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Molecular Characterization of Yogurt Bacteria Isolated from Beans and Lentils

Mesut CAY
Gaziantep University

Ibrahim Halil KILIC
Gaziantep University

Isik Didem KARAGOZ
Gaziantep University

Zeynep KOLOREN
Gaziantep University

Bekir Siddik KURT
Gaziantep University

Sami Serhat TONUS
Gaziantep University

Mehmet OZASLAN
Gaziantep University

Abstract: As yogurt and similar fermented dairy products have proved positive effects on human nutrition and health, its production and consumption are rapidly increasing all over the world. However, efforts continues to improve the production and quality of yogurt with various properties using new techniques. In Turkey production of yogurt form fermented milk is carried out using starter cultures imported from abroad. Lentil and bean were inoculated into milk without UHT to obtain a first culture. Yogurt production was carried out using milk without UHT in the first culture. Fresh pure culture was obtained from the produced yogurt and DNA's were isolated and stored. Molecular characterization was performed using 16s rRNA sequence analysis by next-generation sequencing and MALDI-TOF methods.In this study, we investigated whether or not the obtained bacteria are good candidates to be yogurt starter cultures. This study is a preliminary study for the researchers who will work in this field and will shed light to the scientific community.

Keywords: Lentil, Beans, Yogurt

Introduction

Nowadays yogurt made with various flavors and properties has become a valuable food with rapidly increasing consumption in almost every country.As yogurt and similar fermented dairy products have proved positive effects on human nutrition and health, its production and consumption are rapidly increasing all over the world. However, efforts continues to improve the production and quality of yogurt with various properties using new techniques (İşleten, 2006) In the Turkish Food Codex Communiqué on fermented milk products, yogurt is defined as the fermented dairy product in which the symbiotic cultures of Streptococcus thermophilus and Lactobacillus delbruecki subsp. bulgaricus are generally used during fermentation process.Naturally, many different techniques are widely used for the production of yogurt from milk using lactic acid bacteria. The aim of this study was to identify the microorganisms isolated from beans (Phaseolus vulgaris) and lentil (Lens

culinaris) and used in the production of yogurt using microbiological methods and to obtain preliminary data for industrial use of the identified strains.

Materials and Methods

Species identification of commercial legumes was carried out at the Botany Department of the Faculty of Science and Arts, Gaziantep University. Daily pasteurized milk (without Ultra-High Temperature) supplied from shopping malls was used for the production of yogurt.

Yogurt Production

Commercially available lentils and beans were inoculated into milk without UHT and incubated at 37 ° C for 24 hours. It was designated as the 1st product. The yoghurt was obtained by adding 20gr from the 1st product and inoculating it into 1 litre of milk. Yogurt production scheme is given in Figure 1.1.Produced yoghurts were stored at 4°C. In the 2nd day following storage, the produced yoghurts were diluted and seeded in the medium.

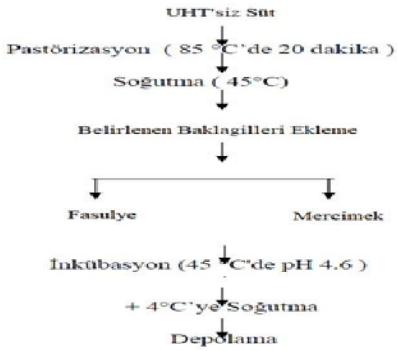

Figure 1.1 Yogurt production flowchart

Microbiological Analyzes of Yoghurt Samples

Under aseptic conditions,the produced yoghurt samples were homogenized by adding 10 g to 90 mL of sterile 0.1% (v/v) dilution solution of Yeast Sucrose Broth (YSB). Homogenization and subsequent dilutions were performed in a ratio of 1: 9. According to Wahr and Frank (2004),the produced yoghurt samples were seeded in MRS Agar and M17 Agar (Lee et al., 1974) (Figure 1.2), and after incubation, gram staining (Figure 1.3) and catalase assays (Table 1.1) were performed.

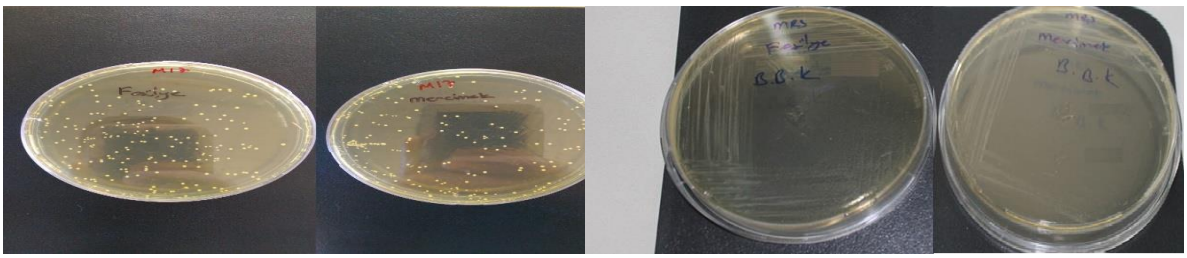

Figure 1.2 Development of microorganisms

Four isolates were isolated from the culture media and observed under microscope. These were short or long rod (Lactobacillus) or round or elliptical (Streptococcus) shaped and were gram-positive (+) when stained with gram stain.Two of the four isolates were of coccus morphology whereas the other two were of basillus morphology.All four isolates were gram positive.

MALDI-TOF Method

Morphological identification of the isolated bacteria was performed using Gram staining, catalase test and microscopic 100x oil immersion objective lense. Identification of the isolated bacteria using MALDI-TOF MS method was carried out in the Central Research Institute of Food and Feed Control Directorate,Bursa,Turkey.

Molecular Characterization of Lactic Acid Bacteria by 16S rRNA Method

Molecular identification of the four isolates isolated from the produced yogurts was confirmed by repeating the 16S rRNA gene sequence analysis method (Sanger et al., 1977) twice in Sentegen,Ankara.

Results

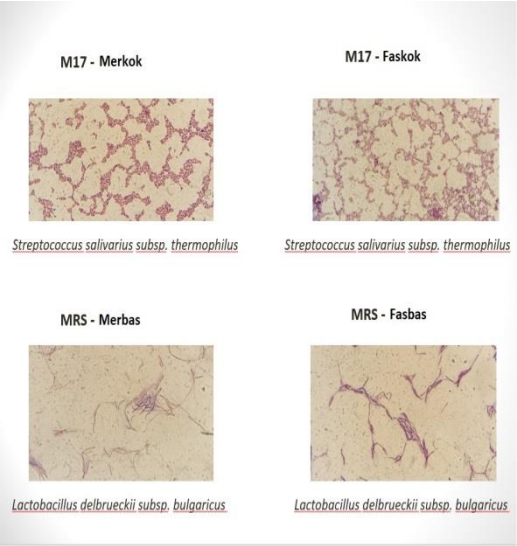

Figure 1.3 Gram staining results

Table 1.1 Catalase test results

Bacteria (M17)	Catalase test results
Merkok (Coccus-shaped bacteria isolated from Lentil)	-
Faskok (Coccus-shaped bacteria isolated from Beans)	-
Bacteria (MRS)	
Merbas (Basillus-shaped bacteria isolated from Lentil)	-
Fasbas (Basillus-shaped bacteria isolated from Beans)	-

Table 1.2 MALDI-TOF results

Number	Isolate code	Morphology/ gram staining	MALDI-TOF MS identification results
1	Merkok (Coccus-shaped bacteria isolated from Lentil)	Coccus (+)	*Streptococcus thermophilus*
2	Faskok (Coccus-shaped bacteria isolated from Beans)	Coccus (+)	*Streptococcus thermophilus*
4	Merbas (Basillus-shaped bacteria isolated from Lentil)	Basillus (+)	*Lactobacillus delbrueckii*
5	Fasbas (Basillus-shaped bacteria isolated from Beans)	Basillus (+)	*Streptococcus mitis/oralis*

Table 1.3 16S rRNA sequence analysis results.

Number	Isolate code	Morphology/gram staining	16s rRNA identification results
1	Merkok (Coccus-shaped bacteria isolated from Lentil)	Coccus (+)	Streptococcus salivarius subsp. thermophilus
2	Faskok (Coccus-shaped bacteria isolated from Beans)	Coccus (+)	Streptococcus salivarius subsp. thermophilus
4	Merbas (Basillus-shaped bacteria isolated from Lentil)	Basillus (+)	Lactobacillus delbrueckii subsp. bulgaricus
5	Fasbas (Basillus-shaped bacteria isolated from Beans)	Basillus (+)	Lactobacillus delbrueckii subsp. bulgaricus

Discussion and Conclusion

Nowdays, the dairy industry is making great efforts to develop new production technologies and active marketing strategies that will add new flavors and improve health benefits of yogurt to meet consumer expectations and to increase the per capita milk consumption. Using standard yogurt culture; we aimed to improve physical, chemical, microbiological and sensory properties of yogurt and to introduce good candidates to be yogurt starter cultures. Taken together our results in general,through fermenting milk without UHT using two different legumes first yeast was obtained then yogurt was produced. All samples were gram positive (+) when stained with gram. It was observed that the colonies that were grown on M17 media were of coccus morphology while those grown on MRS media were of bacillus morphology. According to the results of identification by MALDI-TOF, Merkok- and Faskok-denoted isolates were identified as Streptococcus salivarius subsp. thermophilus whereas Merbas-denoted isolate was identified as Lactobacillus delbrueckii subsp.bulgaricus and Fasbas-denoted isolate was identified as Streptococcus mitis/oralis. According to the results of molecular identification using 16S rRNA gene sequence analysis Merkok- and Faskok-denoted isolates were identified as Streptococcus salivarius subsp.thermophilus, whereas Merbas- and Fasbas-denoted isolates were identified as Lactobacillus delbrueckii subsp.bulgaricus. In our study, we attempted to make robust characterization using 16S rRNA gene sequence analysis.In this study; Merkok- and Faskok-denoted isolates were similar strains whereas Fasbas-denoted isolate was identiifed as different strain.However, 16s rRNA gene sequence anlysis has identified this strain as Lactobacillus delbrueckii.The contribution of these strains to the process of yogurt production may be a potential source of data for future studies. The newly identified strains will definitely make a positive contribution to the production of yogurt under controlled conditions and to the industrial use of strains. The results obtained in our study will constitute a database for researchers working with lactic acid bacteria and therefore the effort and time spent in the identification process will be spared.

References

İşleten, M., 2006. Süt kaynaklı toz bileşenlerin yağsız yoğurdun kalite kriterleri üzerine etkisi. Çanakkale Onsekiz Mart Üniversitesi, Fen Bilimleri Enstitüsü, Gıda Mühendisliği Anabilim Dalı, Yüksek Lisans Tezi, Çanakkale

Lee, S.Y., Vedamuthu, E.R. and Waham, C.J. 1974. An agar medium for the differential enumeration of yogurt starter bacteria. J. or Milk, Food Tech., Vol. **37** (5); pp. 272-276.

Sanger, F., Nicklen, S. and Coulson, A.R. 1977. DNA Sequencing with chainterminating inhibitors. PNAS 74(12), 5463-5467.

Wahr, M.H. and Frank, J.F. 2004. Lactic Acid Bacteria, Standart methods for the examination of dairy products 17th Edition, NW, pp. 235-247, Washington.

Author Information

Mesut Cay
Gaziantep University
Department of Biology 27310, Gaziantep, Türkiye

Ibrahim Halil Kilic
Gaziantep University
Department of Biology 27310, Gaziantep, Türkiye
Contact e-mail: kilic@gantep.edu.tr

Mehmet Ozaslan
Gaziantep University
Department of Biology 27310, Gaziantep, Türkiye

Isik Didem Karagoz
Gaziantep University
Department of Biology 27310, Gaziantep, Türkiye

Zeynep Koloren
Gaziantep University
Department of Biology 27310, Gaziantep, Türkiye

Bekir Siddik Kurt
Gaziantep University
Department of Biology 27310, Gaziantep, Türkiye

Sami Serhat Tonus
Gaziantep University
Department of Biology 27310, Gaziantep, Türkiye