactic acid for skin Health and Melanogenesis Inhibition // *Curr Pharm Biotechnol*, 2020; 21(2), 566-577
12. Jafarov M.M. Morpho-cultural properties of lactic acid bacteria strains isolated from sour milk products used in Kur-Araz agro-climatic region// *News of Baku state University, Series of natural Sciences* 2008; 3, 79-82.
13. Jafarov M.M., Ganbarov Kh.G., Agabeyova R.A. Morpho-cultural and physiological properties of Brettanomyces ontermedium yeast strains isolated from spontaneous sour milk products// *Scientific works of the Institute of Microbiology of ANAS, Baku – “Science”*, 2011; 9, 100-106.
14. Jafarov M.M. Morpho-cultural properties of *Streptococcus lactis* lactic acid bacteria strains / *Republican scientific conference on modern problems of biology, Baku*, 2008; 170-171.
15. Kamil Bostan, Ayla Unver Alcay, Semiha Yalçin et al. Identification and characterization of lactic acid bacteria isolated from traditional cone yoghurt // *Springer, Food science and biotechnology*, 2017; 26(6), 1625-1632, doi: 10.1007/s10068-017-0222-z
16. Karaolis C, Botsaris G., Pantelides I., Tsaltsas D. Potential application of *Saccharomyces boulardii* as a probiotic in goat EËss yoghurt: survival and organoleptic effects // *International Journal of Food Science and Technology*, 2013; 48, 1445-1452.
17. Khelissa S., Chihib N.E., Gharsallaoui A. Conditions of nisin production by *Lactococcus lactis* subsp. Lactis and its main uses as a food preservative // *Archives of Microbiology*, 2021; 203, 465-480.
18. Lee W.J., Lucey J.A. Formation and physical properties of yogurt // *Asian-Australasian Journal of Animal sciences*, 2010; 23(9), 1127-1136.
19. Lodder J. The Vests A taxonomic study / J. Lodder. Amsterdam: -1970. 1358p.
20. Tian X., Chen H., Liu H., Chen J. Recent advances in lactic acid production by lactic acid bacteria // *Applied biochemistry and biotechnology*, 2021; 4151- 4171.
21. Tigistu Goa, Getenet Beyene, Mekidim Mekonnen, Kasahun Gorems. Isolation and Characterization of Lactic Acid Bacteria from Fermented Milk Produced in Jimma Town, Southwest Ethiopia, and Evaluation of their Antimicrobial Activity against Selected Pathogenic Bacteria // *International Journal of Food Science*, 2022; 2022; doi: 10.1155/2022/2076021, <https://doi.org/10.1155/2022/2076021>, 15-29
22. Yu J., Wang W.H., Menghe B.L. et al. Diversity of lactic acid bacteria associated with traditional fermented dairy products in Mongolia // *Pub Med Journal of dairy Science*, 2011; 94(7), 3229-3234, DOI: 10.3168/jds.2010-3727
23. Yörtük G., Güner A. Classification of lactic acid bacteria and the importance of Weissella species in food microbiology // *Atatürk University Vet. Know. Journal*, 2011; 163-176.