

DETECTION OF PROBIOTIC MICROORGANISMS USING RT-PCR, AND
ISOLATION, IDENTIFICATION, PROBIOTIC PROPERTIES OF
LACTOBACILLUS SPECIES FROM KEFIR

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
BIOCHEMISTRY

SEPTEMBER 2020

Approval of the thesis:

**DETECTION OF PROBIOTIC MICROORGANISMS USING RT-PCR,
AND ISOLATION, IDENTIFICATION, PROBIOTIC PROPERTIES OF
LACTOBACILLUS SPECIES FROM KEFIR**

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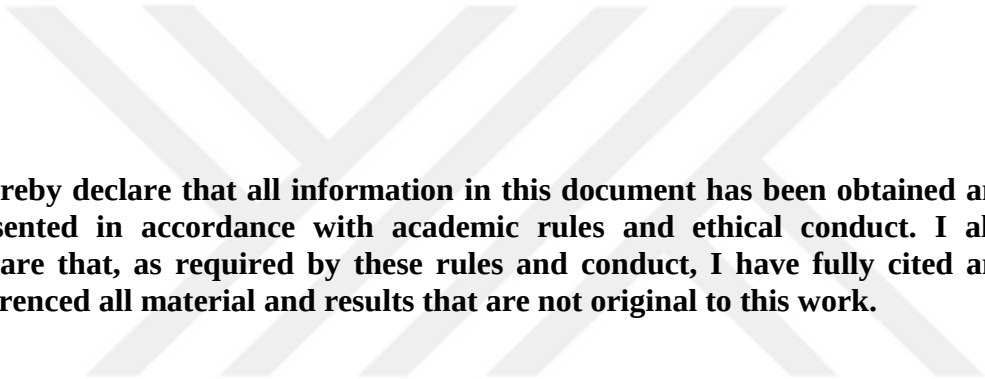
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ABSTRACT

DETECTION OF PROBIOTIC MICROORGANISMS USING RT-PCR, AND ISOLATION, IDENTIFICATION, PROBIOTIC PROPERTIES OF *LACTOBACILLUS* SPECIES FROM KEFIR

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September 2020, 258 pages

Kefir is a dairy product rich in probiotics. In this study, a SYBR green-based real-time PCR (RT PCR) method was used for the detection of kefir probiotic strains. This assay allowed species-specific detection of *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. kefiri*, *L. kefiranofaciens*, *L. fermentum*, *L. plantarum*, *L. amylovorus*, *L. casei*, *L. paracasei*, *Streptococcus thermophilus* and *Saccharomyces cerevisiae*. This method was performed using DNA isolated directly from five different sources of kefir from Turkey and one milk product from Kyrgyzstan. Results of amplification and melting curve from real time PCR assay showed that *L. kefiri*, *L. kefiranofaciens*, *L. casei*, and *L. paracasei* were the dominant bacteria in all kefir samples while *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. fermentum*, *L. plantarum*, *L. amylovorus*, *Streptococcus thermophilus*, and *Saccharomyces cerevisiae* were detected in a few kefir samples. Based on microscopic examination and biochemical tests, 30 of 100 bacterial isolates were confirmed to belong to the genus *Lactobacillus*. It was also indicated that isolated *Lactobacillus* species from kefir samples have potential probiotic properties.

In addition, identification studies for the isolates from the kefir samples were also carried out. These isolates were identified by 16S rRNA sequencing BLAST analysis and alignment. BLAST results showed that 19 isolates had more than 97% similarity with *Lactobacillus paracasei* subsp. *tolerans* strain NBRC 15906, 5 isolates had more than 99% similarity with *Lactobacillus gallinarum* strain ATCC 33199, 2 isolates (k5-14 and k5-15) showed 99.21 and 98.71 % similarity of *Lactobacillus zeae* respectively, and 2 isolates (K4-6a and K6-3a) displayed more than 99.22 % similarity with *Lactobacillus helveticus* strain NBRC 15019. In addition, 2 more isolates were also identified, K6-14 which was identical to *Lactobacillus rhamnosus* strain NBRC 3425 with 99.45% similarity and K2-3 which showed 98.27 % similarity to *Lactobacillus intestinalis* strain TH4. Sequence alignment using the Clustal omega program was useful for identification of some isolates.

Keywords: Kefir, *Lactobacillus*, real-time PCR , *L. kefiri*, *L. kefiranofaciens*, *L. casei*, *L. paracasei*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. fermentum*, *L. plantarum*, *L. amylovorus*, *Streptococcus thermophilus*, *L. gallinarum*, *L. zeae*, *L. helveticus*, *L. rhamnosus*, *L. intestinalis*, *Saccharomyces cerevisiae*, biochemical tests, 16 S rRNA sequencing analysis.

ÖZ

KEFİRDE PROBIOTIC MIKROORGANİZMALARIN RT-PCR İLE TESPİTİ VE *LACTOBACILLUS* TÜRLERİNİN İZOLASYONU TANISI, PROBIOTIC ÖZELLİKLERİ

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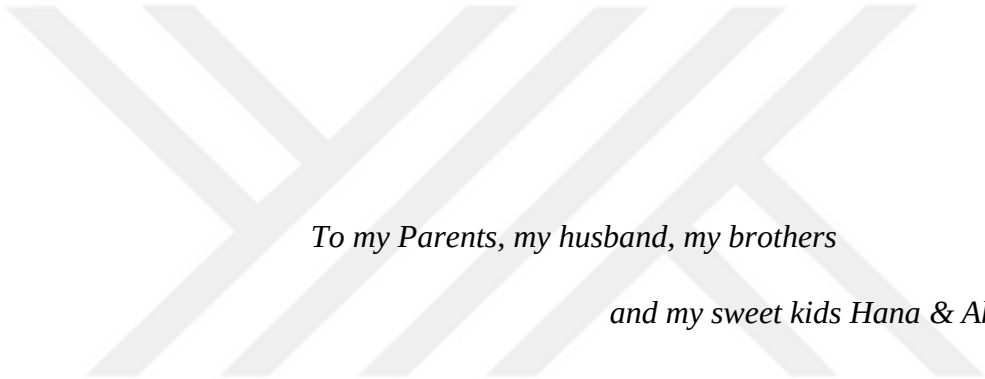
Ortak Tez Yöneticisi: Prof. Dr. Ayşe Gül Gözen

Eylül 2020, 258 sayfa

Kefir, probiyotik bakımından zengin bir süt ürünüdür. Bu çalışmada kefir probiyotik suşlarının saptanması için SYBR green bazlı gerçek zamanlı PZR metodu kullanılmıştır. Bu analiz, *L.acidophilus*, *L.delbrueckii subsp. bulgaricus*, *L. kefiri*, *L. kefiranofaciens*, *L. fermentum*, *L. plantarum*, *L. amylovorus*, *L. casei*, *L. paracasei*, *Streptococcus thermophilus* ve *Saccharomyces cerevisiae* türlerine özgü tespiti olanak sağlamıştır. Bu metot, Kırgızistan'daki bir süt ürününden ve Türkiye'deki beş farklı kefir kaynağından doğrudan izole edilen DNA'yı kullanarak gerçekleştirilmiştir. PZR testinden elde edilen amplifikasyon ve erime eğrisi sonuçları, tüm kefir örneklerinde *L. kefiri*, *L. kefiranofaciens*, *L. casei* ve *L. paracasei*'nin dominant bakteri olurken; *L. acidophilus*, *L. delbrueckii subsp. bulgaricus*, *L. fermentum*, *L. plantarum*, *L. amylovorus*, *Streptococcus thermophilus* ve *Saccharomyces cerevisiae*'ın birkaç kefir örneğinde tespit edildiğini göstermektedir. Mikroskopik inceleme ve biyokimyasal testlere göre, 100 bakteriyel izolattan 30'unun *Lactobacillus* cinsine ait olduğu doğrulanmıştır. Kefir örneklerinden izole edilen *Lactobacillus* türlerinin potansiyel probiyotik özelliklere sahip olduğu da gösterilmiştir.

Ayrıca, kefir örneklerinden elde edilen bu izolatların tanımlama çalışmaları yürütülmüştür. Bu izolatlar 16S rRNA sekanslama analizi ve hizalama ile tanımlanmıştır. BLAST sonuçları, 19 izolatın *Lactobacillus paracasei* subsp. tolerans suşu NBRC 15906 ile % 97'den fazla benzerlik gösterdiğini, 5 izolatın *Lactobacillus gallinarum* suşu ATCC 33199 ile % 99'dan fazla benzerlik gösterdiğini, 2 izolatın (k5-14 ve k5-15) *Lactobacillus zeae* ile sırasıyla % 99.21 ve % 98.71 benzerlik gösterdiğini, ayrıca 2 izolatın (K4-6a ve K6-3a) *Lactobacillus helveticus* suşu NBRC 15019 ile % 99.22'den fazla benzerlik gösterdiğini ortaya koymuştur. Bununla birlikte, *Lactobacillus rhamnosus* suşu NBRC 3425 ile %99,45 benzerlik gösteren (K6-14) ve *Lactobacillus intestinalis* TH4 suşu ile %98,27 benzerlik gösteren K2-3 olmak üzere iki izolat daha tanımlanmıştır. Clustal omega programı ile karşılaştırmalı dizilim hizalama (sequence alignment), bazı izolatların tanısında yararlı olmuştur.

Anahtar Kelimeler: Kefir, *Lactobacillus*, gerçek zamanlı PZR, *L. kefir*, *L. kefiranofaciens*, *L. casei*, *L. paracasei*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. fermentum*, *L. plantarum*, *L. amylovorus*, *Streptococcus thermophilus*, *L. gallinarum*, *L. zeae*, *L. helveticus*, *L. rhamnosus*, *L. intestinalis*, *Saccharomyces cerevisiae*, biyokimyasal testler, 16S rRNA sekans analizi.



To my Parents, my husband, my brothers

and my sweet kids Hana & Ahmed

ACKNOWLEDGMENTS

I would like to express my greatest thanks to my supervisor Prof. Dr. G. Candan Gürakan for her guidance, constructive criticism, comments, positive approach and patient insight throughout the research. Her friendly behaviour let me free to express myself through this work and proceed in my own way.

I would also like to express my gratitude to my co-supervisor Prof. Dr. Ayşe Gül Gözen for her invaluable advices, contributions and help. I should also mention my respect and thank for allocating her valuable time to my research.

I would also like to thank the members of examining committee; Prof. Dr Kamuran Ayhan (Thesis Follow up Committee Member), Prof. Dr. Alev Bayındırlı (Thesis Follow up Committee Member), Assoc. Prof. Dr. Yeşim Soyer and Assist. Prof. Dr. Aysun Cebeci, for their scientific inputs, suggestions, supports and encouragements.

I would like to thank my lab mates Elnaz Monir, Ipek Aktuna, Sefa and Nasim for their help and great cooperation for having such a productive time in the laboratory.

Lastly, I would like to thank my family, my parents, my husband, my brothers and my lovely kids for their unconditional love and support. Words cannot express how grateful to my father, Sami and my mother, Bahia and my husband Mohamed for all of the sacrifices they have made for me over the years.

TABLE OF CONTENTS

ABSTRACT.....	v
ÖZ.....	vii
ACKNOWLEDGMENTS	x
TABLE OF CONTENTS.....	xi
LIST OF TABLES	xvi
LIST OF FIGURES	xvii
LIST OF ABBREVIATIONS	xix
CHAPTERS	
1.INTRODUCTION	1
1.1 Kefir	1
1.1.1. Origin of Kefir	1
1.1.2. Kefir Grain	1
1.1.3 Chemicals Composition of the Kefir	3
1.1.4 Health Benefits of Kefir.....	4
1.2 Lactic Acid Bacteria (LAB).....	7
1.2.1 Taxonomical Classification of Lactic Acid Bacteria	7
1.2.2 Description Of Lactic Acid Bacteria.....	8
1.2.3 Lactic Acid Bacteria Benefiting Health....	9
1.3 Isolation And Identification Of Lactic Acid Bacteria	10
1.3.1 Phenotypic Identification.	11
1.3.1.1 Morphological Methods.....	11
1.3.1.2 Physiological and Biochemical Tests.	11
1.3.2 Genotypic Identification... ..	14

1.3.2.1 The 16Sr RNA Gene Sequencing...	13
1.3.2.2 BLAST Analysis...	15
1.4. Detection of Probiotic Bacteria in Kefir Using Real-Time PCR...	14
1.4.1 Real-time PCR (Species-Specific PCR)...	15
1.4.2. SYBR Green I method.....	17
1.5 Microbiota of Kefir...	18
1.5.1 <i>Lactobacillus casei</i>	20
1.5.2 <i>Lactobacillus paracasei</i>	20
1.5.3 <i>Lactobacillus plantarum</i>	24
1.5.4 <i>Lactobacillus acidophilus</i>	24
1.5.5 <i>Lactobacillus intestinalis</i>	25
1.5.6 <i>Streptococcus thermophilus</i>	25
1.5.7 <i>Saccharomyces cerevisiae</i>	25
1.5.8 <i>Lactobacillus helveticus</i> ...	26
1.5.9 <i>Lactobacillus gallinarum</i>	26
1.5.10 <i>Lactobacillus kefir</i>	27
1.5.11 <i>Lactobacillus kefiranoferiens</i>	28
1.5.12 <i>Lactobacillus fermentum</i>	28
1.5 13 <i>Lactobacillus amylovorus</i>	28
1.5.14 <i>Lactobacillus delbrueckii subsp. bulgaricus</i>	29
1.5.15 <i>Lactobacillus zeae</i> ...	29
1.6 Probiotic of Lactic Acid Bacteria.....	29
1.7 Aim of the study.....	30
2. MATERIALS AND METHODS	33
2.1. Collection of samples.	33

2.2. Kefir Preparation From Kefir Grains.....	34
2.3. Real Time PCR Assay (RT PCR)	34
2.3.1 Selection the Primers of Real Time PCR.....	34
2.3.2 DNA Extraction for Real Time PCR	36
2.3.3 Detection of Different Bacteria and Yeast species in Kefir Using Real Time PCR	36
2.3.4 Preparation of Real time PCR Master Mix	36
2.3.4.1 Dispensing the Master Mix	37
2.3.4.2. Addition of Template DNA	37
2.3.5 Real Time PCR Analysis	38
2.4 Isolation of the Bacteria from Kefir Samples	38
2.4.1 Phenotypic identification of the Isolated Bacterial Strains.....	39
2.4.1.1 Gram staining.....	39
2.4.1.2 Catalase Test	39
2.5 Biochemical Characterization of the Isolated Bacterial Strains.....	39
2.5.1 pH Tolerance test	40
2.5.2 Temperature Tolerance Test	40
2.5.3 NaCl Tolerance Test	40
2.5.4 Phenol Tolerance Test.....	41
2.5.5 Lactose Utilization	41
2.5.6 The Protolytic Activity of Lactic Acid Bacteria	41
2.5.7 Bile Salt Hydrolase	42
2.5.8 Carbohydrastes Fermentation Test.....	42
2.6 Genetic Characterization of Isolates	42
2.6.1 Extraction of Genomic DNA of LAB Isolates.....	43

2.6.1.1 DNA Binding Spin - Column activation	43
2.6.1.2 Bacterial Sample Preparation	43
2.6.1.3 DNA Isolation	43
2.6.2 Polymerase Chain Reaction(PCR).....	44
2.6.2.1 Amplification of 16S r RNA Gene	44
2.6.2.2 Separation the DNA Fragment Using Agarose Gel.....	45
2.6.2.3 DNA Purification from Agarose Gel.....	45
2.6.3 Sequencing of 16S r RNA Gene.....	45
2.6.4 Analysis of 16S r RNA Gene	46
2.6.5 Multiple Sequence Alignment (MSA).....	46
3.RESULTS AND DISSCUSSION	47
3.1. Experimental Strategy.....	47
3.2 Collection of Samples.....	53
3.3 Kefir Preparation	53
3.4 Detection of Probiotic Microorganisms Using Real Time PCR.....	53
3.5 Isolation of Lactic Acid Bacteria.....	80
3.6 Phenotypic Identification of Lactic Acid Bacteria	80
3.6.1 Colony Morphology	80
3.6.2 Microscopic Examination.....	83
3.6.3 Catalase Test.....	83
3.7 Biochemical Tests	85
3.7.1 pH Tolerance Test	85
3.7.2 NaCl Tolerance Test.....	86
3.7.3 Temperature Tolerance Test.....	88
3.7.4 Phenol Tolerance Test	90

3.7.5 Lactose Utilization	91
3.7.6 The Proteolytic Activity of Lactic Acid Bacteria	91
3.7.7 Bile Salt Tolerance (Deconjugation of Bile Salts).....	94
3.7.8 Carbohydrates Fermentation Test	96
3.8 16S rRNA Gene Sequencing of <i>Lactobacillus</i> Isolates	99
3.9 Multiple Sequence Alignment and Blast Analysis	100
4.CONCLUSION.....	121
REFERENCES.....	123
APPENDICES	
A. Real Time PCR Analysis	157
B. 16S rRNA ribosomal RNA Gene Partial Sequence	163
C. Blast Analysis of Partial 16S rRnA Gene of Kefir Isolates	193
CURRICULUM VITAE	257

LIST OF TABLES

TABLES

Table 1.1 Detection of LAB and yeast of kefir, kefir grains and dairy product using real time PCR	16
Table 1.2 Microbial population of kefir grains and beverages from different origins	22
Table 2.1 Origins of homemade kefir and milk samples.....	33
Table 2.2 The oligonucleotides primers	35
Table 2.3 real time PCR materials.....	37
Table 2.4 PCR mixture for 16S rRNA gene sequencing.....	44
Table 3.1 Detection the population of kefir samples using specific primers for real-time PCR	54
Table 3.2 Source of kefir and Colony morphology of isolates.....	81
Table 3.3 Gram staining and catalase test for kefir isolates	84
Table 3.4 The Tolerance of lactobacillus isolates to pH and NaCl salt.....	87
Table 3.5 Temperature, phenol tolerance and lactic acid production test	89
Table 3.6 Proteolytic activity and bile salt tolerance test	93
Table 3.7 Carbohydrates fermentation patterns of isolates	97
Table 3.8 Fermentation of sugars by <i>Lactobacillus</i> isolates and references	99
Table 3.9 BLAST analysis and alignment results for K2 isolates.....	101
Table 3.10 BLAST analysis and alignment results for K3 isolates.....	103
Table 3.11 BLAST analysis and alignment results for K4 isolates.....	104
Table 3.12 BLAST analysis and alignment results for K5 isolates.....	105
Table 3.13 BLAST analysis and alignment results for K6 isolates.....	106
Table 3.14 Results of one and two directions 16s rRNA sequencing and MSA...	111
Table 3.15 Summary for identification of isolates and references.	116
Table 3.16 Shows the results of real time PCR and isolation of kefir isolates.....	118

LIST OF FIGURES

FIGURES

Figure 1.1 Kefir grains	2
Figure 1.2 SYBR Green I real time PCR technique.	17
Figure 1.3 Genomic based phylogenetic analysis of the <i>L. casei</i> group.....	22
Figure 1.4 Phylogenetic placement of <i>L.acidophilus</i> subgroup within the <i>Lactobacillus</i> genus.....	27
Figure 2.1 Real time PCR program.....	38
Figure 3.1 Experimental planing (section 1).Collection of kefir grains and preparation of kefir milk	48
Figure 3.2 Experimental planing (section 2). Determination the population in home made kefir samples.....	49
Figure 3.3 Experimental strategy (section 3). Isolation and microbiological and biochemical identification LAB.....	50
Figure 3.4 Biochemical tests applied in the study (section 4).	51
Figure 3.5 Experimental planing of the blast analysis.	52
Figure 3.6 Amplification and melting curve for Kefir sample (K4) with <i>L. kefiri</i>	56
Figure 3.7 Amplification and melting curve for Kefir sample (K6) with <i>L.</i> <i>kefiranofaciens</i>	58
Figure 3.8 Amplification and melting curve for Kefir sample (K5) with <i>S.</i> <i>cerveisiae</i>	60
Figure 3.9a Amplification and melting curve for <i>L. acidophilus</i> in Kefir (K6).	62
Figure 3.9b Amplification and melting curve for <i>L. acidophilus</i> in Kefir (K2).	63
Figure 3.10 (a) Amplification and melting curve for Kefir (k1) with <i>L. plantarum</i>	65
Figure 3.10 (b) Amplification and melting curve for Kefir (k2) with <i>L.plantarum</i>	66
Figure 3.11 Amplification and melting curve for <i>L. casei</i> in all samples.....	68

Figure 3.12 Amplification and melting curve for <i>L. paracasei</i> in all samples.....	70
Figure 3.13(a) The amplification and melting curve of <i>L. delbrueckii subsp. bulgaricus</i> in K1.....	71
Figure 3.13(b) The amplification and melting curve of <i>L. delbrueckii subsp. bulgaricus</i>	72
Figure 3.14 (a) The amplification and melting curve of <i>L. amylovorus</i> in K4.....	73
Figure 3.15 The amplification and melting curve of <i>L. fermentum</i> k4.....	75
Figure 3.16 The amplification and melting curve of <i>S. thermophilus</i> in k6.....	77
Figure 3.17 (a) Amplification curves of kefir sample (K5 and K6) with specific primers (<i>L. Kefiri</i> , <i>L. acidophilus</i> , <i>L. kefiranofaciens</i> , <i>L. fermentum</i> , <i>L. plantarum</i> , <i>L. delbrueckii subsp. bulgaricus</i> , <i>S. cerevisiae</i> , <i>S. thermophilus</i> and <i>L. sobris \amylovorus</i>).	78
Figure 3.17 (b) Melting curves of kefir sample (K5 and K6) with specific primers (<i>L. Kefiri</i> , <i>L. acidophilus</i> , <i>L. delbrueckii subsp. bulgaricus</i> , <i>L. kefiranofaciens</i> , <i>L. fermentum</i> , <i>L. plantarum</i> , <i>S. cerevisiae</i> , <i>S. thermophilus</i> and <i>L.sobris \amylovorus</i>	78
Figure 3.18 Gram staining.....	83
Figure 3.19 Proteolysis activity for <i>Lactobacillus</i> isolates	92
Figure 3.20 Bile salt hydrolase activity on solid MRS.....	96
Figure 3.21 Agarose gel electrophoresis of PCR	100
Figure 3.22 The alignment of the 16S rRNA gene of <i>Lactobacillus</i> isolate k6-3a with bases of <i>L. helveticus</i> and <i>L. gallinarum</i>	108
Figure 3.23 The alignment of the 16S rRNA gene of <i>Lactobacillus</i> isolate k2-3a with bases of <i>L. helveticus</i> , <i>L. intestinalis</i> and <i>L. gallinarum</i>	109
Figure 3.24 The alignment of the 16S rRNA gene of <i>Lactobacillus</i> isolate K5-14 with bases of <i>L. casei</i> , <i>L. paracasei</i> and <i>L. zeae</i>	109
Figure 3.25 The alignment of the 16S rRNA gene of <i>Lactobacillus</i> isolate K6-14 with bases of <i>L. rhamnosus</i> , <i>L. paracasei</i> and <i>L. zeae</i>	110
Figure 3.26 The alignment of the 16S rRNA gene of <i>Lactobacillus</i> isolate K5-15 with bases of <i>L. casei</i> , <i>L. paracasei</i> and <i>L. zeae</i>	110

Figure 3.27 Phylogenetic tree of aligned 16S rRNA gene sequences for the known species	112
Figure 3.28 Phylogenetic tree of aligned 16S rRNA gene sequences for some isolates.....	112
Figure 3.29 Phylogenetic tree of aligned 16S rRNA gene sequences for K6-14 with related known species	112



LIST OF ABBREVIATIONS

ABBREVIATIONS

Ribosomal RNA: r RNA

Polymerase Chain Reaction : (PCR)

Lactic Acid Bacteria : (LAB)

Base Pair : (bp)

Real Time Polymerase Chain Reaction : (Real Time PCR)

Real Time PCR : (RT PCR)

Lactobacillus : (*L.*)

Saccharomyces: (S.)

Streptococcus: (*Str*)

Temperature : (Temp)

Melting Temperature: (Tm)

Multiple Sequence Alignment : (MSA)

Ataturk Orman çiftliği : (AOC)

De Man, Rogosa, and Sharp : (MRS)

Threshold Cycle : (CT)

Biological Resource Center,NITE : (NBRC)

American Type Culture Collection: (ATCC)

German Collection of Microorganisms and Cell Cultures : (DSM)

CHAPTER 1

INTRODUCTION

1.1 Kefir

1.1.1 Origin of Kefir

The name “Kefir” derived from Turkish source “keyif” means good pleasure. Kefir beverage consumed in Southwest Asia, Russia and Eastern Europe (Gaware et al., 2011). Wszolek et al (2006) has stated that, the common way of preparing kefir is an inoculation of grains into the milk. The starter culture used to produce kefir is small, gelatinous, yellowish, and irregularly shaped as explained by Witthuhn et al., 2005.

People living in the countries of the former Soviet Union have been using Kefir for its health benefits for a long time. Health experts in those countries always recommend the consumption of kefir. Prado et al (2015) underline many health benefits related to the drinking of this fermented milk and these health benefits are related to the existence of metabolic substances as organic acids and its microflora. Hospitals in the former Soviet Union used Kefir to treat cancer, digestive disorders, and even atherosclerosis and tuberculosis (Shavit, 2008).

1.1.2 Kefir Grains

During the preparation of kefir beverage, kefir grains use as fermentation starter by incubation with the milk. Kefir grains have an irregular surface, smooth, viscous, shiny and yellowish-white color as described by Magalhães et al (2011) and Rattray and O’Connell (2011). If wet kefir grains does not inoculate into fresh milk, will keep

activity for 8-10 days only, however, dried grains can keep their activity for about 12-18 months (O'Brien, 2012).



Figure 1.1 Kefir grains (Otles and Cagindi, 2003)

Kefir grains represent a symbiotic relationship between lactobacilli and lactococci bacteria, acetic bacteria and yeasts. A complex microbial population of kefir grains consists of different species of bacteria and yeasts and also several species of filamentous molds (Sarkar et al., 2008; Wang et al., 2008).

Kefiran is the main polysaccharide of Kefir grains that is a complex structure containing equal amounts of glucose and galactose and is essentially generated by *L. kefiranofaciens*. Moreover, Kefiran may be used as a supplement in fermented products because it develops the viscosity properties of acidic milk gels. Also, kefiran can activate the characteristic of skim milk which promote the viscosity of Kefir milk (Prado et al., 2015). The milk of the most mammals as sheep, cow, and goat can be fermented by kefir grains and each one has different nutritional qualities and organoleptic properties. Kefir grains can ferment milk alternatives like coconut milk, soy milk, and rice milk (Gaware et al., 2011).

1.1.3 Chemicals Composition of Kefir

Sarkar (2007) has indicated that Kefir contains 89–90% moisture, 6.0% sugar, 3.0% protein, 0.7% ash, 0.2% lipid and 1% each of lactic acid with alcohol.

Puerari et al (2012) and Ahmed et al (2013) classified many secondary metabolites during fermentation as flavor, taste, and aroma forming compounds synthesized such as carbonyl substances (diacetyl, acetaldehyde, ethyl acetate, acetoin and ethanol), volatile and non volatile organic acids.

Farnworth (2005) found that lactic acid was the maximal concentration organic acid produced from lactose in milk. The quantities of ethanol and CO₂ synthesized depend on the conditions of production process. Depending on the species or strains present in the milk, the amounts and kind of flavor compounds formed by these strains vary and can affect the quality of the final beverage (Maurellio et al. 2001).

Acetone is a flavorless and odorless compound, it has a considerable effect on the flavor of kefir (Aghlara et al, 2009). Acetone produces from lactose and citrate metabolism and its formation depends on the strain. Some lactobacilli strains such as *L. helveticus* and some streptococci cultures such as *S. lactis* can produce acetone in low amounts (Beshkova et al., 2003).

Ethanol is synthesized from acetaldehyde, by alcohol dehydrogenase enzyme. Yeasts are the most ethanol maker according to Guzel-Seydim et al (2000). Two kinds of yeasts may be found in kefir: they are lactose and non lactose fermenting yeasts. The fermented product prepared by using non-lactose fermenting yeast. It has a stronger yeast flavor than the product obtained by lactose-fermenting yeast (Simova et al., 2002; Beshkova et al. 2003).

Carbon dioxide is produced via alcoholic fermentation and it gives the slight ebullition of kefir (Liu et al., 2002).

Acetic acid is a fatty acid that has been determined at low concentrations than 800 mg in kefir (Garrote et al., 2001). In spite of, Guzel-Seydim et al (2000) could not find any acetic acid in kefir. Magalhães et al (2011) detected low acetic acid ratios in kefir milk without any effect on the organoleptic properties of the beverage.

Formation of acetic acid needs different amino acids; for example, *S. diacetylactis* can make acetic acid from leucine, glycine, and alanine (Liu et al., 2002). Also, acetate can be produced from pyruvate in the presence or absence of oxygen. (Garrote et al., 2001).

Zubeyde et al., 2010 discussed the properties of kefir produced using different milk samples and types of fermentation culture. They concluded that starter culture type and storage duration affected the pH changes.

1.1.4 Health Benefits of Kefir

Kefir has many significant health benefits as physiological and therapeutic properties due to a wide diversity of metabolic bioactive substances produced during fermentation. The diversity of the microbial population also influences these health care (Leite et al ., 2013). Kefir is known to contain enzymes, amino acids, mineral (magnesium, phosphorus, calcium), and vitamins (B12, B2, A, D, K) (Gaware et al., 2011).

- **Heart health:**

Kefir aids in caring the heart since it regulates the blood pressure and clears the blood vessels. Drinking kefir helps to clean the blood vessels and the blood pressure can be regulated (Gaware et al., 2011).

- Reduces the cholesterol levels:

Kefir aids in reducing high cholesterol levels. It is very beneficial for eliminating many cardiovascular diseases like heart attack (Shavit, 2008).

- Digestion:

Kefir enhances digestion, prevents constipation, regulates the bowel movements and cleaning the intestines.

- Brain-enhancement:

Kefir can develop the working of the brain and it is important for a healthy nervous system. It helps to fight the stress. The minerals in Kefir also improve the focus and the power of memory-retention of the brain (Gaware et al., 2011).

- Respiratory system:

Kefir plays an important part in the medication of asthma and bronchitis.

- Weight loss:

Kefir probiotics increase the body's metabolism leading to weight loss.

- Prevention against toxins:

Kefir can protect the human body from the toxic effects of radiation and other harmful pollutants and can enhance the immune function.

- The Lactose Intolerance:

Ahmed et al. (2013) highlight that some of the kefir's bacteria can break lactose down, kefir aids people who has lactase enzyme deficiency to digest lactose products.

- A Healthier Immune System:

Probiotics of kefir have stimulatory reactions on the immune system by increasing T-lymphocyte numbers and improving phagocytosis. Kefir produces bioactive peptides in the fermentation process which have an indirect effect on the immune system as mentioned by Shavit (2008).

- Antibiotic and antifungal properties:

Prado et al. (2015) overviewed that kefir has certain antifungal compounds that treat yeast infections like candidiasis and eczema. Moreover, Kefir microorganisms synthesize lactic acid, bactericides and antibiotics that help to kill pathogenic bacteria such as *Helicobacter*, *Salmonella*, *Shigella*, *Escherichia coli*, *Staphylococcus*, *Micrococcus luteus*, *Bacillus subtilis*, *Streptococcus pyrogens*, and *Listeria monocytogenes*.

- Anti-cancer agent:

Many studies summarized by Shavit (2008) have demonstrated that kefir has conservative effects against some types of cancer and reduce the size of tumors by inhibiting the growth of bacteria in the digestive system that convert procarcinogens into carcinogens.

- Anti-Diabetic:

Kefir has an important role in diabetics as it decreases the glucose concentration and control the regular sugar level in the blood (Shavit, 2008).

- Anti-inflammatory:

Kefir is useful in treating several disorders like gastritis, pancreatitis and other inflammatory diseases (Prado et al., 2015).

- Wound healing properties:

Hassan et al (2012) discussed that acetic acid, lactic acid, sugar compounds and other chemical compounds found in kefir were important factors for wound healing properties.

1.2 Lactic Acid Bacteria (LAB)

1.2.1 Taxonomical Classification of Lactic Acid Bacteria:

(LAB) include various bacterial species within the phylum firmicutes. The genera *Lactococcus*, *Lactosphaera*, *Leuconostoc*, *Lactobacillus*, *Milissococcus*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Enterococcus*, *Weissella* and *Vagococcus* are identified as lactic acid bacteria (Tadesse et al., 2005). Only few of them are dairy related. They are *Enterococcus*, *Lactococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc* and *Lactobacillus* (Axelsson, 1998)

Taxonomical classification of LAB depends on sequencing analysis for 16S ribosomal RNA (rRNA). It has detected that some classification created based on phenotypical characterization do not match with the phylogenetic relations. Molecular assays, mainly polymerase chain reaction (PCR) based techniques such as rep-PCR finger printing and pulse-field gel electrophoresis are remarked useful for probiotic strain differentiation, identification and discrimination (Gevers et al., 2001; Holzapfel et al., 2001).

Species-specific (real time PCR) was developed by Monique and Jan (2006) to detect and quantify different *Lactobacillus* species such as *L. casei*, *L. delbrueckii*, *L. rhamnosus*, *L. plantarum*, *L. reuteri*, *L. acidophilus*, *L. fermentum* and *L. paracasei* in breast-fed infants.

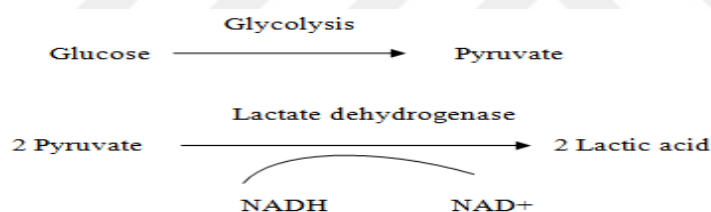
1.2.2 Description of Lactic Acid Bacteria

The LAB are common and prevalent in nature, rich in carbohydrates and contain protein breakdown products, vitamins, essential enzymes, antibacterial peptides, hydrogen peroxide and aromatic compounds, all of which contribute to probiotic properties (Leroy and de Vuyst, 2004). The LAB play a significant role in food fermentation that affects acidity, texture, and flavor of the end products in addition to many benefits on human health (Sun, 2010).

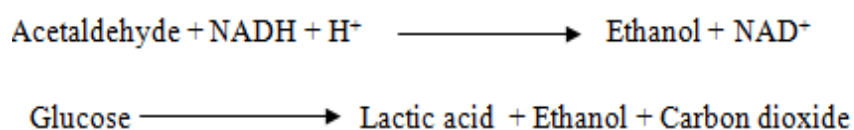
The LAB consist of a great group of rod, gram positive, catalase negative and aerotolerant bacteria. These bacteria yield lactic acid as the basic end products in sugar fermentation.

Regarding carbohydrate metabolism, they are classified into 2 major groups:

1. Homofermentative LAB



2. Heterofermentative LAB



Wessels et al (2004) reviewed the power of LAB to produce the chemicals substances of respiratory chains as cytochromes and porphyrins. Lactic acid bacteria can gain ATP only by sugar fermentation. They can survive under anaerobic

conditions easily, but also they can survive in the presence of oxygen by producing peroxidases.

1.2.3 Lactic Acid Bacteria Benefiting Health

LAB are considered a major class of probiotic bacteria (Collins et al., 1998; Schrezenmeir and de Vrese, 2001). Fuller (1989) defined the probiotic word as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial action”. Also, Salminen et al (1999) suggested that probiotics are microorganisms that have a helpful effect on human health.

Many lactobacilli, lactococci and bifidobacteria are probiotic bacteria (Rolfe, 2000; Tuohy et al., 2003). LAB have a significant part in prolongation the storage life and improving the safety of basic food products by producing bacteriocins that inhibit foodborne pathogens and spoilage microorganisms. They also have a beneficial effect on nutritional and sensory characteristics (Ravi et al., 2011).

LAB applied in probiotic construction, are obtained from the gastrointestinal tract and they are tested and identified with a history of safe use and non-pathogenic or antibiotic resistance (Stolarczyk, 2002). The capability of lactic acid bacteria to form lactic acid from lactose, it contributes to the effective treating of lactose intolerance. People are unable to digest lactose in milk due to the absence or dysfunction of the essential enzyme systems.

Besides, Jack (1995) investigated that LAB reduce the pH of the intestinal medium which eliminate the growth and survival of some organisms normally requiring high pH for their growth. Some LAB produce substances with a bacteriostatic effect such as H_2O_2 and bacteriocins thus preventing the development of pathogenic microorganisms as *E.coli*.

LAB represent an whole part of the gastrointestinal environment (Fernandes et al., 1987). The metabolism of probiotics depends on the fermentation mechanism. As well as other gut microflora, LAB ferments different types of substrates as lactose (Gibson and Fuller, 2000). Intestinal LAB have a beneficial function in the metabolism and detoxification of harmful components entering the body by producing antioxidants and bacteriocins (Salminen, 1990).

Grangette et al (2001) and Cross (2002) found that LAB control intestinal disorders due to antibodies that enhance the immune response. LAB can eliminate harmful bacteria from adhesion to the intestinal epithelial cells or by secretion of antimicrobial compounds such as bacteriocins and some organic acids (Reid, 2001).

Boris et al (1998) explained vaginal LAB strains are capable of attaching or self-aggregating with pathogen by surface proteins or lipoproteins. Both may help to the ejection of pathogens from the vaginal mucous membrane.

1.3 Isolation and Identification of Lactic Acid Bacteria

LAB applied as starter substances in the food industry, and their dietary needs are very complicated. Hence, their predominant environments of being rich in sugars, having amino acids, vitamins and living in environments with weak oxygen, their frequency in milk products create health benefits for the consumer and enhance the flavor, texture and the nutritional quality of the food products (Stiles and Holzapfel, 1997).

The current study deals with the isolation, studying probiotic properties and identification of lactobacilli from dairy products and kefir samples collected from different sources in Turkey and Kyrgyzstan. For identification species of bacteria,

both genotypic and phenotypic techniques were utilized to get accurate identifications.

1.3.1 Phenotypic Identification

Even if genetic identification methods are time saving and give very accurate results, phenotypic identification methods are very helpful especially in the differentiation at the genus level and decrease the number of isolates for genetic identification.

To determine LAB, phenotypic techniques, which incorporate morphological examinations and physiological and biochemical tests are generally applied.

1.3.1.1 Morphological Methods

Microscopic investigation is the initial criterion that provides data about the genus level and clarity of lactic acid bacteria. There are several staining tests to distinguish the bacterial cells as simple staining, endospore staining, capsule staining, acid fast staining and gram staining. The useful method for *Lactobacillus* identification is the Gram staining. According to Gram staining reaction, bacteria can be divided into Gram-positive and Gram-negative organisms. The LAB join to the Gram-positive, rod shape group. Round cells are named cocci, elongated rod cells are named bacilli and intermediary shape between cocci and bacilli are named cocobacilli (Garvie, 1984).

1.3.1.2 Physiological and Biochemical Tests

The rule of classification includes physiological and biochemical tests (Stiles and Holzapfel, 1997). LAB were known as catalase negative and cannot arrange the degradation of H_2O_2 to water and oxygen. The absence of catalase activity is demonstrated by the absence of O_2 production when cells are added to a drop of

diluted H₂O₂. This examination is one of the most helpful tests for the identification of LAB.

Examination of growth ability at several temperatures is beneficial for the recognition of LAB and other species. The optimal temperature for growth changes between genera from 15°C to 45° C.

The pH tolerance test is another test to characterize probiotic LAB, which are tolerant of the gastrointestinal medium, capable of adhering to the intestinal tissues, and compete with gastric pathogens. The ideal pH for lactic acid production differs between 5.0 and 7.0. Their ability to tolerate low acidic conditions gives them an advantage over other bacteria (Wood and Holzapfel, 1995).

Lactobacillus must have the proficiency to resist bile salts to survive through the intestinal tract and provide their therapeutic effect in the intestine of the host. Therefore, LAB strains need to be tolerant to low acidic environment and digestive enzymes. Also they could be able to grow when the concentration of bile salt is 0.3% (w/v).

Some *Lactobacillus* species can tolerate 6.5 % NaCl concentration. NaCl inhibits the growth and survival of some bacteria (Hoque et al., 2010)

Lactose utilization is one of the biochemical tests that is helpful to narrow the isolates number for the next biochemical tests and genetic identification (Klaenhammer and de Vos, 2011). The fermentation of lactose is called glycolysis or glycolytic pathway. Obligatory homo-fermentative LAB, that ferment lactose into lactic acid. The formation of lactic acid causes changes in the color of the phenol red indicator.

Generally, LAB can be defined as a facultative anaerobe that produces lactic acid as the basic final product from the fermentation of sugars. So, the structure of the final product is very essential for the discrimination of *Lactobacillus* species.

Hoier, (1992) and Suskovic et al (1997) demonstrated that phenols have a bacteriostatic property and are one of the toxic metabolites produced during the digestion process. They are produced from some aromatic amino acids of nutritional compounds and proteins that undergo deamination by gut bacteria. So, the ability of *Lactobacillus* to tolerate phenol is one of the vital properties for probiotic LAB.

LAB have many technological properties. Proteolytic activity is one of these properties and a very useful characteristic for lactic acid bacteria. Proteases are the enzymes that hydrolyze proteins and catalyze peptide synthesis (Ishtiaq Ahmed et al. 2010)

Casein→ peptone→ peptides→ amino acids

LAB are known by their potent need for basic growth factors as some peptides and amino acids. However, there are not sufficient amounts of amino acids and peptides in milk to help the growth of bacteria (Abu-Tarboush, 1996). Therefore, these microorganisms perform their proteolytic analysis, to digest casein as nitrogen source.

The reactions of proteolysis in milk improve the structure, nutritional quality of these products (El-Fattah, 2013). It has been discussed that proteolysis has significant effect in flavor and texture enhancing by the breakdown of proteins (Ávila et al., 2005).

1.3.2 Genotypic Identification

Traditional microbiological examinations for phenotypical descriptions are considered inappropriate as they have restrictions in differentiating great numbers of isolates with identical physiological descriptions. Various DNA-based methods have been used to solve this problem (Mohania et al., 2008). Genetic assays performed alone or in association with each other for determination of LAB provide more accurate results in 16S rRNA gene sequencing and species-specific PCR (Rosseti and Giraffa, 2005).

1.3.2.1 The 16S rRNA Gene Sequencing

Ribosomes have minor subunit (30S) and major subunit (50S) in prokaryotes. The minor subunit has 16S rRNA while the major subunit has two RNA molecules (23S and 5S) rRNA. Completely or partially sequencing of the 16S rRNA gene is commonly applied for the determination of LAB. Ribosomes consist of proteins combined with rRNAs. This gene is approximately 1500 base pairs (bp) long. It makes 16S rRNA sequencing assay is fast and cheap (Mizrahi-Man et al., 2013). (Sacchi et al., 2002) demonstrated that even if sequencing of the whole gene is occasionally necessary, partially sequencing of variable regions is mostly common for identification.

1.3.2.2 BLAST Analysis

BLAST represents for Basic Local Alignment Search Tool, presented by the (NCBI), aligns request sequences versus those found in a chosen target databank. It can be obtained from (NCBI) BLAST website (<http://blast.ncbi.nlm.nih.gov>). The BLAST analyses were achieved for aligned sequences of each DNA.

In conclusion, phenotypic methods should be applied with genotypic techniques for precise identification. For limiting the number of isolates and rapid identification of bacteria in kefir. The phenotypic methods such as catalase test, Gram staining, acid and bile salt tolerance, temperature, phenol test, proteolytic activity, and carbohydrates fermentation patterns can be employed for genus identification, following by a genotypic examinations as partially or completely (16S rRNA) sequencing. Multiple sequence alignments can perform with the Clustal Omega program submitted by The European Bioinformatics Institute.

1.4. Detection of Probiotic Bacteria in Kefir Using Real-Time PCR

1.4.1 Real-time PCR (Species-Specific PCR)

The real-time PCR method was performed because of its higher specificity and accuracy in the quantitative analysis (Higuchi et al., 1993). The real-time PCR method is faster to perform and contains fewer steps limiting cross-contamination than competitive PCR. In real-time PCR, dye molecules of fluorescent reporters attached to primers or double-stranded DNA binding dyes to hybridize with PCR products during the process of amplification.

The alteration in fluorescence throughout the PCR is determined by a device that integrates thermal cycling with dye. There is a relationship between the amount of initial DNA and the amount of PCR product at each cycle. (Lipsky et al., 2001; Bonfini et al., 2007). The small diversity in gene expression between samples can be detected by real time PCR (Wong and Medrano, 2005). In microbial studies, the real-time PCR technique is frequently used to detect abundance of bacterial groups or even a specific species in a bacterial community. Also, real time PCR used for detection microbial population in Kefir and milk samples as shown in Table 1.1

Real time PCR analyses were done to detect the different populations in kefir samples and SYBR Green I method was used. In that, the amount of fluorescence signals increases after certain PCR cycle and this cycle is named as “threshold cycle” or “Ct” value. To set the Ct values, the baseline needs to be adjust by the device’s software. To determine the threshold level, the amplification curves are presented in logarithmic mode (Ahmed et al., 2017).

Melting curve reaction is an estimation of dissociation mechanism of dsDNA during the increasing in temperature, draws the change in the fluorescence detected when double stranded DNA with integrated dye molecules separates into single-stranded DNA. The temperature that half of DNA is denatured, pointed to the melting temperature (T_m).

Table 1.1 Detection of lactic acid bacteria and yeast of kefir, kefir grains and dairy product using real time PCR.

Target	Source	References
<i>Lactobacillus/Lactococcus</i> spp., <i>Lactic acid bacteria</i> , <i>Acetic acid bacteria</i> , <i>Enterococcus</i> spp., <i>Streptococcus</i> spp., <i>Candida</i> spp <i>Saccharomyces</i> spp	Kefir Grain and Fermented Kefir Milk	Kim D. H et al., 2015a
<i>L. kefiranofaciens</i>	Kefir Grain and Kefir Milk	Kim D. H et al., 2015b
<i>L. kefiri</i>	kefir milk	Kim D. H et al., 2016
<i>L. helveticus</i>	Dairy products	Moser A. et al., 2017

<i>L. acidophilus</i> <i>L. delbreuckii</i> <i>L. casei</i>	Taiwan dairy products	Kao Y. T. et al., 2007
<i>L. acidophilus</i> , <i>L. brevis</i> , <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> , <i>L. helveticus</i> , <i>L. reuteri</i>	yoghurt	Herbel R.S. et al., 2013

1.4.2. SYBR Green I method

SYBR Green dye presents a simple and credible assay in real-time PCR. It binds to (dsDNA) and the strength of the fluorescent increases at each step of the real time PCR (Howell et al.,1999). However, SYPR green may detect any dsDNA as non-specific amplicons or primer dimers.

In this technique, melting analysis are performed to ensure the accurately of the real time PCR using SYBR Green dye. By increasing the temperature, the DNA product dissociates into a sdDNA that causes the release of SYBR Green lowering the fluorescent signal. Melting plots are determined as the relation between fluorescence emission against the temperature. A peak formation can be used for recognition of specific amplicon in the melting curve (Querici et al., 2010).

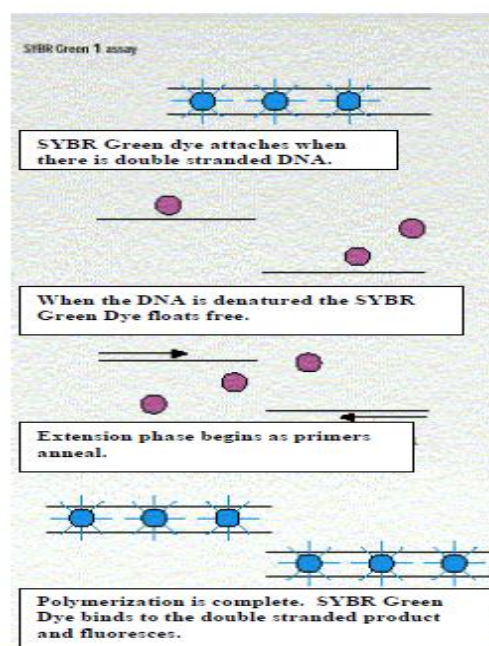


Figure 1.2 SYBR Green I real-time PCR assay (applied biosystems)

1.5 Microbiota of Kefir

Kefir grains consist of many microbial compositions like yeasts, lactic acid bacteria (LAB) and acetic acid bacteria. These microbial species are categorized into 4 groups: heterofermentative LAB, homofermentative LAB, non-lactose, and lactose assimilating yeasts (Prado et al., 2015).

Homofermentative LAB including *L. kefiranofaciens*, *L. delbrueckii* ssp. *bulgaricus*, *L. paracasei* ssp. *paracasei*, *L. acidophilus* and *L. plantarum* are predominant species. However, these species are detected at 20% level of total *Lactobacillus* in the final kefir product while the rest is the *L. kefiri* (Zanirati et al., 2015), heterofermentative LAB, combining *L. parakefiri*, *L. kefiri*, *L. brevis* and *L. fermentum* and strains of *L. lactis* (Leite et al., 2012; Leite et al., 2013).

The mixture of *lactococci*, *lactobacilli*, yeasts and acetic acid bacteria are adhere together by a protein-polysaccharide matrix (Antoniou and Dimitreli, 2008). Garrote et al (2001), Simova et al (2002), Zhou et al (2009) and Miguel et al., (2010) discussed that the predominate lactobacilli detected from kefir grains were *L. kefirgranum*, *L. kefiranofaciens*, *L. parakefiri*, *L. kefiri*, *L. delbrueckii*, *L. acidophilus*, *L. brevis*, *L. casei*, *L. paracasei*, *L. plantarum*, *L. fermentum*, *L. helveticus* .

Magalhães et al (2011) and Rattray and O'Connell (2011) outlined that LAB is primarily responsible for lactose production from lactic acid present in kefir beverage, which decreases pH and preserves milk. The other microbial population in kefir includes lactose fermenting yeasts that form ethanol with carbon dioxide. Also,

acetic acid bacteria and non lactose fermenting yeast have a role in the fermentation process.

Furthermore, over 23 different yeast species have been detected from grains of kefir and many fermented products of various regions. However, *S. unisporus*, *Candida kefir* and *S. cerevisiae* are the predominant microorganisms (Witthuhn et al., 2004; Zanirati et al., 2015).

The grains swell in about 5.0 -7.0 % of their mass after fermentation process. The ratio of microorganisms in the grains varies in the final product during their growth in milk. There are different conditions for fermentation processes such as type of milk, fermentation temperature, fermentation time, grain and inoculum proportion and the distribution of microorganisms explained by (Rattray and O'Connell, 2011; Ray and Montet, 2017).

Gao et al (2012) and Altay et al (2013) conclude that the microbial population differs depends on the substrate, origin of kefir and the methods of culturing. Taiwan kefir, Russian kefir, Irish kefir and Turkish kefir composition varies from that of Tibetan kefir.

Yeasts and lactococci are present in the exterior layer of the kefir grain. However, more yeasts cells were found in the interior layer and the number of lactobacilli was much higher (Prado et al., 2015), then hypothesis proposed about grain formation mechanisms that *S. turicensis* and *L. kefiranofaciens* aggregate to small granules and *L. kefiri* and *Kluyveromyces marxianus* attach to the surface of these granules to form thin biofilms. Then the yeasts and *Lactobacillus* associate with the biofilm to form the kefir grains (Wang et al., 2012; Hamet et al., 2013). The survival of

microorganisms in kefir grains affects by the existence of each other, due to the symbiotic relationship between strains. (Farnworth and Mainville, 2008).

The microbial composition of kefir grains and kefir milk consists of several species of lactic acid bacteria, acetic acid bacteria and yeasts which promote a beneficial symbiotic relationship within a microbial environment (Farnworth, 2005). In addition to the variety of these microbial species is specified by the area of origin (Lin et al., 1999). Microbial population of Kefir grains and beverages from different origins can shown in Table 1.2

1.5.1 *Lactobacillus casei*

L. casei, *L. paracasei*, and *L. rhamnosus* are closely related together, they are regarded as the *L. casei* group. Phylogenetic tree of these isolates shows in Figure 1.3. These strains have very homogeneous physiological characteristics and nutritional needs and survive under the same conditions. *L. casei* is rod, gram positive and facultatively heterofermentative (Salvetti et al., 2012). It is used in fermented dairy products as probiotics to enhance human health (Reid, 2015; Orlando et al., 2016). On the other hand, *L. casei* subsp. *pseudopplantarum* is one of the beneficial certified probiotics to treat the gastrointestinal diseases.

(Collins et al. 1989) demonstrated that according to 16S rRNA gene sequences many subspecies recognized as *L. casei* were reclassified to other species. *L. casei* subsp. *alactosus* and *L. casei* subsp. *pseudopplantarum* were renamed *L. paracasei* subsp. *paracasei*, and *L. casei* subsp. *tolerans* was reassigned to *L. paracasei* subsp. *tolerans*.

1.5.2 *Lactobacillus paracaei*

L. paracasei is rod-shaped, facultatively heterofermentative, and belongs to the phylogenetic group *L. casei*. It can grow between 10°C and 40°C but many strains can grow between 5°C and 45°C (Holzapfel and Wood, 2014). *L. paracasei* can be grow in the digestive tract of humans and in the fermented milk products. The metabolic end products during the growth from these energy sources are actually lactic acid, but ethanol, acetic acid also occur (Makras et al., 2005).

L. paracasei can produce substances with antioxidant activity, it can degrade superoxide anion and hydrogen peroxide. Also it has the ability to prevent free radicles (Ayeni et al., 2011).

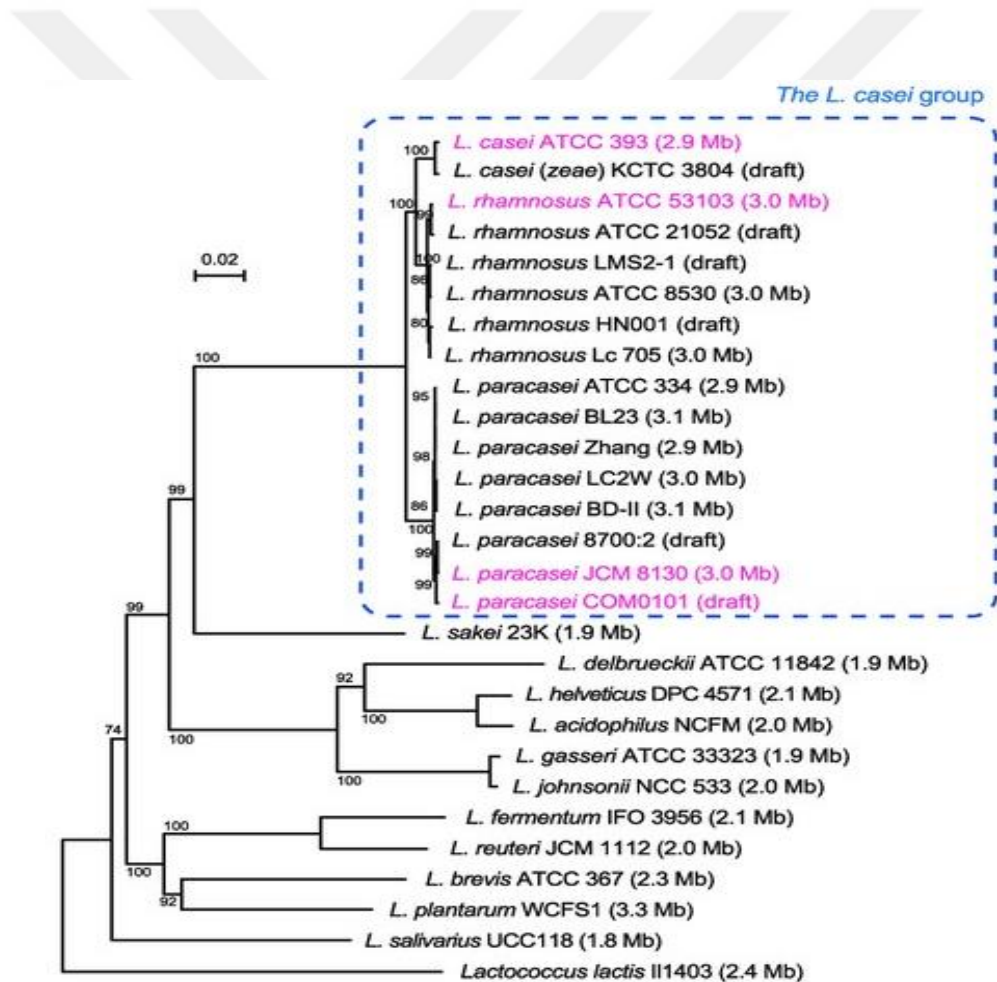


Figure 1.3 Genome-based phylogenetic analysis of the *L. casei* group (Toh H. et al., 2013).

Table 1.2 Microbial population of kefir grains and beverages from different origins.

Source	Country	Species isolated and identified	References
Kefir grains and milk	Argentina	<i>L. kefiranofaciens</i> , <i>L. plantarum</i> , <i>L. lactis</i> ssp. <i>lactis</i> , <i>Kluyveromyces marxianus</i> , <i>S. cerevisiae</i> , <i>L. kefir</i> , <i>Acetobacter</i> sp., <i>L. parakefiri</i> , <i>L. paracasei</i>	Garrote et al., 2001; Hamet et al., 2013
Kefir grains and milk	Belgium	<i>L. kefir</i> , <i>Lactococcus lactis</i> , <i>L. kefiranofaciens</i> , <i>Lactococcus lactis</i> ssp. <i>cremoris</i> , <i>Kluyveromyces marxianus</i> , <i>Kazachastania khefir</i>	Korsak et al., 2015
Kefir grains and milk	Bulgaria	<i>L. brevis</i> , <i>L. helveticus</i> , <i>L. casei</i> ssp. <i>pseudo plantarum</i> , <i>L. delbrueckii</i> ssp. <i>bulgaricus</i> , <i>Str. thermophilus</i> , <i>S. cerevisiae</i> , <i>L. lactis</i>	Simova et al., 2002
Kefir grains	Brazil	<i>L. kefir</i> , <i>Lactococcus lactis</i> , <i>L. paracasei</i> , <i>L. helveticus</i> , <i>L. crispatus</i> , <i>L. kefiranofaciens</i> , <i>S. cerevisiae</i> , <i>Leuconostoc</i> sp., <i>Streptococcus</i> sp., <i>Acetobacter</i> sp., <i>Bifidobacterium</i> sp., <i>L. amylovorus</i> , <i>L. parakefiri</i>	Leite et al., 2012; Zanirati et al., 2015
Kefir milk	Brazil	<i>L. paracasei</i> , <i>L. casei</i> , <i>L. kefir</i> , <i>Lactococcus lactis</i> , <i>Kluyveromyces lactis</i> , <i>L. parabuchneri</i> , <i>kazachastania aerobia</i> , <i>S. cerevisiae</i> , <i>Lachancea meyersii</i>	Magalhaes et al., 2011

Kefir grains	Taiwan	<i>L. kefiranofaciens</i> , <i>L. kefir</i> , <i>Leuconostoc mesenteroides</i> , <i>Saccharomyces turicensis</i> , <i>Lactococcus lactis</i>	Chen et al., 2008; Wang et al., 2012
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Table 1.2 Microbial population of kefir grains and beverages from different origins.
(continued)

Source	Country	Species isolated and identified	Reference
Kefir grains	Russia	<i>L. paracasei</i> , <i>L. casei</i> , <i>L. kefir</i> , <i>L. kefiranofaciens</i> , <i>S. cerevisiae</i> , <i>L. lactis</i> ssp. <i>cremoris</i> , <i>L. lactis</i>	Kotova et al., 2016
Kefir grains	Italy	<i>Bacillus</i> sp., <i>L. kefiranofaciens</i> , <i>Lactococcus lactis</i> , <i>Enterococcus</i> sp., <i>S. thermophilus</i>	Garofalo et al., 2015
Kefir grains	South Africa	<i>Lactococcus lactis</i> , <i>L. brevis</i> , <i>L. delbrueckii</i> ssp. <i>bulgaricus</i> , <i>L. plantarum</i> , <i>L. helveticus</i>	Witthuhn et al., 2004, 2005
Kefir grains	Turkey	<i>L. helveticus</i> , <i>L. kefiranofaciens</i> , <i>L. acidophilus</i> , <i>L. helveticus</i> , <i>L. amylovorus</i> , <i>L. gallinarum</i> , <i>Streptococcus thermophilus</i> , <i>Kluyveromyces marxianus</i>	Kok-Tas et al., 2012; Nalbantoglu et al., 2014
Kefir milk	Turkey	<i>Lactococcus lactis</i> , <i>Lactococcus cremoris</i> , <i>Streptococcus thermophilus</i> , <i>S. durans</i>	Yüksekdağ et al., 2004
Kefir milk and grains	Turkey	<i>Leuconostoc mesenteroides</i> , <i>L. kefir</i> , <i>Lactococcus lactis</i> , <i>L. acidophilus</i> , <i>Streptococcus thermophilus</i> , <i>L. kefiranofaciens</i>	Guzel-Seydim et al., 2005; Kesmen and Kacmaz, 2011
Kefir milk	Turkey	<i>Leuconostoc mesenteroides</i> , <i>L. brevis</i> , <i>L. plantarum</i> , <i>S. cerevisiae</i> , <i>L. paracasei</i>	Merih K. and Evrim Y.,

1.5.3 *Lactobacillus plantarum*

L. plantarum is one of the investigated probiotic microorganism used in the food industry (Sudhanshu et al., 2018). It is straight rods, found singly, paired or in chains, facultatively heterofermentative, grow at 15°C but not at 45°C and non-motile (Holzapfel and Wood, 2014). *L. plantarum* present in most of the foods that are lactic acid fermented and also found in the digestive tract of humans (Molin, 2008). *L. plantarum* can survive in the acidic stomach in humans at pH less than 4.0, so it is highly tolerant to low pH. *L. plantarum* can ferment many carbohydrates indicate that it can adapt to different environments. The microorganism can convert tannins into flavonoids and phenolic acids which has useful antioxidant properties (Molin, 2008).

L. plantarum can not synthesize certain vitamins and amino acids that are important for their growth. A study by Ma et al. (2016) demonstrated that *L. plantarum* needs six amino acids to ferment the milk. Besides, mineral salts had a stimulating effect on growth but were not essential. Since *L. plantarum* is vitamin auxotroph, it requires vitamins for growth from the milk (Ma et al., 2016).

1.5.4 *Lactobacillus acidophilus*

L. acidophilus is one of the most suggested probiotic organisms for dietary use (Shah, 2007). It is a gram positive, rod shape, non flagellated, non motile microorganism and intolerant to salt. Moreover, It is an anaerobic microorganism and contains mainly homofermentative lactobacilli, but some are facultative

heterofermentative (Hutkins, 2006). *L. acidophilus* is distributed in the gastrointestinal and perform an important function of the indigenous microflora of human. It helps in absorption of nutrients and digestion process. Also it aids treating lactose intolerance caused due to the deficiency of lactase enzyme.

1.5.5 *Lactobacillus intestinalis*

L. intestinalis is non spore forming rods, gram-positive bacteria and facultatively anaerobic rods. It has low G+C contents which is a factor that can differentiate this species from other homofermentative species (Fujisawa et al., 1990).

Lauer et al. (1980) found that *L. intestinalis* strain CNRZ 219 had a DNA homology group which did not show high similarity with any strain of *L. acidophilus* group.

1.5.6 *Streptococcus thermophilus*

Streptococcus thermophilus used in food fermentations. It has been used with *L. delbrueckii* subsp *bulgaricus* to make yogurt for long time. Moreover, *S. thermophilus* has also been applied in different industrial products such as fermented milk products and cheese. It helps to accelerate the acidification rate and enhance the flavor and texture quality of these dairy products. In addition to, *S. thermophilus* has different probiotic effects such as antioxidant activities and inhibition of specific pathogens (Adolfsson et al., 2004; Iyer et al., 2010).

1.5.7 *Saccharomyces cerevisiae*

Pilar et al (2018) proposed that many *Saccharomyces* yeast species have strains with probiotic potential. Also meire et al (2017) reported that *S. cerevisiae* has the ability

to digest nutrients, antagonism to pathogen, anti-oxidant effect, adhesion to epithelial cells.

S. cerevisiae is the most lactose-negative strain of the kefir yeast isolates. It promotes an alcoholic aroma along with a refreshing taste and improves the sensory qualities of the kefir beverage (O'Brien, 2012).

1.5.8 *Lactobacillus helveticus*

L. helveticus is a significant industrial organism and is mostly used in the fermentation of milk for manufacturing different kinds of cheese. Various studies found that *L. helveticus* has many probiotic characteristics such as the capability to adhere to the epithelial cells, survive gastrointestinal passage and inhibit pathogens. Also, *L. helveticus* could benefit the host by promoting the bioavailability of nutrients, eliminating allergens and other unuseful molecules from food and producing bioactive compounds (Valentina and Simone, 2012).

L. helveticus is a member of the *L. acidophilus* subgroup. It is hardly recognizable by physiological tests and biochemical tests from other closely related homofermentative lactobacilli such as *L. amylovorus*, *L. crispatus*, *L. acidophilus*, *L. gasseri*, *L. johnsonii* and *L. gallinarum* (Holzapfel and Wood, 1998). The Phylogenetic tree between these *Lactobacillus* species can shown in Figure 1.4.

1.5.9 *Lactobacillus gallinarum*

L. gallinarum is a rod-shaped microorganism, obligate homofermentative and it is tolerant to 4.0% sodium chloride. It is mainly found in dairy and non-dairy products with *L. helveticus* (Guan et al., 2003; Meroth et al., 2004; Moroni et al., 2011).

L. gallinarum, *L. acidophilus*, *L. johnsonii*, *L. crispatus*, *L. amylovorus* and *L. gasseri* are suggested as proper species in the *L. acidophilus* complex group (Klein

et al., 1998). *L. gallinarum* is not a significant species from a technological view and its potential existence in dairy products was considered (Van Hoorde et al., 2008; Bujnakova et al., 2012). Identification of *L. gallinarum* may become essential in the future, because of its antimicrobial properties.

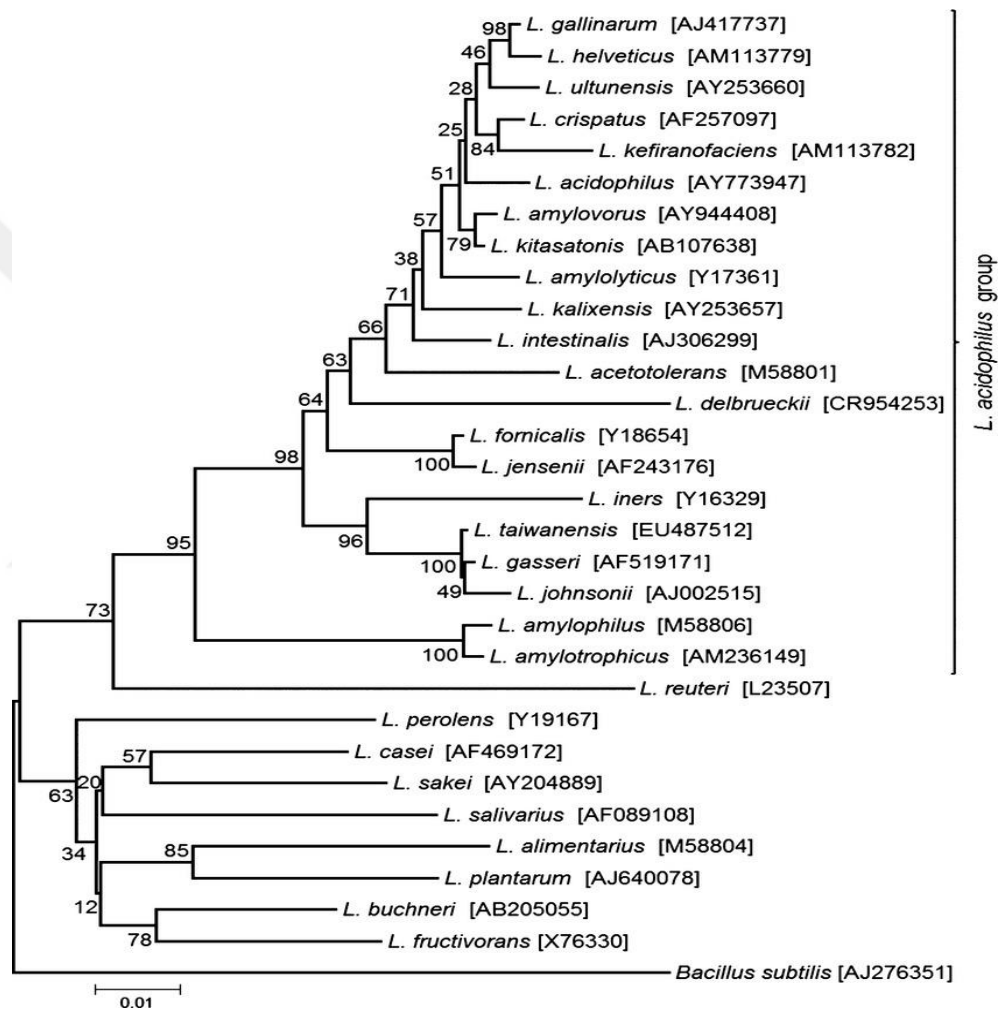


Figure 1.4 Phylogenetic placement of *L. acidophilus* subgroup within the *Lactobacillus* genus (Matthew B, 2013).

1.5.10 *Lactobacillus kefir*

L. kefir is a *Lactobacillus* strain, it has some antibiotic resistance, safe for human consumption and has anti-inflammatory properties (Drago et al., 2016).

(Kandler and Kunath, 1983) discussed several useful effects including toxin neutralization, antimicrobial activity, suppression of food borne pathogens, modulation of the immune response and elimination of hyper cholesterolemia have been detected by *L. kefir*.

1.5.11 *Lactobacillus kefiranofaciens*

L. kefiranofaciens is one of the most extensively investigated species among kefir microbiota because of its functional properties and beneficial health effects as antitumor and anti-inflammatory activity (Ahmed et al., 2013; Chen 2012 and 2013).

L. kefiranofaciens isolated from the grains produce kefiran, it contains approximately equal amounts of glucose and galactose. Kefiran is used as a thickener, stabilizer to improve the quality and taste of kefir.

1.5.12 *Lactobacillus fermentum*

L. fermentum is a heterofermentative inhabitant of the digestive system and is often isolated from human biological samples (López-Huertas 2015). This species was previously declared for its various probiotic properties (Barretto et al., 2016). The consumption of *L. fermentum* and *L. amylovorus* modifies the microbial population exist in the gut and it provides certain metabolic effects influencing energy production and body fattiness (Dibaise et al., 2008). The results of Xiao et al (2011) indicate that *L. fermentum* has the ability to decrease cholesterol, tolerate acid and bile salt and inhibit pathogens.

1.5.13 *Lactobacillus amylovorus*

L. amylovorus presents features typical of homofermentative *Lactobacillus* species. It is an anaerobic, rod, Gram positive, nonmotile bacteria. It belongs to the *L. acidophilus* group according to taxonomic studies. It requires several vitamins for growth (Kant, 2011).

L. amylovorus is an plentiful *Lactobacillus* species survive in the intestines of piglets and it exhibits different probiotic properties such as antimicrobial efficiency versus gastric pathogenic microorganisms (Konstantinov et al., 2006, 2008; Roselli et al., 2007).

1.5.14 *Lactobacillus delbrueckii* subsp. *bulgaricus*

L. delbrueckii subsp. *bulgaricus* and *Streptococcus thermophilus* are thermophilic bacteria and they are highly adapted to grow on lactose and convert it into lactic acid. *L. bulgaricus* performs proteolytic activity that are useful in the protein rich substrate (Klaenhammer et al., 2008), because it has good adaptability to milk substrates and low pH values (Delley and Germont, 2002).

1.5.15 *Lactobacillus zeae*

Lactobacillus casei group, which contains several probiotic bacteria, as the species *L. casei*, *L. paracasei*, *L. rhamnosus*, and *L. zeae* (Collins et al., 1989).

A marked finding from (Tao et al., 2020) study, indicating that *L. zeae* could effectively reduce diarrhoea in piglets. And the consumption of *L. zeae* had effects on controlling expressions of genes related to inflammation and anti-oxidation, which can relieve the inflammatory response and improve intestinal health.

1.6 Probiotic Properties of Lactic Acid Bacteria

Lactic acid bacteria are isolated from different food products and they are used as probiotics, confer health benefits on the host when consumed in sufficient amounts (Quinto et al., 2014).

They are skilful in suppressing the growth of pathogenic organisms through various techniques such as attachment to epithelial cells, modification of the immune system and production of antimicrobial substances. They have the ability to grow and survive under various conditions (Palachum et al., 2018). The isolates should be able to tolerate low pH of gastric juice with resistance to bile salts. They also have some health benefits like antimicrobial actions, toxin reducing agents, and promoting immune system (Chiang and Pan, 2012; Berardi et al., 2013).

LAB enhance the balance of the microbial community in the intestine, allow protection against pathogen, and prevent or treat intestinal diseases (Gionchetti et al., 2000). These effects are performed by secretion of antimicrobial substances and competition with harmful bacteria (Fons et al., 2000). LAB have many technological skills such as proteolytic, lipolytic, acidification, texturizing, thickening coagulation, aroma production (Pitino et al., 2010).

1.7 The aim of the study

Kefir is a fermented milk beverage, weakly acidic and slightly alcoholic taste and has been a traditional beverage in Turkey. It is fermented by the reaction of probiotic strains that found in symbiotic associations in the kefir grains. Probiotic strains are microorganisms that are intended to have health benefits when consumed in a sufficient quantity. Probiotics are non-toxic, non-pathogenic and resistant to

pancreatic secretions and stomach acids such as bile and digestive enzymes and this property would be important in the small intestines for probiotics having to survive in high numbers.

Kefir is utilized by the fermentation of milk using kefir grains that have a complex microbial symbiosis of LAB, some yeasts and acetic acid bacteria. The interest in kefir consumption or probiotic strains is growing day after day because people become more conscious of its beneficial and medical effects. This growing desire for probiotics leads to the improvement in the food industry and finding new strains with probiotic properties that are favorable for kefir production.

The study aimed to characterize the probiotic properties of the LAB from five different Turkish kefir samples and one milk product from Kyrgyzstan. Turkish kefir was chosen for studying its strains since the microorganisms exist in kefir depend on the origin of kefir grains and differ from one country to another (Guzel S. 2005).

This study included mainly four parts. In the first part, four home-made kefir grain samples were collected from Ankara, Istanbul in Turkey and AOC, a commercial kefir sample and one milk product from Kyrgyzstan for comparison. Kefir milk was prepared from these kefir grains. In the second part, different LAB and yeasts were determined by real time PCR with specific primers. In the third part, lactic acid bacteria were isolated from five kefir and milk samples and the isolates were identified using microscopic and biochemical methods. The fourth part included the genotypic identification for 30 LAB isolates using the 16S rRNA method. In our study, we focused on isolation lactic acid bacteria, particularly lactobacilli since *Lactobacillus* species are among the most prevalent microorganisms found in kefir (Slattery C. et al ., 2019). Therefore, this study intended to have information about the genotypic variety of probiotics in the homemade kefir.





CHAPTER 2

MATERIALS AND METHODS

2.1 Collection of Samples

Homemade kefir grains were collected from Istanbul and Ankara in Turkey. In addition to these grains, one milk product sample (semi-solid) was kindly brought from Kyrgyzstan by Prof. Dr Kamuran Ayhan (Ankara University) and one commercially kefir sample was available from the manufacturer, Ataturk Orman Çiftliği (AOC), it was used for comparison of microbial populations with the homemade samples. The samples were collected and transported to our lab in the Food Engineering Department at the Middle East Technical University (METU). The origins of kefir and milk samples are given in Table 2.1.

Table 2.1 Origins of homemade kefir and milk samples

No	Sample name	Source	Origin
1	(K1)	Commercial kefir sample (AOC)	Turkey, Market
2	(K2)	Homemade kefir grains from Ankara	Bey pazari
3	(K3)	Homemade kefir grains from Ankara	Malikoy
4	(K4)	*Milk product sample from Kyrgyzstan	Kyrgyzstan
5	(K5)	Homemade kefir grains from Istanbul	Çatalca
6	(K6)	Homemade kefir grains from Istanbul	Çerkezköy

*Semi-solid milk product

2.2 Kefir Preparation from Kefir Grains

Otles and Cagindi (2003) discussed the traditional method of producing kefir:

The active kefir grains were transferred to pasteurized milk. The milk was covered, placed in warm conditions (30°C). After that, the milk was incubated until lightly thickened and aroma was pleasant. It takes 24 hours in room temperatures (25-30°C) or less than 24 hours at warmer temperature. After fermentation, the milk texture was changed, the grains were removed from the kefir beverage. The kefir grains were kept in a new batch of distilled water and were preserved at -20°C.

2.3 Real Time PCR Assay (RT PCR)

A real time PCR assay was performed to detect and determine the populations of kefir and milk product samples.

2.3.1 Selection of the Primers for Real-time PCR

In real-time PCR, species-specific primer pairs were used to detect different strains of the species *L. kefir*, *L. casei*, *L. delbrueckii* subsp. *bulgaricus*, *L. kefiranofaciens*, *L. fermentum*, *L. plantarum*, *S. cerevisiae*, *S. thermophilus*, *L. sobrius*, *L. amylovorus*, *L. acidophilus* and *L. paracasei* (Table 2.2).

Table 2.2 The oligonucleotides primers.

Strain	Sequence	References
1 <i>L. acidophilus</i>	For: CTTTGACTCAGGCAATTGCTCGTGAAGGTAG Rev: CAACTTCTTTAGATGCTGAAGAAACAGCAG CTACG	Herbel (2013)
2 <i>L. kefir</i>	For: 5'GGGAGATGCCCCATGTTGGT-3 Rev: 5'AAGCTTTTCGAAGTGCCTGTGA-3	Kim et al., (2016)
3 <i>L. delbrueckii</i> subsp. <i>bulgaricus</i>	For: GAACTTGATGTTGTTGAAGGGATGCAATTCTG Rev: GAGCGGCCTTGTTGCACGATTTTC(439-461)	Herbel (2013)
4 <i>L. kefirano</i> <i>faciens</i>	For: 5CAGTTCGCATGAACAGCTTTTAA-3 Rev: 5'-GCACCGCGGGTCCAT-3'	Dong et al., (2015)
5 <i>L. fermentum</i>	For: AAC CGA GAA CAC CGC GTT AT Rev: ACT TAA CCT TAC TGA TCG TAC ATC AGT CACA	Monique and Jan (2006)
6 <i>L. plantarum</i>	For: TGG ATC ACC TCC TTT CTA AGG AAT Rev: TGT TCT CGG TTT CAT TAT GAA AAA ATA	Monique and Jan (2006)
7 <i>L. sobrius</i> \ <i>amylovorus</i>	For: TTCTGCCTTTTTGGGATCAA Rev: CCTTGTTTATTCAAGTGGGTGA	Romain Marti et al., (2010)
8 <i>Streptococcus</i> <i>thermophilus</i>	For: GGTCCAAGAAGAAGTAATTGA Rev: GACCTTATACAAATCTGGTT	Abd El -Aziz et al., (2014)
9 <i>Saccharomyces</i> <i>cerevisiae</i>	For: ACATATGAAGTATGTTTCTATATAACGGGTG Rev: TGGTGCTGGTGCGGATCTA	Abd El-Aziz et al., (2014)
10 <i>L. casei</i>	For: CTA TAA GTA AGC TTT GAT CCG GAG ATT Rev: CTT CCT GCG GGT ACT GAG ATG T	Monique and Jan (2006)
11 <i>L. paracasei</i>	For: ACATCAGTGTATTGCTTGTCAGTGAATAC Rev: CCTGCGGGTACTGAGATGTTTC	Haarman and Knol (2006)

2.3.2 DNA Extraction for Real-Time PCR

The total DNA from the kefir and milk product samples were extracted using Poland EURX DNA purification kit following the manufacturer's procedures.

Two ml of the kefir and milk product samples were taken to a microcentrifuge tube and centrifuged to separate pellets. A pellet was taken, Res f E 750 µl was added and mixed. 60 µl Lyse FE with 10 µl Proteinase K was added. Short vortex was used. The lysate was centrifuged for 5 min and 400 µl supernatant was transferred to a new tube. 400 µl PR buffer was added, vortexed for 5 seconds, and was incubated on ice for 5 min. 600 µl of the supernatant was transferred to a new 2 ml microcentrifuge tube. 600 µl Sol FE buffer and 600 µl of 96-99% ethanol were added and mixed well. Centrifugation briefly was done after each step. 40 µl of buffer FE was added to the spin column. 600 µl of the supernatant was transferred to the spin column. 500 µl Wash FEx buffer was added. 50-100µl of elution buffer was added. The spin-column collection tube was incubated for 5 min at room conditions. DNA was ready for further analysis.

2.3.3 Detection of Different Bacteria and Yeast Species in Kefir Using Real-Time PCR

Applied Biosystems 7500 Real-time PCR was used. ROX dye was the reference dye used to normalize fluorescent reporter signal.

2.3.4 Preparation of Real-Time PCR Master Mix

A master mix was used for real-time PCR reactions according to standard kit procedures (Table. 2.3).

Table 2.3 Real Time PCR materials

Reagent	Amount (μl)
DNA	2
Master mix (SYBR Green)	5
For. primer, Rev. primer	0.5
H ₂ O	17
Total amount	25

2.3.4.1 Dispensing the Master Mix:

Master Mix was distributed into the wells of a real-time PCR plate.

2.3.4.2 Addition of Template DNA

2 μl of DNA was added, containing the master mix and the tubes were covered with optical adhesive covers. Tubes were centrifuged before placing them into the thermocycler and real-time PCR was performed according to SYBR Green conditions. A control without template (primer control) was run in every set-up.

Program of SYBR Green real time PCR

Step 1: 50°C, 2 min

2: 95°C, 10 min (denaturation) 40 cycles

3: 95°C, 15 sec (annealing)

4: 60°C, 1 min (elongation)

5: (Dissociation step)

Melting plot analysis was measured directly by increasing temperature (65-95°C to determine the specificity of real time PCR reaction.

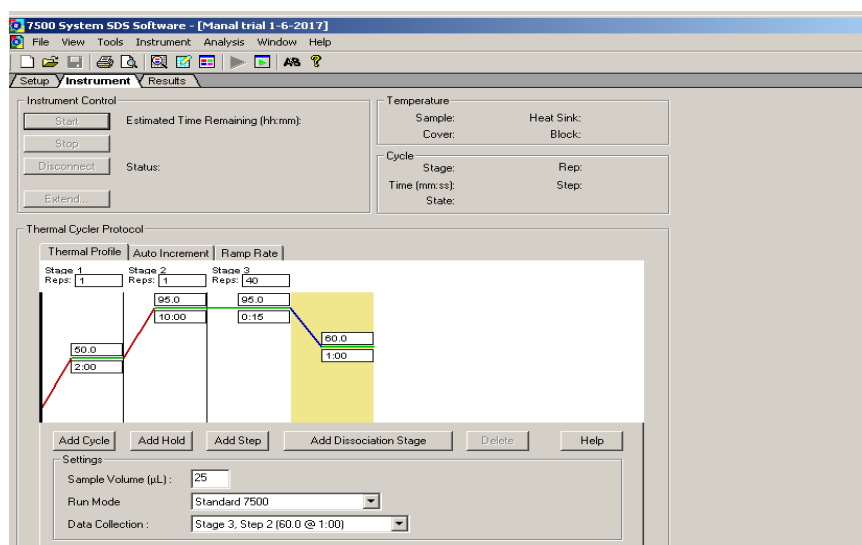


Figure 2.1 Real-time PCR program

2.3.5 Real-Time PCR Analysis

Each real-time PCR was repeated three times and the data were examined using the real-time PCR 7500 system for detection of gene expression. Threshold cycle (Ct) for each gene was determined. Amplification and melting curve were analyzed.

2.4 Isolation of the Lactic Acid Bacteria from Kefir Samples

Serial dilutions of kefir samples were done from 10^{-1} up to 10^{-6} . One milliliter of these dilutions was poured in plates of de Man, Rogosa, and Sharp (MRS) media agar (Oxoid, UK). They were incubated at 37°C for 48 -72 h anaerobically using a gas pack (Anaerocult C, Merck). After incubation, colonies were randomly picked up from each plate. The colonies that showed certain morphological differences in shape, color and size were supposed to be LAB. Streaking method was done for each colony and only a single colony was used to obtain pure cultures (Khalil and Anwar, 2016).

2.4.1 Phenotypic Identification of the Isolated Bacterial Strains

All of the pure isolates were tested for catalase reaction and Gram staining. Gram positive and catalase negative isolates were preserved in broth medium with 20% glycerol at -80°C for next studies.

2.4.1.1 Gram Staining

Gram color staining (Merck) was used for all isolated bacteria. A single colony was put on a clean slide, dry and then heat fixed. It was submerged with crystal violet solution, washed with distilled water after 1 minute and covered with iodine. After that it decolorized with ethyl alcohol and water. After that, safranin was used, washed again with water and dried. The slides observe under oil immersion (100X) (MacFaddin, 2000).

2.4.1.2 Catalase reaction

Few drops of hydrogen peroxide(3%) was added to bacterial culture. Bubbles formation indicated to catalase (+) and no bubble formation indicated to catalase (-) (MacFaddin, 2000).

2.5 Biochemical Characterization of the Isolated Bacterial Strains

Identification of the isolated bacteria as *Lactobacillus* species was done according to their cultural, morphological, physiological and biochemical tests by the methods as explained in Bergey's Manual of Bacteriology. The rod gram positive and negative catalase isolates were examined for their temperature, pH, bile salt, NaCl, and

phenol tolerance test. Also, lactose production, sugar fermentation, and proteolysis activity tests were performed. All experiments were replicated twice.

2.5.1 pH Tolerance Test

The isolated bacterial cultures were transferred into sterile MRS broth of different pH 2, 2.5, 3, 4, and 6 adjusted by 10 N HCl and 1N NaOH, incubated at 37°C for 48 h. Negative control was used. Results were recorded by observing the turbidity of the broth media after 48 h and no growth was observed in the control (Mannan et al., 2017).

2.5.2 Temperature Tolerance Test

The bacterial cultures were inoculated into sterile MRS broth and incubated in a different temperature at 25°C, 37°C, 40 or 45°C for 48h. Only media was used as a negative control. Results were detected by seeing the turbidity of the media after 48 h and no growth was seen in the control (Papamanoli et al., 2003).

2.5.3 NaCl Tolerance Test

MRS broth with 2%, 4%, or 6.5% NaCl concentrations was prepared, inoculated with 1% fresh culture of the bacteria, and incubated for 24- 48 hours. Only media was used as a negative control. Results were detected by observing turbidity after 48 h and no growth was seen in the control (Chakraborty and Bhowal, 2015; Mannan et al., 2017; Aarti et al., 2018).

2.5.4 Phenol Tolerance Test

MRS broth media containing 0.4% of phenol concentration was prepared for the detection of phenol tolerance. The broth was inoculated with bacterial culture and incubated at 37°C for 48 h. Results were confirmed by observing turbidity after 48 h and no growth was observed in the control (Chakraborty and Bhowal, 2015).

2.5.5 Lactose Utilization

The acid production by bacterial cultures was determined by observing the color changing of the medium. The sterilized medium was prepared as followed: 10g peptone, NaCl 15g, phenol red 0.018g, lactose 5g and 1L distilled water, inoculated with bacterial cultures, and kept at 37°C for 48 hours. Change of color to yellow indicates the formation of lactic acid (Pundir et al., 2013).

2.5.6 The Proteolytic Activity of Lactic Acid Bacteria

The proteolytic activity protocol was described by Phyu et al (2015) with modifications. All isolated bacterial cultures were examined for their ability of protease utilization on a skim milk agar plate plate (skim milk 2.8% , casein 0.5%, yeast extract 0.25%, dextrose 0.1% and agar 1.5%). The bacterial culture were used for agar-well diffusion assay. Wells of 5 mm in diameter were done on skim milk agar. To each of the wells, 25 µl from each broth was added. After incubation, the diameter of clear zone was measured in mm (Phyu et al., 2015).

2.5.7 Bile Salt Hydrolase

Bile salt hydrolase activity of lactobacilli isolates was applied by agar plate assay. Agar plates of the MRS medium were prepared with 0.5% (w/v) of the taurodeoxycholic acid (TDCA) and glycolic acid (GCA). All plates were inoculated with an overnight culture by using a 10- μ L loop. Plates were placed in anaerobic conditions at 37°C for 48-72 h. Precipitated acid was observed around colonies detecting highly active strains within 48 h and bile salt hydrolysis was represented at two ways: The formation of precipitate around colonies or the production of granular white colonies (Mahrous, 2011).

2.5.8 Carbohydrate Fermentation Test

The sugar fermentation test was done using 1% (w/v) sugar in MRS broth medium. Different sugar substrates namely glucose, sucrose, maltose, mannitol, and lactose were applied in this test. Phenol red was used as an indicator. Durham's tube was put inversely in each of the broth test tubes. The media without inoculation was used as a negative control. Results were spotted by the color changing from red to yellow color and gas formation in durham's tube (Promot et al., 2018).

2.6 Genetic Characterization of Isolates

The bacterial isolates were identified by sequencing of specific regions of ribosomal DNA (16S rRNA gene). This procedure was performed in BM Laboratuvar Sistemleri in Ankara-Turkey.

2.6.1 Extraction of Genomic DNA of LAB Isolates

Eurx Gene Matrix Tissue and Bacterial DNA isolation kit (Poland) were used for DNA isolation of bacterial samples with the protocol in the link below:

<https://eurx.com.pl/docs/manuals/en/e3551.pdf>

2.6.1.1 DNA Binding Spin-Column Activation

30 µl of activation Buffer T was applied onto the DNA binding spin-column and kept until the lysate was transferred to the spin-column.

2.6.1.2 Bacterial Sample Preparation

The bacterial colony suspended in 300 µl buffer Lyse BG. 50 µl buffer BL and 2 µl RNase A were added to the cell suspension, and was incubated after that at 37°C for 15 min. 20 µl Proteinase K was added to the cell pellet. The sample was incubated at 56°C for 30 min. 350 µl buffer Sol T was added again and it was incubated at 56°C for 5 min and mixed for 15 sec.

2.6.1.3 DNA Isolation

The sample was centrifuged for 1 min at 12 000 x g. The spin-column was removed, discarded flow-through. 50 µl of Wash TX1 buffer was added and centrifuged for 1 min at 11 000 x g, 500 µl of TX2 buffer was added and centrifuged for 1 min to remove traces of Wash TX2 buffer. 50–150 µl of Elution buffer was added to elute bound DNA.

DNA was ready for further analysis. It was stored either at 2–8°C or -20°C. After DNA isolation, spectrophotometric estimation were performed on the Thermo

Scientific Nanodrop 2000 (USA) instrument to check the quantity and purity of the DNAs isolated.

2.6.2 Polymerase Chain Reaction (PCR)

2.6.2.1 Amplification of 16S rRNA Gene

PCR amplification was done using Taq DNA polymerase (Solis Biodyne [Estonia] FIREPol® DNA Polymerase enzyme). PCR amplification was done in a volume of 50 µl reaction mixture prepared as seen in Table 2.4.

Gene regions targeted for species identification were amplified with the primers 27F - 1492R as universal primers. PCR programs included initial denaturation at 94°C for 3 min, denaturation 94°C for 45s, annealing 57°C for 60s, extension 72°C for 1 min (35 cycles) and final extension 72 °C for 5 min.

The temperature was decreased to 4°C and the PCR process was completed. One step PCR was achieved to amplify 1500 base target regions.

Table 2.4 PCR mixture for 16S rRNA gene sequencing.

Reagent	Amount
d H ₂ O	18.9
1.25 units Taq DNA polymerase	10 µl
Template DNA	50 µg
0.2 mM deoxynucleotide triphosphates	10 µl
2.5 mM MgCl ₂	10 ml
DNA primer	0.1 µl

2.6.2.2 Separating the DNA Fragment Using Agarose Gel

PCR products were measured qualitatively using 7% agarose gel electrophoresis at 120 V for 50 min. The amplicons were then removed from the gel.

2.6.3.2 DNA Purification from Agarose Gel

The PCR product was purified following the kit procedures using the purification enzyme "ExoSAP-IT™ PCR Product Cleanup Reagent" (Thermo Fisher Scientific, USA) for the single-band samples obtained.

5 µL of PCR product was mixed with 2 µL of ExoSAPIT™ reagent for a combined 7 µL reaction volume. The mixture was incubated at 37°C for 15 minutes. After that, it was incubated at 80°C for 15 minutes to inactivate ExoSAP-IT™ reagent. Final PCR products were kept at –20°C until further study.

2.6.3 Sequencing of 16S rRNA Gene

For Sanger Sequencing samples, the ABI 3730XL Sanger sequencing device (Applied Biosystems, Foster City, CA) and BigDye Terminator v3.1 Cycle Sequencing Kit were used in the Macrogen Netherlands laboratory (Applied Biosystems, Foster City, CA). The forward and reverse readings obtained with the primers 27F-1492R were contigued to form a consensus sequence.

2.6.4 Analysis of 16S rRNA Gene

The full identification of the isolates were then gained by searching the produced nucleotide sequences of 16S rRNA genes in the database of the gene bank.

Finch TY version 1.4.0 (<https://digitalworldbiology.com/FinchTV>) was performed for seeing DNA sequence chromatograms. Analysis of the nucleotide sequences was done using online BLASTN (<https://blast.ncbi.nlm.nih.gov/Blast>).

2.6.5 Multiple Sequence Alignment (MSA)

Multiple sequence alignments and phylogenetic tree were performed with the Clustal Omega program (1.2.1) presented by The European Bioinformatics Institute at this link: <http://www.ebi.ac.uk/Tools/msa/clustalo/>.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Experimental Design

The current study includes four basic experimental sections: 1) collection of kefir grains and kefir milk preparation, 2) determination of the population in different homemade kefir samples by real-time PCR, 3) lactic acid bacteria isolation, and 4) identification of the isolates using 16S rRNA gene sequencing.

In the first section, four different homemade kefir grains were collected from Istanbul and Ankara in Turkey, one milk product from Kyrgyzstan, and one product of commercial kefir milk (AOC) from Turkey. Kefir milk was prepared from kefir grains as mentioned in Chapter 2.

Figure 3.1 demonstrates the flow chart of Section 1.

In the second section, real-time PCR technique was performed for the detection of LAB and yeast in kefir milk using specific primers. The flow chart of the experimental section 2 is seen in Figure 3.2

In the third section, 100 bacteria isolates were isolated from five different homemade kefir samples. Previously, they were identified using both microbiological and biochemical methods. Temperature tolerance, acid tolerance, bile salt tolerance, phenol tolerance, sugar fermentation, and proteolysis tests were carried out for the probiotic characterization of these isolates. The flow chart and the diagram of the section 3 is shown in Figure 3.3 and Figure 3.4

In the fourth section, 30 isolates were identified using molecular techniques. The 16S rRNA gene sequencing and BLAST analysis were carried out. The flow chart of

the section 4 is shown in Figure 3.5. In addition, the partial sequences of the 16S rRNA gene of kefir isolates were compared with the type strains of *Lactobacillus* to differentiate these closely related strains.

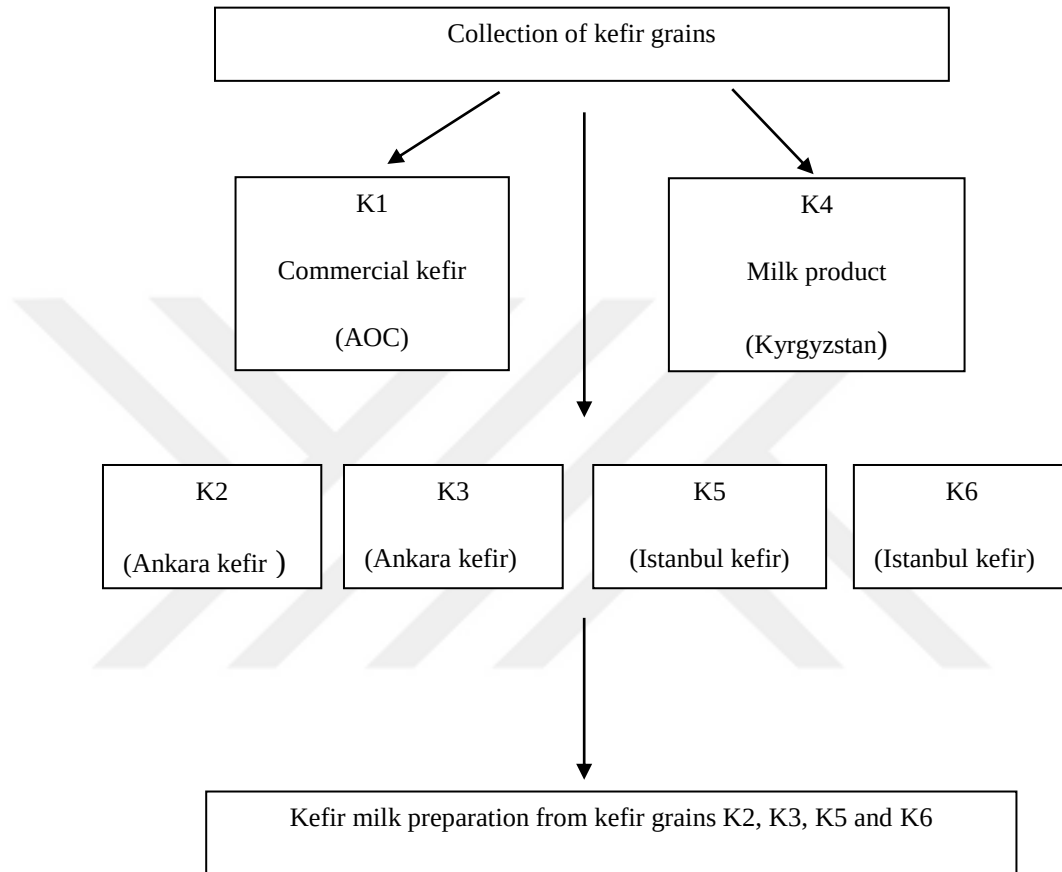


Figure 3.1 Experimental planing (Section 1); collections of kefir grains and preparation kefir milk.

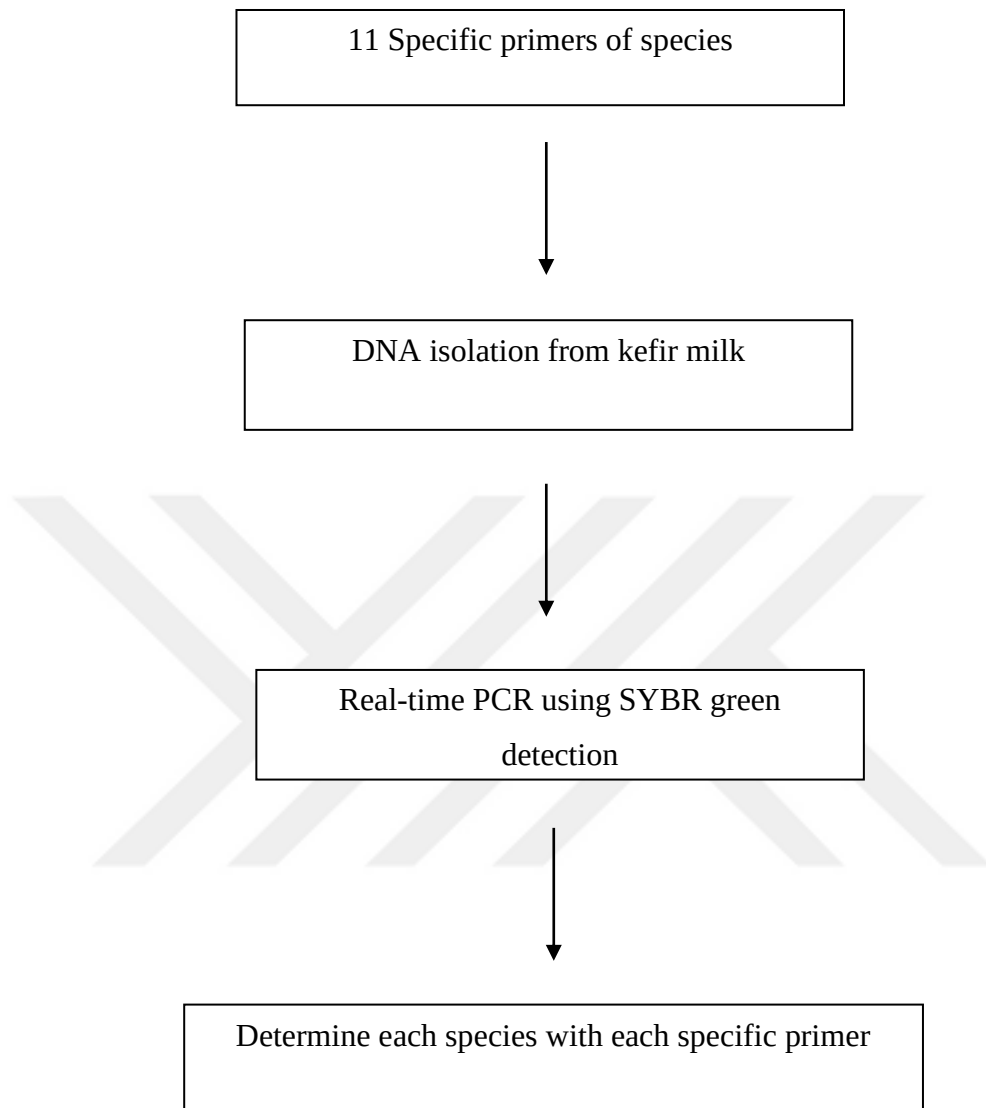


Figure 3.2 Experimental planning (Section 2); determination of the population in homemade kefir samples

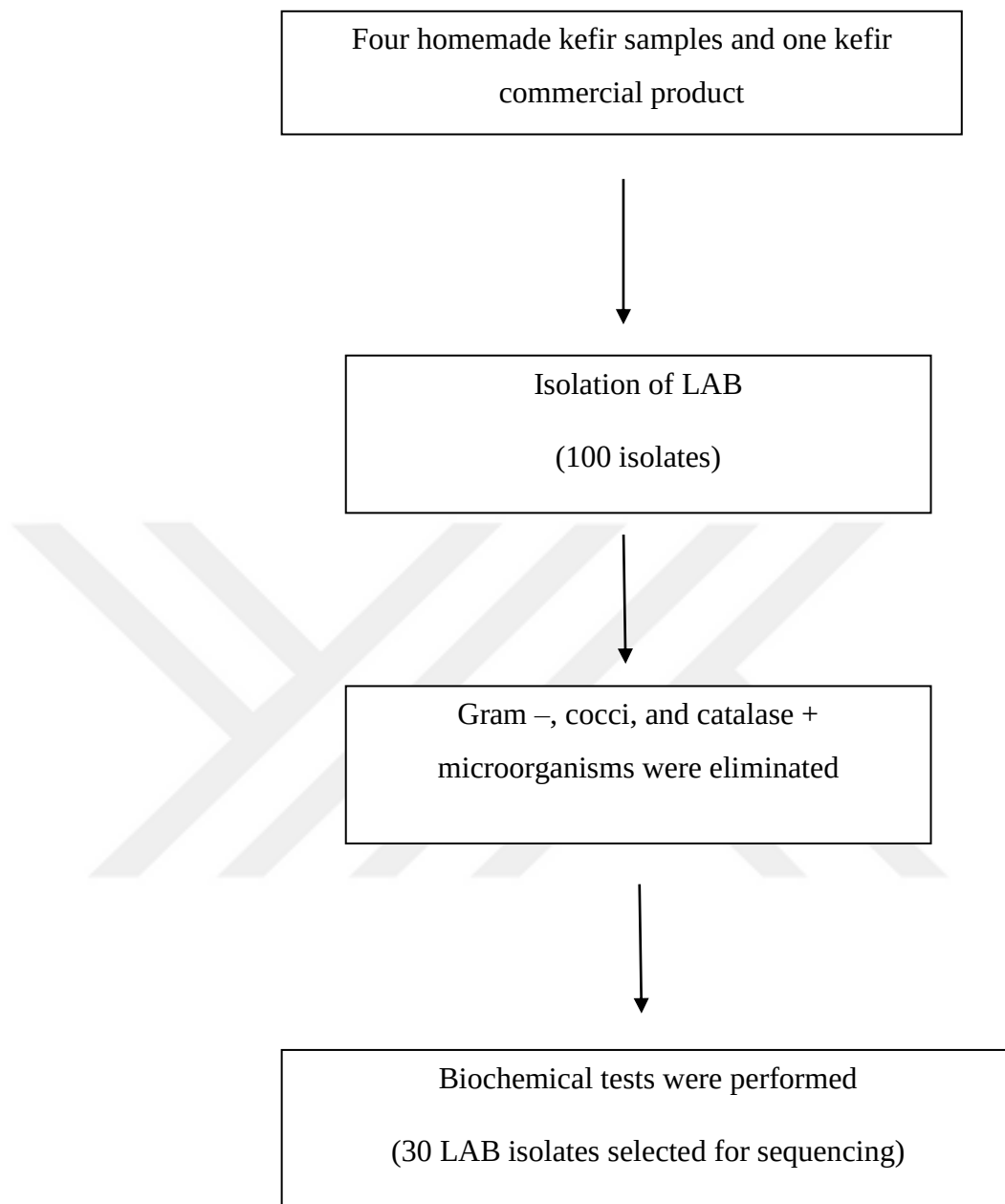


Figure 3.3 Experimental planning (Section 3); isolation and microbiological / biochemical identifications of LAB.

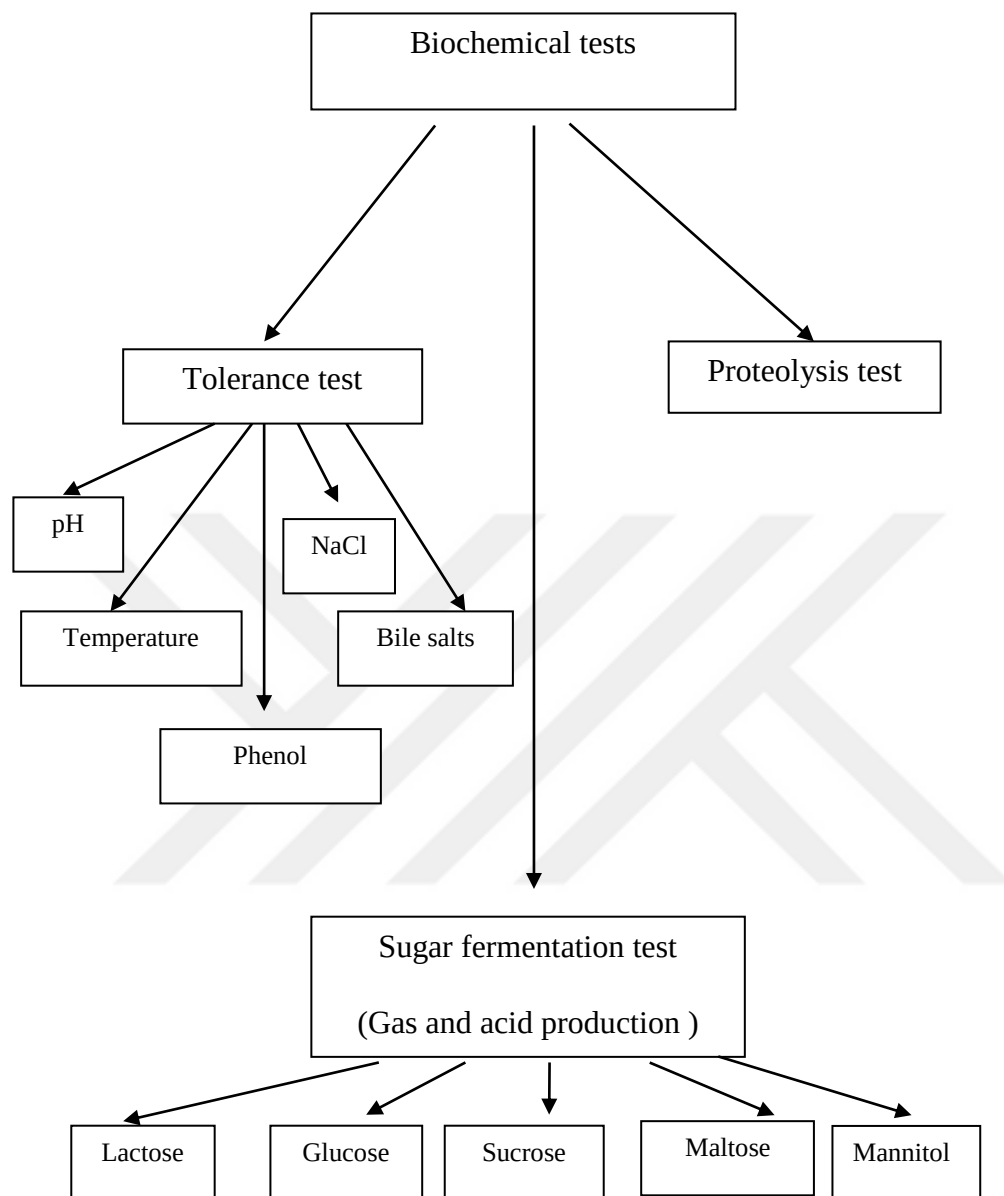


Figure 3.4 Biochemical tests performed in the study (Section 4)

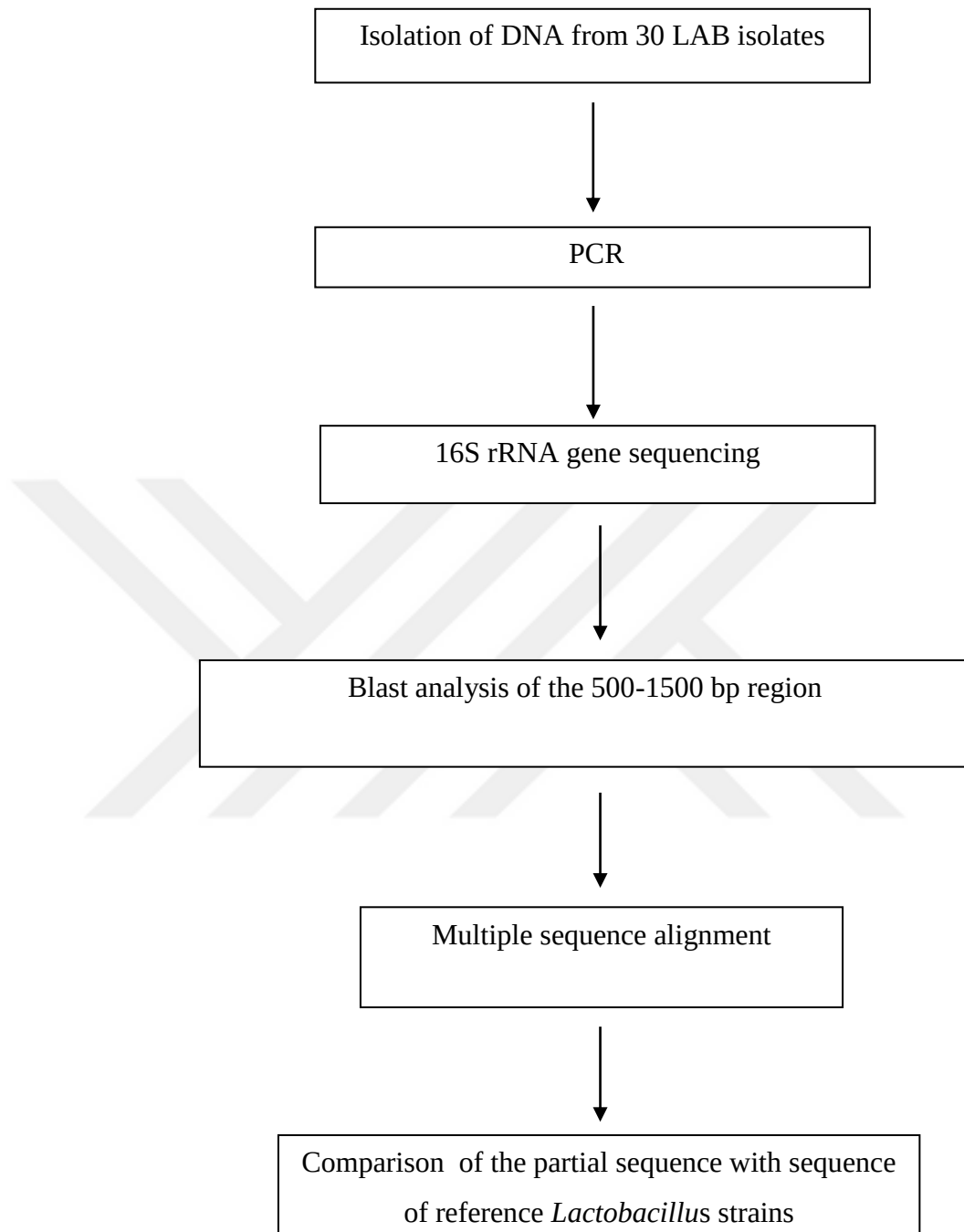


Figure 3.5 Experimental planning of the blast analysis

3.2 Collection of Samples

The grains of homemade kefir were brought from various regions of Ankara and Istanbul, Turkey. The milk product sample was collected from Kyrgyzstan. The samples were transferred to the Laboratory in the Food Microbiology Department at Middle East Technical University (METU) to be analyzed and studied. (Table 2.1)

3.3 Kefir Preparation

As mentioned before in the previous chapter, kefir was prepared from kefir grains by inoculating in pasteurized milk and incubating at 30°C for 24 hours followed by the separation of kefir milk from the grains. The kefir beverage were used for further analysis and the grains were stored at -20°C for the next usage.

3.4 Detection of Probiotic Microorganisms Using Real-Time PCR

Lactobacilli are difficult to detect and identify by traditional microbiological tests (Kao et al. 2007; Poltronieri et al. 2008). The real-time PCR assay is an accurate, rapid, time-saving, and powerful tool for monitoring specific microorganisms within 4 to 5 h (Park et al., 2009). This assay could be a useful tool for differentiating *Lactobacillus* species from other yeast and bacteria in kefir (Dopson et al., 2011).

In this study, the microbial composition of kefir was investigated by real-time PCR using SYBR green dye and specific primers for selected microorganisms (*L. kefir*, *L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus*, *L. kefiranofaciens*, *L. fermentum*, *L. plantarum*, *S. cerevisiae*, *S. thermophilus*, *L. sobri* \amylovorus, *L. casei*, and *L. paracasei*). Most of the primers produced positive signals with DNA from the kefir and milk samples on the real-time PCR run.

L. kefir, *L. casei*, *L. kefiranofaciens*, and *L. paracasei* were detected in all kefir samples. *L. acidophilus* was determined only in 5 kefir samples, *L. delbrueckii* subsp. *bulgaricus* was determined in 5 kefir samples, however, *L. fermentum*, *L. sobris amylovorus*, and *L. plantarum* were detected only in 2 samples, *S. cerevisiae* and *S. thermophilus* were found in 5 kefir samples. The results of detection using real-time PCR are presented in Table 3.1

Table 3.1 Detection the population of kefir samples using specific primers for real-time PCR.

No of kefir sample	Name	P1 <i>L. kefir</i>	P2 <i>L. acidophilus</i>	P3 <i>L. delbrueckii</i> subsp <i>bulgaricus</i>	P4 <i>L. kefiranofaciens</i>	P5 <i>L. fermentum</i>	P6 <i>L. plantarum</i>	P7 <i>S. cerevisiae</i>	P8 <i>S. thermophilus</i>	P9 <i>L. sobris amylovorus</i>	P10 <i>L. casei</i>	P11 <i>L. paracasei</i>
1	K1	+	+	+	+	-	+	-	+	-	+	+
2	K2	+	+	+	+	-	+	+	-	-	+	+
3	K3	+	+	-	+	-	-	+	+	-	+	+
4	K4	+	+	+	+	+	-	+	+	+	+	+
5	K5	+	+	-	+	-	-	+	+	+	+	+
6	K6	+	-	+	+	+	-	+	+	-	+	+

In real-time PCR, DNA is measured after each cycle through fluorescent dyes, the fluorescent signal increases in the same ratio with the number of PCR products produced. The real-time PCR instrument produces an amplification curve and shows the fluorescent signals (ΔR_n) against the cycle number. The threshold cycle (C_t) is

the fractional cycle number at which the fluorescence passes the threshold. There is a relation between Ct and the concentration of the target in the PCR reaction. The Ct value is inversely related to the starting amount of the target DNA as described by Heid et al (1996). In the current study, all strains generated Ct values ranging from 19 to 36 threshold cycles and unspecific signals developed were easily identifiable by melting curve analysis. Herbel et al (2013) found that all reference *Lactobacillus* strains and isolates from yogurt had Ct values ranging from 14 to 28 threshold cycles.

As the template amount decreases, the cycle number of the amplification increases. It is supposed that the real-time PCR is operating at high efficiency when the number of product doubles during each cycle.

The specificity of the reaction was checked by the melting curve analysis (T_m) in which the amplified PCR products were denatured and cooled to 5-10°C. The T_m depends on GC content (Edwards, 2004). The change in fluorescence to change in temperature ratio ($-\Delta F/\Delta T$) is plotted against temperature to get a clear melting curve.

Melting curves helped distinguish primer-dimers from specific PCR products and to confirm the amplified products. The primer dimers melt at lower temperatures than the amplicon (PCR products). Primer-dimers take place when two PCR primers join to each other instead of the target. Also, the presence of these primer-dimers may be related to the deficient primer annealing time or the unsuitable annealing temperature. According to the recent study, the T_m for *Lactobacillus* spp that was identified from kefir and milk samples using real-time PCR was between 78-85.5°C. The present results are similar to the results by Abdulmir et al (2010) in which the

melting curve for *Lactobacillus* spp. in six samples of fish and shrimp sauces had a peak at $T_m = 85.5^\circ\text{C}$.

Lactobacillus kefir

In the current study, *L. kefir* was successfully detected in all kefir milk samples in the real-time PCR assay. The same result was found in the previous study by Kim et al (2016). They easily utilized a real-time PCR assay to detect *L. kefir* in the kefir milk for monitoring the quality of kefir probiotics. It is significant for kefiran production in the kefir grain structure. Kefiran has been supposed to be effective against a diversity of diseases (Kim et al., 2016).

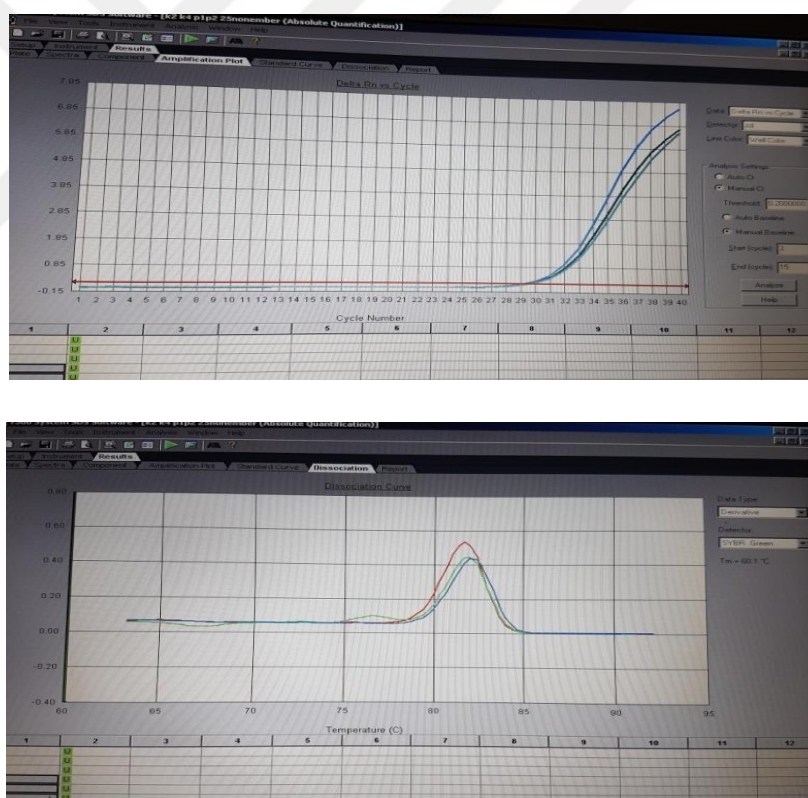


Figure 3.6 Amplification and melting curve for Kefir sample (K4) with *L. kefir*.

L. kefir was specified as a starter culture and determines one of the flavor properties of kefir beverages. The presence of *L. kefir* in all samples in a recent study was demonstrated with real-time PCR analysis. Also, *L. kefir* is presented as kefir and kefir grain constituents in, for example, Argentinean, Taiwanese, Turkish, Brazilian, and Canadian samples. These results were presented by Chen et al (2008), Miguel et al (2010) and Kesmen and Kacmaz (2011). It was shown in Figure 3.6 that the melting curve of *L. kefir* in the K4 sample had Ct value started from 32 and a specific peak at $T_m = 82^\circ\text{C}$.

Lactobacillus kefiranofaciens

Chon et al (2013) studied that the level of *L. kefiranofaciens* was strongly correlated with the amount of kefir produced in kefir so it is a key indicator bacterium of kefir milk. In this study, *L. kefiranofaciens* was detected by real-time PCR in all samples. These results are consistent with the reports in the literature (Kim et al., 2015b). They reported a real-time PCR primer and probe set for the fast detection of *L. kefiranofaciens* in kefir grain and kefir milk. In addition, our results are similar to the reports for isolation of *L. kefiranofaciens* in Italia, Argentina, Belgium, Brazil, China, and Taiwan from the kefir and kefir grains (Garrote et al., 2001; Chen et al., 2008; Magalhaes et al., 2011; Wang et al., 2012; Gao et al., 2013; Hamet et al., 2013; Korsak et al., 2015; Zanirati et al., 2015; Garofalo et al., 2015).

Kefiran is a polysaccharide produced by *L. kefiranofaciens*. The production of this polysaccharide is increased when *L. kefiranofaciens* survives with *S. cerevisiae* (Cheirsilp et al., 2003). This can be explained and was supported by our results that *L. kefiranofaciens* and *S. cerevisiae* in all kefir samples except commercial one, It may be to avoid gas production in the product during the marketing. In the current

study, the amplification curve had a Ct value at 19 and one peak of the melting curve was shown at 83 °C in K6 sample (Figure 3.7)

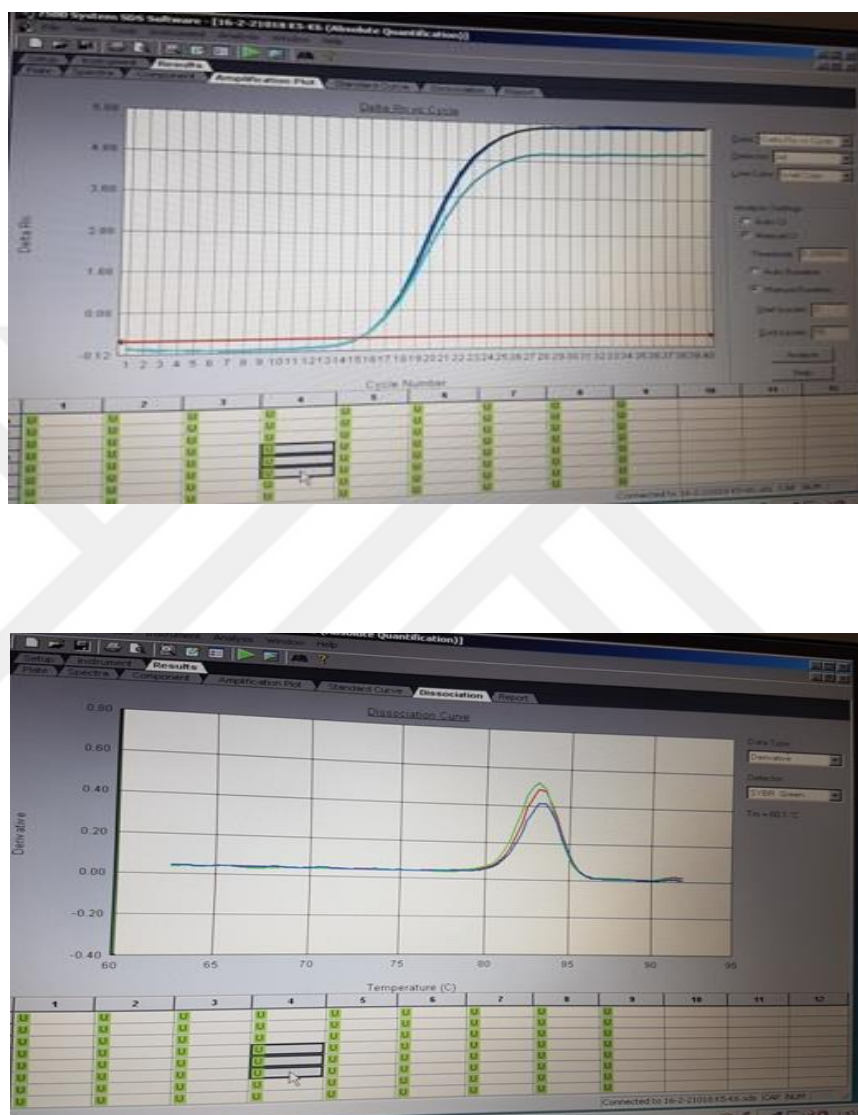


Figure 3.7 Amplification and melting curve for Kefir sample (K6) with *L. kefiranofaciens*.

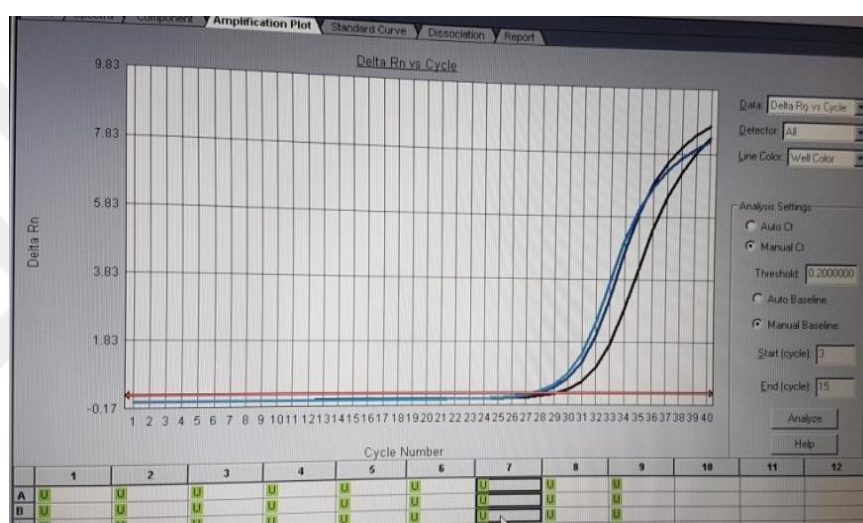
Saccharomyces cerevisiae

DNA extracted from kefir and milk samples was examined by real-time PCR using *Saccharomyces cerevisiae* primers which yielded positive results for all samples except for K1 (commercial kefir product). It agrees with the results reported by Kim et al (2015a) that *Saccharomyces* spp was easily detected from kefir grains and fermented milk kefir using real-time PCR. *S. cerevisiae* was also identified in kefir beverages by Beshkova et al (2003) as the yeast species were able to produce alcohol. However, some *Lactobacillus* strains can also produce ethanol.

S. cerevisiae is an eukaryotic microorganism and a facultative anaerobe. It can use sugars to produce water and CO₂ gas in the presence of oxygen, or it can do fermentation in anaerobic conditions to produce ethanol and CO₂ gas (Berg et al., 2012). The absence of *S. cerevisiae* only in K1 (commercial kefir product) may be to avoid the production of gases from its growth in kefir milk. Onaran and Çufaoğlu (2017) observed that homemade kefir samples contained yeast more than the commercial samples. However, the addition of *Saccharomyces* spp. to the milk kefir culture enhances the quality of kefir, explaining the cruciality of the symbiosis between the bacteria and yeast (Cheirsilp et al., 2003). Also, the yeasts are important for the microbiological properties and enhancement of the physical and chemical characteristics of the fermented product. According to Farnworth (2005), yeasts have an important role in the production of kefir with essential amino acids, vitamins, and produce metabolites that contribute to the flavor of kefir.

S. cerevisiae was found in kefir grains and kefir beverage of various origins such as Argentina, Bulgaria, Brazil and China (Simova et al., 2003; Hamet et al., 2013; Gao et al., 2013 and Zainirati et al., 2015). These findings indicated that Turkish homemade kefir samples of the present study are similar to other samples from different regions.

Figure 3.8 shows the amplification and melting curves for *S.cerevisiae* in sample K5, *S. cerevisiae* had a Ct value at 32 and a specific peak for the melting curve at 82°C.



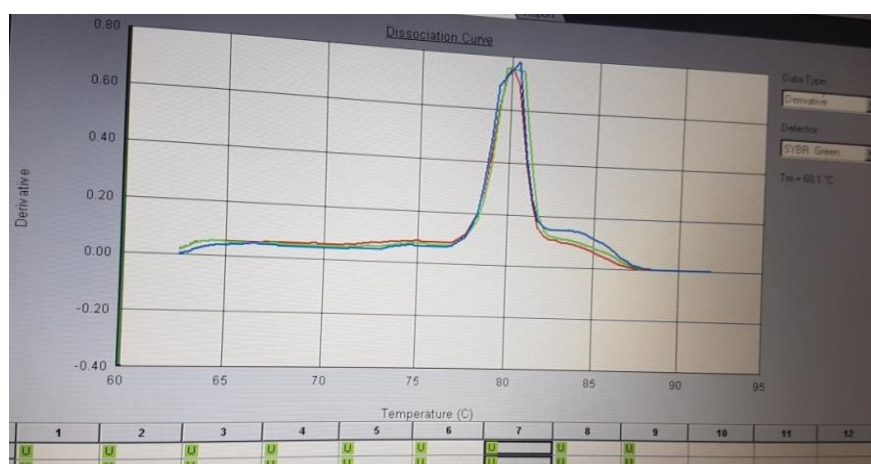


Figure 3.8 Amplification and melting curve for Kefir sample (K5) with *S. cerevisiae*.

Lactobacillus acidophilus

L. acidophilus is one of the significant species in kefir and it has a wide range of health benefits in cholesterol metabolism, antimicrobial activity, immune modulation, and tumor suppression.

Guzel S. et al (2005), Kesmen and Kacmaz (2011), Kok-Tas et al (2012) and Nalbantoglu et al (2014) reported that *L. acidophilus* was one of the probiotic species found in kefir grains and beverages in Turkey. These results were compatible with our results in which *L. acidophilus* was detected in 5 samples using a real-time PCR approach.

The melting curve analysis of *L. acidophilus* in K6 appears to be a case of primer dimers due to the presence of additional peaks at low temperature to the left of the peak for the amplified product at smaller T_m as shown in Figure 3.9 (a). It was considered as a negative result because of the presence of nonspecific products.

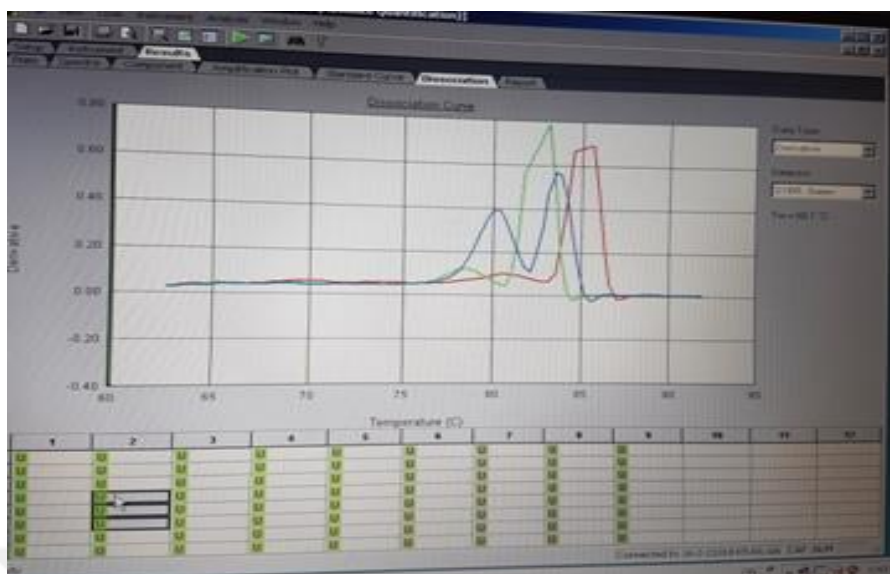
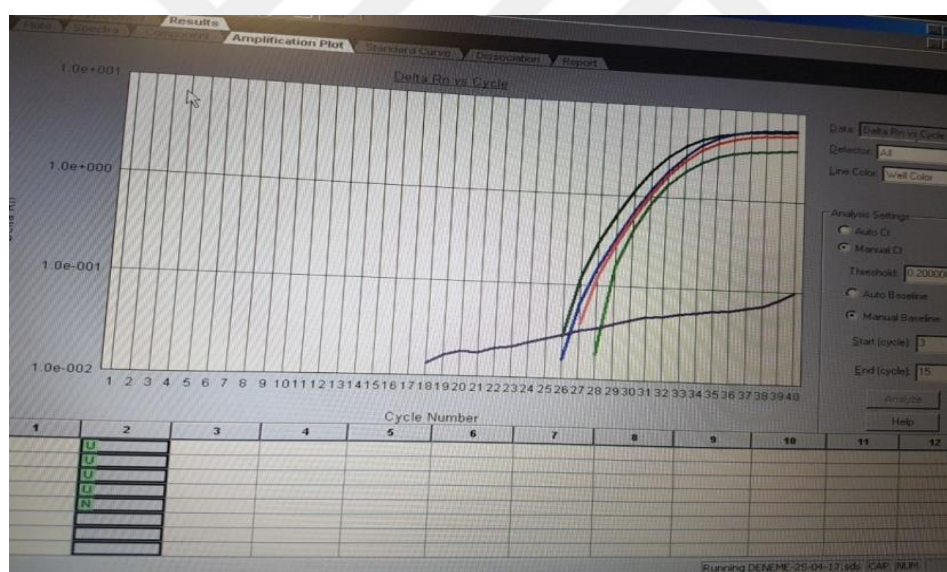


Figure 3.9 (a) Amplification and melting curve for *L. acidophilus* in Kefir (K6), case of primer dimer.



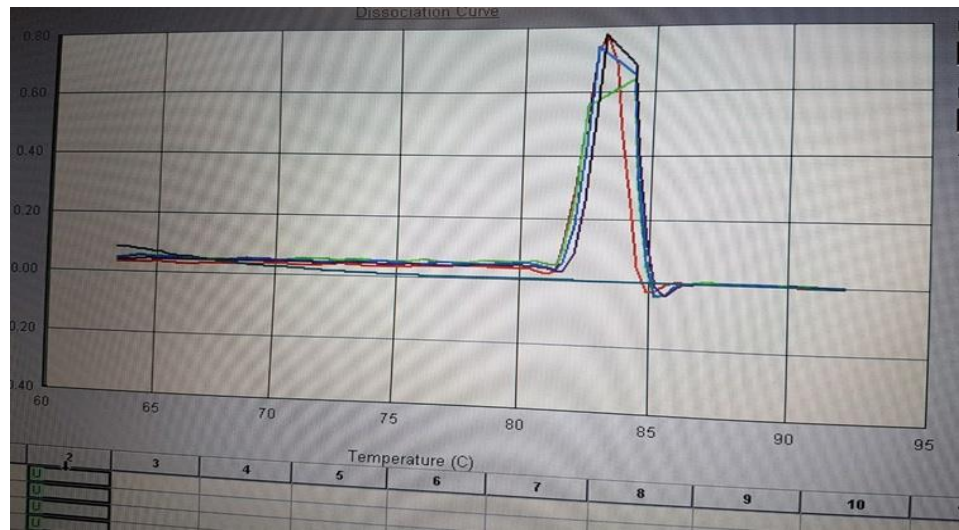


Figure 3.9 (b) Amplification and melting curves for *L. acidophilus* in K2.

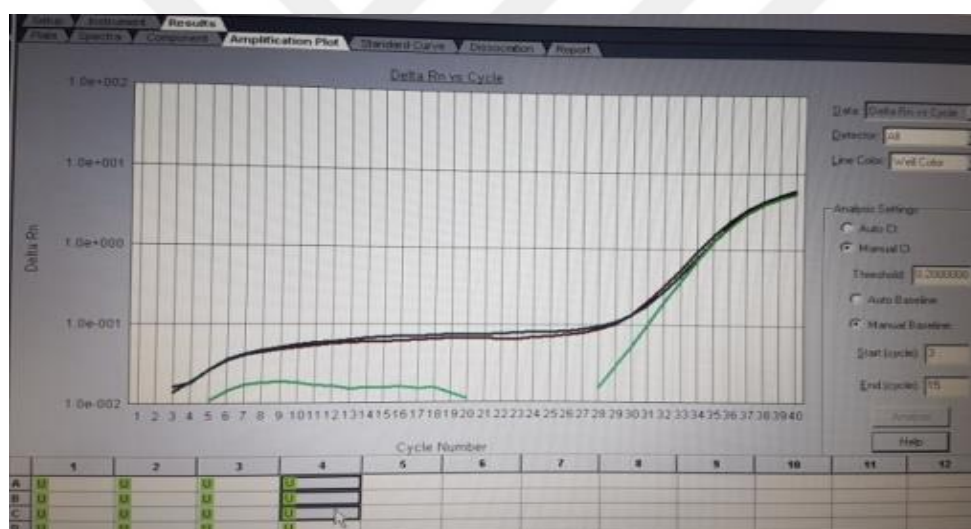
Lactobacillus plantarum

L. plantarum was previously isolated from kefir of different origins such as Argentina, China, South Africa, and Turkey and it was characterized as a potential probiotic strain as discussed in the previous studies (Garrote et al., 2001; Hamet et al., 2013; Gao et al., 2012 and 2013; Witthuhn et al., 2004; Merih and Evrim, 2015). In addition, De Montijo-Prieto (2015) found that *L. plantarum* isolated from kefir could increase resistance to intestinal infections through its immunomodulatory activity. The capability of *L. plantarum* to antagonize the cytotoxic effects of *E.coli* (Hugo et al., 2008) and Shiga2 toxin (Kakisu et al., 2013) were studied.

Kıvanç and Yapıcı (2016) found that *L. plantarum* was the dominant species and some species like *L. kefiranofaciens*, *L. kefir*, *L. kefirgranum*, and *L. parakefir* were not found in their study. These findings are inconsistent with our study because *L.*

plantarum was detected in commercial kefir (K1) and Ankara kefir (K2). However, *L. kefir*, *L. kefiranofaciens* were the most dominant species in kefir using real-time PCR and this can be explained by the difference in geographical origins of the kefir grains.

It was shown that the Ct value for *L. plantarum* in K1 and K2 was 31 and had a specific peak in the melting curve analysis at 78°C. In contrast, Herbel (2013) detected *L. plantarum* in a yoghurt sample at a melting temperature of about 84°C. The amplification and melting curve for *L. plantarum* in K1 and K2 can be seen in Figure 3.10(a,b).



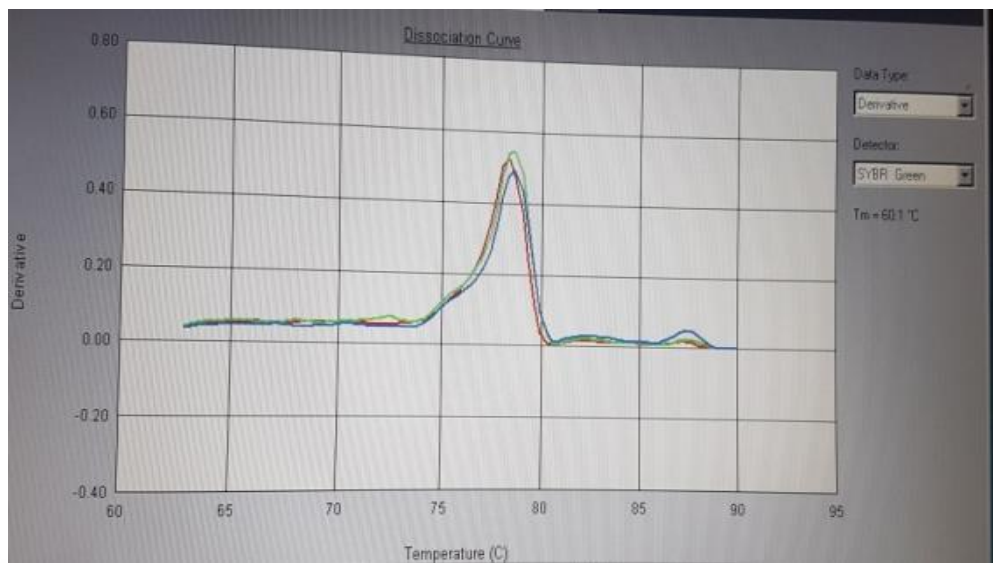
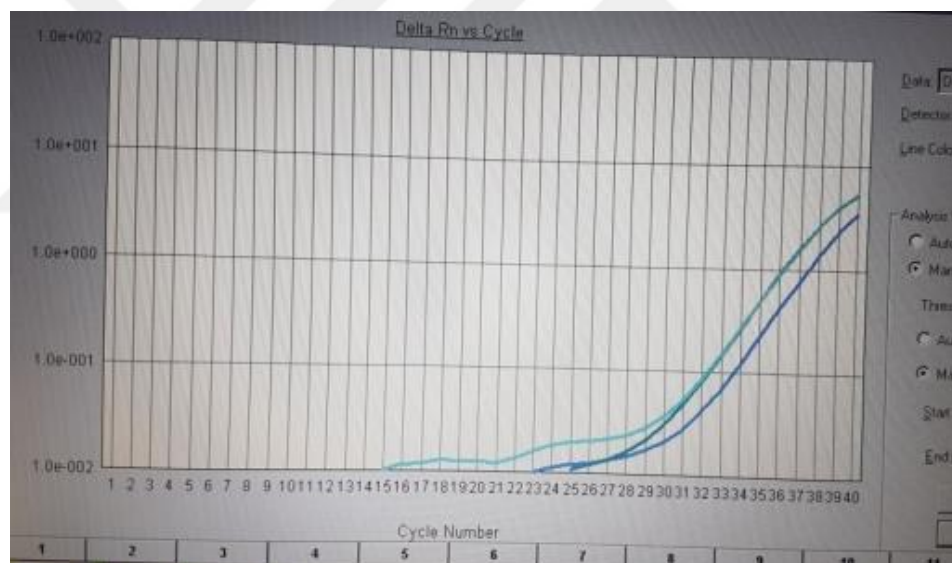


Figure 3.10 (a) Amplification and melting curve for Kefir (K1) with *L. plantarum*.



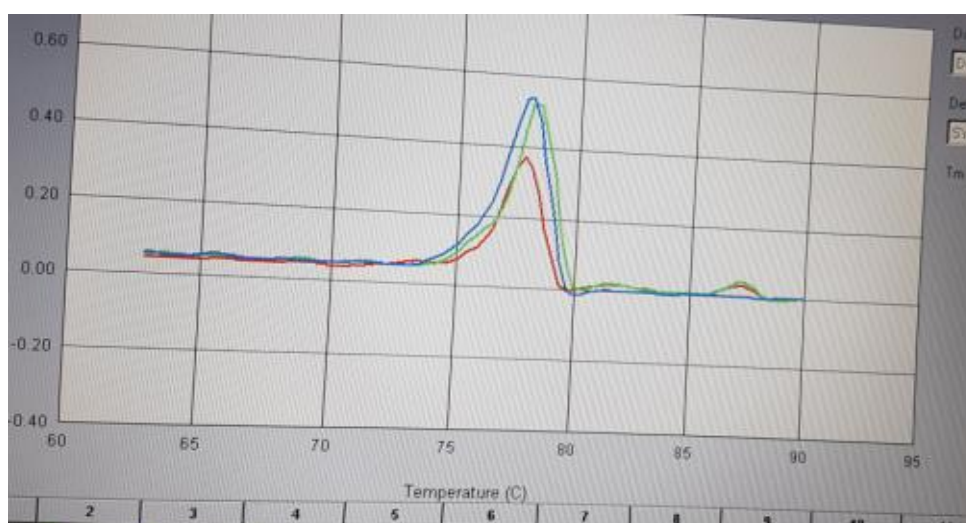


Figure 3.10 (b) Amplification and melting curve for Kefir (K2) with *L. plantarum*.

Lactobacillus casei

L. casei was one of the dominant homofermentative *Lactobacillus* species in the current study and it was found in all kefir samples from Turkey and milk sample from Kyrgyzstan. In addition, the presence of *L. casei* in kefir grains and beverage had been shown in samples from Russia, Argentina, Bulgaria, Brazil, China, and Turkey by previous reports (Garrote et al., 2001; Leite et al., 2012; Hamet et al., 2013; Zanirati et al., 2015; Magalhaes et al., 2011; Gao et al., 2012 and 2013a; Merih and Evrim, 2015; Kotova et al., 2016).

L. casei is used as probiotics for the production of fermented products due to its ability to form good amounts of lactic acid which can enhance the aroma and flavor of a product. In addition to its high bile salt and acidity tolerance and antimicrobial

effects, many beneficial health effects of *L. casei* strains have been studied such as accelerating cancer's cure duration, stimulating the immune system, and decreasing cholesterol levels (Somer et al., 2012).

As it can be seen in Figure 3.11, *L. casei* was recognized by real-time PCR and had a Ct value at 28 and its T_m value was located between 82°-85°C. Kao et al (2007) found that T_m of *L. casei* group including *L. casei* and *L. paracasei* were located at about 63.94 °C. Our results are in accordance with these results reported in the literature. The amplification and melting curve for *L. casei* in all samples can be seen in Figure 3.11.



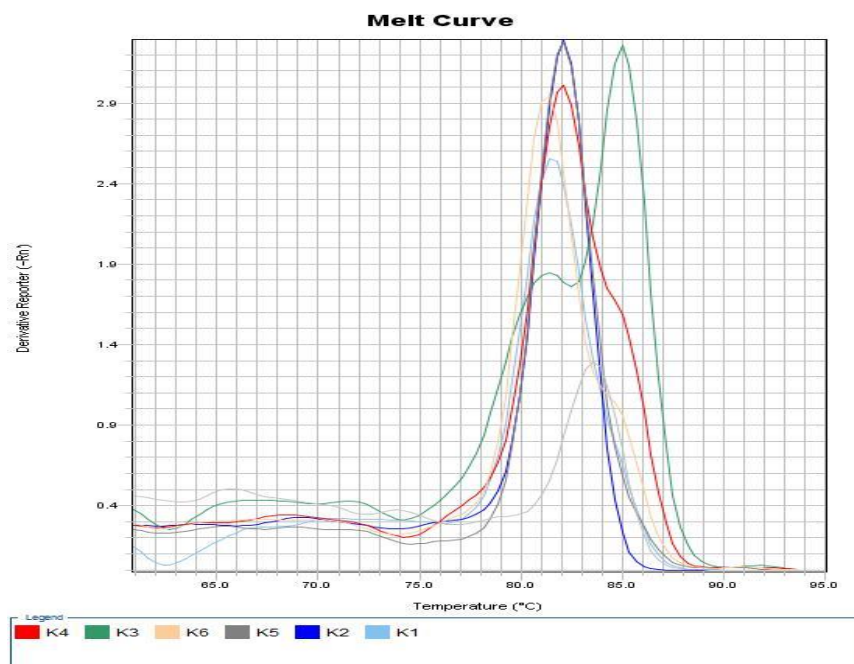


Figure 3.11 Amplification and melting curve for *L. casei* in all samples.

Lactobacillus paracasei

L. paracasei is a probiotic, useful at improving the intestinal environment, and produce lactic acid to improve the taste and aroma of dairy products (Xiao and Dong, 2003). They have an effective role in decreasing cholesterol and controlling blood pressure, elimination of gastric mucosal lesions, alleviation of allergies, and suppression of fat tissue growth (Chiang and Pan 2012). Also, Xue et al (2015) found that *L. paracasei* M7 prevents the adhesion of Salmonella to epithelial tissues. In a recent study, *L. paracasei* was displayed to be one of the most abundant bacterial strains in all samples. These are similar to the results announced by Magalhães et al (2011) that *L. paracasei* was one of the most abundant Brazilian kefir samples. Also, it was found in kefir grains and beverages of Russia, Argentina, Bulgaria, Brazil, China, and Turkey (Garrote et al., 2001; Leite et al., 2012; Hamet et al., 2013; Zanirati et al., 2015; Magalhaes et al., 2011; Gao et al., 2012 and 2013a; Merih and Evrim, 2015; Kotova et al., 2016).

L. paracasei had Ct values at 30 and peaks ranging from 78-85°C in the melting curve for all kefir samples. However, there were two peaks for K5 in the melting curve but it does not always indicate a primer dimer problem. Ahmed et al (2017) discussed that SYBR Green dye gives fluorescence signal when bound to double-stranded DNA (ds DNA). As the temperature increases, dsDNA denatures becoming ssDNA. This is supposed to be an intermediate phase that it is neither dsDNA nor ssDNA (two phases in which the DNA does not melt directly and the DNA melt). Mackey et al (1988) found in a limited investigation that the temperature (T_{max}) at which peak was greater was strongly correlated with the G + C content of the DNA. Additional reasons such as amplicon misalignment and secondary structure in the amplicon region are also possible. Thus, the appearance of double peaks during melting curve analysis does not always point to non specific amplification (Wittwer et al., 2009; Dwight et al., 2011). In the K5 case, both peaks appear at a high temperature which may refer to positive result for *L. paracasei* detection (Figure 3.12). In addition, the presence of *L. paracasei* in K5 has also been proven in the results section of isolation and identification.

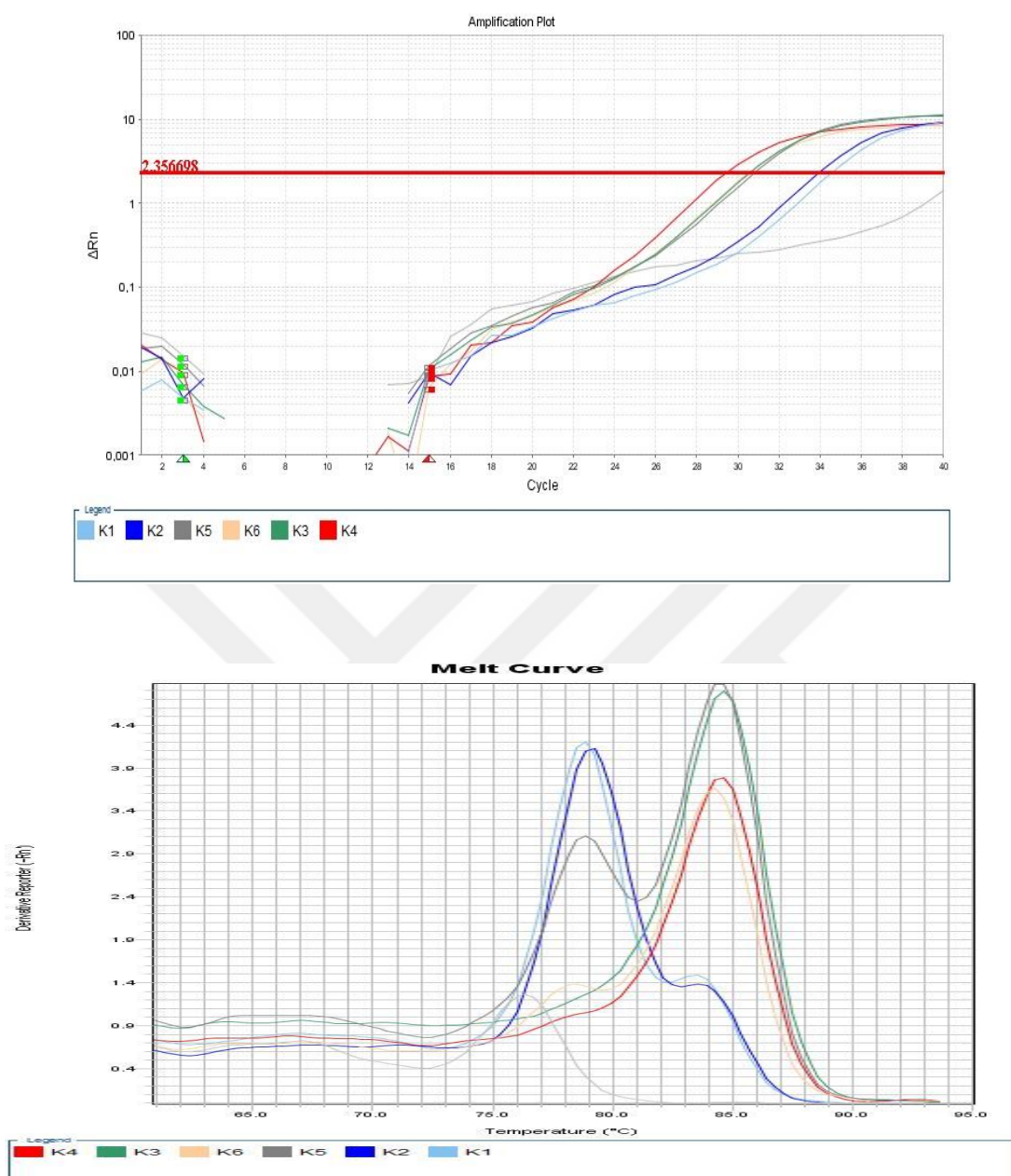


Figure 3.12 Amplification and melting curve for *L. paracasei* in all samples.

Lactobacillus delbrueckii* subsp. *bulgaricus

Simova et al (2002) identified *L. delbrueckii* subsp. *bulgaricus* from Bulgarian kefir grains and beverages. Also, Kao et al (2007) and Harbel et al (2013) detected *L.*

delbrueckii subsp. *bulgaricus* in milk and yoghurt samples using real-time PCR. Similar to our study, *L. delbrueckii* subsp. *bulgaricus* was found and detected in all kefir samples except K3 and K5 as their real-time PCR run did not yield an amplification or melting curve for *L. delbrueckii* subsp. *bulgaricus*.

Figure 3.13(a) shows Ct value at 27 and one peak appears at 85°C in the melting curve of *L. delbrueckii* subsp. *bulgaricus* in commercial Kefir.

As it can be shown from Figure 3.13(b), Tm for *L. delbrueckii* subsp. *bulgaricus* in the melting curve analysis of kefir samples ranged from 82°C to 85°C.

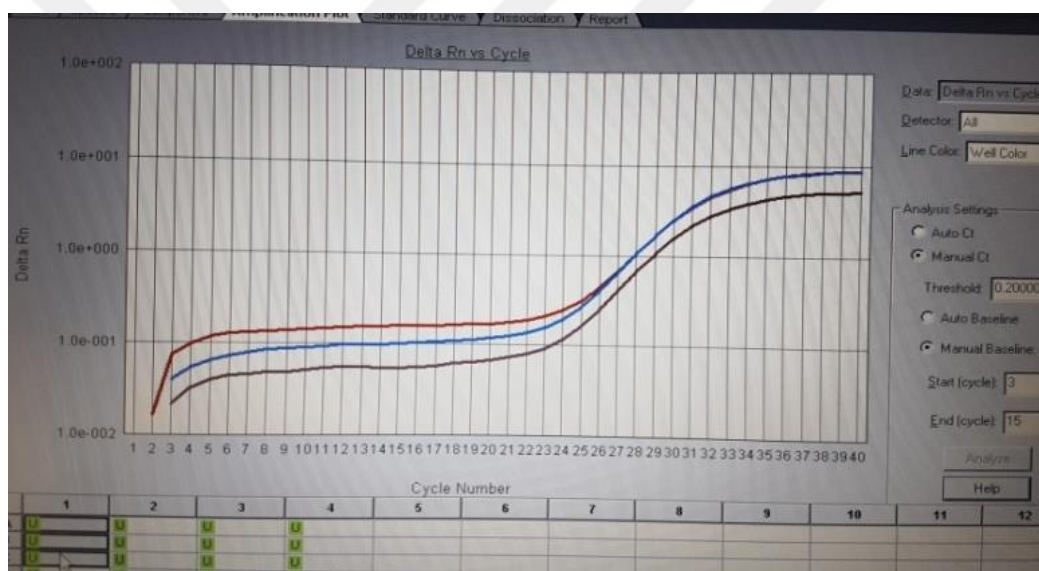


Figure 3.13a The amplification curve of *L. delbrueckii* subsp. *bulgaricus* in k1.

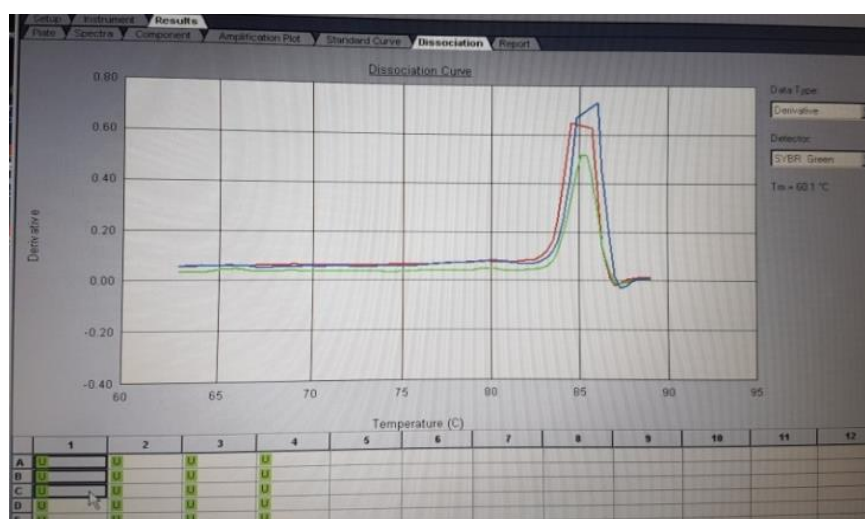


Figure 3.13b The melting curve of *L. delbrueckii* subsp. *bulgaricus* in k1.

Lactobacillus sobrius\amylovorus

Magalhaes et al (2011) and Nalbantoglu et al (2014) demonstrated the presence of *L. amylovorus* in Brazilian and Turkish kefir samples. In the current study, *L. sobrius\amylovorus* was detected in two kefir samples K4 and K5, using real-time PCR. The absence of this species in the commercial and other kefir samples may be due to the fact that some *L. amylovorus* strains form bacteriocins effective against some strains of the *Lactobacillus* such as *L. delbrueckii* subsp. *bulgaricus* (Gawhen and Bergmeyer, 1974).

As underlined by Laiño et al (2014), *L. amylovorus* CRL 887 is able to produce not only folates but also vitamins that it needs for its growth. The importance of using *L. amylovorus* in the food industry as phytase production (Sreeramulu et al., 1996), to reduce cholesterol level (Grill et al., 2000) and its production for antifungal compounds was also demonstrated (Ryan et al., 2011).

L. sobrius\amylovorus was detected in K4 and K5 with high Ct value of 38. This high Ct is indicative of very low amounts of target DNA or showing a state of contamination (Heid C. et al., 1996).

T_m for *L.sobrius\amylovorus* in the current study was 74-78°C. The amplification and melting curve of *L. amylovorus* in k4 can be shown in Figure 3.14.

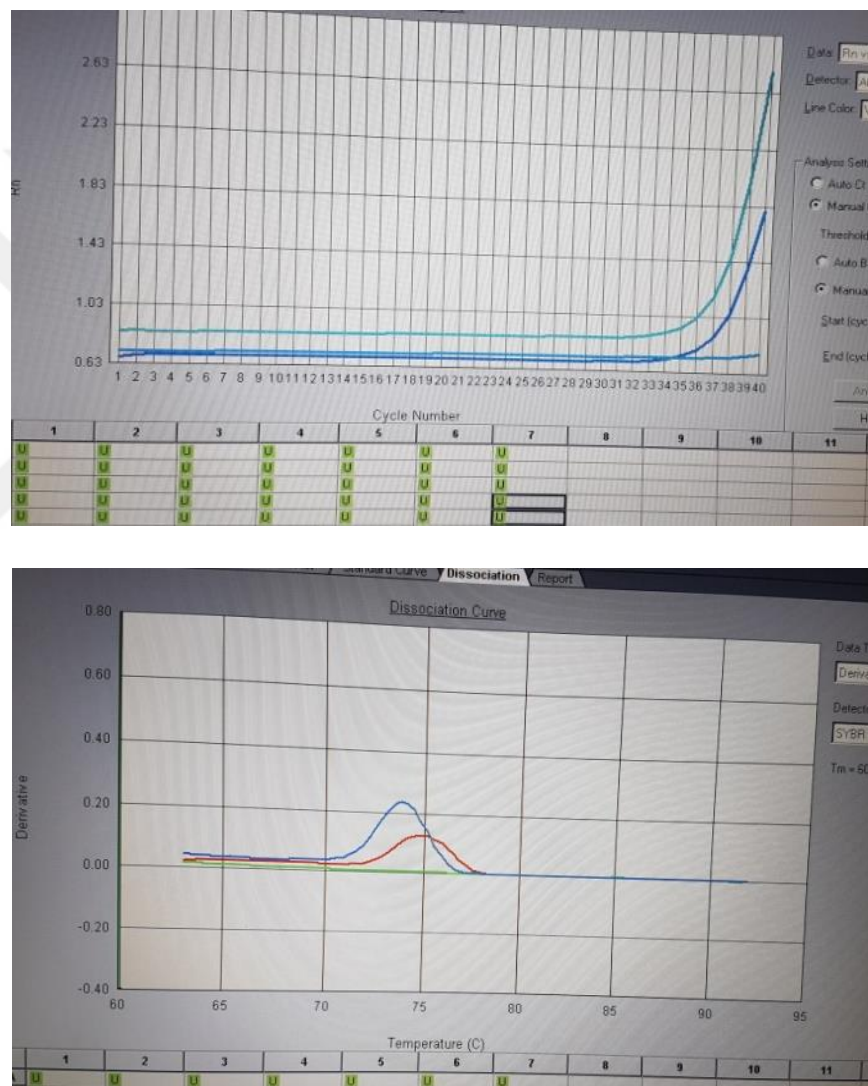


Figure 3.14 The amplification and melting curve of *L. amylovorus* in K4.

Lactobacillus fermentum

Witthuhn et al (2004, 2005) detected *L. fermentum* in South African kefir grains. In this study, *L. fermentum* was detected using real-time PCR in K4 and K6. It had a Ct value at 32 and peak at 82°C in the melting temperature. The amplification and melting curve for K4 can be seen in Figure 3.15. *L. fermentum* is obligately heterofermentative lactobacilli producing lactic, succinic, and acetic acids (Mikelsaar and Zilmer, 2009). *L. fermentum* has both antioxidative and antimicrobial properties (Kullisaar Z. 2016; Kumar et al., 2017). Antioxidant *L. fermentum* can lower the risk of some diseases due to the diversity of gut microbiota and colorectal cancer (Sepp et al., 2018).

Ukrainian homemade kefir beverage includes *Saccharomyces* spp. and bacteria such as *L. fermentum* almost 90% of the microbial association, with *Leuconostoc lactis*, and acetic bacteria *Acetobacter* (Vichko et al. 2013). In traditional kefir produced from grains, some lactobacilli like the species of *L. delbrueckii* subsp. *bulgaricus* grow together with *L. fermentum* comprised up to 98.2% of the *Lactobacillus* population (Witthuhn et al., 2005).

In the current study, *L. fermentum* and *L. delbrueckii* subsp. *bulgaricus* were present together in kefir samples K4 and K6, which proves the high diversity of kefir microflora and indicates the effects of kefir microbiota on each other in the kefir environment.

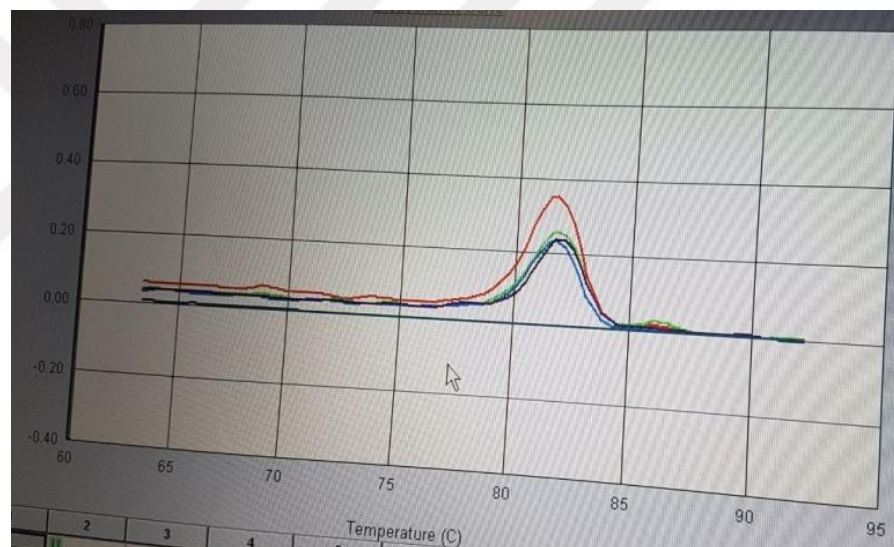
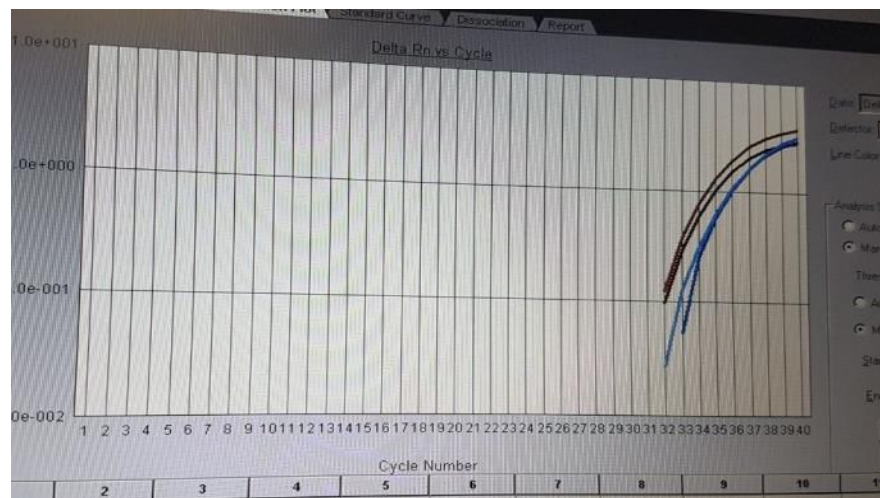


Figure 3.15 The amplification and melting curve of *L. fermentum* in K4.

Streptococcus thermophilus

Bulgarian, Brazilian, Turkish, and Tibetan kefir grains and beverages contain the probiotic *S. thermophilus*. It is used in food fermentation and many industrial dairy products. It can enhance the structure and aroma properties of these dairy products. Besides, *S. thermophilus* has various beneficial effects including antioxidant actions, modification of intestinal microbiota, and suppression of specific pathogens. (Adolfsson et al., 2004; Iyer et al., 2010).

Our findings showed the occurrence of *S. thermophilus* in all kefir samples except K2. It had a Ct value at 32 and melting temperature ranging from 84-87°C for K1, K5, and K6, but it was 73°C for K3 and K4. The amplification and melting curve for K5 can be seen in Figure 3.16.

As mentioned by Simova et al (2002), *Streptococcus lactis* and *Streptococcus thermophilus* were the dominant microflora in all kefir grains. These results differ from the results of Neve (1992) that they found a very rare presence of streptococci in the grain. In Turkish kefir, *Streptococcus thermophilus* were identified by Yüksekdağ et al (2004) and their results are similar to our results.

Laiño et al (2013) discussed an aggregation of *L. bulgaricus* and *S. thermophilus* to the commercial yoghurt and resulted in a twice as much increase in folate levels. In the present study, *L. bulgaricus* and *S. thermophilus* were found together in four kefir samples K1, K4, K5, and K6. It confirms the importance of the combination to improve the health benefits of kefir by increasing folate level.

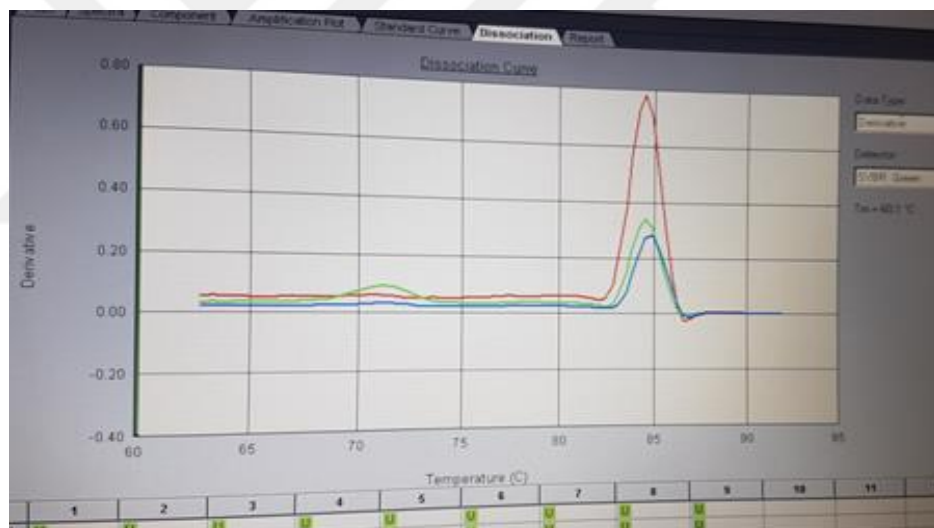
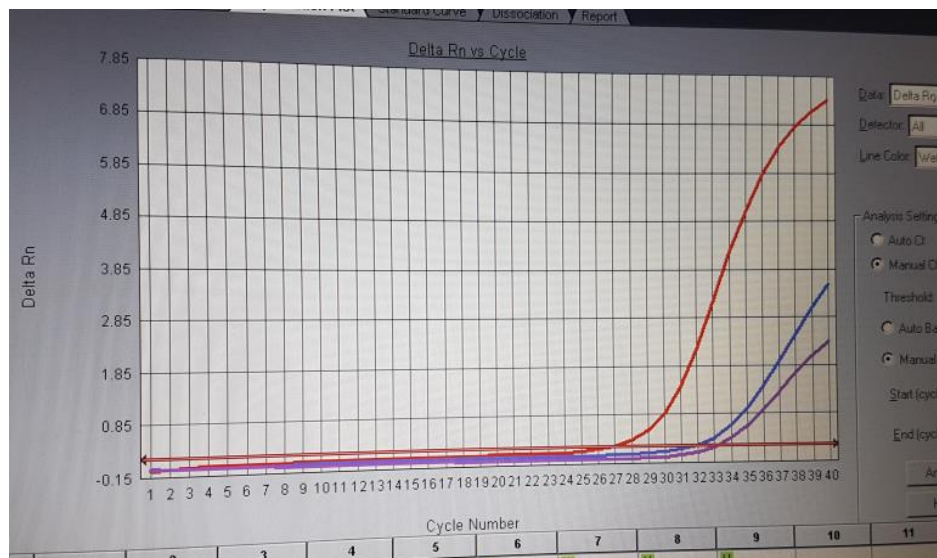


Figure 3.16 The amplification and melting curve of *S. thermophilus* in K6.

The amplification and melting curve of kefir 5 and 6 with specific primers (*L. kefir*, *L. acidophilus*, *L. kefiranofaciens*, *L. delbrueckii* subsp. *bulgaricus*, *L. fermentum*, *L. plantarum*, *S. cerevisiae*, *S. thermophilus*, *L. sobri* \amylovorus) were given in Figure 3.17(a and b).

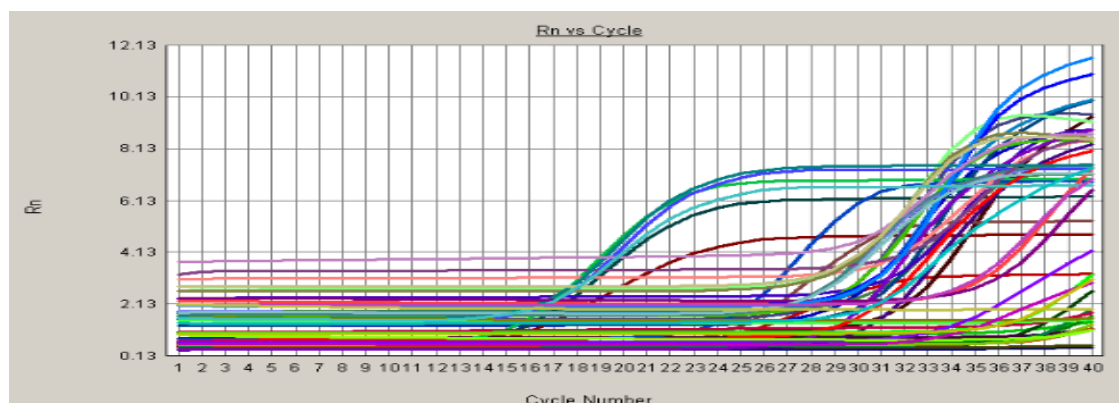


Figure 3.17 (a) Amplification curves of kefir sample (K5 and K6) with specific primers (*L. kefir*, *L. acidophilus*, *L. kefiranofaciens*, *L. fermentum*, *L. plantarum*, *L. delbrueckii* subsp. *bulgaricus*, *S. cerevisiae*, *S. thermophilus* and *L. sobri* \amylovorus).

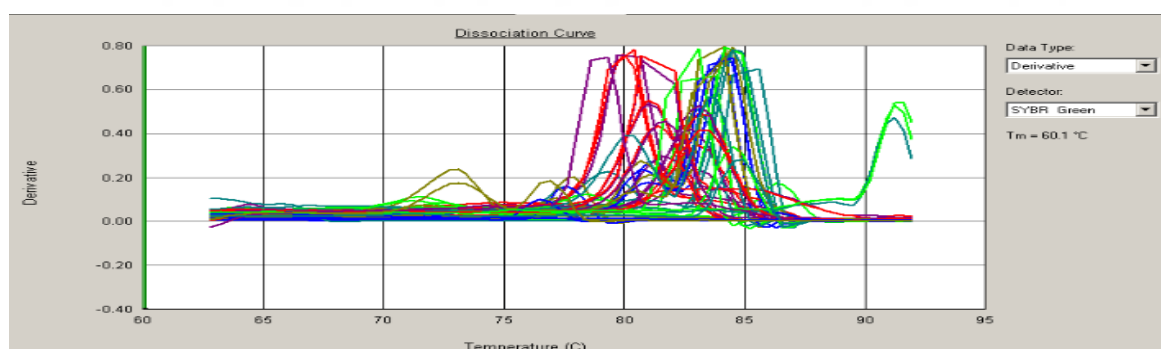


Figure 3.17 (b) Melting curves of kefir sample (K5 and K6) with specific primers (*L. kefir*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. kefiranofaciens*, *L. fermentum*, *L. plantarum*, *S. cerevisiae*, *S. thermophilus*, and *L. sobri* \amylovorus).

In this point, real-time PCR was a simple and accurate assay to successfully identify *L. kefiri*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. kefiranofaciens*, *L. fermentum*, *L. plantarum*, *S. cerevisiae*, *S. thermophilus*, *L. sobrius*, *L. amylovorus*, *L. casei* and *L. paracasei* in different kefir and milk samples. In addition, determination of the predominant species in all samples like *L. Kefir*, *L. kefiranofaciens*, *L. casei* and *L. paracasei* could be useful for monitoring the quality of kefir probiotics to produce our kefir in further studies.

Our recent study agreed with Herbel et al (2013) that used the same method successfully to identify *Lactobacillus* species by species-specific primers for *S. thermophilus*, *L. delbrueckii*, *L. casei*, *L. paracasei*, *L. rhamnosus*, *L. acidophilus*, and *L. johnsonii* in milk products. On the other hand, Kao et al (2007) explained that real-time PCR could detect *L. acidophilus* and *L. delbrueckii* by species-specific primers, but it could not be used to detect *L. casei*, *L. paracasei*, and *L. rhamnosus* of probiotic products in Taiwan, which is in agreement with our results. Using real-time PCR could easily identify *L. casei*, *L. paracasei* in our samples.

The microbial population of kefir is dependent on its source which can differ from origin to origin. Therefore, it is very important to make species-based identification of the microorganisms in kefir to determine the health benefits of the products.

It can be concluded from real-time PCR assay that our kefir and milk samples also have several important organisms as the other samples from other countries. In this study, one milk product sample from Kyrgyzstan (K4) was rich in probiotic *Lactobacillus* strains as well as kefir samples collected from Ankara and Istanbul. Besides, most of *Lactobacillus* strains detected in real-time PCR assay from our samples were similar to the microbial population of Argentinean, Taiwanese,

Turkish, Brazilian, and Chinese kefir grains and beverages (Garrote et al., 2001; Hamet et al., 2013, Chen et al., 2008; Wang et al., 2012; Kok-Tas et al., 2012; Nalbantoglu et al., 2014; Leite et al., 2012; Zanirati et al., 2015; Gao et al., 2012, 2013a).

All of the amplification and melting curves of real-time PCR for the kefir samples with 11 primers are given in the appendix chapter.

3.5 Isolation of Lactic Acid Bacteria

Lactobacillus species are among the most prevalent microorganisms found in kefir (Slattery et al., 2019). This analysis focuses on the isolation *Lactobacillus* strains from kefir and milk samples.

A total of 100 isolates were isolated from homemade kefir and milk product sample, subcultured on MRS medium, and incubated at 37°C for 48 hours under anaerobic condition. The purity of the isolates was examined by the streaking method. The pure colony was picked up and preserved with in a 20% glycerol solution at -20 and -80°C.

3.6 Phenotypic Identification of LAB

3.6.1 Colony Morphology

All of the isolates were identified based on the characteristic morphology and colony morphology of *Lactobacillus* strains was evaluated and the properties of the colony such as form, size, and color are shown in Table 3.2.

Colonies appeared creamy, white-colored, circular, and belonged to the genus *Lactobacillus*. They varied in size and for some marginal characteristics. These findings were similar to the characters of *Lactobacillus* strains isolated by Kandler and Weiss (2005).

Table 3.2 Sources of kefir and colony morphology of isolates.

No	Isolate name	Source of kefir	Form of colony	Color of colony	Size
1	K2-2a	Ankara	Circular, irregular	Creamy	Small
2	K2-3	Ankara	Circular, regular	Creamy	Small
3	K2-3a	Ankara	Circular, irregular	Creamy	Small
4	K2-4	Ankara	Circular, irregular	Creamy	Small
5	K2-4a	Ankara	Circular, irregular	Creamy	Small
6	K2-14	Ankara	Circular, irregular	Creamy	Small
7	K3-2a	Ankara (2)	Circular,regular	White	Large
8	K3-3a	Ankara (2)	Circular,irregular	Creamy	Small
9	K3-8b2	Ankara (2)	Circular,regular	Creamy	Small
10	K3-13b	Ankara (2)	Circular,regular	Creamy	Small
11	k3-20	Ankara (2)	Circular,regular	Creamy	Small
12	K3-20a	Ankara (2)	Circular,regular	Creamy	Small
13	K3-28	Ankara (2)	Circular,regular	Creamy	Small
14	K4-6a	Kyrgyzstan	Circular,regular	White	Large
15	k4-16	Kyrgyzstan	Circular,regular	Creamy	Small
16	K4-17	Kyrgyzstan	Circular,regular	Creamy	Small
17	K4-28	Kyrgyzstan	Circular,regular	Creamy	Small

Table 3.2 Sources of kefir and colony morphology of isolates (continued).

No	Isolate name	Source of kefir	Form of colony	Color of colony	Size
18	K5-3	Istanbul	Circular, regular	Creamy	Small
19	K5-3a	Istanbul	Circular, regular	White	Small
20	K5-5a	Istanbul	Circular, regular	Creamy	Small
21	K5-11	Istanbul	Circular, regular	White	Small
22	K5-14	Istanbul	Circular, regular	Creamy	Small
23	K5-15	Istanbul	Circular, regular	White	Small
24	K6-1	Istanbul (2)	Circular, regular	Creamy	Small
25	K6-3a	Istanbul (2)	Circular, regular	White	large
26	K6-6a	Istanbul (2)	Circular, regular	White	Small
27	K6-7	Istanbul (2)	Circular, regular	Creamy	Small
28	K6-9a	Istanbul (2)	Circular, regular	White	large
29	K6-12	Istanbul (2)	Circular, regular	Creamy	Small
30	K6-14	Istanbul (2)	Circular, regular	White	Large

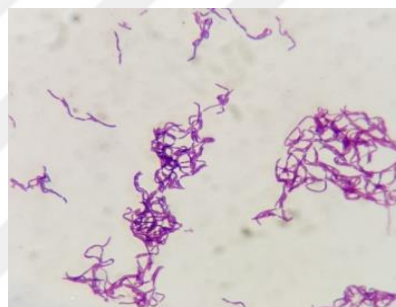
Results are expressed as small for colony diameter 2 mm and large for colony diameter 2-5 mm.

3.6.2 Microscopic Examination

The isolates were subjected to Gram's staining and were evaluated under a light microscope. The results are shown in Table 3.3. Staining was used to distinguish the bacterial isolates depends on their cell wall properties. The strains stained purple color indicated that they were gram-positive, rod-shaped as single or in chain forms as seen in Figure 3.18. These results determined that the isolated bacteria could be identified as *Lactobacillus* species (Salvetti et al., 2012; Rao et al., 2015).



k4-6a



k5-14

Figure 3.18 Gram staining

3.6.3 Catalase Test

This test is a diagnostic tests for the differentiation of bacteria. Since the isolates were gram-positive bacteria, they were examined for catalase activity. No bubble was noticed indicating catalase-negative and these bacteria could not degrade H_2O_2 to produce O_2 . It is recognized that *Lactobacillus* is catalase-negative.(Table 3.3)

Table 3.3 Gram staining and catalase test for kefir isolates.

Isolate No	Isolate name	Gram stain	Shape	Catalase test
1	K2-2a	+ ve	long rod & in chain	-ve
2	K2-3	+ ve	short rod ,single & in chain	-ve
3	K2-3a	+ ve	long rod & in chain	-ve
4	K2-4	+ ve	long rod & in chain	-ve
5	K2-4a	+ve	short rod & single	-ve
6	K2-14	+ ve	short rod & single	-ve
7	K3-2a	+ ve	short rod & single	-ve
8	K3-3a	+ ve	long rod & in chain	-ve
9	K3-8b2	+ ve	short rod & single	-ve
10	K3-13b	+ ve	short rod & single	-ve
11	k3-20	+ ve	short rod & single	-ve
12	K3-20a	+ ve	short rod & single	-ve
13	K3-28	+ ve	short rod & single	-ve
14	K4-6a	+ ve	long rod & in chain	-ve
15	k4-16	+ ve	short rod & single	-ve
16	K4-17	+ ve	short rod & single	-ve
17	K4-28	+ ve	short rod & single	-ve
18	K5-3	+ ve	short rod & single	-ve
19	K5-3a	+ ve	short rod & single	-ve
20	K5-5a	+ ve	short rod & single	-ve
21	K5-11	+ ve	short rod & single	-ve
22	K5-14	+ ve	short rod & in chain	-ve
23	K5-15	+ ve	short rod & in chain	-ve

Table 3.3 Gram staining and catalase test for kefir isolates (Continued).

Isolate No	Isolate name	Gram stain	Shape	Catalase test
24	K6-1	+ ve	short rod & single	-ve
25	K6-3a	+ ve	long rod & in chain	-ve
26	K6-6a	+ ve	short rod & in chain	-ve
27	K6-7	+ ve	short rod & single	-ve
28	K6-9a	+ ve	short rod & single	-ve
29	K6-12	+ ve	short rod & single	-ve
30	K6-14	+ ve	long rod & in chain	-ve

3.7 Biochemical Tests

Biochemical characteristics of LAB isolates were examined as follow.

3.7.1 pH Tolerance Test (Acid tolerans)

The resistance of the probiotic strains to low pH is one of the important criteria (Çakır 2003). They must pass through the extreme conditions of the stomach, at a pH lower than 0.1, to reach the small intestine (Chou and Weimer 1999; Çakır 2003). A single isolated colony was transferred into MRS broth at various pH values 2,2.5,3,4, and 6 and incubated at 37 °C for 24 h to observe the ability of the growth of *Lactobacillus* isolates (Table 3.4). From this experiment, isolated *Lactobacillus* showed that the optimum pH value for good growth was at pH 4.0 and 6.0, moderate growth at pH2.5 and 3.0 with no or less growth at pH 2.0. Therefore, it was demonstrated that the isolated *Lactobacillus* species were able to survive in stressful acidic and neutral conditions. Similar results were reported by Pyar and Peh (2014) who observed the turbidity for *Lactobacillus* growth in broth with different pH values arranging from 4.0 to 7.0. Hoque et al (2010) found that a major decrease in

the viability of strains was often noticed at $\leq$ pH 2.0. Besides, *Lactobacillus* growth was detected at pH 2.5 to pH 8.5. These earlier results are similar to ones shown by our study.

Shivram and Vishwanath (2012) explained that the isolated *Lactobacillus* strains had potential to survive at pH 2.0 and 3.0. Srinu et al (2013) displayed that all the selected lactic acid bacterial strains as *L. plantarum* and *L. casei* survived in the tested acidic pH range (1.5, 2.0, 3.0 and 3.5).

3.7.2 NaCl Tolerance Test

Commonly, NaCl is an inhibitory substance that can suppress bacterial growth. In the current study, the NaCl tolerance test was applied at 2%, 4% and 6.5% NaCl concentrations. The results explained that all *Lactobacillus* spp. isolated from kefir were able to survive at 6.5% of NaCl and optimal growth was detected at 2- 4% of NaCl (Table 3.4). These results are similar to those by Hoque et al (2010) that the *Lactobacillus* spp isolated from yoghurts could grow at 1-9 % of NaCl and optimal growth was detected at 1-5 % NaCl.

Forhad et al (2015) found that *Lactobacillus* species were able to survive at 1-6% NaCl concentration where as they could not grow at high NaCl concentrations. High salt tolerance is a useful property for an organism to be used as a probiotic. High osmotolerance would be an important character for LAB strains because when lactic acid production causes alkali to be pumped, and the free acid would be converted to its salt form. This causes an increase in osmotic pressure on bacterial cells. It would then affect their metabolism, water activity, enzyme activity, and physiology. The results for the pH and NaCl tolerance test are given in Table 3.4

Table 3.4 The tolerance of *Lactobacillus* isolates to pH and NaCl salt.

No	Isolate name	NaCl tolerance			pH tolerance				
		2%	4%	6.5%	pH= 2	pH= 2.5	pH= 3	pH =4	pH= 6
1	K2-2a	++	++	+	-	-	+	++	++
2	K2-3	++	++	+	-	+	+	++	++
3	K2-3a	++	++	+	-	-	+	++	++
4	K2-4	++	++	+	-	+	+	++	++
5	K2-4a	++	++	+	-	+	+	++	++
6	K2-14	++	++	+	-	+	+	++	++
7	K3-2a	++	++	+	-	+	+	++	++
8	K3-3a	++	++	+	-	+	+	++	++
9	K3-8b2	++	++	+	-	+	+	++	++
10	K3-13b	++	++	+	-	+	+	++	++
11	K3-20	++	++	+	-	+	+	++	++
12	K3-20a	++	++	+	-	+	+	++	++
13	K3-28	++	++	+	-	+	+	++	++
14	K4-6a	++	++	+	-	+	+	+	++
15	K4-16	++	++	+	-	+	+	++	++
16	K4-17	++	++	+	-	+	+	++	++
17	K4-28	++	++	+	-	+	+	++	++
18	K5-3	++	++	+	-	+	+	++	++
19	K5-3a	++	++	+	-	+	+	++	++
20	K5-5a	++	++	+	-	+	+	++	++
21	K5-11	++	++	+	-	+	+	++	++
22	K5-14	++	++	+	-	+	+	++	++
23	K5-15	++	++	+	-	+	+	++	++

Table 3.4 The tolerance of *Lactobacillus* isolates to pH and NaCl salt (Continued).

No	Isolate name	NaCl tolerance			pH tolerance				
		2%	4%	6.5%	pH= 2	pH= 2.5	pH= 3	pH =4	pH=6
24	K6-1	++	++	+	-	+	+	++	++
25	K6-3a	++	++	+	-	+	+	++	++
26	K6-6a	++	++	+	-	+	+	++	++
27	K6-7	++	++	+	-	+	+	++	++
28	K6-9a	++	++	+	-	+	+	++	++
29	K6-12	++	++	+	-	+	+	++	++
30	K6-14	++	++	+	-	-	-	++	++

(++) good growth, (+) less growth, (-) No growth

3.7.3 Temperature Tolerance Test

Temperature is an essential factor which can affect bacterial growth and the reason for doing this test is to detect ability of the isolated cultures to survive within normal body temperature. In the current study, the isolates were screened for their capacity to tolerate different temperatures of 25, 37, 40, and 45°C (Table 3.5). According to the results, all of the isolates easily grow at 25, 37, and 40°C. These results are similar to those of Pundir et al (2013). However, some isolates could not grow at 45°C. According to Chakraborty and Bhowal (2015), the isolated *Lactobacillus* species were able to survive with in 30-50°C and the optimum temperature for maximum growth was 37°C. Also, Yavuzdurmaz (2007) showed that after 7 days of observation all of the *Lactobacillus* isolates survived at 45 °C, however, they could not grow at 10 °C and 15 °C. These results differ from our results.

Table 3.5 Temperature, phenol tolerance, and lactic acid production test.

No	Isolate name	Temperature tolerance				Phenol tolerance	Lactic acid production
		25°C	37°C	40°C	45°C	(0.4%)	
1	K2-2a	+	++	+	+	+ve	+ve
2	K2-3	+	++	+	+	+ve	+ve
3	K2-3a	+	++	+	+	+ve	+ve
4	K2-4	+	++	+	-	+ve	+ve
5	K2-4a	++	++	+	-	+ve	+ve
6	K2-14	+	++	+	+	+ve	+ve
7	K3-2a	++	++	+	+	+ve	+ve
8	K3-3a	++	++	+	-	+ve	+ve
9	K3-8b2	++	++	+	-	+ve	+ve
10	K3-13b	++	++	+	-	+ve	+ve
11	K3-20	++	++	+	-	+ve	+ve
12	K3-20a	++	++	+	-	+ve	+ve
13	K3-28	+	++	+	+	+ve	+ve
14	K4-6a	+	++	+	+	+ve	+ve
15	K4-16	+	++	+	+	+ve	+ve
16	K4-17	+	++	+	+	+ve	+ve
17	K4-28	++	++	+	-	+ve	+ve
18	K5-3	++	++	+	-	+ve	+ve
19	K5-3a	++	++	+	-	+ve	+ve
20	K5-5a	++	++	+	-	+ve	+ve

Table 3.5 Temperature, phenol tolerance, and lactic acid production test (continued).

No	Isolate name	Temperature tolerance				Phenol tolerance	Lactic acid production
		25°C	37°C	40°C	45°C	(0.4%)	
21	K5-11	++	++	+	-	+ve	+ve
22	K5-14	+	++	+	+	+ve	+ve
23	K5-15	+	++	+	+	+ve	+ve
24	K6-1	+	++	+	-	+ve	+ve
25	K6-3a	+	++	+	+	+ve	+ve
26	K6-6a	+	++	+	+	+ve	+ve
27	K6-7	+	++	+	-	+ve	+ve
28	K6-9a	+	++	+	-	+ve	+ve
29	K6-12	+	++	+	-	+ve	+ve
30	K6-14	+	++	+	+	+ve	+ve

(++) good growth, (+) less growth, (-) No growth

3.7.4 Phenol Tolerance Test

Probiotic strains survive low concentrations of inhibitory substances such as phenol since it is bacteriostatic. Certain aromatic amino acids originated from proteins would be deaminated in the gut by bacteria which cause the formation of phenols that have bacteriostatic effects (Suskovic et al., 1997).

As seen from Table 3.5, the isolated candidates were examined for their aptitude to tolerate the phenolic environment. A phenol tolerance test was performed in 0.4% of phenol concentration. It was observed that *Lactobacillus* isolated from kefir samples

could endure 0.4% phenol. These results have the similarities with results of Elizete and Carlos (2005) and Hoque et al (2010).

Vizoso Pinto et al (2006) found different ratios of sensitivity for 4 strains of *L. johnsonii* and 6 strains of *L. plantarum* against 0.4% phenol concentration while *L. plantarum* strains were less sensitive. Our results indicate a good tolerance of all tested strains towards phenol even if the growth in the presence of phenol is lower than the growth without phenol.

3.7.5 Lactose Utilization

The isolates were screened for their capacity to grow in medium supplemented with lactose. Change in color from red to yellow indicates the conversion of lactose to lactic acid and all the tested isolates can ferment lactose as shown in (Table 3.5).

Lactose utilization of isolated LAB was described by Ahmed and Kanwal (2004). This test detects the importance of probiotic bacteria for lactose-intolerant people who have a deficiency of enzyme galactosidase. When they use lactose-containing products, symptoms including cramps, diarrhoea, abdominal pain can happen. Most of the studies conclude that the addition of certain probiotics to milk products and kefir will help these people to ingest those products without having any symptoms (Scheinbach 1998; Fooks et al., 1999).

3.7.6 The Proteolytic Activity of LAB

Proteins and peptides are the basic structures and composed of 20 amino acids. Proteases are enzymes that stimulate the hydrolysis of peptide bonds in proteins and

polypeptide forming peptides and amino acids for bacterial growth (Christensen et al., 1999).

All of the isolated bacteria that gave positive results in the previous microscopic and biochemical tests belonged to the genus *Lactobacillus*. They were examined for their protease activity by screening on skim milk agar by agar-well diffusion method at 37°C after 48 h (Phyu et al., 2015). Proteolytic activity is a very essential characteristic of the LAB. It could be detected by measuring the diameter of the clear zone on skim milk agar. In the current study, the diameter of the clear zone confirmed that all isolates could hydrolyze casein by production of a protease enzyme. Similar results were noted by Atanasova et al (2014) and Phyu et al., (2015). However, they varied in the efficiency of casein hydrolysis. The strains K2-3, K2-14, K4-6a, K5-3, K5-3a, K5-14, K5-15, and K6-14 formed a large clear zone from 31 to 33 mm around the colonies. The strain K5-14 produced the largest visible zone. These results were demonstrated by quantitative determination of the proteolytic activity as shown in Table 3.6 and Figure 3.19.

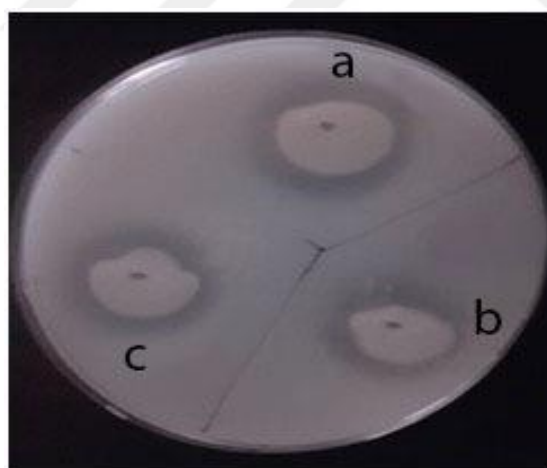


Figure 3.19 Proteolysis activity for *Lactobacillus* isolates.

a (K6-14), b (K2-4a), c (K5-3)

Table 3.6 Proteolytic activity and bile salt tolerance test.

No	Isolate name	Bile salt tolerance test		Proteolysis (diameter of clear zone mm)
		Taurodeoxycholic acid 0.5% (w/v)	Glycocholic acid 0.5% (w/v)	
1	K2-2a	+	+	29
2	K2-3	++	+	34
3	K2-3a	+	+	28
4	K2-4	++	++	29
5	K2-4a	++	++	28
6	K2-14	++	++	33
7	K3-2a	+	+	25
8	K3-3a	+	+	28
9	K3-8b2	+	+	24
10	K3-13b	+	+	27
11	K3-20	++	++	23
12	K3-20a	+	+	21
13	K3-28	+	+	23
14	K4-6a	++	+	30
15	K4-16	+	+	25
16	K4-17	++	++	25
17	K4-28	++	+	20
18	K5-3	++	+	30
19	K5-3a	+	++	31
20	K5-5a	+	+	25
21	K5-11	+	+	30
22	K5-14	++	+	35
23	K5-15	+	+	33

Table 3.6 Proteolytic activity and bile salt tolerance test (continued).

No	Isolate name	Bile salt tolerance test		Proteolysis (diameter of clear zone mm)
		Taurodeoxycholic acid 0.5% (w/v)	Glycocholic acid 0.5% (w/v)	
24	K6-1	+	++	25
25	K6-3a	+	+	30
26	K6-6a	+	+	25
27	K6-7	+	+	29
28	K6-9a	+	+	23
29	K6-12	+	++	21
30	K6-14	++	+	32

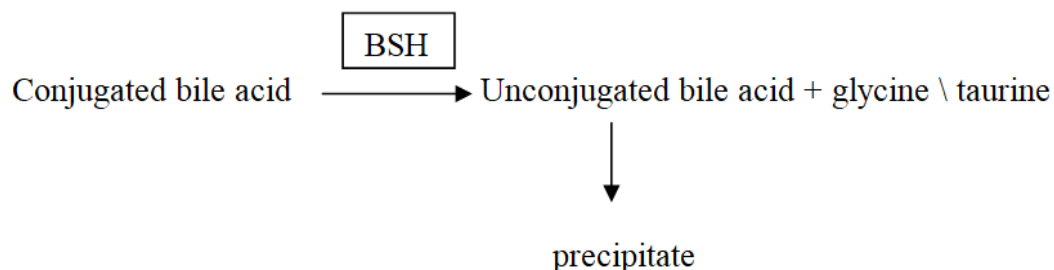
(++) good growth, (+) less growth, (-) No growth

3.7.7 Bile Salt Tolerance Test

Bile salt is found in bile and secreted by liver cells to help in digestion. It can play a significant role in fat emulsification and cause segregation of the lipid bilayer, integral protein, and phospholipids of bacterial cell membranes resulting in a decrease in bacterial content and cell death (Burns et al., 2008).

The ability of LAB to survive in the small intestine and grow in the presence of bile salts is one of the most crucial properties to play their effective role as probiotics. In the small intestine, the bile salt concentration is about 0.2% to 0.3%, and it can increase up to 2% (w/v). It depends on the type and amount of the ingested food (Bakari et al., 2011). Bile resistance of some strains is related to the activity of bile

salt hydrolase enzyme which helps hydrolyze conjugated bile to decrease its toxic effect (Du Toit et al., 1998).



In a recent study, all *Lactobacillus* cultures were grown on bile salt–MRS agar plate containing 0.5% (w/v) taurodeoxycholic and glycholic acid. They produced free cholic acid with white precipitate around active colonies and spreaded into the medium. These results are in accordance with other work that checked the activity of bile salt hydrolase activity (BSH) for some probiotic lactobacilli and all tested strains gave BSH-positive (Ahn 2003; Begley 2006; Deshpande et al.,2014).

Results for bile salts tolerance test are seen in Table (3.6). Based on the result, these isolates may be beneficial for use as probiotic organisms because all the isolates can tolerate and grow in 0.5 % bile salt concentration. A concentration of 0.3% of bile salts is close nearly to the bile level of the gastrointestinal tract (Golden and Gorbach, 1992). The presence of the food matrix may also maintain the bacteria from the bile effect and leads to improving the bile resistance of the strains (Begley et al. 2005). The bile salt tolerance test of *Lactobacilluis* colonies is demonstrated (Figure 3.20).

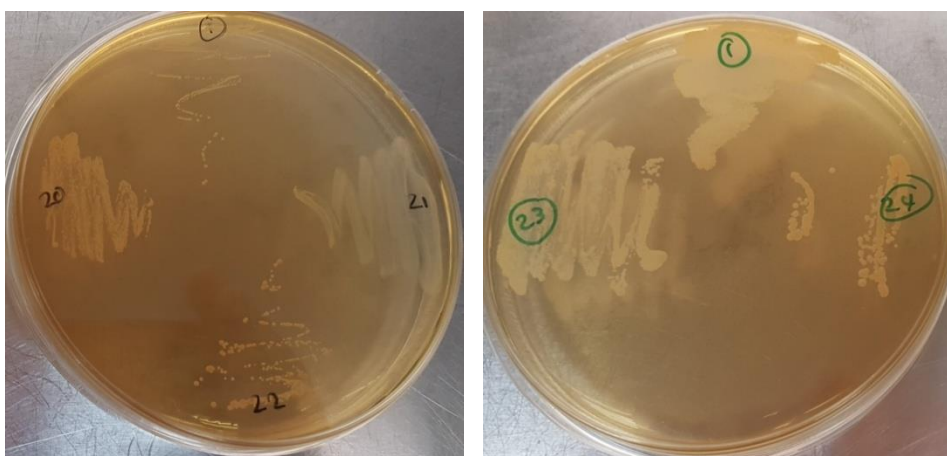


Figure 3.20 Bile salt hydrolase activity by lactobacilli on solid MRS. The greatest precipitations were by 1 (K2-4) , 21 (K4-17), and 23 (k3-13b)

The greatest precipitation for both bile salts was in the case of K2-4, k3-13b, and k4-17. According to Xanthopoulos et al (1997), the ability to tolerate bile salts varies a lot between the LAB species and among strains themselves. This is similar to the results in the recent study.

Leite (2015) tested 37 isolates by a plate assay for resistance to different bile concentrations ranging from 0.3 to 2% and 34 isolates were grown in 0.3%. These results are in accordance with the results of the recent study.

3.7.8 Carbohydrates Fermentation Test

The main task of the carbohydrate fermentation test is to examine the ability of bacteria to ferment different types of sugars. This test was done using 1% (w/v) sugar in MRS broth (sucrose, glucose, lactose, maltose, and mannitol). Phenol red broth base medium was prepared as an indicator to identify the bacteria according to their sugars fermentation. Table 3.7 shows that all the isolated bacteria fermented

sucrose, glucose, and lactose, but some isolates could not ferment mannitol and maltose. 8 of 30 isolates could ferment all types of sugars with gas production from glucose fermentation as K2-2a, K2-4, K3-2a, K3-20, K5-5a, K5-11, K6-3a and K6-14.

Gas production was detected in durham tubes from glucose fermentation. Some isolates could not produce gas from glucose fermentation. Bogdan et al (2014) discovered that all the isolates belonging to *Lactobacillus paracasei* ssp *paracasei* strains could ferment ribose, galactose, glucose, fructose, mannose, lactose, mannitol, and maltose. Our *L. paracasei* isolates which were identified by 16 S rDNA sequencing showed similar results in a way that they fermented glucose, lactose, sucrose, maltose and mannitol.

Phyu and Aye (2015) also examined the ability of LAB isolated from various yogurts and fermented products to ferment sugars consisting dextrose, glucose, lactose, maltose, fructose, raffinose, galactose, and xylose.

Table 3.7 Carbohydrates fermentation patterns of isolates

Isolate No	Isolate name	Glucose		Sucrose	Lactose	Maltose	Mannitol
		Acid	Gas	Acid	Acid	Acid	Acid
1	K2-2a	+	+	+	+	+	+
2	K2-3	+	-	+	+	+	+
3	K2-3a	+	-	+	+	+	-
4	K2-4	+	+	+	+	+	+
5	K2-4a	+	-	+	+	+	+
6	K2-14	+	-	+	+	+	+
7	K3-2a	+	+	+	+	+	+
8	K3-3a	+	-	+	+	+	+
9	K3-8b2	+	+	+	+	-	+

10	K3-13b	+	-	+	+	-	-
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Table 3.7 Carbohydrates fermentation patterns of isolates (continued)

Isolate No	Isolate name	Glucose		Sucrose	Lactose	Maltose	Mannitol
		Acid	Gas	Acid	Acid	Acid	Acid
11	K3-20	+	+	+	+	+	+
12	K3-20a	+	+	+	+	-	-
13	K3-28	+	+	+	+	-	+
14	K4-6a	+	-	+	+	+	-
15	K4-16	+	+	+	+	-	-
16	K4-17	+	+	+	+	-	-
17	K4-28	+	-	+	+	-	-
18	K5-3	+	+	+	+	-	-
19	K5-3a	+	+	+	+	-	-
20	K5-5a	+	+	+	+	+	+
21	K5-11	+	+	+	+	+	+
22	K5-14	+	+	+	+	-	-
23	K5-15	+	+	+	+	-	-
24	K6-1	+	+	+	+	-	+
25	K6-3a	+	+	+	+	+	+
26	K6-6a	+	+	+	+	+	-
27	K6-7	+	+	+	+	-	-
28	K6-9a	+	+	+	+	-	-
29	K6-12	+	+	+	+	-	-
30	K6-14	+	+	+	+	+	+

Mangalore (2015) used 22 different carbohydrates for identifying the LAB. They showed positive results for acid fermentation of different sugars such as glucose, sucrose, maltose, mannitol, and lactose. These results have some similarity with our results. Also, Khedid et al (2009) examined 12 isolates of *L. helveticus* which fermented glucose, lactose, and galactose, 45% fermented mannose, 38% of isolates used maltose, and only a few fermented trehalose. Table 3.8 shows fermentation of

different sugars by our *Lactobacillus* isolates and identification according to the sugar fermentation results of other studies.

Table 3.8 Fermentation of sugars by *Lactobacillus* isolates and references

Isolates	Fermented sugars	LAB	References
K2-2a, k2-3, k2-4, k2-4a, k2-14, k3-2a, k3-3a, k3-20 k5-5a, k5-11, k6-3a, k6-14	Glucose (acid formation), sucrose, lactose, maltose, mannitol	<i>L. casei</i> <i>L. plantarum</i> <i>L. paracasei</i>	Shafakatullah and Chandra 2015 Asmahan, A. (2011).
K2-2a, k2-4, k3-2a, k3-20, k5-5a, k5-11, k6-3a, k6-14	Glucose(acid and gas formation), sucrose, lactose, maltose, mannitol	<i>L.brevis</i>	Abdel-Rahman et al. 2011
K2-3, k2-4a, k2-14, k3-3a	Glucose (acid and no gas formation), sucrose, lactose, maltose, mannitol	<i>L. acidophilus</i>	Hassan et al., 2014
K2-3a, K4-6a, K6-6a	Glucose, sucrose, lactose, maltose	<i>L. mesenteroides</i>	Gebreselassie et al., 2016
K3-8b2, k3-28, k6-1	Glucose, sucrose, lactose, mannitol	<i>L. bulgaricus</i>	Askari M. et al., 2019
K3-13b, k3-20a, k4-16, k4-17, k4-28, k5-3, k5-3a, k5-14, k5-15, k6-7, k6-9a, k6-12	Glucose, sucrose, lactose	<i>L. brevis</i>	Gebreselassie et al., 2016

3.8 16S rRNA Gene Sequencing of *Lactobacillus* Isolates

There are some difficulties in the identification of LAB with phenotypic methods because strains of the same species can display phenotypic change (Drancourt et al., 2000). Therefore, it is essential to perform genotypic identification of the results after the phenotypic identification methods.

After the confirmation of the amplification of 500-1000 bp amplified region of 16S rRNA gene with gel electrophoresis, the PCR products were subjected to sequencing analysis. This procedure was done by BM Laboratuvar Sistemleri in Ankara, Turkey. Figure 3.21 shows the gel electrophoresis of genomic DNA extracted from *Lactobacillus* isolates.

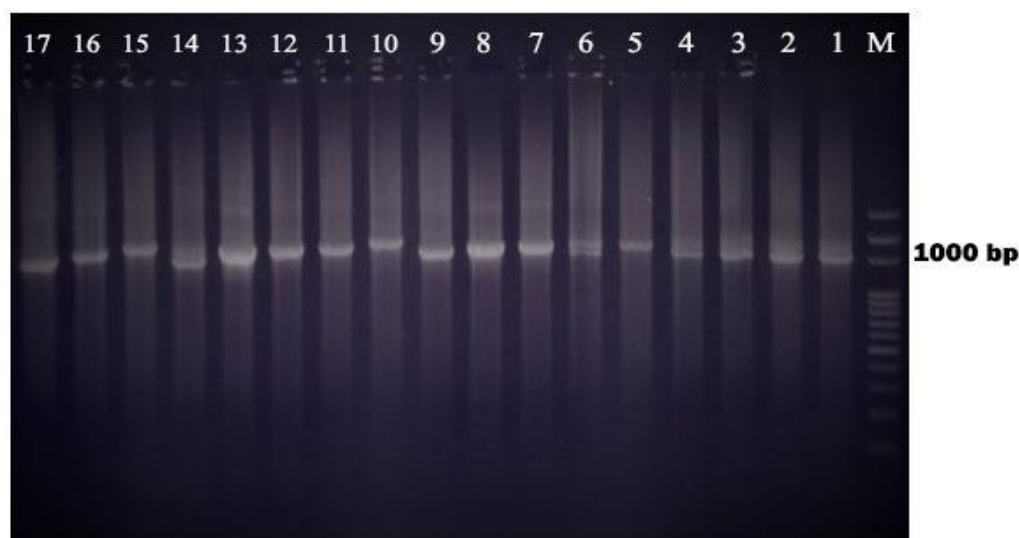


Figure 3.21 Agarose gel electrophoreses of PCR products using 27F - 1492R as universal primers and the marker is the 100 bp DNA. 1(K3-8b2), 2(K6-7), 3(K4-16), 4(K5-3), 5(K3-13b), 6(K4-28), 7(K6-12), 8(K3-20a), 9(K2-4), 10(K3-28), 11(K2-3), 12(K5-11), 13(K3-20a), 14(K4-17), 15(K6-14), 16(K5-15), 17(K2-14).

3.9 BLAST Analysis and Multiple Sequence Alignment

Thirty isolates from 5 samples of kefir and milk samples (K2, K3, K4, K5, and K6) were identified using 16S rRNA gene sequencing. Partial 16S rRNA was obtained after sequencing, recovered in FASTA format, and subjected for BLAST search in The National Center for Biotechnology Information Gen Bank (NCBI). The partial 16S rRNA sequences in the FASTA format are shown in the appendix.

BLAST result identified 22 of 30 isolates as *L. paracasei* subsp. *tolerans* strain NBRC 15906, 7 isolates were *L. gallinarum* strain ATCC 33199 and one isolate was *L. helveticus* strain NBRC 15019. The percent identity between the 22 isolates and *L. paracasei* subsp. *tolerans* strain NBRC 15906 is in range of 97% -100%.

The BLAST analysis results of all isolates are shown in Table (3.9), (3.10), (3.11), (3.12) and (3.13).

Table 3.9 BLAST analysis and alignment results for K2 (Ankara kefir) isolates

No	Query strain	Reference strain	Accession number (Sequence ID)	Identification percentage %
1	K2-2a	<i>L. gallinarum</i> strain ATCC 33199	NR_042111.1	99.22
		<i>L. helveticus</i> strain NBRC 15019	NR_1137191.1	99.05
		<i>L. acidophilus</i> strain NBRC 13951	NR_113638.1	97.93
2	*K2-3	<i>L. intestinalis</i> strain TH4	NR_117071.1	98.27
		<i>L. gallinarum</i> strain ATCC 33199	NR_042111.1	99.19
		<i>L. helveticus</i> strain NBRC 15019	NR_1137191.1	99.01
3	K2-3a	<i>L. gallinarum</i> strain ATCC 33199	NR_042111.1	99.45
		<i>L. helveticus</i> strain NBRC 15019	NR_1137191.1	99.36
		<i>L. acidophilus</i> strain NBRC 13951	NR_113618.1	98.08
4	K2-4	<i>L. paracasei</i> subsp. <i>tolerans</i> strain NBRC 15906	NR_041054.1	99.67
		<i>L. zeae</i> strain RIA 482	NR_037122.1	98.69
		<i>L. casei</i> strain NBRC 15883	NR_113333.1	98.60

(*isolates identified by alignment using the Clustal omega program)

Table 3.9 BLAST analysis and alignment results for K2 (Ankara kefir) isolates (continued)

No	Query strain	Reference strain	Accession number (Sequence ID)	Identification percentage %
5	K2-4a	<i>L. gallinarum</i> strain ATCC 33199	NR_042111.1	99.05
		<i>L. helveticus</i> strain NBRC 15019	NR_113719.1	99.87
		<i>L. crispatus</i> strain DSM 20584	NR_119274.1	98.30
		<i>L. amylovorus</i> DSM 20531	NR_117064.1	98.20
6	K2-14	<i>L. gallinarum</i> strain ATCC 33199	NR_042111.1	99.43
		<i>L. helveticus</i> strain NBRC 15019	NR_113719.1	99.24
		<i>L. crispatus</i> strain DSM 20584	NR_119274.1	98.67

Table 3.10 BLAST analysis and alignment results for K3 (Ankara kefir) isolates

No	Query strain	Reference strain	Accession number (Sequence ID)	Identification percentage %
7	K3-2a	<i>L. paracasei</i> subsp. <i>tolerans</i> strain	NR_041054.1	99.73
		NBRC 15906	NR_037122.1	98.64
		<i>L. zeae</i> strain RIA 482	NR_113333.1	98.55
		<i>L. casei</i> strain NBRC 15883		
8	K3-3a	<i>L. gallinarum</i> strain ATCC 33199	NR_042111.1	99.19
		<i>L. helveticus</i> strain NBRC 15019	NR_113719.1	99.01
		<i>L. acidophilus</i> strain NBRC	NR_113638.1	89.20
		13951		

Table 3.10 BLAST analysis and alignment results for K3 (Ankara kefir) isolates (continued)

No	Query strain	Reference strain	Accession number (Sequence ID)	Identification percentage %
9	K3-8b2	<i>L. paracasei</i> subsp. <i>tolerans</i> strain NBRC 15906	NR_041054.1	99.51
		<i>L. zae</i> strain RIA 482	NR_037122.1	98.52
		<i>L. casei</i> strain NBRC15883	NR_113333.1	98.44
		<i>L. rhamnosus</i> strain NBRC 3425	NR 113332	98.25
10	K3-13b	<i>L. paracasei</i> subsp. <i>tolerans</i> strain NBRC 15906	NR_041054.1	99.23
			NR_037122.1	98.44
		<i>L. zae</i> strain RIA 482	NR_113333.1	98.35
		<i>L. casei</i> strain NBRC 15883	NR 113332.1	98.34
		<i>L. rhamnosus</i> strain NBRC 3425		
11	K3-20	<i>L. paracasei</i> subsp. <i>tolerans</i> strain NBRC 15889	NR_113337.1	99.80
		<i>L. zae</i> strain RIA 482	NR_037122.1	99.69
		<i>L. casei</i> strain NBRC 15883	NR_113333.1	99.69
		<i>L. rhamnosus</i> strain NBRC 3425	NR 113332	99.69
12	K3-20a	<i>L. paracasei</i> subsp. <i>tolerans</i> strain NBRC 15906	NR_041054.1	99.75
		<i>L. zae</i> strain RIA 482	NR_037122.1	98.86
		<i>L. casei</i> strain NBRC 15883	NR_113333.1	98.74
		<i>L. rhamnosus</i> strain NBRC 3425	NR 113332.1	98.36

Table 3.10 BLAST analysis and alignment results for K3 (Ankara kefir) isolates (continued)

No	Query strain	Reference strain	Accession number (Sequence ID)	Identification percentage %
13	K3- 28	<i>L. paracasei</i> subsp. <i>tolerans</i> strain NBRC 15906	NR_041054.1	99.92
		<i>L. zae</i> strain RIA 482	NR_037122.1	98.91
		<i>L. casei</i> strain NBRC 15883	NR_113333.1	98.82
		<i>L. rhamnosus</i> strain NBRC 3425	NR_113332.1	98.49

Table 3.11 BLAST analysis and alignment results for K4 (Kyrgyzstan) isolates

No	Query strain	Reference strain	Accession number (Sequence ID)	Identification percentage %
14	K4-6a	<i>L. helveticus</i> NBRC 15019	NR_113719.1	99.66
		<i>L. gallinarum</i> strain ATCC 33199	NR_042111.1	99.66
		<i>L. acidophilus</i> strain NBRC 13951	NR_113638.1	98.37
15	K4-16	<i>L. paracasei</i> strain ATCC 25302	NR_117987	99.73
		<i>L. zae</i> strain RIA 482	NR_037122.1	98.98
		<i>L. casei</i> strain NBRC 15883	NR_113333.1	98.65
16	K4-17	<i>L. paracasei</i> subsp. <i>tolerans</i> strain NBRC 15889	NR_113337.1	99.81
		<i>L. zae</i> strain RIA 482	NR_037122.1	99.72
		<i>L. casei</i> strain NBRC 15883	NR_113333.1	99.72
17	K4-28	<i>L. paracasei</i> strain NBRC 15906	NR_041054.1	99.50
		<i>L. zae</i> strain RIA 482	NR_037122.1	98.49
		<i>L. casei</i> strain NBRC 15883	NR_113333.1	98.41

Table 3.12 BLAST analysis and alignment results for K5 (Istanbul kefir) isolates

No	Query strain	Reference strain	Accession number (Sequence ID)	Identification percentage %
18	K5-3	<i>L. paracasei</i> strain NBRC 15906	NR_041054.1	99.43
		<i>L. zae</i> strain RIA 482	NR_037122.1	98.45
		<i>L. casei</i> strain NBRC 15883	NR_113333.1	98.37
		<i>L. rhamnosus</i> strain NBRC 3425	NR_113332.1	98.05
19	K5-3a	<i>L. paracasei</i> strain NBRC 15889	NR_113337.1	99.75
		<i>L. zae</i> strain RIA 482	NR_037122.1	99.00
		<i>L. casei</i> strain NBRC 15883	NR_113333.1	98.92
		<i>L. rhamnosus</i> strain NBRC 3425	NR_113332.1	98.59
20	K5-5a	<i>L. paracasei</i> strain NBRC15889	NR_113337.1	99.91
		<i>L. zae</i> strain RIA 482	NR_037122.1	99.09
		<i>L. casei</i> strain NBRC 15883	NR_113333.1	99.00
		<i>L. rhamnosus</i> strain NBRC 3425	NR_113332.1	98.54
21	K5-11	<i>L. paracasei</i> strain NBRC 15889	NR_113337.1	99.65
		<i>L. zae</i> strain RIA 482	NR_037122.1	99.47
		<i>L. casei</i> strain NBRC 15883	NR_113333.1	99.47
		<i>L. rhamnosus</i> strain NBRC 3425	NR_113332.1	99.47
22	*K5-14	<i>L. zae</i> strain RIA 482	NR_037122.1	99.21
		<i>L. paracasei</i> strain NBRC 15889	NR_113337.1	99.30
		<i>L. casei</i> strain NBRC 15883	NR_113333.1	99.21
23	*K5-15	<i>L. zae</i> strain RIA 482	NR_037122.1	98.71
		<i>L. paracasei</i> strain NBRC 15889	NR_113337.1	98.79
		<i>L. casei</i> strain NBRC 15883	NR_113333.1	98.71

(*isolates identified by alignment using the Clustal omega program)

Table 3.13 BLAST analysis and alignment results for K6 (Istanbul kefir) isolates

No	Query strain	Reference strain	Accession number (Sequence ID)	Identification percentage %
24	K6-1	<i>L. paracasei</i> subsp. <i>tolerans</i> strain NBRC 15906	NR_041054.1	97.08
		<i>L. zae</i> strain RIA 482	NR_037122.1	96.06
		<i>L. casei</i> strain NBRC 15883	NR_113333.1	95.96
25	*K6-3a	<i>L. helveticus</i> strain NBRC 15019	NR_113719.1	99.22
		<i>L. gallinarum</i> strain ATCC 33199	NR_042111.1	99.39
		<i>L. acidophilus</i> strain NBRC 13951	NR_113638.1	98.09
26	K6-6a	<i>L. paracasei</i> subsp. <i>tolerans</i> strain NBRC 15906	NR_0410541.1	99.52
		<i>L. zae</i> strain RIA 482	NR_037122.1	98.45
		<i>L. casei</i> strain NBRC 15883	NR_113333.1	98.36
27	K6-7	<i>L. paracasei</i> subsp. <i>tolerans</i> strain NBRC 15906	NR_0410541.1	99.42
		<i>L. zae</i> strain RIA 482	NR_037122.1	98.44
		<i>L. casei</i> strain NBRC 15883	NR_113333.1	98.36
28	K6-9a	<i>L. paracasei</i> subsp. <i>tolerans</i> strain NBRC 15906	NR_0410541.1	99.98
		<i>L. zae</i> strain RIA 482	NR_037122.1	98.04
		<i>L. casei</i> strain NBRC 15883	NR_113333.1	97.96

Table 3.13 BLAST analysis and alignment results for K6 (Istanbul kefir) isolates (continued)

No	Query strain	Reference strain	Accession number (Sequence ID)	Identification percentage %
29	K6-12	<i>L. paracasei</i> subsp. <i>tolerans</i> strain ATCC 25302	NR_117987.1	99.25 98.41
		<i>L. zae</i> strain RIA 482	NR_037122.1	98.23
		<i>L. casei</i> strain NBRC 15883	NR_113333.1	
30	*K6-14	<i>L. rhamnosus</i> strain NBRC 3425	NR_113332.1	99.45
		<i>L. paracasei</i> subsp. <i>tolerans</i> strain NBRC 15906	NR_0410541.1	99.54
		<i>L. zae</i> strain RIA 482	NR_037122.1	99.45
		<i>L. casei</i> strain NBRC 15883	NR_113333.1	99.45

(*isolates identified by alignment using the Clustal omega program)

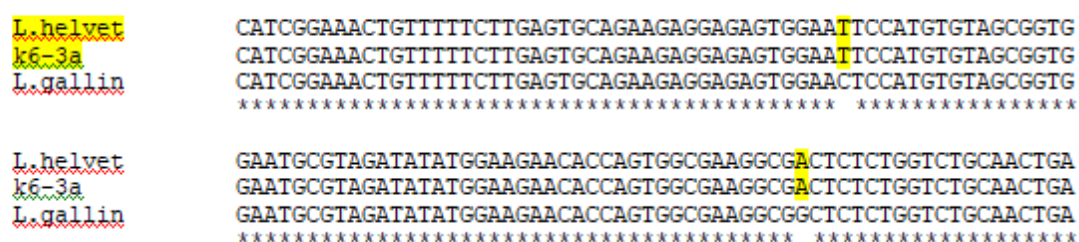
Phylogenetic relationship of all isolated microorganisms was analyzed with other partial 16S rRNA sequences of similar microorganisms that exist in the gene bank database. The sequences of closely related *Lactobacillus* sp. were matched under Multiple Sequence Alignment (MSA) using the Clustal Omega program to identify the differences in DNA base sequences. The identical nucleotides for a given position were marked with * (asterisk). The output data is shown in the Appendix.

This method suffers from the difficulties during identification methods especially for strains of the same species. Performing the 16S rRNA sequencing in one direction is acceptable for species identification, however it is not successful on differentiation at the strain level.

The MSA was performed after the BLAST analysis to all isolates because of the high similarity between them. This alignment was useful to differentiate some

isolates as (K2-3, K5-14, K5-15, K6-3a, and K6-14). Finally, after performing BLAST and MSA, it was found that 19 out of 30 isolates had 97-100% similarity under the group of *L. paracasei* sp. and closely related to identified organism *L. paracasei* subsp. *tolerans* strain NBRC 15906. Five isolates had homology with *L. gallinarum* strain ATCC 33199, two isolates were identified according to alignment as *L. helveticus* strain NBRC 15019, two isolates were closely related to *L. zeae* strain RIA 482, one isolate had homology to *L. intestinalis* strain TH4 while the last one isolate was identified as *L. rhamnosus* strain NBRC 3425.

Multiple Sequence Alignment was successfully used to compare between k6-3a, *L. helveticus* strain NBRC 15019, and *L. gallinarum* strain ATCC 33199. k6-3a has indicated highest similarity to *L. gallinarum* strain ATCC 33199 using Blast analysis. However, it was closely related to *L. helveticus* sequence using Clustal Omega program as shown in Figure 3.22



```

L.helvet  CATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAAATCCATGTGTAGCGGTG
k6-3a    CATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAAATCCATGTGTAGCGGTG
L.gallin  CATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTG
*****

L.helvet  GAATGCGTAGATATATGGAAGAACACCAAGTGGCGAAGGCGACTCTCTGGTCTGCAACTGA
k6-3a    GAATGCGTAGATATATGGAAGAACACCAAGTGGCGAAGGCGACTCTCTGGTCTGCAACTGA
L.gallin  GAATGCGTAGATATATGGAAGAACACCAAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGA
*****

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Figure 3.22 The alignment of the 16S rRNA gene of *Lactobacillus* isolate k6-3a with bases of *L. helveticus* and *L. gallinarum*.

The MSA identification of k6-3a as *L. helveticus* was confirmed by 2 ways 16s rRNA sequencing (Table 3. 14)

In addition, k2-3 isolate was aligned with the sequences of *L. gallinarum* strain ATCC 33199 adapted from NCIB blast by using Clustal Omega program. K2-3 is

similar to *L. intestinalis* more than *L. gallinarum* and *L. helveticus* by Multiple Sequence Alignment as seen in Figure 3.23



Figure 3.23. The alignment of the 16S rRNA gene of k2-3 isolate with bases of *L. helveticus*, *L. intestinalis*, and *L. gallinarum*.

MSA was also used to compare isolate K5-14, *L. casei*, *L. paracasei* and *L. zeae*. It was useful to find single nucleotide match between K5-14 and *L. zeae* (Figure 3.24)

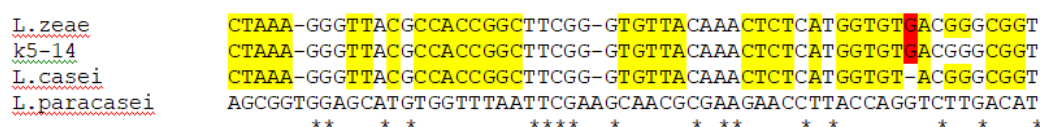


Figure 3.24 The alignment of the 16S rRNA gene of K5-14 isolate with bases of *L. zeae*, *L. casei*, and *L. paracasei*.

MSA was also performed to compare K6-14, *L. paracasei*, *L. zeae* and *L. rhamnosus*. One more nucleotide similarity was observed between k6-14 and *L. rhamnosus*. However, low similarity was obtained with all species (Figure 3.25). Therefore, this isolate could be a closely related new specie.

```

L.paracasei      CGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGG
L.zeae          CGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGG
L.rhamonsus     CGGCTTAACCGAGGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGG
k6-14           TGGTGTGAC--GGGCGGTGTGTACAAGGCCCGGGAACGTATTACCGCGGCGTGC--TGA
                ** * ** * * * * * * * * * * * * * * * * * *

```

Figure 3.25 The alignment of the 16S rRNA gene of K6-14 isolate with bases of *L. zeae*, *L. rhamnosus* and *L. paracsei*.

MSA of K5-15, *L. casei*, *L. paracasei* and *L. zeae* has shown that K5-15 was closely related to *L. zeae* with 3 nucleotides while similarity with 2 nucleotides was observed with *L. paracasei*. As shown in Figure 3.26.

```

k5-15           TTACGCC-ACCGGCTGCGT-GTTACAACTCTCA-TGG--TGACGGGCGGTGT-TACAAGGCCCGGGAACGTATT
l.zeae          GCAAGTCGAACGAGTTTGTGCG--ATGACGGTGCTTGATCGTGA--TTCAACTTAAACGAGTGCGGACGGGTGAG
l.paracasei     GCAAGTCGAACGAGTTTCGTTG--ATGATCGGTGCTTGACCGAGA--TTCAACATGAACGAGTGCGGACGGGTGAG
                * * * * * * * * * * * * * * * * * * * * * *

```

Figure 3.26 The alignment of the 16S rRNA gene of K5-15 isolate with bases of *L. zeae*, *L. casei*, and *L. paracsei*.

Thus, allignement for K5-15 isolate gave contraversial result. This problem was solved with 2 ways sequencing which allowed identification of this isolate as *L. paracasei* (Table 3.14).

Some isolates were selected for two-directions sequencing of 16s rRNA as shown in Table 3.14.

Table 3.14 Results of one and two directions 16S rRNA sequencing and MSA

Isolate name	One direction of 16S rRNA sequencing	Multiple Sequence Alignment (MSA)	two directions of 16S rRNA sequencing
K2-3	<i>L. gallinarum</i>	<i>L. intestinalis</i>	<i>L. gallinarum</i>
K5-14	<i>L. paracasei</i>	<i>L. zeae</i>	<i>L. paracasei</i>
K5-15	<i>L. paracasei</i>	<i>L. zeae</i> and/or <i>L. paracasei</i>	<i>L. paracasei</i>
K6-3a	<i>L. gallinarum</i>	<i>L. helveticus</i>	<i>L. helveticus</i>
K6-14	<i>L. paracasei</i>	<i>L. rhamnosus</i>	<i>L. paracasei</i>

It is highly difficult to differentiate closely related species of *Lactobacillus*. In our study, K2-3, K5-14, K5-15 and K6-14 were identified by 16S rRNA sequencing in both one and two directions. On the other hand, K6-3a resulted in identification as same species by MSA and sequencing in two directions. Meanwhile, MSA for K5-15 indicated an ambiguous result in between two similar species of *L. zeae* and *L. paracasei* whereas sequencing in two directions resulted in one of these species, *L. paracasei*. These results demonstrated that MSA could also provide reliable results in identification of some species.

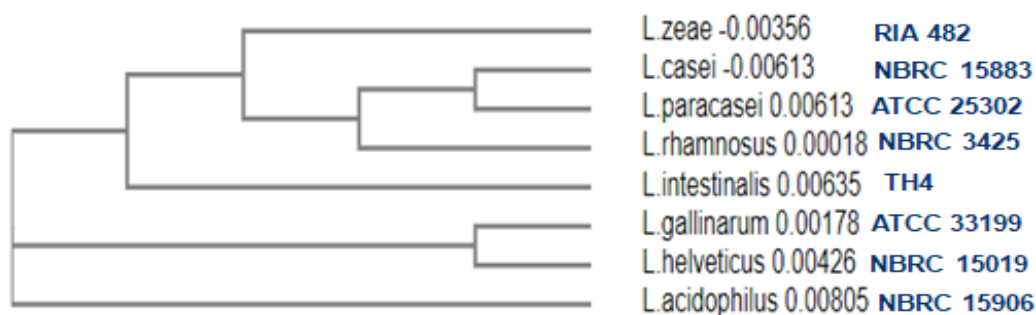


Figure 3.27 Phylogenetic tree of aligned 16S rRNA gene sequences for the known species.

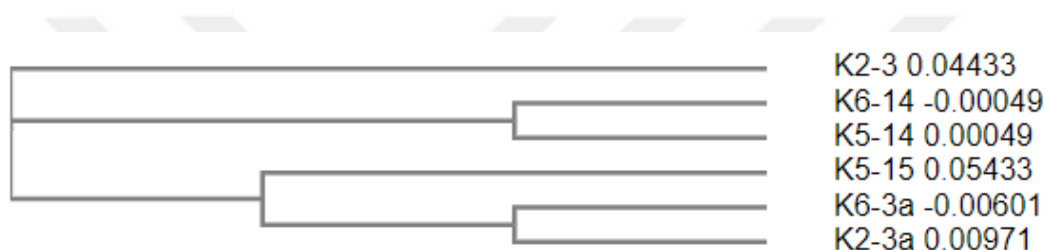


Figure 3.28 Phylogenetic tree of aligned 16S rRNA gene sequences for some isolates.



Figure 3.29 Phylogenetic tree of aligned 16S rRNA gene sequences for K6-14 with related known species.

In fact, It is also hard to differentiate *L. paracasei* subsp. *tolerans* strain NBRC 15906, *L. casei* strain NBRC 15883, and *L. zae* strain RIA 482 strains since they are closely related. Salvetti et al (2012) and Huang et al (2018) demonstrated that *L. casei*, *L. paracasei*, and *L. rhamnosus* comprise the *L. casei* group and these strains are closely related in the phenotypic and genotypic properties. Also, there are difficulties in identifying these species by the most used genotypic assay of 16S rRNA gene sequencing. That was the reason for controversial taxonomic status and nomenclature of this group. Furthermore, Desai (2006) presented that *L. casei*, *L. paracasei*, *L. zae* , and *L. rhamnosus* form a taxonomic group and had difficulty to differentiate from each other using traditional methods.

16S rRNA gene sequences in many *Lactobacillus* species are too similar to be easily distinguished. In particular, closely related species within the *L. acidophilus* group (*L. acidophilus*, *L. gallinarum*, and *L. helveticus*), the *L. casei* group (*L. casei*, *L. paracasei*, and *L. rhamnosus*), the *L. plantarum* group (*L. plantarum*, *L. paraplantarum*, and *L. pentosus*), and the *L. sakei* group (*L. sakei*, *L. curvatus*, and *L. graminis*) are hard to identify by 16S rRNA gene sequences. (Hung C., 2017 and 2018).

Based on 16S rRNA gene sequences, many subspecies previously known as *L. casei* were reassigned into the other species. *L. casei* subsp. *alactosus* and *L. casei* subsp. *pseudoplantarum* were rearranged to *L. paracasei* subsp. *paracasei*, and *L. casei* subsp. *tolerans* was renamed as *L. paracasei* subsp. *tolerans* (Collins et al. 1989). This may be explain the presence of many isolates of *L. paracasei* isolated from kefir samples using partial 16S rRNA gene sequencing.

Kao et al (2007) outlined that both *L. paracasei* and *L. rhamnosus* were organized in the *L. casei* group and their 16S rRNA sequence has 98% similarity so it is difficult to differentiate them by conventional PCR procedure. Also, it was investigated that

the classification of *L. paracasei* group (including *L. casei*, *L. paracasei* and *L. zeae*) were regarded as members of the same group according to 16S rRNA gene sequencing.

Skerman et al (1980) demonstrated that all *L. casei* strains in their study were identified as *L. paracasei* or *L. casei* by the result of 16S rRNA sequencing.

According to Stackebrandt and Goebel (1994), the strains presenting homology of at least 97% might be identified as the same species. In our work, BLAST results of the most isolates showed 99% or higher percentage of identity with both *L. paracasei*, *L. casei*, *L. zeae*, and *L. rhamnosus*. The same was happening between *L. helveticus*, *L. gallinarum*, *L. crispatus*, and *L. amylovorus*. Fujisawa et al (1990) and Klein et al (1998) classified the species *L. acidophilus*, *L. crispatus*, *L. gallinarum*, *L. gasseri*, *L. amylovorus*, and *L. johnsonii* within the *L. acidophilus*/*L. delbrueckii* group.

(Kim et al., 2020) accomplished a genomic analysis of *L. helveticus* and *L. gallinarum* and the result proved that *L. gallinarum* strain containing a unique gene of *L. helveticus*). This result confirmed the similarity between *L. helveticus* and *L. gallinarum* in our results of 16S rRNA sequencing.

The results of different identification methods are often ambiguous, due to the close genetic relationship between species of *L. acidophilus* group and *L. casei* group. So the development of a suitable method to identify these bacteria is necessary. Yu et al (2012) have demonstrated a strategy using elongation factor (*tuf* gene), heat shock protein gene and phenylalanyl-tRNA synthase to discriminate the closely related species in *L. acidophilus* group, *L. casei* group and *L. plantarum* group.

The present results demonstrate that *L. paracasei* subsp. *tolerans* strain NBRC 15906 was the predominant species in Turkish and Kyrgyzstan samples and are in line with the study of Magalhães et al (2011) that *L. paracasei* was the most

abundant bacterium in the Brazilian kefir sample. *L. paracasei* was also isolated and identified from Turkish, Russian, Brazilian, and Argentinian kefir samples (Merih and Evrim, 2015; Kotova et al., 2016; Magallhaes et al., 2011; Zanirati et al., 2015; Garrote et al., 2001). These results confirmed the similarity between Turkish kefir beverage with kefir samples from other regions. (Table 3.15)

L. gallinarum strain ATCC 33199 was the predominant species in K2 kefir from Ankara. Four isolates (K2-2a), (K2-3a), (K2-4a), and (K2-14) were identified as *L. gallinarum* from K2 and one isolate from K3 (K3-3a) was identified as *L. gallinarum*. These results have some similarities with results of Kok-Tas et al (2012) and Nalbantoglu et al (2014) that detected *L. gallinarum* in Turkish kefir grains.

From Taiwanese, Bulgarian, Brazilian, South African, and Turkish kefir grains and beverages, *L. helveticus* were isolated and identified (Chin et al., 1999; Simova et al., 2002; Leite et al., 2012; Zanirati et al., 2015; Witthuhn et al., 2004, 2005; Kok-Tas et al., 2012; Nalbantoglu et al., 2014). This is in good agreement with our findings for isolation of *L. helveticus* from (K4-6a) Kyrgyzstan milk product and (K6-3a) from Istanbul kefir sample.

Table 3.16 shows a summary for the results of real-time PCR and results of identification by 16S rRNA sequencing. *L. paracasei* was significant positive as predominant probiotic bacteria in kefir with both assays. In the recent study, *L. paracasei* was isolated and identified from all kefir samples and also it was detected using real-time PCR in all samples including the commercial one, Turkish homemade kefir samples, and Kyrgyzstan milk product. However, *L. kefir* and *L. kefiranofaciens* were predominant in real-time PCR in kefir samples, but our investigations could not obtain any of them from the *Lactobacillus* isolation.

Table 3.15 Summary for identification of isolates and references

Isolates	Reference strain	Identification percentage %	Similar kefir samples	References
K2-4, K3-2a, K3-8b2, K3-13b, K3-20, K3-20a, K3- 28, K4-16, K4-17, K4-28, K5-3, K5-3a, K5-5a, K5-11, K6-1, K6-6a, K6-7, K6-9a, and K6-12	<i>L. paracasei</i> subsp. <i>tolerans</i> strain NBRC 15906	79.08-99.91%	Brazilian, Turkish, Russian, Argentinian	Merih and Evrim,2015; Kotova et al., 2016; Magallhaes et al., 2011; Zanirati et al., 2015; Garrote et al., 2001
K2-2a, K2-3a, K2-4a, K2-14, K3-3a	<i>L. gallinarum</i> strain ATCC 33199	99.19-99.45%	dairy products	Tamime A Y. et al, 1005
*K2-3	<i>L. intestinalis</i> strain TH4	98.27%	-	-
K4-6a, *K6-3a	<i>L. helveticus</i> strain NBRC 15019	99.23-99.66%	Bulgarian Brazilian South African Turkish	Simova et al., 2002 Leite et al., 2012; Zanirati et al., 2015 Witthuhn et al., 2004,2005 Kok-Tas et al., 2012; Nalbantoglu et al., 2014
*K5-14, *K5-15	<i>L. zeae</i> strain RIA 482	99.21-99.71	-	-
*K6-14	<i>L. rhamnosus</i> strain NBRC 3425	99.45%	Dairy products	Wouters et al. (2002)

Some differences were found between the variety of *Lactobacillus* species detected using real-time PCR and isolates identification according to 16S rRNA sequencing. These differences are related to the high sensitivity and accuracy of real-time PCR for detecting very minute amounts of bacterial cells which can not be isolated by traditional cultivation methods (Mark et al., 2005).

In the present study, The microbial population of kefir and milk product samples was detected, this proved that our samples had great variety in probiotic microorganisms.

Homemade kefir from Ankara (K2) had *L. kefiri*, *L. kefiranofaciens*, *L. acidophilus*, *L. delbrueckii subsp. bulgaricus*, *L. casei*, *L. paracasei*, *Saccharomyces cerevisiae*, *L. Plantarum*, *L. gallinarium* and *L. zeae*. The K2 is more rich in probiotic strains than K3 from Ankara since *L. delbrueckii subsp. bulgaricus*, *L. zeae*, and *L. plantarum* were not detected in K3 .

Table 3.16 shows the results of real time PCR and isolation of kefir isolates

	<i>Lactobacillus</i> isolates	K1 (AOC)	K2 (Ankara kefir)		K3 (Ankara kefir)		K4 (Kyrgyzstan)		K5(Istanbul kefir)		K6 (Istanbul kefir)	
		RT. PCR	RT. PCR	Isolation	RT. PCR	Isolation	RT. PCR	Isolation	RT. PCR	Isolation	RT. PCR	Isolation
1	<i>L. acidophilus</i> (<i>L. gallinarium</i>)	+	+	+	+	+	+	-	+	-	-	-
				(k2-2a) (k2-3a) (k2-4a) (k2-14) (<i>L.gallinarium</i>)		(k3-3a) (<i>L.gallinarium</i>)						
2	<i>L. casei</i>	+	+	-	+	-	+	-	+	-	+	-
3	<i>L. paracasei</i>	+	+	+	+	+	+	+	+	+	+	+
				(k2-4)		(k3-2a) (k3-8b2) (k3-13b) (k3-20) (k3-20a) (k3-28)		(k4-16) (k4-17) (k4-28)		(k5-3) (k5-3a) (k5-5a) (k5-11)		(k6-1) (k6-6a) (k6-7) (k6-9a) (k6-12)
4	<i>L.delbrueckii</i> subsp. <i>bulgaricus</i>	+	+	-	-	-	+	-	+	-	+	-
5	<i>L .kefiri</i>	+	+	-	+	-	+	-	+	-	+	-
6	<i>L. kefiranofaciens</i>	+	+	-	+	-	+	-	+	-	+	-
7	<i>L. fermentum</i>	-	-	-	-	-	+	-	-	-	+	-
8	<i>L. plantarum</i>	+	+	-	-	-	-	-	-	-	-	-
9	<i>S. cerevisiae</i>	-	+	-	+	-	+	-	+	-	+	-
10	<i>S. thermophilus</i>	+	-	-	+	-	+	-	+	-	+	-
11	<i>L. sobris</i> <i>\amylovorus</i>	-	-	-	-	-	+	-	+	-	-	-
12	<i>L. helveticus</i>	NT	NT	-	NT	-	NT	+	NT	-	NT	+
								(k4-6a)				(k6-3a)
13	<i>L .intestinalis</i>	NT	NT	+	NT	-	NT	-	NT	-	NT	-
				(K2-3)								
14	<i>L. zeae</i>	NT	NT	-	NT	-	NT	-	NT	+	NT	-
										(k5-14) (k5-15)		
15	<i>L .rhamnosus</i>	NT	NT	-	NT	-	NT	-	NT	-	NT	+
												(k6-14)

+, detected and/or isolated, -, not detected \or not isolated; NT, not tested

Milk sample from Kyrgyzstan (K4) was the richest sample of bacteria and yeast using real-time PCR, even than the commercial one. It was composed of *L. kefir*, *L. kefiranofaciens*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. casei*, *L. paracasei*, *Saccharomyces cerevisiae*, *Streptococcus thermophilus*, *L. amylovorus*, *L. fermentum*, and *L. helveticus*.

Homemade kefir from Istanbul (K5) had *L. kefir*, *L. kefiranofaciens*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. casei*, *L. paracasei*, *Saccharomyces cerevisiae*, *Streptococcus thermophilus*, *L. amylovorus*, and *L. intestinalis*.

In addition, the kefir sample from Istanbul (K6) was the only sample that lacked *L. acidophilus* using real-time PCR assay. However, it had *L. kefir*, *L. kefiranofaciens*, *L. delbrueckii* subsp. *bulgaricus*, *L. casei*, *L. paracasei*, *Saccharomyces cerevisiae*, *Streptococcus thermophilus*, *L. fermentum*, *L. helveticus*, and *L. rhamnosus*.

The microbial population in our samples had significant similarity with the microbial population of other kefir samples from Turkey, China, Brazil, Bulgaria, Taiwan, and Argentina as shown in Chapter 1 (Table1.1).

All of these probiotic strains might have beneficial roles in reducing cholesterol, improving digestion, enhancing the immune system, and cancer prevention.(Shavit 2008; Chiang and Pan, 2012; Berardi et al., 2013). Therefore, the most investigations are focused on lactobacilli for the isolation, selection, identification, and development of new strains with desired properties.



CHAPTER 4

CONCLUSION

Kefir is a fermented milk product, produced by kefir grains. It has always been traditionally consumed in Turkey. Kefir is rich in probiotics of LAB, acetic acid bacteria, and yeasts. These LAB play an important role in the kefir production, in addition to some benefits for human health. Therefore, in this study, four samples of Kefir grains were collected from different regions in Turkey and Kefir beverage was prepared from them for detection and isolation of probiotics. In addition, one milk product from Kyrgyzstan and one commercial kefir product (AOC) were used for the detection and identification of the kefir population.

In the first part of the study, a SYBR green based real-time PCR assay (real-time PCR) was performed for detection of kefir probiotic bacteria and a yeast with specific primers for the species *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. kefiri*, *L. kefiranofaciens*, *L. fermentum*, *L. plantarum*, *L. amylovorus*, *Streptococcus thermophilus*, *Saccharomyces cerevisiae*, *L. casei* and *L. paracasei* using DNA directly isolated from kefir sources. The results of real time PCR were summarized according to the amplification and melting curves. *L. kefiri*, *L. kefiranofaciens*, *L. casei*, and *L. paracasei* were detected in all kefir samples. In addition to the presence of *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. fermentum*, *L. plantarum*, *L. amylovorus*, *Streptococcus thermophilus*, and *Saccharomyces cerevisiae* in some kefir samples. The predominance of some lactic acid bacteria and yeast in homemade kefir supports the importance of these probiotics in commercial kefir production.

In the second part of the current study, LAB were isolated from kefir samples. Isolates were identified using microscopic and biochemical identification methods as

temperature, pH, bile salt, NaCl, and phenol tolerance tests. Also, lactose production, sugar fermentation, and proteolysis activity tests were performed.

All the kefir isolates showed resistance to acid, temperature, phenol, and bile salts in addition to their proteolytic activity.

L. acidophilus group and *L. casei* group isolates are difficult to differentiate. However, thirty bacterial isolates were identified by partial 16S rRNA sequencing (one or two ways) and the results of sequencing were determined by BLAST analysis and Multiple Sequence Alignment.

All isolates were identified according to sequencing and alignment. It was determined that 19 isolates were *L. paracasei* subsp. *tolerans* strain NBRC 15906, 5 isolates were *L. gallinarum* strain ATCC 33199, two were *L. helveticus* strain NBRC 15019, two were *L. zae* strain RIA 482, one was *L. intestinalis* strain TH4 and one was *L. rhamnosus* strain NBRC 3425.

As a result, it was concluded that real-time PCR is an accurate method for detection of the kefir population and there are many probiotic bacteria in examined kefir samples with good probiotic properties and proteolytic activity. The identified isolates in this study, have potential to be used for the production of kefir.

Further studies are required to select some of these dominant probiotics with specific benefits from kefir to produce lyophilized kefir.

REFERENCES

- Aarti, C., Khusro, A., Varghese, R., Arasu, M. V., Agastian, P., Al-Dhabi, N.A., Ilavenil, S. and Choi, K. C. (2018). "In vitro investigation on probiotic, anti-candida, and antibiofilm properties of *Lactobacillus pentosus* strain LAP1". Arch. oral. biol., 89: 99-106.
- Abdel Aziz. M Shadia. (2014) . "Strategies Inhibition and Dispersal: Biofilm B" Biotechnology SAJ 1 (1) :105
- Abdel-Rahman M.A., Tashiro Y., Zendo T., Hanada K., Shibata K. And Sonomoto K. (2011) "Efficient homofermentative L-(+)- lactic acid production from xylose by a novel lactic acid bacterium *Enterococcus mundtii*" QU 25. Applied and Environmental Microbiology, 77: 1892–5
- Abd El-Fattah A.M. Sally Samir Sakr, Samia Mahmoud El-Dieb & Hany Abdel Satar Elkashef (2017) "Bioactive peptides with ACE-I and antioxidant activity produced from milk proteolysis" International Journal of Food Properties, 20:12
- Abdulmir A. S., Yoke T., Nordin N. and Abu Bakar F. (2010) "Detection and quantification of probiotic bacteria using optimized DNA extraction, traditional and real-time PCR methods in complex microbial communities" African Journal of Biotechnology Vol. 9 (10), pp. 1481-1492
- Abu-Tarboush, H. M. (1996). "Comparison of associative growth and proteolytic activity of yoghurt starters in whole milk from camels and cows". Journal of Dairy Science, 79, 366 -371.
- Adolfsson, O.; Meydani, S.N.; Russell, R.M. (2004) " Yogurt and gut function". Am. J. Clin. Nutr., 80, 245–256.

- Adriana, P., & Socaciu, C. (2008). "Probiotic activity of mixed cultures of kefir's lactobacilli and non-lactose fermenting yeasts". *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca - Agriculture*, 65(2), 329–334.
- Aghlara, & et al. (2009). " Characterization of headspace volatile flavor compounds formed during kefir production" *Application of solid phase Microextraction. International Journal of Food Properties*, 12(4), 808–818.
- Ahmed, F. E., Gouda, M. M., Hussein, L. A., Ahmed, N. C., Vos, P. W., & Mohammad, M. A. (2017). "Role of melt curve analysis in interpretation of nutrigenomics" *MicroRNA expression data. Cancer Genomics and Proteomics*, 14(6), 469–481.
- Ahmed, T. and Kanwal, R. "Biochemical characteristics of lactic acid producing bacteria and preparation of camel milk cheese by using starter culture" *Pakistan Vet. J.* 2004; 24:87-91.
- Ahmed, Z., Y. Wang. N. Anjum, A. Ahmad, and S. T. Khan. 2013. "Characterization of exopolysaccharide produced by *Lactobacillus kefirifaciens* ZW3 isolated from Tibet kefir" *Part II. Food Hydrocolloid*. 30:343-350.
- Ahmed Z, Wang Y, Ahmad A, et al. (2013) "Kefir and health: a contemporary perspective". *Crit Rev Food Sci Nutr* 53, 422–434.(13)
- Ahn, Y. T., G. B. Kim, K. S. Lim, Y. J. Baek, and H. U. Kim, (2003) "Deconjugation of bile salts by *Lactobacillus acidophilus* isolates," *International Dairy Journal*, vol. 13, no. 4, pp. 303–311,.
- Altay, F., Karbancioglu- Güler, F., Daskaya- Dikmen, C., and Heperkan, D. (2013). "A review on traditional Turkish fermented non-alcoholic beverages:

- microbiota, fermentation process and quality characteristics". *Int. J. Food Microbiol.* 167, 44–56
- Antoniou, K. D., G., T., & G. Dimitreli, G. T. and. (2008). "Effect of Starter Culture, Milk Fat and Storage Time on the Rheological Behaviour of Kefir".
- Askari Zakariah M., Ratmawati Malaka, Amran Laga, and Ambo Ako (2019) "Isolation and Identification of Lactic Acid Bacteria from Dangke a White Soft Traditional Cheese from Enrekang Regency" . *International Journal of Recent Technology and Engineering* ISSN: 2277-3878, (8)2.
- Asmahan, A.A. (2011). "Isolation and Identification of Lactic acid bacteria isolated from traditional drinking yoghurt in Khartoum State, Sudan". *Current research in bacteriology.* 4(1): 16-22.
- Atanasova, J., Moncheva, P. and Ivanova, I. (2014). "Proteolytic and antimicrobial activity of lactic acid bacteria grown in goat milk". *Biotechnol Biotechnol Equip.* 28, 1073-1078.
- Ávila, M.; Garde, S.; Medina, M.; Nuñez, M.(2005) . "Effect of milk inoculation with bacteriocin-producing lactic acid bacteria on a *Lactobacillus helveticus* adjunct cheese culture". *J. Food Prot.* 68, 1026–1033
- Axelsson ,L. *Lactic acid Bacteria* (1998) "Classification and Physiology in lactic acid Bacteria, Microbiology and functional Aspects "(Marcel Dekker Inc ,NEW YORK, pp.1-7.
- Ayeni F A , B Sánchez, BA Adeniyi, G Clara, A Margolles, P Ruas-Madiedo. (2011). "Evaluation of the functional potential of *Weissella* and *Lactobacillus* isolates obtained from Nigerian traditional fermented foods and cow's intestine". *International Journal of Food Microbiology* 147 (2), 97-104

- Bakar, F. A., Nordin, N., Yoke, T. S., & Abdulmir, A. S. (2010). "Detection and quantification of probiotic bacteria using optimized DNA extraction , traditional and real-time PCR methods in complex microbial communities". *African Journal of Biotechnology*, 9(10), 1481–1492.
- Bakari D, Tatsadjieu NL, Mbawala A, Mbofung CM. (2011). "Assessment of physiological properties of some lactic acid bacteria isolated from the intestine of chickens use as probiotics and antimicrobial agents against enteropathogenic bacteria". *Innov Rom Food Biotechnol* 8: 33–40.
- Barretto C, Ngom-Bru C, Genevaz A, Fournier C, Moine D, Kassam M, et al. (2016). "Genome sequence of *Lactobacillus fermentum* strain NCC2970 (CNCM I-5068)". *Genome Announc.* 4: e 01254- e 01216.
- Begley, M., Gahan, C.G.M. and Hill, C. (2005). "The interaction between bacteria and bile". *FEMS Microbiology Reviews* 29: 625-651.
- Begley M., Hill C., Gahan C.G.M. (2006): "Bile salt hydrolase activity in probiotics". *Applied and Environmental Microbiology*, 72: 1729–1738
- Berardi, C. W., Solovey, E. T., and Cummings, M. L. (2013). “Investigating the efficacy of network visualizations for intelligence tasks,” in *Proceedings of the IEEE International Conference on Intelligence and Security Informatics*, (Seattle, WA), 278–283.
- Berg, R.D. (1995). "Bacterial translocation from the gastrointestinal tract". *Trends Microbiol.* 3, 139-154.
- Berg, J.M., Tymoczko, J.L., and Stryer, L. (2012). *Biochemistry*, 7th edition. W. H. Freeman and Company, New York, NY.
- Beshkova D, Simova ED, Simov ZI, Frengova GI, Spasov Z N (2002) "Pure cultures for making kefir". *Food Microbiol* 19:537-544.

- Beshkova, D.; Simova, E.D.; Frengova, G.I.; Simov, Z.i.; Dimitrov, Z.H.P.(2003).
"Production of volatile aroma compounds by kefir starter cultures".
International Dairy Journal, v.13, p.529-535
- Bogdan N. , Ovidiu T. , Mihai N. , Mihaela T. , Mirela H. , Ionela M. (2014)
"Identification of probiotic strains from human milk in breastfed infants with
respiratory infections". Food technology Vol. xviii , no. 2
- Bonfini, L., W. Moens, E. Ben, M. Querci, B. Aygun, P. Corbisier, D. Morisset, J.
Zel, and G. Van den Eede. (2007). "Analytes and related PCR primers used for
GMO detection and quantification". JRC Sci. Technol. Rep. EUR 23059 EN.
1-51.
- Boris, S., J.E. Suárez, F. Vázquez, Barbés. (1998). "Adherence of human vaginal
lactobacilli to vaginal epithelial cells and interaction with uropathogens".
Infect. Immun. 66, 1985- 1989
- Bujnakova D, Kmet V (2012). "Functional properties of *Lactobacillus* strains
isolated from dairy products". Folia Microbiol 57:263–267
- Burns P, Patrignani P, Serrazanetti D, Vinderola GC, Reinheimer JA, Lanciotti R,
Guerzoni ME. (2008). "Probiotic Crescenza Cheese containing *Lactobacillus*
casei and *Lactobacillus acidophilus* manufactured with high-pressure
homogenized milk". J Dairy Sci, 91 (2) 500-512.
- Çarasi P, Racedo SM, Jacquot C, et al. (2015). "Impact of kefir derived
Lactobacillus kefir on the mucosal immune response and gut microbiota".
Journal of Immunology Research;2015:361604.
- Çakır, İ. (2003). "Determination of some probiotic properties on *Lactobacilli* and
Bifidobacteria". Ankara University Thesis of Ph.D.

- Chakraborty, A., & Bhowal, J. (2015). "Original Research Article Isolation , Identification and Analysis of Probiotic Properties of *Lactobacillus* Spp . from Selected Regional Dairy Product ". *Int.J.Curr.Microbiol.App.Sci*, 4(6), 621–628.
- Chen, Y. P., and M. J. Chen. (2013). "Effects of *Lactobacillus kefiranofaciens* M1 isolated from kefir grains on germ-free mice". *PLoS One* 8:e78789.
- Chen, Y. P., P. J. Hsiao, W. S. Hong, T. Y. Dai. and M. J. Chen. (2012). "Lactobacillus kefiranofaciens M1 isolated from milk kefir grains ameliorates experimental colitis in vitro and in vivo". *J. Dairy Sci.* 95:63-74.
- Chen X, Wu RN, Meng H, Zhang HP.(2008)." Review on the mechanism of acid tolerance of lactic acid bacteria". *China Dairy Ind*; 3: 30-34.
- Cheirsilp B, Shimizu H, Shioya S .(2003). Enhanced kefir production by mixed culture of *Lactobacillus kefiranofaciens* and *Saccharomyces cerevisiae*". *J. Biotechnol* 100:43-53.
- Chiang, S.S. and Pan, T.M. (2012). "Beneficial effects of *Lactobacillus paracasei* subsp. *paracasei* NTU 101 and its fermented products". *Appl. Microbiol. Biotechnol.* 93, 903-916.
- Chin Wen, L., HsiaoLing, C., & JeRuei, L. (1999). "Identification and characterization of lactic acid bacteria and yeasts isolated from kefir grains in Taiwan". *Australian Journal of Dairy Technology*, 54(1), 14-18.
- Chou, L.S and Weimer, B. (1999). "Isolation and characterization of acid and bile tolerant isolates from strains of *Lactobacillus acidophilus*". *Journal of Dairy Science* 82:23- 31.
- Chon, J. W., H. S. Kim, K. Y. Song, D. H. Kim, H. S. Kim, J. H. Yim, D. Choi, D. G. Hwang, Y. G. Kim, S. K. Lee, and K. H. Seo. (2013). "Functional

- characteristics of kefir as a fermented dairy product "a review. Korean J. Dairy Sci. Technol. 31:99-108
- Christensen JE, Dudley EG, Pederson JA, Steele JL (1999) "Peptidases and amino acid catabolism in lactic acid bacteria". Antonie Van Leeuwenhoek, 76:217-246.
- Christian A. Heid, Junko Stevens, Kenneth J. Livak, and P. Mickey Williams. "Real Time Quantitative PCR". (1996) . Cold spring Harbor laboratory press.
- Collins M.D., Phillips B.A., and Zannoni P. (1989). "Deoxyribonucleic acid homology studies of *Lactobacillus casei*, *Lactobacillus paracasei* sp. nov., subsp. *paracasei* and subsp. *tolerans*, and *Lactobacillus rhamnosus* sp. nov., comb. nov." Int. J. Syst. Bacteriol. 39 :105-108.
- Collins, J.K., G. Thornton, G.O. Sullivan. (1998). "Selection of probiotic strains for human applications". Int. Dairy J. 8, 487-490.
- Cross, M.L. (2002). "Microbes versus microbes: immune signals generated by probiotic lactobacilli and their role in protection against microbial pathogens". FEMS Immunol. Med. Microbiol. 34, 245-253.
- Da Cruz Pedrozo Miguel, M. G., P. Gomes Cardoso, L. de Assis Lago, and R. F. Schwan. (2010). "Diversity of bacteria present in milk kefir grains using culture-dependent and culture-independent methods". Food Res. Int. 43:1523–1528
- Delley, M and Germont, JE (2002). "Differentiation of *Lactobacillus helveticus*, *Lactobacillus delbrueckii* subsp *bulgaricus*, subsp *lactis* and subsp *delbrueckii* using physiological and genetic tools and reclassification of some strains from the ATCC collection". Syst. Appl. Microbiol., 25: 228-231.

- De Montijo-Prieto, S., Moreno, E., Bergillos-Meca, , Lasserrot, A., Ruiz-López, M.D., Ruiz-Bravo, A., Jiménez-Valera, M.A. (2015) "*Lactobacillus plantarum* strain isolated from kefir protects against intestinal infection with *Yersinia enterocolitica* O9 and modulates immunity in mice". Res. Microbiol. 166(8) pp. 626–32
- Deshpande K.G, C.B. Dolas, and N.S. Chavan. (2014) " Investigation of tolerance of *Lactobacillus casei* to the presence of acids, bile salts and Deconjugation of bile salts". Int. J.Curr. Microbiol. App.Sci 3 (7) 600 - 612
- Desai. A. R, N. P. Shah, and I. B. Powell. (2006). "Discrimination of Dairy Industry Isolates of the *Lactobacillus casei* Group" . J. Dairy Sci. 89:3345–3351
- De Vuyst, L. and P. Neysens (2005). "Biodiversity and identification of sourdough lactic acid bacteria". Food Microbiol., 24(2): 120-127
- DiBaise, J. K., Zhang, H., Crowell, M., Krajmalnik-Brown, R., Decker, G. A., & Ritmann, B. E. (2008). "Gut microbiota and its possible relationship with obesity". Mayo Clinic Proceedings, 83,460–469
- Dicks LMT, Du Plessis EM, Dellaglio F, Lauer E. (1996). "Reclassification of *Lactobacillus casei* subsp. *casei* ATCC 393 and *Lactobacillus rhamnosus* ATCC 15820 as *Lactobacillus zeae* nom. rev., designation of ATCC 334 as the neotype of *L. casei* subsp. *casei*, and rejection of the name *Lactobacillus paracasei*". Int J Syst Bacteriol; 46: 337–40.
- Dobson, A., O. O’Sullivan, P. D. Cotter, P. Ross, and C. Hill. (2011). "High-through put sequence-based analysis of the bacterial composition of kefir and an associated kefir grain". FEMS Microbiol. Lett. 320: 56-62.
- DONG-Hyeon Kim ; JUNG-Whan Chon; HONG Seok Kim; JIN Hyeok Yim; HYUNSOOK KIM; KUN-Ho S. (2015) "Rapid Detection of *Lactobacillus*

kefiranofaciens in Kefir Grain and Kefir Milk Using newly developed Real time PCR" . J Food Prot (2015) 78 (4): 855–858.

Drago L, De Grandi R, De Vecchi E, (2016)." Antibiotic susceptibility profile of a new *Lactobacillus kefiri* strain". Journal of Global Antimicrobial Resistance.4:74–5.

Drancourt M., Claude Bollet, Antoine Carlouz. (2000). "16S Ribosomal DNA Sequence Analysis of a Large Collection of Environmental and Clinical Unidentifiable Bacterial Isolates". Journal of Clinical Microbiology 38(10):3623-30

Du Toit, M., Franz, C., Schillinger, U., Warles, B. and Holzapfel, W. (1998). "Characterization and selection of probiotic lactobacilli for a preliminary mini pigfeeding trail and their effect on serum cholesterol level, faeces moisture contents". International Journal of Food Microbiology 40: 93-104.

Dwight Z, Palais R, Wittwer CT (2011) "uMELT: prediction of high-resolution melting curves and dynamic melting profiles of PCR products in a rich web application". Bioinformatics, 27, 1019–1020.

Edward K, Logan J, and Saunders N.(2004)." Real time PCR : an Essential Guide. Norfolk, UK ": Horizon Bioscience.

Ehrmann, M. A., and Vogel, R. F. (2005). "Molecular taxonomy and genetics of sourdough lactic acid bacteria". Trends in Food Science and Technology. 16: 31-42.

Elizete D.F.R.P. and Carlos R. S. (2005). "Biochemical characterization and identification of probiotic *Lactobacillus* for swine ". B. CEPPA, Curitiba, 23: 299-310

- Farnworth ER (2005) "Kefir - a complex probiotic" .Food Sci Technol Bull .Functional Foods2:1-17
- Farnworth ER, Mainville I (2008). "Kefir –A Fermented Milk Product". Handbook of Fermented .Functional Foods (2 ed). CRC Press Taylor & Francis Group, Boca Raton, London, New York, p. 89-127
- Fernandes, C.F., Shahani, K.M. and Amer, M.A. (1987) . "Therapeutic role of dietary lactobacilli and lactobacillic fermented dairy products". FEMS Microbiol Rev46, 343–356.
- Fijałkowska M. , Sebastian Przemieniecki W. , Cezary Purwin, .(2020). " The effect of an additive containing three Lactobacillus species on the fermentation pattern and microbiological status of silage". J Sci Food Agric 2020; 100: 1174–1184
- Fons M. , Ana Gomez, Tuomo Karjalainen (2000). " Mechanisms of Colonisation and Colonisation Resistance of the Digestive Tract" . Microbial Ecology in Health and Disease, 12:2, 240-246.
- Fooks, L. J., Fuller, R., and Gibson G. R. (1999) . "Prebiotics probiotics and human gut microbiology" . International Dairy Journal.1999; 9:53-61.
- Forhad M. H. , S.M. Khaledur Rahman¹, Md. Shahedur Rahman, Forhad Karim Saikot¹ and Krishno Chandra Biswas.(2015)." Probiotic Properties Analysis of Isolated Lactic Acid Bacteria from Buffalo Milk". Vol. 7 No. 1: 5
- Fuller, R. (1989). "A review: Probiotics in man and animals". J. Appl. Bact. 66, 365-378.
- Fujisawa, T., K., Benno, Y., & Mitsuoka, A. N. D. T. (1990). "Lactobacillus intestinalis (ex Hemme 1974) Isolated from the Intestines of Mice and Rats". 302–304.

- Fujisawa T, Benno Y, Yaeshima T, Mitsuoka T (1992) "Taxonomic study of the *Lactobacillus acidophilus* group, with recognition of *Lactobacillus gallinarum* sp. nov. and *Lactobacillus johnsonii* sp. nov. and synonymy of *Lactobacillus acidophilus* group A3 with the type strain of *Lactobacillus amylovorus*". Int J Syst Bacteriol 42:487–491
- Gandhi, D.N., Patel, R.S., Wadhwa, B.K., Bansal, N., Kaur, M. and Kumar, G. (2000). "Effect of agro-based by-products on production of lactic acid in whey permeate medium". J. Food Sci. Technol., 37, 292-295.
- Gao, J., Gu, F., Abdella, N.H., Ruan, H., and He, G. (2012). "Investigation on culturable microflora in Tibetan kefir grains from different areas of China". J. Food Sci. 77, 425–433.
- Gao, J., Gu, F., He, J., Xiao, J., Chen, Q., Ruan, H., et al. (2013a). "Metagenome analysis of bacterial diversity in Tibetan kefir grains". Eur. Food Res. Technol. 236, 549–556.
- Gao, J., Gu, F., Ruan, H., Chen, Q., He, J., and He, G. (2013b). "Induction of apoptosis of gastric cancer cells SGC 7901 in vitro by a cell-free fraction of Tibetan kefir". Int. Dairy J. 30, 14–18.
- Garofalo, C., Osimani, A., Milanović, V., Aquilanti, L., De Filippis, F., Stellato, G. (2015). "Bacteria and yeast microbiota in milk kefir grains from different Italian regions". Food Microbiol. 49, 123–133.
- Garrote, G.L., Abraham, A.G. and De Antoni, G.L. (2001). "Chemical and microbiological characterisation of kefir grains". J Dairy Res 68, 639–652
- Garvie, E. I. (1984). "Separation of species of the genus *Leuconostoc* and differentiation of the *leuconostocs* from other lactic acid bacteria". Methods Microbiol. 16:147

- Gaware, V., K., K., Dolas, R., Somwanshi, S., & Nikam, V. (2011). "THE MAGIC OF KEFIR" : A REVIEW Pharmacology online 1 : 376-386 .
- Gawhen K ,Bergmeyer, H. U. & BERNT, E. (1974). "UV-assay with pyruvate and NADH. In Methods of Enzymatic Analysis" BERGMEYER, H.U., vol. 2, pp. 574–579.
- Gebreselassie, N., Abay, F., & Beyene, F. (2016). Biochemical and molecular identification and characterization of lactic acid bacteria and yeasts isolated from Ethiopian naturally fermented buttermilk. *Journal of Food Science and Technology*, 53(1), 184-196
- Gevers, D., G. Huys, J. Swings. (2001). "Applicability of rep-PCR fingerprinting for identification of *Lactobacillus* species". *FEMS Microbiol. Lett.* 205, 31-36.
- Gibson, G.R., R. Fuller. (2000). "Aspects of in vitro and in vivo research approaches directed toward identifying probiotics and probiotics for human use". *J. Nutr.* 130, 391S- 395S.
- Gionchetti P, Rizzello F, Venturi A et al (2000) ."Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis :a double blind , placebo- controlled trial". *Gastroenterology* 119:305-309
- Girum Tadesse, Eden Ephraim and Mogessie Ashenafr. (2011). "Assessment of the antimicrobial activity of lactic acid bacteria isolated from Borde and Shamitta traditional Ethiopian fermented beverages, on some food - borne pathogens and effect of growth medium on the inhibitory activity". *International Journal of Food Safety*, 5: 13 - 20.
- Golden B.R. and S.L.Gorbach (1992). "Probiotics for humans In :Fuller, R(Ed), *Probiotics*" , The scientific Basis ,Chapman and Hall ,London ,335-376.

- Guan LL, Hagen KE, Tannock GW, Korver DR, Fasenko GM, Allison GE (2003) "Detection and identification of *Lactobacillus* species in crops of broilers of different ages by using PCR denaturing gradient gel electrophoresis and amplified ribosomal DNA restriction analysis". *Appl Environ Microbiol* 69:6750–6757
- Guzel-Seydim, Z. B., A. C. Seydim, and A. K. Greene. (2000). "Organic acids and volatile flavor components evolved during refrigerated storage of kefir". *J. Dairy Sci.* 83:275–277
- Guzel-Seydim Z, Wyffels JT, Seydim AC, Greene AK (2005). "Turkish kefir and kefir grains: microbial enumeration and electron microscobic observation". *Int J Dairy Technol* 58:25-29
- Grangette, C., H. Müller-Alouf, D. Goudercourt, M.-C. Geoffroy, M. Turneer, A. Mercenier. (2001). "Mucosal immune responses and protection against tetanus toxin after intranasal immunization with recombinant *Lactobacillus plantarum*. *Infect*". *Immun.* 69, 1547-1553.
- Grill, J.P., Cayuela, C., Antoine, J.M., Schneider, F., (2000). "Effects of *Lactobacillus amylovorus* and *Bifidobacterium breve* on cholesterol". *Lett. Appl. Microbiol.* 31, 154–156.
- Haarman M. and Jan Knol. (2006). "Quantitative Real-Time PCR Analysis of Fecal *Lactobacillus* Species in Infants Receiving a Prebiotic Infant Formula". *Applied and environmental microbiology* vol. 72, No. 4p. 2359–2365.
- Hamet, M.F., Londero, A., Medrano, M., Vercammen, E., VanHoorde, K., Garrote, G.L., (2013). "Application of culture-dependent and culture-independent methods for the identification of *Lactobacillus kefirifaciens* in microbial consortia present in kefir grains" *Food Microbiol.* 36, 327–334.

- Hassan, F.H., Golnar, R., Mohammad, R.F., Mitra, M. and Mitra, S. (2012). "Evaluation of wound healing activities of kefir products". Elsevier. 38, 719-723
- Hassan P, Peh KK. (2014). "Characterization and identification of *Lactobacillus acidophilus* using biolog rapid identification system". International Journal of Pharmacy and Pharmaceutical Sciences 6, 189-193.
- Heid, C. A., Stevens, J., Livak, K. J. & Williams, P. M. (1996). "Real time quantitative PCR". Genome Res 6, 986-994.
- Herbel R. Stefan, Vahjen Wilfried, Wieler Lothar H and Guenther Sebastian; (2013) "Timely Approaches to Identify Probiotic Species of the Genus *Lactobacillus*. Gut Pathogens" 5(1).
- Higuchi, R., Fockler, C., Dollinger, G., Watson R., Kinetic (1993). "PCR analysis: Real-time monitoring of DNA amplification reactions" Biotechnology, 11: 1026–1030 .
- Hoier, E., (1992). "Use of probiotic starter cultures in dairy products". In: The 25th Annual Convention, Australian Institute of Food Science Technology, Sydney, Australia
- Holzapfel WH, Wood BJB (1998). "The genera of lactic acid bacteria". Blackie Academic and Professional, London
- Holzapfel, W.H., P. Haberer, R. Geisen, J. Björkroth, U. Schillinger. (2001). "Taxonomy and important features of probiotic microorganisms in food nutrition". Am. J. Clin. Nutr. 73, 365S-373S.
- Holzapfel, W.H. (2006). "Introduction to Prebiotics and Probiotics. In: Probiotics in Food Safety and Human health", Eds. I. Goktepe, V.K. Juneja ve M. Ahmedna, Taylor and Francis Group, CRC Press Boca Raton, 1-35.

- Holzappel , W. H. & Wood, B. J., (2014). "Lactic Acid Bacteria: Biodiversity and Taxonomy". Chichester: John Wiley & Sons.
- Howell, W. M., Jobs, M., Gyllensten U., Brookes, A. J., (1999) ."Dynamic allele-specific hybridization, a new method for scoring single nucleotide polymorphisms", Nat. Biotechnol., 17: 87–88 .
- Hoque M. Z., Akter F., Hossain M.K., Rahman M.S.M., Mbillah M. and ISLAMK. M. D.(2010)." Isolation, identification and analysis of probiotic properties of *Lactobacillus* Spp. from selective regional yogurts".World J. Dairy and Food Sci., 5(1): 39-46.
- Huang CH, Liou JS, Huang L, Watanabe K.(2017). "Developing novel species-specific DNA markers for PCR-based species identification of the *Lactobacillus sakei* group". Lett Appl Microbiol.;66:138 –44.
- Huang CH, Huang L. (2018). "Rapid species- and subspecies-specific level classification and identification of *Lactobacillus casei* group members using MALDI Biotyper combined with Clin Pro Tools". J Dairy Sci.;101:979 –91.
- Hugo, A., Kakisu, E., De Antoni, G., and Perez, P. (2008). "Lactobacilli antagonize biological effects of enterohaemorrhagic *Escherichia coli* in vitro". Lett. Appl. Microbiol. 46, 613–619.
- Hujanen M. ,Y.Y.Linko (1996) ."Effect of temperature and various nitrogen sources on L(+)-lactic acid production by *Lactobacillus casei*". Applied and Microbiology and Technology, Volume 45, Issue 3, pp 307–313.
- Hutkins R. (2006). "Cultured dairy products". Microbiology and Technology of Fermented Foods, Blackwell Publishing, Ames, Iowa .Pages 107–114
- Idris, A., & Suzana, W. (2006). "Effect of sodium alginate concentration, bead diameter, initial pH and temperature on lactic acid production from pineapple

- waste using immobilized *Lactobacillus delbrueckii*". Progress Biochemistry 41(5):1117-1123
- Ishtiaq ahmed et al.,(2010). "Optimization of media and environmental conditions for alkaline protease production using *Bacillus subtilis* in submerged fermentation process". IJAVMS. Vol.4,issue 4,105-113.
- Iyer, R., Thomas, S.K., Kapila, S., Mani, J., Singh, R., (2010). "Probiotic properties of folate producing *Streptococcus thermophilus* strains". Food Res. Int. 43, 103–110.
- Iyer, R.; Tomar, S.K.; Maheswari, T.U.; Singh, R. (2010) "*Streptococcus thermophilus* strains: Multifunctional lactic acid bacteria". Int. Dairy J. 20, 133–141.
- Jack RW, Tagg JR, Ray B (1995) "Bacteriocins of Gram -positive bacteria" Microbiol Rev 59:171-200.
- Kakisu, E., Abraham, A.G., Tironi Farinati, C., Ibarra, C. and De Antoni, G.L. (2013) "*Lactobacillus plantarum* isolated from kefir protects vero cells from cytotoxicity by type-II shiga toxin from Escherichia coli O157" H7. J Dairy Res 80, 64–71
- Kao Y-T, Liu Y-S, Shyu Y-T (2007). "Identification of *Lactobacillus* spp. in probiotic products by real-time PCR and melting curve analysis". Food Res Int 40:71–79. doi
- Kandler, O., & Kunath, P. (1983). "*Lactobacillus kefir* sp.nov, a component of the microflora of Kefir". Systematic and Applied Microbiology, 4, 286e294.
- Kandler, O., & Weiss, N. (2005). "Genus *Lactobacillus*". In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, J. G. Holt (Eds.), Bergey's Manual of Systematic Bacteriology (vol. 2, pp. 1209 – 1234).

- Kant, R., L. Paulin, E. Alatalo, W. M. de Vos, and A. Palva. (2011). "Genome Sequence of *Lactobacillus amylovorus* GRL1112". J. Bacteriol. 193:789–790.
- Kesmen Z, Kacmaz N.(2011)." Determination of lactic microflora of kefir grains and kefir beverage by using culture-dependent and culture- independent methods". J Food Sci; 76: 276-83.
- Khalil, M. I., & Anwar, M. N. (2016). "Isolation, Identification and Characterization of Lactic Acid Bacteria from Milk and Yoghurts". Research & Reviews: Journal of Food and Dairy Technology, 4(3), 17–26.
- Khedid, K., Faïd, M., Mokhtari, A., Soulaymani, A., & Zinedine, A. (2009). "Characterization of lactic acid bacteria isolated from the one humped camel milk produced in Morocco". Microbiological Research, 164(1), 81–91.
- Kim, D. H. Chon J. W. , Kim H., Kim H. S. , Choi D. Hwang D. G. and Seo K. H. (2015)a . "Detection and enumeration of lactic acid bacteria ,acetic acid bacteria and yeast in kefir grain and milk using quantitative real -time PCR. Journal of Food Safety" ISSN 1745-4565
- Kim D. H. ; Chon J. W. ; Kim H. S. ; YIM J. H. ; Kim H. S. ; Seo K. H. (2015)b. "Rapid Detection of *Lactobacillus kefiranoferiens* in Kefir Grain and Kefir Milk Using Newly Developed Real-Time PCR ". J Food Prot. 78 (4): 855–858.
- Kim, D. H., Kang, I. B., Jeong, D., Kim, H., Kim, H. S., Lee, S. K., Seo, K. H. (2016). "Development of rapid and highly specific TaqMan probe-based real-time PCR assay for the identification and enumeration of *Lactobacillus kefir* in kefir milk". International Dairy Journal, 61, 18–21.
- Kim E., Seung-Min Yang , Bora Lim, Si Hong Park , Bryna Rackerby and Hae-Yeong Kim .(2020). "Design of PCR assays to specifically detect and identify

- 37 *Lactobacillus* species in a single 96 well plate " Kim et al. BMC Microbiology 20:96
- Kıvanç, M., & Yapıcı, E. (2016). "Kefir as a Probiotic Dairy Beverage: Determination Lactic Acid Bacteria and Yeast". ETP International Journal of Food Engineering, 1(1), 55–60.
- Klein G, Park A, Bonaparte C, Reuter G (1998). "Taxonomy and physiology of probiotic lactic acid bacteria". Int J Food Microbiol 41:105–125
- Klaenhammer, T; Altermann, E; Pfeiler, E; Buck, BL; Goh, YJ; O'Flaherty, S; Barrangou, R and Duong, T (2008). "Functional genomics of probiotic lactobacilli". J. Clin. Gastroenterol., 42: 160-161.
- Klaenhammer T.R. and de Vos W.M. (2011). "An incredible scientific journey. The evolutionary tale of the lactic acid bacteria". In: Ledeboer A, Hugenholtz J, Kok J, Konings W, Wouters J (ed.) The 10th LAB symposium. Thirty years of research on lactic acid bacteria. 24 Media Labs, pp. 1-11.
- Kok-Tas,T., Ekinci, F.Y.,and Guzele Seydim, Z. B. (2012)." Identification of microbial flora in kefir grains produced in Turkey using PCR". Int. J. Dairy Technol. 65, 126–131.
- Kondyli, E., Katsiari, M.C., Masouras, T., and Voutsinas, L.P. (2002). "Free fatty acids and volatile compounds of low-fat feta-type cheese made with a commercial adjunct culture". Food Chemistry 79: 199–205
- Konstantinov, S. R., et al. (2006). "*Lactobacillus sobrius* sp. nov., abundant in the intestine of weaning piglets". Int. J. Syst. Evol. Microbiol. 56:29–32.
- Konstantinov, S. R., et al. (2008). "Feeding of *Lactobacillus sobrius* reduces *Escherichia coli* F4 levels in the gut and promotes growth of infected piglets". FEMS Microbiol. Ecol. 66:599–607

- Korsak N. B. Taminiau, M. Leclercq, C. Nezer, S. Crevecœur, C. Ferauche, E. Detry, V. Delcenserie, and G. Daube. (2014). "Short communication: Evaluation of the microbiota of kefir samples using metagenetic analysis targeting the 16S and 26S ribosomal DNA fragments" . J. Dairy Sci. 98:3684–3689
- Kotova I.B, T.A. Cherdyntseva, and A.I. Netrusov.(2016). "Russian Kefir Grains" Microbial Composition 6 and Its Changes during Production.
- Kullisaar, T., Zilmer, K., Salum, T., Rehema, A., & Zilmer, M. (2016). "The use of probiotic *L. fermentum* ME-3 containing Reg'Activ Cholesterol supplement for 4 weeks has a positive influence on blood lipoprotein profiles and inflammatory cytokines" An open-label preliminary study. Nutrition Journal, 15(1), 93
- Kumar and Chordia. (2017) "Role of Microbes in Human Health". Appli Microbiol Volume 3 • Issue 2 ISSN:2471-9315
- Laiño, J.E., Juarez del Valle, M., Savoy de Giori, G., LeBlanc, J.G., (2013). "Development of a high folate concentration yogurt naturally bio-enriched using selected lactic acid bacteria". LWT Food Sci. Technol. 54, 1–5
- Laiño, J. E., Juarez del Valle, M., Savoy de Giori, G., & LeBlanc, J. G. J. (2014). "Applicability of a *Lactobacillus amylovorus* strain as co-culture for natural folate bio-enrichment of fermented milk". International Journal of Food Microbiology, 191, 10–16.
- Lauer, E., C. Helming, and O. Kandler. (1980) "Heterogeneity of the species *Lactobacillus acidophilus* (Moro) Hansen and Moquot as revealed by biochemical characteristics and DNA-DNA hybridisation". Zentralbl. Bakteriologie, Parasitenkunde, Infektionskrankheiten, Hygiene, Abteilung 1, Originalreihe C 1:15&168.

- Le Blanc, J.G., Laiño, J.E., Juarez del Vale, M., Vannini, V., van Sinderen, D., Taranto, M.P., de Valdez, G.F., Savoy de Giori, G., Sesma, F., (2011). "B-group vitamin production by lactic acid bacteria—current knowledge and potential applications". *J. Appl. Microbiol.* 111, 1297–1309.
- Leite AMO, Mayo B, Rachid CTCC, Peixoto RS, Silva JT, Paschoalin VMF, Delgado S (2012) "Assessment of the microbial diversity of Brazilian kefir grains by PCR DGGE and pyro sequencing analysis". *Food Microbiol*, 31, 215-221.
- Leite, A. M. de Oliveira et al . (2013)" Microbiological, technological and therapeutic properties of kefir" A natural probiotic beverage. *Brazilian Journal of Microbiology*, 44(2), 341–349.
- Leite A. M. O., Miguel, R. S. Peixoto,§ P. Ruas-Madiedo, V. M. F. Paschoalin, 1 B. Mayo, and S. Delgado.(2015)" Probiotic potential of selected lactic acid bacteria strains isolated from Brazilian kefir grains" *J. Dairy Sci.* 98:3622–3632.
- Lerory F. and Luc De Vuyst (2004). "Lactic acid bacteria as functional starter cultures for the food fermentation industry". *Trends in Food Science and Technology*, 15: 67-68.
- Lipsky, R. H., Mazzanti, C. M., Rudolph, J. G., Xu, K., Vyas, G., Bozak, D., Radcl, M. Q., Goldman, D., (2001). "DNA Melting analysis for detection of single nucleotide polymorphisms", *Clinical Chemistry*, 47: 635-644.
- Lin CW, Chen HL, Liu JR (1999) "Identification and characterization of lactic acid bacteria and yeasts isolated from kefir grains in Taiwann". *Aust Journal of Dairy Tech* 54:14-18.

- Liu, S.Q. (2002). "Malolactic fermentation in wine - beyond deacidification". J. Appl. Microbiol. 92: 589-601
- Liutkevicius, A., & Sarkinas, A. (2004). "Studies on the growth conditions and composition of kefir grains – as a food and forage biomass". Veterinarija ir Zootechnika, 25, 64–70
- López-Huertas E. (2015). "Safety and efficacy of human breast milk *Lactobacillus fermentum* CECT 5716". A mini-review of studies with infant formulae. Benef. Microbes 6: 219-224
- Ma, Chenjie. et al., (2016). "Determination of the essential nutrients required for milk fermentation by *Lactobacillus plantarum*". LWT - Food Science and Technology, 01, Volume 65, pp. 884-889
- MacFaddin, J.F. (2000). "Biochemical Tests for Identification of Medical Bacteria". 3rd ed. Philadelphia: Lippincott Williams and Wilkins. USA. pp. 363-367.
- Mackey B. , Susane. Parsons,' Christopher A. Miles and Robert J. Owen. (1988) "The Relationship between the Base Composition of Bacterial DNA and Its Intracellular Melting Temperature As Determined by Differential Scanning Calorimetry" . Journal of General Microbiology, 134, 1185-1195.
- Magalhães, K. T., de Melo Pereira, G. V., Campos, C. R., Dragone, G., & Schwan, R. F. (2011). "Brazilian kefir: Structure, microbial communities and chemical composition". Brazilian Journal of Microbiology, 42(2), 693–702.
- Mahrous, H. (2011). "Probiotics Bacteria from Egyptian Infants cause Cholesterol Removal in Media and Survive in Yoghurt". Food and Nutrition Sciences, 02(02), 150–155.
- Makras, L., Van Acker, G. & De Vuyst, L., (2005). "*Lactobacillus paracasei* subsp. *paracasei* 8700:2 Degrades Inulin-Type Fructans Exhibiting Different Degrees

- of Polymerization". Applied and Environmental Microbiology, 11, 71(11), pp. 6531-6537.
- Mangalore, S. N. (2015). "Biocompatibility study of *Lactobacillus casei* isolated from cucumber and evaluation of probiotic effects in the human gut". Vol. 1 ISSN: - 2349-2724
- Mannan, S. J., Rezwan, R., Rahman, M. S., & Begum, K. (2017). "Isolation and Biochemical Characterization of *Lactobacillus* species from Yogurt and Cheese samples in Dhaka Metropolitan Area". Bangladesh Pharmaceutical Journal, 20(1), 27-33.
- Marco Toscano, Roberta De Grandi, Enzo Grossi and Lorenzo Drago. Role of the Human Breast Milk-Associated Microbiota on the Newborns' Immune System .2017 Front. Microbiol., volume 8.
- Mark A. Valasek and Joyce J. Repa. (2005)." The power of real-time PCR "Physiol Educ 29: 151-159
- Maurellio .G.Moio I., Moschetti G., Piompino p., addeo .,F and Coppola S.(2001) . "Characterization of lactic acid bacteria strains on the basis of neutral volatile compounds produced in whey". Journal Of Applied Microbiology .90 :928 - 942
- Matthew Bull. , Sue Plummer , Julian Marchesi & Eshwar . (2013). "The life history of *Lactobacillus acidophilus* as a probiotic: a tale of revisionary taxonomy, misidentification and commercial success". FEMS Microbiol Lett 349 77-87
- Meire dos Santos F., Karoline M. Soares, Wendell Wagner C. A. , Jose Antonio C. , Maria Taciana Holanda C. Ana Lúcia F.(2017). "*Saccharomyces cerevisiae* from Brazilian kefir-fermented milk: An in vitro evaluation of probiotic properties". P. Microbia; pathogenesis . Volume 110, Pages 670-677

- Merih Kıvanç & Evrim Yapıcı. (2015). "Kefir as a Probiotic Dairy Beverage: Determination Lactic Acid Bacteria and Yeast" . International Journal of Food Engineering Vol. 1, No. 1
- Meroth CB, Hammes WP, Hertel C (2004). "Characterisation of the microbiota of rice sourdoughs and description of *L. spicheri* sp. nov." Syst Appl Microbiol 27:151–159 21.
- Miguel, M.G.d.C.P., Cardoso, P.G., Lago, L.d.A. and Schwan, R.F. (2010). "Diversity of bacteria present in milk kefir grains using culture-dependent and culture-independent methods". Food Res Int 43, 1523–1528.
- Mikelsaar, M., & Zilmer, M. (2009). "Lactobacillus fermentum ME-3 an antimicrobial and anti-oxidative probiotic". Microb. Ecol. Health Dis, 21, 1-27.
- Mizrahi-Man O, Davenport ER, Gilad Y (2013). "Taxonomic Classification of Bacterial 16S rRNA Genes Using Short Sequencing Reads" Evaluation of Effective Study Designs. PLoS ONE 8(1)
- Mohania, D., R. Nagpal, M. Kumar, A. Bhardwaj, M. Yadav, S. Jain, F. Marotta, V. Sing, O. Parkash, and H. Yadav. (2008). "Molecular approaches for identification and characterization of lactic acid bacteria". J. Dig. Dis. 9:190–198 enzymatischen Analyse, 3. Aufl, Bd II. Verlag Chemic, Weinheim, S 1538-1541.
- Molin, Göran. (2008). "Lactobacillus plantarum: the role in foods and in human health". Farnworth, E.R (ed.). Handbook of Fermented Functional Foods. CRC Press. 353-393.

- Monique Haarman and Jan Knol. (2006) ."Quantitative Real-Time PCR Analysis of Fecal *Lactobacillus* Species in Infants Receiving a Prebiotic Infant Formula" *Applied and Environmental Microbiology*, p. 2359–2365.
- Moroni AV, Arendt EK, Bello FD (2011). "Biodiversity of lactic acid bacteria and yeasts in spontaneously-fermented buckwheat and teff sourdoughs". *Food Microbiol* 28:497–502
- Moser, A., Berthoud, H., Eugster, E., Meile, L., and Irmeler, S. (2017a). "Detection and enumeration of *Lactobacillus helveticus* in dairy products". *Int. Dairy J.* 68, 52–59
- Motaghi M, Mazaheri M, Moazami N, Farkhondeh A, Fooladi MH, Goltapeh EM. .(1997) "Short Communication: Kefir production in Iran". *World J Microbiol Biotechnol.* 13(5):579–81.
- Nalbantoglu,U., Cakar,A., Dogan,H., Abaci,N., Ustek,D., Sayood,K., (2014). "Metagenomic analysis of the microbial community in kefir grains". *Food Microbiol.* 41, 42–51.
- Nakamura, L. K. (1981). "*Lactobacillus amylovorus*, a new starch hydrolyzing species from cattle waste-corn fermentations". *Int. J. Syst. Bacteriol.* 31:56–63.
- Nannu Shafakatullah and M. Chandra. (2015). " Biocompatibility study of *Lactobacillus casei* isolated from cucumber and evaluation of probiotic effects in the human gut". *IJCBS research paper* vol. 1 (12) 2349–2724
- Neve H. (1992). "analysis of kefir grain starter cultures by scanning electron microscopy". *Milchwissenschaft* 47: 275 – 278.
- O'Brien, K. V. (2012). "The Effect of Frozen Storage on the Survival of Probiotic". *Journal of Dairy Science* Volume 99, Issue 9, September 2016, Pages 7043-7048

- Onaran, B., & Çufaoğlu, G. (2017). "Comparison of microbial population of household and commercial kefirs in Ankara , Turkey". 88(1), 52–58.
- Orlando, A., Linsalata, M., and Russo, F. (2016). "Antiproliferative effects on colon adenocarcinoma cells induced by co-administration of vitamin K1 and *Lactobacillus rhamnosus* GG". Int. J. Oncol. 48, 2629–2638.
- Otles S. and Cagindi O. (2003). "Kefir :A probiotic diary –composition , nutritional and therapeutic aspects" . journal of nutrition. pp. 54–59.
- Palachum, W., Chisti, Y., Choorit, W., (2018). "In-vitro assessment of probiotic potential of *Lactobacillus plantarum* WU-P19 isolated from a traditional fermented herb". Annals of Microbiology 68(2): 79- 91.
- Papamanoli E., N Tzanetakis , E Litopoulou- Tzanetaki , P Kotzekidou."(2003) Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties". 65(2):859-67.
- Park, E. J., H. W. Chang, K. H. Kim, Y. D. Nam, S. W. Roh, and J. W. Bae. (2009). "Application of quantitative real-time PCR for enumeration of total bacterial, archaeal, and yeast populations in kimchi". J. Microbiol. 47: 682-685.
- Pilar Fernandez-Pacheco, María Arévalo-Villena, Antonio Bevilacqua, Maria Rosaria Corbo, Ana Briones Pérez (2018). "Probiotic characteristics in *Saccharomyces cerevisiae* strains: Properties for application in food industries" . Food science and technology 97. 332-340.
- Pitino et al. (2010). "Survival of *Lactobacillus rhamnosus* strains in the upper gastrointestinal tract". Food Microbiology 27:1121–1127 .
- Phyu, H., Oo, Z., and Aye, K. N. (2015). "Screening on Proteolytic Activity of Lactic Acid". Advances in Science Engineering and Technology, (5), 34–37.

- Poltronieri P, D'Urso OF, Blaiotta G, Morea M (2008). "DNA arrays and membrane hybridization methods for screening of six *Lactobacillus* species common in food products". *Food Anal Methods* 1:171–180
- Prasad, J., Gill, H., Smart, J., and Gopal, P.K. (1998). "Selection and Characterization of *Lactobacillus* and *Bifidobacterium* strains for use as probiotic". *International Dairy Journal* 8:993-1002.
- Prado, M. R., Blandón, L. M., Vandenberghe, L. P. S., Rodrigues, C., Castro, G. R., Thomaz-Soccol, V., & Soccol, C. R. (2015). "Milk kefir: Composition, microbial cultures, biological activities, and related products". *Frontiers in Microbiology*, 6(OCT), 1–10.
- Promot, s. k., kamel, w., rahman, s. s., hossain, m. m. and choudhary, n. (2018) "Biotechnol production using vegetables peaks medium and the effective role of cellulolytic bacterial (*Bacillus subtilis*) pre-treatment". *versus*, 7:27.
- Pundir, R. K., Rana, S., Kashyap, N., & Kaur, A. (2013). "Probiotic potential of lactic acid bacteria isolated from food samples An in vitro study". *Journal of Applied Pharmaceutical Science*, 3(3), 85–93.
- Puerari, C., Magalhães, K.T., and Schwan, R.F. (2012). "New cocoa pulp-based kefir beverages: Microbiological, chemical composition and sensory analysis " *.Food Research International* 48: 634–640.
- Pyar, H., & Peh, K. K. (2014). "Characterization and identification of *Lactobacillus acidophilus* using biolog rapid identification system". *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(1), 189–193.
- Querci, M., Van den Bulcke, M., Zel, J., Van den Eede, G., Broll, H. (2010). "New approaches in GMO detection" *Anal Bioanal Chem.*, 396:1991–2002.

- Rao P., K., Chennappa, G., Suraj, U., Nagaraja, H., Charith Raj, A. P., and Sreenivasa, M. Y. (2015). "Probiotic potential of *Lactobacillus* strains isolated from sorghum-based traditional fermented food". *Probiotics Antimicrob. Proteins* 7, 146–156.
- Rattray FP, O'Connell MJ (2011). "Fermented Milks Kefir" In:Fukay, J. W. (ed.), *Encyclopedia of Dairy Sciences* (2th ed).Academic Press , San Diego, USA, p.518-524.
- Ravi V, Prabhu M and Subramanyam D (2011). "Isolation of Bacteriocin Producing Bacteria from Mango Pulp and its Antimicrobial Activity". *J Microbiol Biotech Res.* 1: 54-63
- Ray, R. C., & Montet, D. (2017). "Kefir and koumiss origin , Health Benefits and current status of knowledge". *Fermented Foods: Part II: Technological Interventions*, 1–513.
- Reid, G. (2001). "Probiotic agents to protect the urogenital tract against infection". *Am. J.Clin. Nutr.* 73, 437S-443S.
- Reid Gregor .(2015) "The growth potential for dairy probiotics". *International Dairy Journal* 49.16-22.
- Remacle C, Reusens B. (2004) "Functional foods, ageing and degenerative disease". Cambridge UK: Woodhead Publishing for Technology & Engineering.
- Rolfe, R.D. (2000). "The role of probiotic cultures in the control of gastrointestinal health". *J. Nutr.* 130, 396S-402S
- Romain Marti, Patrick Dabert, Christine Ziebal, and Anne-Marie Pourcher. (2010) . "Evaluation of *Lactobacillus sobrius*/*L. amylovorus* as a New Microbial Marker of Pig Manure". *Applied and environmental microbiology*, p. 1456–1461.

- Roselli, M., et al. (2007). "The novel porcine *Lactobacillus sobrius* strain protects intestinal cells from enterotoxigenic *Escherichia coli* K88 infection and prevents membrane barrier damage". J. Nutr. 137: 2709–2716
- Rossetti, L. and Giraffa, G. (2005). "Rapid identification of dairy lactic acid bacteria by M13- generated ,RAPD-PCR fingerprint databases". Journal of Microbiological Methods 63:135-144
- Ryan, L.A., Zannini, E., Dal Bello, F., Pawlowska, A., Koehler, P., Arendt, E.K., (2011). "*Lactobacillus amylovorus* DSM19280 as a novel food-grade antifungal agent for bakery products". Int. J.Food Microbiol. 146, 276–283.
- Sacchi,C.T., Whitney, A.M, Mayer, L. Whitney, A. M, Mayer, L. W., Morey , R., Steigerwalt, A., Boras, A., Weyant, R. S.,and Popovic,T. (2002). "Sequencing of 16S rRNA gene:a rapid tool for identification of *Bacillus anthracis* " Emerging infectious diseases. 8:1117-1123.
- Salvetti, E., Torriani, S., and Felis, G. E. (2012). "The genus *Lactobacillus*: a taxonomic update". Probiotics Antimicrob. Proteins 4, 217–226
- Sameh Ali G., Rehab Mahmoud Abd El-Baky, Abo Bakr F A. and Gamal F. Mahmoud G. (2016). "In vitro evaluation of probiotic potential of five lactic acid bacteria and their antimicrobial activity against some enteric and food-borne pathogens". African Journal of Microbiology Research Vol. 10(12), pp. 400-409.
- Sarkar, S. (2007). "Potential of kefir as a dietetic beverage—A review". British Journal of Nutrition, 109, 280–290
- Sarkar, S. (2008)" Biotechnological innovations in kefir production: a review". British Food Journal 110, 283- 295

- Schrezenmeir, J. and de Vrese, M. (2001). "Probiotics, prebiotics, and synbiotics-approaching a definition". Am. J. Clin. Nutr. 73, 361S-364S.
- Salminen, S. (1990). "The role of intestinal microbiota in preserving intestinal integrity and health with reference to lactic acid bacteria and med 22, 42.
- Salminen, S., A. Ouwehand, Y. Benno, Y.K. Lee. (1999). "Probiotics: how should they be defined? "Trends Food Sci. Techn. 10, 107-110
- Savage D C., S. G. Lundeen and L. T. O'Connor,(1995). "Mechanisms by which Indigenous Microorganisms Colonise Epithelial Surfaces as a Reservoir of the Luminal Microflora in the Gastrointestinal Tract "Microecology Terminology, Vol. 21, pp. 27-36.
- Sepp, E., Smidt, I., Štšepetova, J., Rööp, T., Hütt, P., Rätsep, M., & Mikelsaar, M. (2018). "The effect of *Lactobacillus fermentum* ME-3 on the intestinal microbiota and urine polyamines content: A double-blind placebo-controlled pilot trial". Journal of Functional Foods, (48)430–438.
- Severijnen, R., Naber, T., Tolboom, J., & Bongaerts, G. (2005). "The role of heterolactic lactobacilli in diarrhoea of short small bowel patients". Microbial Ecology in Health and Disease, 17(2), 88–93.
- Scheinbach, S. (1998). "Probiotics: functionality and commercial status". Biotech Adv 16, 581–608.
- Shah NP (2007) "Functional cultures and health benefits". Int Dairy J 17: 1262–1277.
- Shafakatullah N, Chandra M. (2015). "Isolation of lactic acid bacteria from *Allium cepa* var. *aggregatum* and study of their probiotic properties". International Journal of Pharma Sciences and Research 6, 749-757.

- Shavit, E. (2008). "Renewed interest in kefir, the ancient elixir of longevity". *Fungi*, 1(2)
- Shivram, P. L. and P.P. Vishwanath, (2012). "Assessment of probiotic potential of *Lactobacillus* sp. isolated from cheese and preparation of probiotic ice-cream". *IJRAP*, 3 (4): 532 – 536.
- Simova, E., Beshkova, D., Angelov, A., Hristozova, T., Frengova, G., & Spasov, Z. (2002). "Lactic acid bacteria and yeasts in kefir grains and kefir made from them". *Journal of Industrial Microbiology and Biotechnology*, 28(1), 1–6.
- Skerman, V. B. D., McGowan, V., & Sneath, P. H. A. (1980). "Approved lists of bacterial names". *International Journal of Systematic Bacteriology*, 30, 225–420.
- Slattery C. , Paul D. Cotter and Paul W. O .Toole. (2019). "Analysis of Health Benefits Conferred by *Lactobacillus* Species from Kefir" . *Nutrients* 11, 1252
- Sömer V. F., AkpınarD. , Başığit Kılıç G. (2012). "The effects of *Lactobacillus casei* on health and it is usage in food industry" . *GIDA* 37 (3): 165-172
- Sudhanshu S. Behera, Ramesh C. Ray , and Nevijo Zdolec (2018) . "*Lactobacillus plantarum* with Functional Properties: An Approach to Increase Safety and Shelf-Life of Fermented Foods" . *BioMed Research International*, Article ID 9361614, 18 pages
- Sun, Z. L. et al., (2010). "Identification and characterization of the Dominant Lactic Acid Bacteria Isolated from Traditional Fermented Milk in Mongolia". *Folia Microbiology*, pp. 55(3) 270-276.
- Suskovic, J., Brkic, B., Matosic, S. and Maric, V. (1997). "*Lactobacillus acidophilus* M92 as potential probiotic strain". *Milchwissenschaft*. 52, 430-435.

- Srinu. B., T. Madhava Rao, P. V. Mallikarjuna Reddy and K. Kondal Reddy, (2013). "Evaluation of different lactic acid bacterial strains for probiotic characteristics". *Veterinary World*, EISSN, 6: 785- 788.
- Sreeramulu G., D.S. Srinivasa, K. Nand and R. Joseph. (1996). "*Lactobacillus amylovorus* as a phytase producer in submerged culture". *Letters in Applied Microbiology*, 23, 385-388
- Stackebrandt, E. & Goebel, B. M. (1994). "Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology". *Int J Syst Bacteriol* 44, 846– 849.
- Stolarczyk A, Socha P, Socha R (2002) "Probiotics and prebiotics in the prevention and treatment of diseases in children". *Terapia* 116: 39-42
- Tadesse, G., Ephraim, E., & Ashenafi, M. (2005). "Assessment of the antimicrobial activity of lactic acid bacteria isolated from Borde and Shamita, traditional Ethiopian fermented beverages, on some foodborne". *Journal of Food Safety*, 5(5), 13–20.
- Tambekar, D.H. and Bhutada, S.A. (2010) "An evaluation of probiotic potential of *Lactobacillus* sp. from milk of domestic animals and commercial available probiotic preparations in prevention of enteric bacterial infections". *Recent Research in Science and Technology*. 2: 82-88.
- Tao Wu, Yutao Shi, Yanyan Zhang et al. (2020) "Beneficial Effects of *Lactobacillus zae* on the Gut Health of Piglets Challenged with Enterotoxigenic *Escherichia Coli*", PREPRINT (Version 1) available at Research Square.
- Toh H, Oshima K, Nakano A, Takahata M, Murakami M, et al. (2013). "Genomic Adaptation of the *Lactobacillus casei* Group". *PLoS ONE* 8(10).

- Tuohy, K.M., H.M. Probert, C.W. Smejkal, G.R. Gibson. (2003). "Review: Using probiotics and probiotics to improve gut health". *Drug Discov. Today* 8, 692-700.
- Valentina T. and Simone Guglielmetti. (2012) "Health-promoting properties of *Lactobacillus helveticus*". Division of Food Microbiology and Bioprocesses, Department of Food Environmental and Nutritional Sciences
- VASILEAN, I., SEGAL, R. and VASILE, A. (2011). "Obtaining fermented dairy products with the yogurt culture" *Food Technology* 35, 92–99.
- Vandamme, P., Pot, B., Gillis, M., de Vos, P., Kersters, K., and Swings, J. (1996). "Polyphasic taxonomy, a consensus approach to bacterial systematics". *Microbiology Reviews*. 60: 407-438.
- Van Hoorde K, Verstraete T, Vandamme P, Huys G (2008) "Diversity of lactic acid bacteria in two Flemish artisan raw milk Gouda-type cheeses". *Food Microbiol* 25:929–935
- Vichko O, Chervetsova V, Novikov V (2013) "Microbiological characteristics of sour-milk feed supplements and their influence on intestinal micro-biocenosis of piglets". *Res J Pharm Biol Chem Sci* 4:1404–1410
- Vizoso Pinto, M.G., Franz, C.M.A.P., Schillinger, U., Holzapfel, W., (2006). "*Lactobacillus* spp. With in vitro probiotic properties from human faeces and traditional fermented products". *Int. J. Food Microbiol.* 109, 205–214.
- Wang, S. Y., Chen, H. C., Liu, J. R., Lin, Y. C., & Chen, M. J. (2008). "Identification of yeasts and evaluation of their distribution in Taiwanese kefir and viili starters". *Journal of Dairy Science*, 91(10), 3798–3805.

- Wang,S.Y.,Chen, K. N., Lo,Y.M.,Chiang, M. L., Chen, H. C., Liu, J. R., et al. (2012) "Investigation of microorganisms involved in biosynthesis of the kefir grain". *Food Microbiol.* 32, 274–285
- Wessels, S., Axelsson, L., Bech Hansen, E., De Vuyst, L., Laulund, S., Lihteenmiki, L., Lindgren, S., Mollet, B., Salminen, S., and von Wright, A. (2004). "The lactic acid bacteria, the food chain, and their regulation". *Trends in Food Science and Technology.* 15: 498-505.
- Witthuhn RC, Schoeman T, Britz TJ. (2004). "Characterization of the microbial population at different stages of kefir production and kefir grain mass cultivation". *Int Dairy J*, 15: 383-389
- Witthuhn, R. C., Schoeman, T., & Britz, T. J. (2005). "Characterisation of the microbial population at different stages of Kefir production and Kefir grain mass cultivation". *International Dairy Journal*, 15(4), 383–389.
- Wittwer C.T .(2009). "High-resolution DNA melting analysis: Advancements and limitations". *Hum Mutat* 30: 857-859.
- Wood BJB, Holzapel WH (1995). "The Genera of lactic acid bacteria" Blackie Academic & Professional, Glasgow, vol. 2.
- Wong, M. L., & Medrano, J. F. (2005). "Real-time PCR for mRNA quantitation". *Biotechniques*, 39, 1, 75-85.
- Wszolek M., Kupiec-Teahan B., Skov Guldager H. and Tamime A.Y. (2006). "Production of Kefir, Koumiss and Other Related Products". In A. Y. Tamime (Ed.), *Fermented Milks* (pp. 174-216). Ayr, UK: Blackwell Science.
- Wyder, M.T., Bachmann, H.P. and Puhon, Z. (1999). "Role of selected yeasts in cheese ripening: An evaluation in foil wrapped Raclette cheese". *Lebensmittel-Wissenschaft & Technologie* (32)333–343.

- Xanthopoulos V, Litopoulou-Tzanetaki E, Tzanetakis N. (1997). "Lactic acid bacteria". Caen Presses Universitaires de Canada.
- Xiao, L.L., Dong, M.S., (2003). "Screening and cholesterol-degrading activity of *Lactobacillus casei* KM-16". China Dairy Industry 31, 7–10.
- Xiao Qun Zeng . Dao Dong Pan . Pei Dong Zhou (2011). "Functional Characteristics of *Lactobacillus fermentum* F1". Curr Microbiol (2011) 62:27–31
- Xue C, Zhang L, Fan R, Wang S, Li H, Luo X, Liu W, Song W (2015) "Protective action of S-layer proteins from *Lactobacillus paracasei* M7 against Salmonella infection and mediated inhibition of Salmonella-induced apoptosis". Eur Food Res Technol 240:923– 929.
- Yüksekdağ, Z. N., Beyatli, Y., & Aslim, B. (2004). "Determination of some characteristics coccoid forms of lactic acid bacteria isolated from Turkish kefir with natural probiotic". LWT-Food Science and Technology, 37(6), 663-667.
- Yavuzdurmaz H. (2007). "Isolation, Characterization, Determination Of Probiotic Properties Of Lactic Acid Bacteria From Human Milk", School Of Engineering And Sciences Of İzmir Institute Of Technology Thesis Of Master of Science. (October).
- Yu, J., Z. Sun, W. Liu, Q. Bao, J. Zhang, and H. Zhang. (2012)." Phy-logenetic study of *Lactobacillus acidophilus* group, *L. casei* group and *L. plantarum* group based on partial hsp60, pheS and tuf gene sequences". Eur. Food Res. Technol. 234:927–934.
- Yuwono, S.D. and Kokugan, T. (2008). "Study of the Effects of Temperature and pH on Lactic Acid Production from Fresh Cassava Roots in Tofu Liquid Waste by *Streptococcus bovis*". Biochemical Engineering Journal, 40, 175-183.

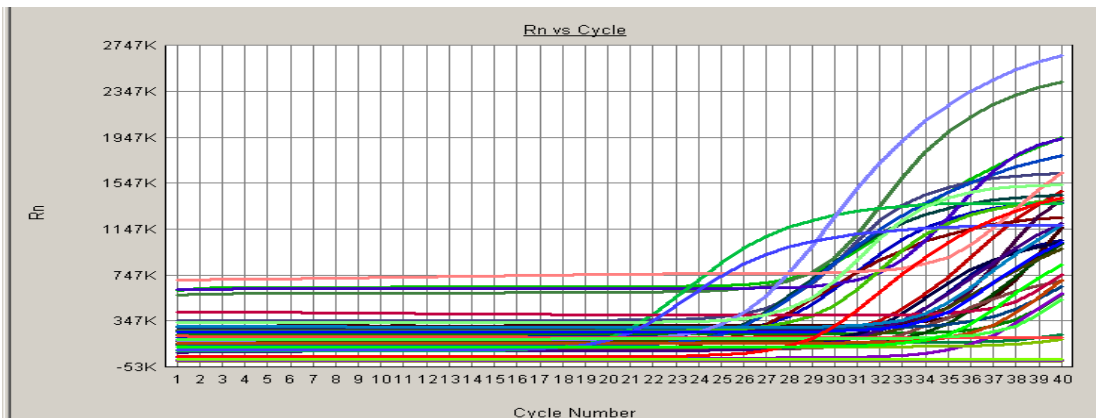
- Zanirati, D.F., Abatemarco, M., Cicco Sandesb, S.H., Nicolia, J.R., Nunes, A.C., and Neumann E. (2015). "Selection of lactic acid bacteria from Brazilian kefir grains for potential use as starter or probiotic cultures". *Anarobe* 32, 70–76.
- Zhou, J., X. Liu, H. Jiang, and M. Dong. (2009). "Analysis of the microflora in Tibetan kefir grains using denaturing gradient gel electrophoresis". *Food Microbiol.* 26:770-775
- Zubeyde, O., Aynur, K., & Lutfu, C. M. (2010). "Effects of Different Milk Types and Starter Cultures on Kefir " *Gida*, 35(3), 177–182.



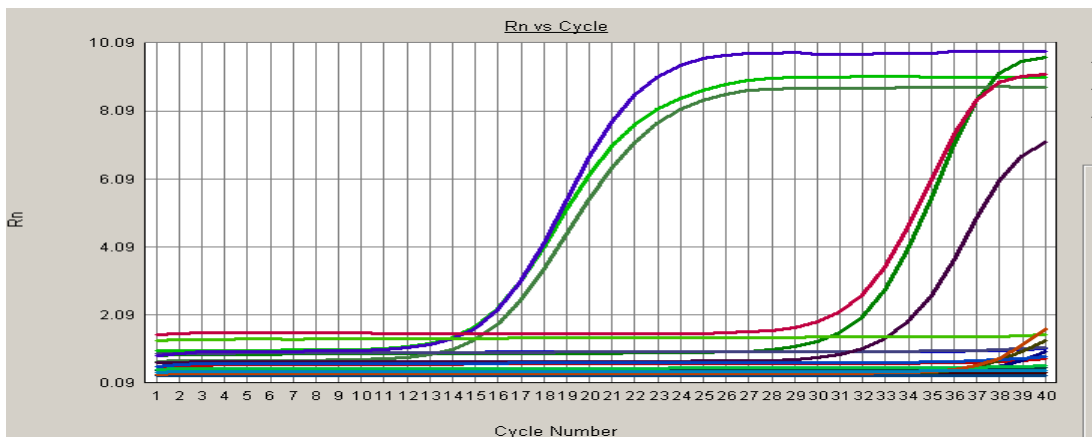
APPENDICES

APPENDIX A. Real Time PCR Results

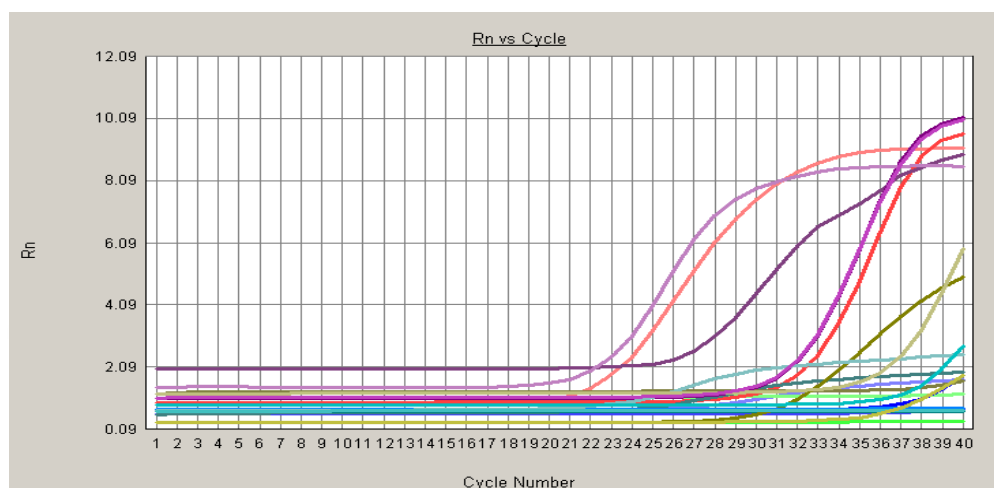
A.1 Amplification curves of kefir sample K1, K2 DNA isolate with specific primers (*L. Kefiri* , *L. acidophilus* ,*L. delbrueckii subsp. bulgaricus* ,*L. kefiranofaciens*, *L. fermentum* , *L. plantarum* , *S. cerevisiae* , *S. thermophilus* , *L. sobris \amylovorus*



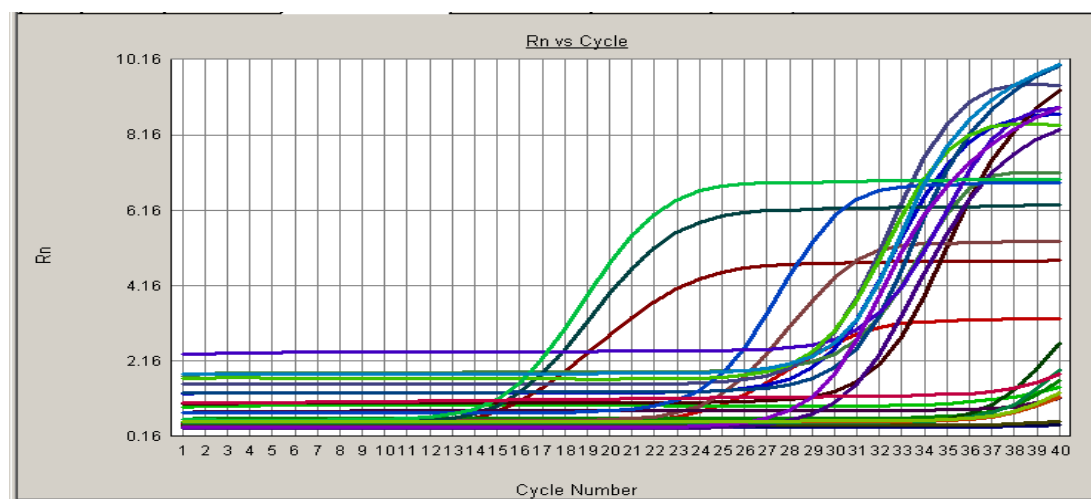
A.2 Amplification curves of kefir sample k3 DNA isolate with specific primers (*L. delbrueckii subsp. bulgaricus* ,*L. kefiranofaciens*, *L. fermentum* , *L. plantarum* , *S. cerevisiae* , *S. thermophilus* ,*L. sobris \amylovorus*)



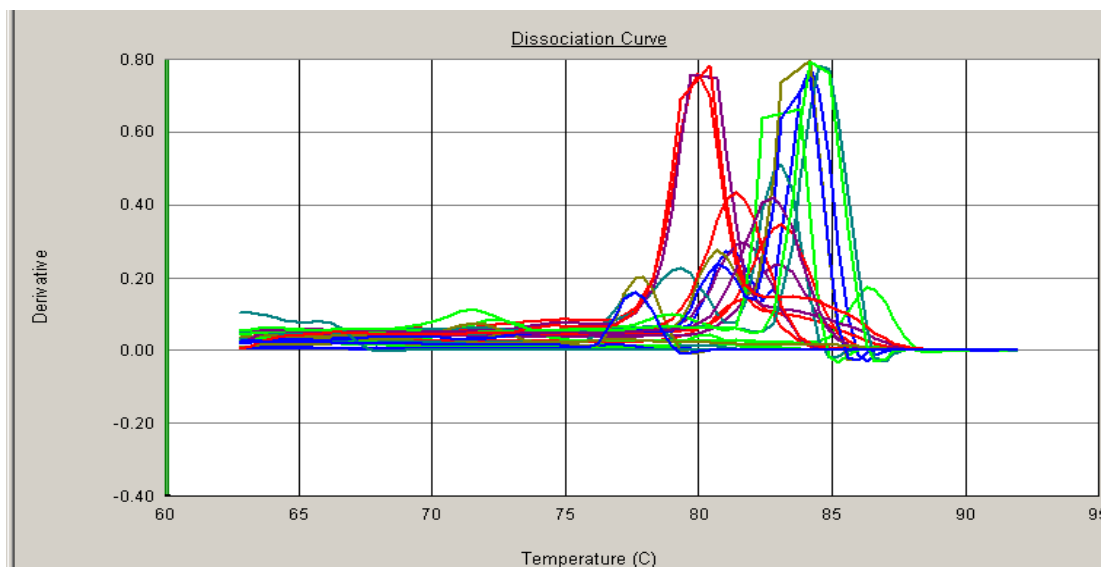
A.3 Amplification curves of kefir sample k4 DNA isolate with specific primers (*L. delbrueckii* subsp. *bulgaricus* , *L. kefiranofaciens*, *L. fermentum* , *L. plantarum* , *S. cerevisiae* , *S. thermophilus* , *L. sobris \amylovorus*)



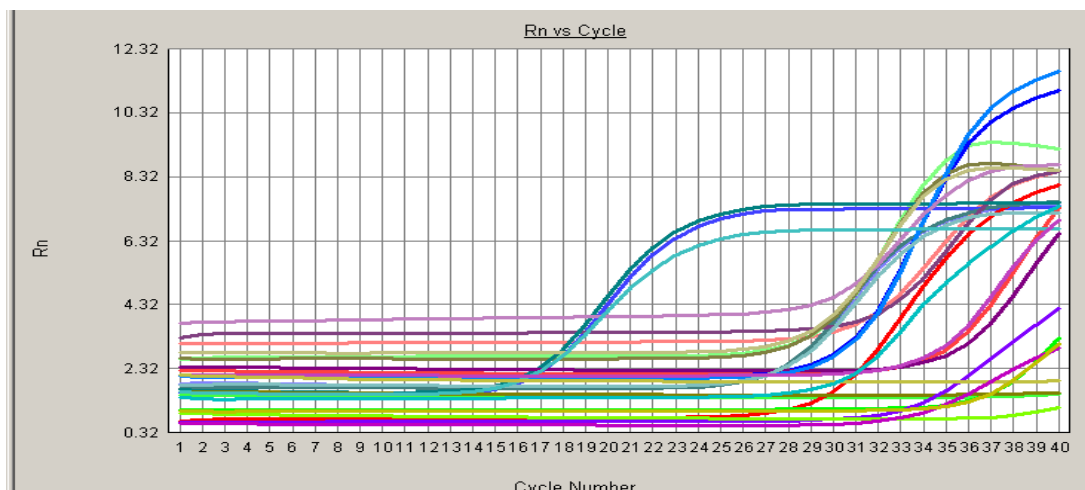
A.4 Amplification curves of kefir sample K5 DNA isolate with specific primers (*L. Kefiri* , *L. acidophilus* , *L. delbrueckii* subsp. *bulgaricus* , *L. kefiranofaciens*, *L. fermentum* , *L. plantarum* , *S. cerevisiae* , *S. thermophilus* , *L. sobris \amylovorus*)



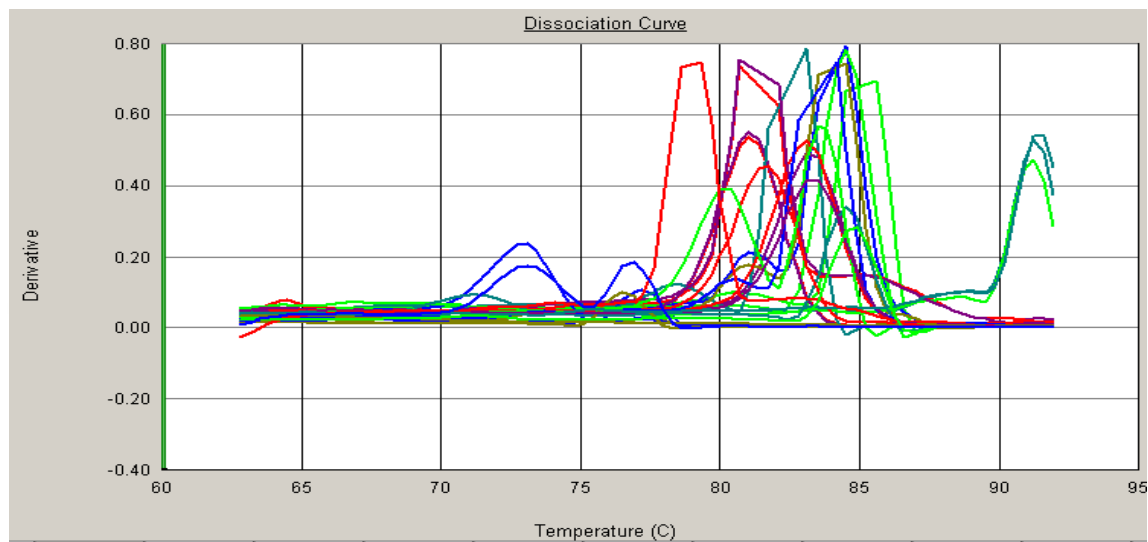
Melting curves of kefir sample (K5 and K6) DNA isolate with specific primers (*L. Kefiri* , *L. acidophilus* , *L.delbrueckii subsp. bulgaricus* , *L. kefiranofaciens*, *L. fermentum* , *L. plantarum* , *S. cerevisiae* , *S. thermophilus* , *L.sobris* \amylovorus



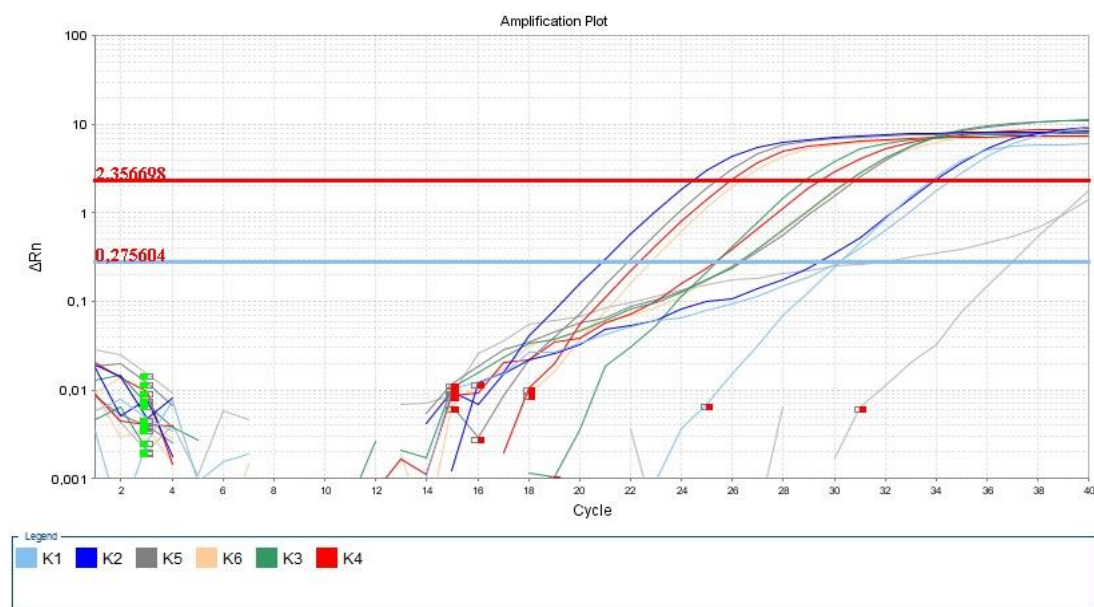
A.5 Amplification curves of kefir sample K6 DNA isolate with specific primers (*L. Kefiri* , *L.acidophilus* , *L.delbrueckii subsp. bulgaricus* , *L.kefiranofaciens*, *L.fermentum* , *L.plantarum* , *S.cerevisiae* , *S.thermophilus* , *L.sobris* \amylovorus



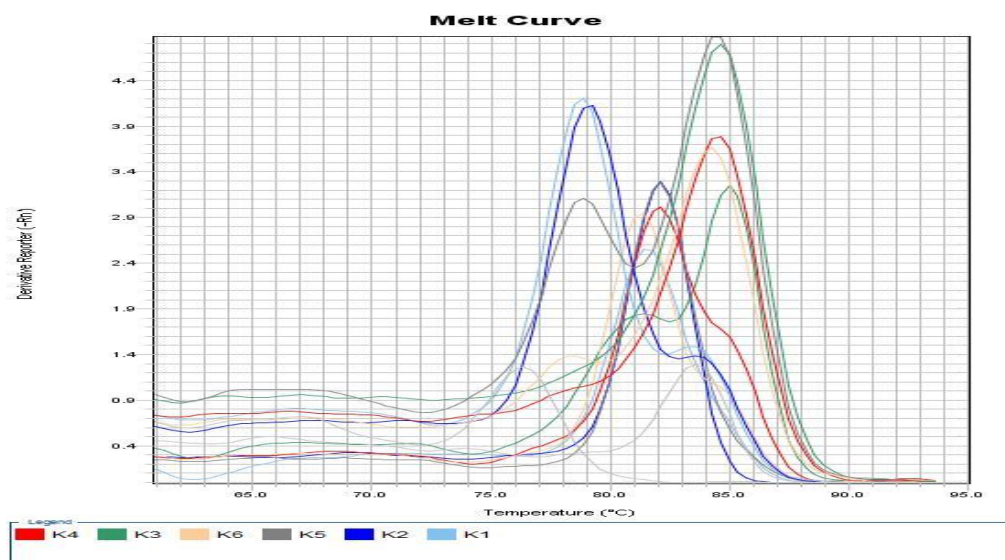
Melting curves of kefir sample K6 DNA isolate with specific primers (*L. Kefiri* , *L.acidophilus*, *L.delbrueckii subsp. bulgaricu* ,*L.kefiranofaciens*, *L .fermentum* , *L. plantarum* , *S.cerevisiae* , *S.thermophilu* ,*L.sobris \amylovorus*



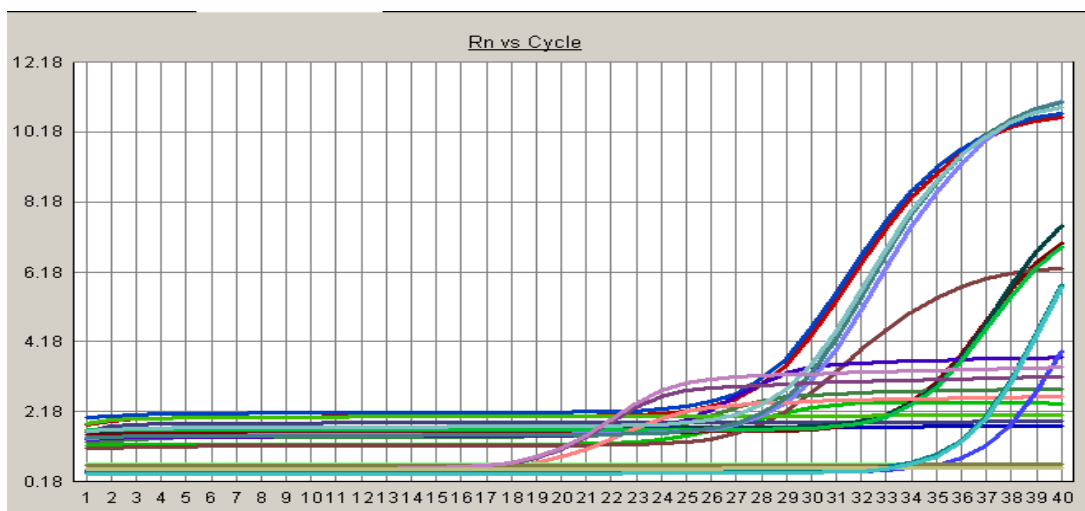
A.6 Amplification curves of kefir sample K1,K2 ,K3 , K4 ,K5, K6 DNA isolate with specific primers (*L. casei* and *L. paracasei*)



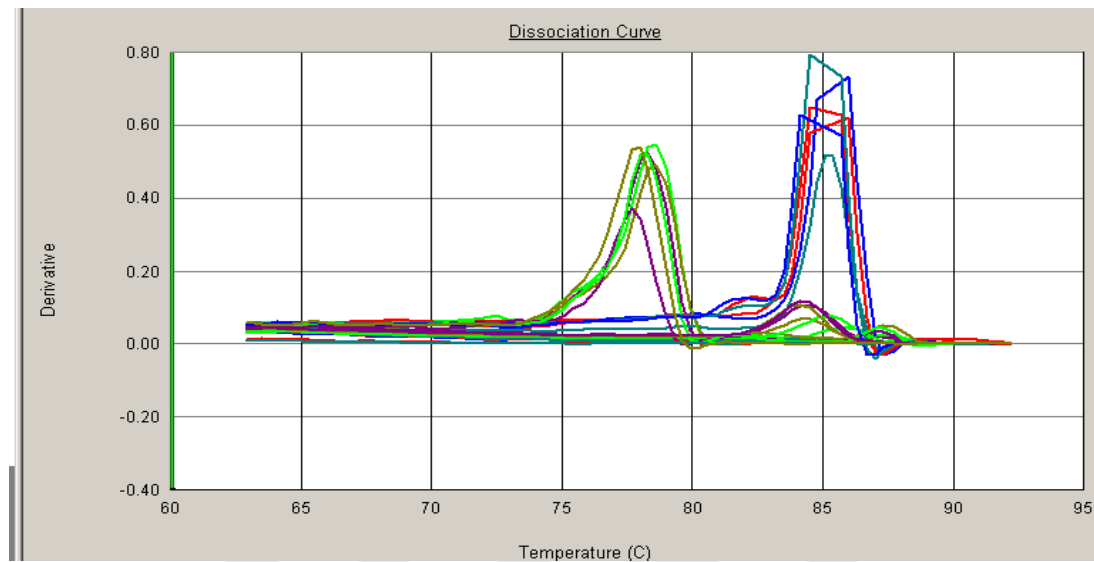
Melting curves of kefir sample K1,K2 ,K3 , K4 ,K5, K6 DNA isolate with specific primers (*L. casei* and *L. paracasei*)



A.7 Amplification curves of kefir sample K1 and K2 DNA isolate with specific primers (*L. delbrueckii subsp. bulgaricus* ,*L. kefiranofaciens*, *L. fermentum* , *L. plantarum*)



Melting curves of kefir sample K1 and K2 DNA isolate with specific primers (*L. delbrueckii* subsp. *bulgaricus* ,*L. kefiranofaciens*, *L. fermentum* , *L. plantarum*)



APPENDIX B

B. 16 S ribosomal RNA gene partial sequence

B.1 16 S ribosomal RNA gene partial sequence for k2-2a

CGAGCGAGCAGAACCAGCAGATTTACTTCGGTAATGACGCTGGGGACGCGAGCGG
CGGATGGGTGAGTAACACGTGGGGAACCTGCCCCATAGTCTAGGATACCACTTGGA
AACAGGTGCTAATACCGGATAATAAAGCAGATCGCATGATCAGCTTATAAAAGGC
GGCGTAAGCTGTCGCTATGGGATGGCCCCGCGGTGCATTAGCTAGTTGGTAAGGTA
ACGGCTTACCAAGGCAATGATGCATAGCCGAGTTGAGAGACTGAACGGCCACATT
GGGACTGAGACACGGCCCAAACCTCCTACGGGAGGCAGCAGTAGGGAATCTTCCAC
AATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTTCGGATC
GTAAAGCTCTGTTGTTGGTGAAGAAGGATAGAGGTAGTAACTGGCCTTTATTTGAC
GGTAATCAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT
AGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGAAGAA
TAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATTGCATCGGAAACTGTTTT
TCTTGAGTGCAGAAGAGGAGAGTGGAACCTCCATGTGTAGCGGTGGAATGCGTAGA
TATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGACGCTGAG
GCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAA
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TAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACG
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TTACCAGGTCTTGACATCTAGTGCCATCCTAAGAGATTAGGAGTTCCCTTCGGGGA
CGCTAAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCTGTGAGATGTTGGGTTA
AGTCCCGCAACGAGCGCAACCCTTATTATTAGTTGCCAGCATTAAGTTGGGCACTC
TAATGAGACTGCCGGGGACAAACCGGAGGAAGGGGGGGATGACGCCAAGCCACTT
GCCCCTATGACCGGGGCAACC

B.2 16 S ribosomal RNA gene partial sequence for k2-3

GGCTCCTTCCGAAGGTTAGGCCACCGGCTTTGGGCATTGCAGACTTCCATGG
TGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTTCT
GATCCGCGATTACTAGCGATTCCAGCTTCGTGCAGTCGAGTTGCAGACTGC
AGTCCGAAGTGAAGAACAGCTTTCAGAGATTGCTTGCCTTCGCAGGCTCGCT
TCTCGTTGTACTGCCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCA
TGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTACCGGCAGTCTCA
TTAGAGTGCCCAACTTAATGCTGGCAACTAATAATAAGGGTTGCGCTCGTT
GCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCACC
ACCTGTCTTAGCGTCCCCGAAGGGAACCTCCTAATCTCTTAGGATGGCACTAG
ATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCT
CCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTGCGGTC
GTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTGAGAGGCGG
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TCTAATCCTGTTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAGACC
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ATGCAATTCCTCGGTTAAGCCGAGGGCTTTCACATCAGACTTATTCTTCCGC
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TACCGCGGCTGCTGGCACGTATTTAGCCGTGACTTTCTGGTTGATTACCGTC
AAATAAAGGCCAGTTACTACCTCTATCCTTCTTCACCAACAACAGAGCTTTA
CGATCCAAAACCTTCTTCACTCAGGCGGCGTTGCTCCATCAAACCTTGCGTCC
ATTGTGGAAGATTCCCTACTGCTGCCCT

B.3 16 S ribosomal RNA gene partial sequence for k2-3a

ACCTGCAAGTCGAGCGAGCAGAACCAGCAGATTTACTTCGGTAATGACGCT
GGGGACGCGAGCGGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCCAT
AGTCTAGGATAACCACTTGGAACAGGTGCTAATACCGGATAATAAAGCAGA
TCGCATGATCAGCTTATAAAAGGCGGCGTAAGCTGTCGCTATGGGATGGCC
CCGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAATGATG
CATAGCCGAGTTGAGAGACTGAACGGCCACATTGGGACTGAGACACGGCCC
AAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTC
TGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTTCGGATCGTAAAGCTCT
GTTGTTGGTGAAGAAGGATAGAGGTAGTAACTGGCCTTTATTTGACGGTAA
TCAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTA
GGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGAA
GAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATTGCATCGGAA
ACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAAGTCCATGTGTAGCGGT
GGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGT
CTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATA
CCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGTTTCC
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CCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCCGCACAAGCGGTGG
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TCTAGTGCCATCCTAAGAGATTAGGAGTTCCTTCGGGGACGCTAAGACAG
GTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTAAAGTCCCG
CAACGAGCGCAACCCTTATTATTAGTTGCCAGCATTAAAGTTGGGCACTCTAA
TGAGACTGCCGGTGACAAACCGGAGGAAGGGGG

B.4 16 S ribosomal RNA gene partial sequence for k2-4

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CATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAA
GTGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAAC
CGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCC
GCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACG
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ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTG
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TTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAA
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TGTAACCTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATAC
CCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCG
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CGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCTGAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT
CTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGG
TGGTGCATGGTTGTCGTCAGCTCGTGTCTGAGATGTTGGGTAAAGTCCCGC
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AAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATC
ATGCCCCTTATGACCTGGGCTACCACGTGCTACATGGATGGTACAACGAGT
TGCGAGACCGCGAGGTCAGCTAATCCTTAAAGCCTTTCCAGTTCGGACTGT
AGGCTGCACTCGCCTACCCAAATCGGAATCCCTAGTA

B.5 16 S ribosomal RNA gene partial sequence for k2-4a

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GCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCACC
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TACCGCGGCTGCTGGCACGTATTTAGCCGTGACTTTCTGGTTGATTACCGTC
AAATAAAGGCCAGTTACTACCTCTATCCTTCTTCCCAACAACAAAACCTTTAC
AATCCAAAAACCTTCTTCACTCAGGCGGCGTTGCTCCCTCAAACCTTGCGTCC
ATTGGGGAAAAATTCCCTACTGCTT

B.6 16 S ribosomal RNA gene partial sequence for k2-14

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GTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTTC
TGATCCGCGATTACTAGCGATTCCAGCTTCGTGCAGTCGAGTTGCAGACTGC
AGTCCGAAGTGAAGAACAGCTTTCAGAGATTCGCTTGCCTTCGCAGGCTCGCT
TCTCGTTGTACTGCCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCA
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TTAGAGTGCCCAACTTAATGCTGGCAACTAATAATAAGGGTTGCGCTCGTT
GCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCACC
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ATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCT
CCACCGCTTGTGCGGGCCCCCGTCAATTCTTTGAGTTTCAACCTTGCGGTC
GTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTGAGAGGCGG
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AAATAAAGGCCAGTTACTACCTCTATCCTTCTTCACCAACAACAAAGCTTTA
CGATCCGAAAACCTTCTTCACTCACGCGGCGTTGCTCCATCAAACCTTGCGTC
CATGGGGAAGATTCCCTACTGCAC

B.7 16 S ribosomal RNA gene partial sequence for K3-2a

ACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACGA
GTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGATA
ACATTTGGAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTC
TTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGCGGCGTAT
TAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACGTAGCCGAAC
TGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACCTCCTACG
GGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCA
ACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAG
AAGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCCAACCAGAA
AGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGC
GTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTG
ATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAAACT
TGAGTGCAGAAGAGGACAGTGGAACCTCATGTGTAGCGGTGAAATGCGTA
GATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACCTGA
CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATAACCCTGGTAGT
CCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGT
GCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTT
GAAACTCAAAGGAATTGACGGGGGCCCCGCACAAGCGGTGGAGCATGTGGT
TTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTTTTGATCA
CCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGTGGTGCATGG
TTGTCGTCAGCTCGTGTCTGAAATGTTGGGTAAAGTCCCGCAACGAGCGCA
ACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTTTAGTAAGACGGCCG
GTGACAAACCGGAGGAAGGGGGGGGATGACGTCGG

B.8 16 S ribosomal RNA gene partial sequence for k3-3a

GAGTTGATCCTGGATCAGAGAAGACCCAACAAAGTTACCTCAGAAATGACG
CTGGGGACGCGAGCGGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCC
ATAGTCTAGGATACCACTTGGAAACAGGTGCTAATACCGGATAATAAAGCA
GATCGCATGATCAGCTTATAAAAGGCGGCGTAAGCTGTCGCTATGGGATGG
CCCCGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAATGA
TGCATAGCCGAGTTGAGAGACTGAACGGCCACATTGGGACTGAGACACGGC
CCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAG
TCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTTCGGATCGTAAAGCT
CTGTTGTTGGTGAAGAAGGATAGAGGTAGTAACTGGCCTTTATTTGACGGT
AATCAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACG
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GTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGA
TACCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGTTT
CCGCCTCTCAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTAC
GACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGGCCCGCACAAGCGGT
GGAGCATGTGGTTTAATTCTGAAGCAACGCGAAGAACCTTACCAGGTCTTGA
CATCTAGTGCCATCCTAAGAGATTAGGAGTTCTCTTCGGGGACGCTAAGAC
AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTAAAGTCC
CGCAACGAGCGCAACCCTTATTATTAGTTGCCAGCATTAAAGTTGGGCACTCT
AATGAGACTGCCGGTGACAAACCGGAGGAAAGGGGGGGATGACGTCAAGT
CATCCGGCCCC

B.9 16 S ribosomal RNA gene partial sequence for k3-8b2

TGCAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAAC
ATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAG
TGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAACC
GCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCC
GCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACG
TAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAA
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTG
ATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGT
TGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCC
AACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG
GTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTT
TTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAA
CTGGGAACTTGAGTGCAGAAGAGGACAGTGGAATCCATGTGTAGCGGTG
AAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTC
TGTAAGTACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATAC
CCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCG
CCCTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGAC
CGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT
CTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAATGACAGG
TGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTAAAGTCCCGC
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AAGACTGCCGGTGACAACCGGAGGAAGGTGGGGATGACGTCAATCATCAT
GCCCCTTATGACCTGGGCTACCACGTGCTACATGGATGGTACACGAGTTGC
GAGACGCGAGGTCAGCTAATCCTTAAAGCCTTTCCAGTTCGGCTGTAGGCT
GCACCCCCTAACAAATCGGA

B.10 16 S ribosomal RNA gene partial sequence for k3-13b

AGTGTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACGAGT
GGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGATAAC
ATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTT
GGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTA
GCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACGTAGCCGAAC TG
AGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAAC TCCTACGGG
AGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAAC
GCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAGAA
GAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCCAACCAGAAAG
CCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGT
TATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGAT
GTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAAACTTG
AGTGCAGAAGAGGACAGTGGAAC TCCATGTGTAGCGGTGAAATGCGTAGA
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CGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGA
AACTCAAAGGAATTGACGGGGGCCCCGCACAAGCGGTGGAGCATGTGGTTTA
ATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTTTTGATCACCT
GAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGTGGTGCATGGTTG
TCGTCAGCTCGTGTCTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC
CCTTATGACTAGTTGCCAGCATTTAGTTGGGGCACTCTAGTAAGAACTGCCG
GGTGACAAACCGGGAAGGAAGGTGGGGGATGACGTCCAAATCCTCTTGCCC
CCTTTAGGAACCTGGGGCTAACCACCGTGCTAACAAGGGAAGGGTAAAA

B11. 16 S ribosomal RNA gene partial sequence for k3-20

GCTCGCTCCCTAAAAGGGTTACGCCACCGGCTTCGGGTGTTACAACTCTCA
TGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGT
GCTGATCCGCGATTACTAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCT
ACAGTCCGAAGTGAAGAATGGCTTTAAGAGATTAGCTTGACCTCGCGGTCTC
GCAACTCGTTGTACCATCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGG
GGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTACCGGCAGT
CTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGCT
CGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATG
CACCACCTGTCATTTTGCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCA
AAAGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCAC
ATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTG
CGGTCGTACTCCCCAGGCGGAATGCTTAATGCGTTAGCTGCGGCACTGAAG
GGCGGAAACCCTCCAACACCTAGCATTTCATCGTTTACGGCATGGACTACCA
GGGTATCTAATCCTGTTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTAC
AGACCAGACAGCCGCCTTCGCCACTGGTGTTCCTTCATATATCTACGCATTT
CACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGT
TTCCGATGCGCTTCCTCGGTAAAGCCGAGGGCTTTCACATCAGACTTAAAAA
ACCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTTGCCACCTA
CATATTACCGCGGCTGCTGGCACGTAATTAGCCGTGGCTTTCTGGTTGGATA
CCGTCACGCCAACAACAATCA

B.12 16 S ribosomal RNA gene partial sequence for k3-20a

GAGTGTCTCGGTGATGATCGGTGCTTGCACCGAGATTCAACATGGGAACGA
GTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATA
ACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTC
TTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGCGGCGTAT
TAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACGTAGCCGAAC
TGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCCAACTCCTACG
GGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCA
ACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGAG
AAGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCCAACCAGAA
AGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGC
GTTATCCGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTG
ATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAAACT
TGAGTGCAGAAGAGGACAGTGGAATCCATGTGTAGCGGTGAAATGCGTA
GATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAGTGA
CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGT
CCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAT

B.13 16 S ribosomal RNA gene partial sequence for k3-28

TGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAA
CATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAA
GTGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAAC
CGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCC
GCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACG
TAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAA
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTG
ATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGT
TGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCC
AACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG
GTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTT
TTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAA
CTGGGAACTTGAGTGCAGAAGAGGACAGTGGAATCCATGTGTAGCGGTG
AAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTC
TGTAAGTACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATAC
CCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCG
CCCTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGAC
CGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT
CTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGG
TGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTAAAGTCCCGC
AACGAGCGCAACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGT
AAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATC
ATGCCCCTTATGACCTGGGCTACCACGTGCTACATGGATGGTACAACGAGT
TGCGAGACCCGAGGTCAGCTAATCCTTAAGCCATTCCAATTCGGATGTAGG
CTGAAC

B.14 16 S ribosomal RNA gene partial sequence for k4-6a

TGCAAGTCGAGCGAGCAGAACCAGCAGATTTACTTCGGTAATGACGCTGGG
GACGCGAGCGGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCCATAGT
CTGGGATAACCACTTGGAAACAGGTGCTAATACCGGATAAGAAAGCAGATCG
CATGATCAGCTTATAAAAGGCGGCGTAAGCTGTCGCTATGGGATGGCCCCG
CGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAATGATGCAT
AGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGCCCAA
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTG
ATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTTCGGATCGTAAAGCTCTGT
TGTTGGTGAAGAAGGATAGAGGCAGTAACTGGCCTTTATTTGACGGTAATC
AACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGG
TGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGAAGA
ATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAACTGCATCGGAAAC
TGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGA ACTCCATGTGTAGCGGTGG
AATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGACTCTCTGGTCT
GCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATAC
CCTGGTAGTCCATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGTTTCCG
CCTCTCAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGAC
CGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCTGAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT
CTAGTGCCATCCTAAGAGATTAGGAGTTCCCTTCGGGGACGCTAAGACAGG
TGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTAAAGTCCCGC
AACGAGCGCAACCCTTGTTATTAGTTGCCAGCATTAAGTTGGGCACTCTAAT
GAAACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATC
AGCCCCTTAGGACCTGGGCTACCCCGTGCTACATGGGAAAGTACACCGGAA
GCGAGCCTGG

B.15 16 S ribosomal RNA gene partial sequence for k4-16

GCTTGCACCGAGATTCAACATGGAACGAGTGGCGGACGGGTGAGTAACAC
GTGGGTAACTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATGCTAAT
ACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGTAAGC
TATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGC
TCACCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTG
GGACTGAGACACGGCCCAAACCTACGGGAGGCAGCAGTAGGGAATCTTC
CACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCT
TTCGGGTCGTAAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACTG
TTGCCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAG
CAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTA
AAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAAC
CGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTG
GAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTG
GCGAAGGCGGCTGTCTGGTCTGTAAGTACGCTGAGGCTCGAAAGCATGGG
TAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAATGC
TAGGTGTTGGAGGGTTTCCGCCCTTCATTGCCGCATACTAACGCATAAGCGT
TCCACCTGGGCGGTTC

B 16 . 16 S ribosomal RNA gene partial sequence for k4-17

GCTCGCTCCCTAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCAT
GGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTG
CTGATCCGCGATTACTAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTA
CAGTCCGAACCTGAGAATGGCTTTAAGAGATTAGCTTGACCTCGCGGTCTCG
CAACTCGTTGTACCATCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGG
GCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTACCGGCAGTC
T TACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGCTC
GTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGC
ACCACCTGTCATTTTGCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCA
AAAGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCAC
ATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTG
CGGTCGTACTCCCCAGGCGGAATGCTTAATGCGTTAGCTGCGGCACTGAAG
GGCGGAAACCCTCCAACACCTAGCATTTCATCGTTTACGGCATGGACTACCA
GGGTATCTAATCCTGTTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTAC
AGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTT
CACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGT
TTCCGATGCGCTTCCTCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAA
ACCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTTGCCACCTA
CGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTGGATA
CCGTCACGCCGGCAACAGTTACTCTGCCGACCATTCTTCTCCAACAACAGA
GTTTTTACGACCCGAAAGCCTTCTTCCTCACGCGGCGTTGCTCCATCAAACCTT
GCGTCCATTGGGGATG

B17. 16 S ribosomal RNA gene partial sequence for k4-28

GCAGTCGACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACAT
GGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTG
GGGGATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAACCGC
ATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGC
GGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACGTA
GCCGAAGTGAAGAGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAAC
TCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT
GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAACTCTGTTG
TTGGAGAAGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCCAA
CCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTG
GCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTT
AAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTG
GGAAACTTGAGTGCAGAAGAGGACAGTGGAAGTCCATGTGTAGCGGTGAA
ATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTG
TAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCC
TGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCC
CTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCG
CAAGGTTGAAACTCAAAGGAATTGACGGGGGGCCCGCACAAGCGGTGGAGC
ATGTGGTTTAATTCTGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTT
TTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGTGG
TGCATGGTTGTCGTCAGCTCGTGTCTGAGATGTTGGGTAAAGTCCCGCAAC
GAGCGCAACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAA
GACTGCCGGTGACAAACCGGAGGAAGGGGGGGATGACGTCAAATCATCAT
GCCCCTTATGACTGGGGCTACCCACGTGCCTACAATGGGATGGTACAACCA
AGTTGCCAGAACCGCCGAGGTCAAGCTAATCTCTTAA

B.18 16 S ribosomal RNA gene partial sequence for k5-3

TGCAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAAC
ATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAG
TGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAACC
GCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCC
GCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACG
TAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAA
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTG
ATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGT
TGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCC
AACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG
GTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTT
TTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAA
CTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAATCCATGTGTAGCGGTG
AAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTC
TGTAAGTACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATAC
CCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCG
CCCTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGAC
CGCAAGGTTGAAACTCAAAGGAATTGACGGGGGGCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCTGAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT
CTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGG
TGGTGCATGGTTGTCGTCAGCTCGTGTCTGAGATGTTGGGTAAAGTCCCGC
AACGAGCGCAACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGT
AAGACTGCCGGTGACAAACCGGAGGAAGGTGGGATGACGTCAATCATCAT
GCCCCTTATGACCTGGGCTACCACGTGCTACATGGATGGTACACGAGTTGC
GAGACCCCAGGTCAGCTAATCCTTAAGCCTTTCCAGTTCGGACGTAGGCTG
CACTCCCTACCAAGTCGGATCGCAGTATCCGGATCAC

B.19 16 S ribosomal RNA gene partial sequence for k5-3a

GCCTAATACATGCAAGTCGAACGAGTTCTTGTTGATGATCGGTGCTTGCACC
GAGATTCAACATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAAAC
CTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAG
ATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTT
GGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGG
CGATGATACGTAGCCGAACCTGAGAGGTTGATCGGCCACATTGGGACTGAGA
CACGGCCCCAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGA
CGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGT
AAAACCTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGTCGGCGT
GACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGT
AATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGC
AGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCG
CATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACCTCCATG
TGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCG
GCTGTCTGGTCTGTAACCTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACA
GGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTG
GAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGG
GGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGGCCCGCAC
AAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAG
GTCTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAA
AATGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCTGTGAGATGTTGGGTT
AAGTCCCGCAACGAGCGCAACCCTTATGACTAGTTGCCAGCATTTAGTTGG
GCACTCTAGTAAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACG
TCAAATATCATGCCCCTTATGACCTGGGCTACCCCCGTGCTACAATGGAGG
GACAACGAGTTGCAGACCGGGAGTCAAGCTAATCTTTAAGGCCTTTTTAGT
TCGGACTGGAGGGTGGAACCTGGC

B.20 16 S ribosomal RNA gene partial sequence for k5-5a

TGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAA
CATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAA
GTGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAAC
CGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCC
GCGGCGTATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGATGATACG
TAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAA
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTG
ATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGT
TGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGTCGGCGTGACGGTATCC
AACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG
GTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTT
TTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAA
CTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAATCCATGTGTAGCGGTG
AAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTC
TGTAAGTACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATAC
CCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCG
CCCTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGAC
CGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCTGAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT
CTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGG
TGGTGCATGGTTGTCGTCAGCTCGTGTCTGAGATGTTGGGTAAAGTCCCGC
AACGAGCGCAACCCTTATGACTAGTTGAC

B.21 16 S ribosomal RNA gene partial sequence for k5-11

GGCTCGCTCCCTAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCA
TGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGT
GCTGATCCGCGATTACTAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCT
ACAGTCCGAACCTGAGAATGGCTTTAAGAGATTAGCTTGACCTCGCGGTCTC
GCAACTCGTTGTACCATCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGG
GGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTACCGGCAGT
CTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGCT
CGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATG
CACCACCTGTCATTTTGCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCA
AAAGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCAC
ATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTG
CGGTCGTACTCCCCAGGCGGAATGCTTAATGCGTTAGCTGCGGCACTGAAG
GGCGGAAACCCTCCAACACCTAGCATTTCATCGTTTACGGCATGGACTACCA
GGGTATCTAATCCTGTTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTAC
AGACCAGACAGCCGCCTTCGCCACTGGTGTCTTCCATATATCTACGCATTT
CACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGT
TTCCGATGCGCTTCCTCGGTAAAGCCGAGGGCTTTCACATCAGACTTAAAAA
ACCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTTGCCACCTA
CGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTGGATA
CCGTCACGCCGGCAACAGTTACTCTGCCGACCATTCTTCTCCAACAACAGA
GTTTTACGACCCGAAAGCCTTCTTCACTCAGGCGGCGTTGCTCCATCAAACCT
TGCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAATTTGGGCC
GGGTCTCATTCCCAATGTGGCCAATCAACCTTCAATTCGGCTACGTATCACC
GCCTTGGTGAACCCTTACCTCCCCACCAAGCTAAACCCCCCGGGTTCCATCC
AAAGCGATAACTTACCCCTTCTTTACCCAC

B.22 16 S ribosomal RNA gene partial sequence for k5-14

GCTCGCTCCCTAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCAT
GGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTG
CTGATCCGCGATTACTAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTA
CAGTCCGAACCTGAGAATGGCTTTAAGAGATTAGCTTGACCTCGCGGTCTCG
CAACTCGTTGTACCATCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGG
GCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTACCGGCAGTC
TACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGCTC
GTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGC
ACCACCTGTCATTTTGCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCA
AAAGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCAC
ATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTG
CGGTCGTACTCCCCAGGCGGAATGCTTAATGCGTTAGCTGCGGCACTGAAG
GGCGGAAACCCTCCAACACCTAGCATTTCATCGTTTACGGCATGGACTACCA
GGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTAC
AGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTT
CACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGT
TTCCGATGCGCTTCCTCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAA
ACCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTTGCCACCTA
CGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTGGATA
CCGTCACGCCGGCAACAGTTACTCTGCCGACCATTCTTCTCCAACAACAGA
GTTTTTACAACCCCAAAGCCTTCTTCACTCAGGCGGCGTTGCTCCATCAAAC
TGCGTCCATTGTGGAAGAATCCCTACTGCTGCCTCCCGTAGGATTTGGGCCG
GGTCCCCTCCCAAGGGGGCCAATCAACCTCCCAGTTCGGCG

B.23 16 S ribosomal RNA gene partial sequence for k5-15

GGCTCGCTCCCTAAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTC
ATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCG
TGCTGATCCGCGATTACTAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCC
TACAGTCCGAACTGAGAATGGCTTTAAGAGATTAGCTTGACCTCGCGGTCT
CGCAACTCGTTGTACCATCCATTGTAGCACGTGTGTAGCCCAGGTCATAAG
GGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTACCGGCAG
TCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGC
TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCAT
GCACCACCTGTCATTTTGCCCCGAAGGGGAAACCTGATCTCTCAGGTGATC
AAAAGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCA
CATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTT
GCGGTGCTACTCCCCAGGCGGAATGCTTAATGCGTTAGCTGCGGCACTGAA
GGGCGGAAACCCTCCAACACCTAGCATTTCATCGTTTACGGCATGGACTACC
AGGGTATCTAATCCTGTTGCTACCCATGCTTTGAGCCTCAGCGTCAGTTA
CAGACCAGACAGCCGCCTTCGCCACTGGTGTCTTCCATATATCTACGCATT
TCACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAG
TTTCCGATGCGCTTCCTCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAA
AACCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTTGCCACCT
ACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTGGAT
ACCGTCACGCCGACAACAGTACTCTGCCGACCATTCTTCTCCAACAACAG
AGTTTTACGACCCGAAAGCCTTCTTCACTCAGGCGGCGTTGCTCCATCAGAC
TTGCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCCTAAGAATTTGGGC
CGTGTCTCAGTCCCAATGTGGCCGATCAACCTCTCAATTCGGCTACATATCA
TCCCCTTGGTGAACCCTTACCTCCCCACTAGCTTAAACCCCCGGGTCCCTCC
AAAAGGAATGCTTACGCCTCTTTTACCCAAAAACATGGCGGGTTTCTGGATT
TTGCGGTTTAACCTCTTTTCCAAGTTATCCCCCTTAGGGGG

B.24 16 S ribosomal RNA gene partial sequence for k6-1

GCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAAC
ATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAG
TGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAACC
GCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCC
GCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACG
TAGCCGAACCTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAA
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTG
ATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGT
TGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCC
AACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG
GTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTT
TTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAA
CTGGGAAACTTGAGTGCAGAAGAAGACAGTGGAATCCATGTGTAGCGGTG
AAATGCGTAGATATATGGAAGAACACCAGTGGCGAAAGCGGCTGTCTGGTC
TGTAACCTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATAC
CCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCG
CCCTTCAATGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGAC
CGCCAGGTTGAAACTCAAAGGAATTGACGGGGGGCCCCCACAACCGGTGGA
GCATGTGGTTTAATTCAAGCAACCCGAGAAACCTTACCAGGGCTTGACTTCT
TTTGATCACCTGAAAAATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGGG
GGGCATGGTTGTCCTCCACCCCGTCCGGGAAATTTGGGTAAATCCCCCAC
CAAGGCAACCCTTTTAACAATTGCCGCCATTTATTTGGGCCCTCTTTAAAAA
TGCCGGGGAAAACCCGAGGAAGGGGGGGTAAAGTCAAAATCCTTGGCCCC
TTTAAACCGGGGTCACCCCGGTCCACTGGAGGGGAAACCAAGTTTGGGAAC
CCGGGGTAAGCTAATTCTTTAAGGCCTTTCCTTTGGGAGGGGGGGGGCAC
CCCCCTCCCAAACGGGAAATCTGGTAATTCGGAAAAAACCCCCGGGGAA
AATTTCCCG

B.25 16 S ribosomal RNA gene partial sequence for k6-3a

TGCAAGTCGAGCGAGCAGAACCAGCAGATTTACTTCGGTAATGACGCTGGG
GACGCGAGCGGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCCATAGT
CTAGGATAACCACTTGGAACAGGTGCTAATACCGGATAATAAAGCAGATCG
CATGATCAGCTTATAAAAGGCGGCGTAAGCTGTCGCTATGGGATGGCCCCG
CGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAATGATGCAT
AGCCGAGTTGAGAGACTGAACGGCCACATTGGGACTGAGACACGGCCCCAA
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTG
ATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTTCGGATCGTAAAGCTCTGT
TGTTGGTGAAGAAGGATAGAGGTAGTAACTGGCCTTTATTTGACGGTAATC
AACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGG
TGGCAAGCGTTGTCCGATTTATTGGGCGTAAAGCGAGCGCAGGCGGAAGA
ATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATTGCATCGGAAAC
TGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAAGTCCATGTGTAGCGGTGG
AATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCT
GCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATAC
CCTGGTAGTCCATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGTTTCCG
CCTCTCAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGAC
CGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT
CTAGTGCCATCCTAAGAGATTAGGAGTTCCCTTCGGGGACGCTAAGACAGG
TGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTAAAGTCCCGC
AACGAGCGCAACCCTTATTATTAGTTGCCAGCATTAAAGTTGGGCACTCTAAT
GAGACTGCCGGTGACAAACCGGAGGAAGGGGGGGATGACCTCAAGCATCA
TGCCCCCTT

B.26 16 S ribosomal RNA gene partial sequence for k6-6a

TCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGA
ACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGG
GATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAACCGCATG
GTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGCGGC
GTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACGTAGCC
GAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACCTCC
TACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGA
GCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACCTCTGTTGTTG
GAGAAGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCCAACCA
GAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCA
AGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAG
TCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTGGGA
AACTTGAGTGCAGAAGAGGACAGTGGAACCTCCATGTGTAGCGGTGAAATGC
GTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC
TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGT
AGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTC
AGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAG
GTTGAAACTCAAAGGAATTGACGGGGGGCCCGCACAAAGCGGTGGAGCATGT
GGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTTTTGA
TCACCTGAAAGATCAGGTTTCCCCTTCGGGGGGCAAATGACAGGGGGTGCAT
GGTTGCCGTCAGCCCGGGGCAGGAGAGTTTGGGGTTAAGTCCTGAACTG

B.27 16 S ribosomal RNA gene partial sequence for k6-7

GCAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACA
TGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAGT
GGGGGATAACATTTGGAAACAGATGCTAATAACCGCATAGATCCAAGAACCG
CATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGC
GGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACGTA
GCCGAACGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAAC
TCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT
GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACCTCTGTTG
TTGGAGAAGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCCAA
CCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTG
GCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTT
AAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTG
GGAAACTTGAGTGCAGAAGAGGACAGTGGAACCTCATGTGTAGCGGTGAA
ATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTG
TAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCC
TGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCC
CTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGACCG
CAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCCGCACAAGCGGTGGAGC
ATGTGGTTTAATTGGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTT
TTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGTGG
TGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTAAAGTCCCGCAAC
GAGCGCAACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAA
GACTGCCGGTGACAACCGGAGGAAGGTGGGGATGACGTCAATCATCATGCC
CC

B.28 16 S ribosomal RNA gene partial sequence for k6-9a

TGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAA
CATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAA
GTGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAAC
CGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCC
GCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACG
TAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAA
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTG
ATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGT
TGTTGGAGAAGAATGGTCGGCAGAGTAACTGTTGCCGGCGTGACGGTATCC
AACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG
GTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTT
TTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAA
CTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAATCCATGTGTAGCGGTG
AAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTC
TGTAAGTACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATAC
CCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCG
CCCTTCAGTGCCGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGAC
CGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTGGAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT
CTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGG
TGGTGCATGGTTGTCGTCAGCTCGTGTCTGAGATGTTGGGTAAAGTCCCGC
AACGAGCGCAACCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGT
AAGACTGCCGGTGACAACCGGAAGGAAGGTGGGGATGACGTCAAATCATC
ATGCCCCCTTATGACCTGG

B.29 16 S ribosomal RNA gene partial sequence for k6-12

TGCTTGCACCGAGATTCAACATGGGAACGAGTGGCGGACGGGTGAGTAACA
CGTGGGTAACTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATGCTAA
TACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCGTAAG
CTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAACG
GCTCACCAAGGCGATGATACGTAGCCGAAGTGGAGAGGTTGATCGGCCACAT
TGGGACTGAGACACGGCCCAAACCTCCTACGGGAGGCAGCAGTAGGGAATC
TTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAG
GCTTTCGGGTCGTAAAACCTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAA
CTGTTGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGC
CAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGC
GTAAAGCGAGCGCAGGCGGTTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTT
AACCGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACA
GTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCA
GTGGCGAAGGCGGCTGTCTGGTCTGTAAGTACGCTGAGGCTCGAAAGCAT
GGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAA
TGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAG
CATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAA
GAACCTTACCAGGTCTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCC
CTTCGGGGGCAAAATGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGT
GAGATGTTGGGTAAAGTCCCGCAACGAGCGCAACCCTTATGACTAGTTG

B.30 16 S ribosomal RNA gene partial sequence for k6-14

GGCTCGCTCCCTAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCA
TGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGT
GCTGATCCGCGATTACTAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCT
ACAGTCCGAAGTGAAGAATGGCTTTAAGAGATTAGCTTGACCTCGCGGTCTC
GCAACTCGTTGTACCATCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGG
GGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTACACCGGCAGT
CTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGCT
CGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATG
CACCACCTGTCATTTTGCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCA
AAAGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCAC
ATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTG
CGGTCGTACTCCCCAGGCGGAATGCTTAATGCGTTAGCTGCGGCACTGAAG
GGCGGAAACCCTCCAACACCTAGCATTTCATCGTTTACGGCATGGACTACCA
GGGTATCTAATCCTGTTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTAC
AGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTT
CACCGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGT
TTCCGATGCGCTTCCTCGGTAAAGCCGAGGGCTTTCACATCAGACTTAAAAA
ACCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTTGCCACCTA
CGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTGGATA
CCGTCACGCCGGCAACAGTTACTCTGCCGACCATTCTTCTCCACAACAAAAT
TTTACGACCCGAAAGCCTT

APPENDIX C

C. BLAST ANALYSIS OF PARTIAL 16S rRNA GENE OF KEFIR ISOLATES

C.1 Blast analysis and alignment results for k2-2a

Sequences producing significant alignments						
Download Manage Columns Show 100						
select all 100 sequences selected						
GenBank Graphics Distance tree of results						
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident
☒	Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	2089	2089	100%	0.0	99.22%
☒	Lactobacillus gallinarum strain JCM 2011 16S ribosomal RNA, partial sequence	2089	2089	100%	0.0	99.22%
☒	Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	2089	2089	100%	0.0	99.22%
☒	Lactobacillus helveticus strain NBRC 15019 16S ribosomal RNA, partial sequence	2078	2078	100%	0.0	99.05%
☒	Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	2078	2078	100%	0.0	99.05%
☒	Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	2078	2078	100%	0.0	99.05%
☒	Lactobacillus acidophilus strain NBRC 13951 16S ribosomal RNA, partial sequence	2006	2006	100%	0.0	97.93%
☒	Lactobacillus acidophilus strain VPI 6032 16S ribosomal RNA, partial sequence	2006	2006	100%	0.0	97.93%
☒	Lactobacillus acidophilus strain JCM 1132 16S ribosomal RNA, partial sequence	2006	2006	100%	0.0	97.93%
☒	Lactobacillus acidophilus strain BCRC10695 16S ribosomal RNA, partial sequence	2006	2006	100%	0.0	97.93%

Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence

Sequence ID: [NR_042111.1](#) Length: 1546 Number of Matches: 1

Range 1: 45 to 1202 [GenBank](#) [Graphics](#)

Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
2089 bits(1131)	0.0	1149/1158(99%)	0/1158(0%)	Plus/Plus
Query 1	CGAGCGAGCAGAACAGCAGATTTACTTCGGTAATGACGCTGGGGACGCGAGCGGCGGAT	60		
Sbjct 45	CGAGCGAGCAGAACAGCAGATTTACTTCGGTAATGACGCTGGGGACGCGAGCGGCGGAT	104		
Query 61	GGGTGAGTAACACGTGGGGAACCTGCCCATAGTCTAGGATACCACTTGGAACAGGTGC	120		
Sbjct 105	GGGTGAGTAACACGTGGGGAACCTGCCCATAGTCTGGGATACCACTTGGAACAGGTGC	164		
Query 121	TAATACCGGATAATAAAGCAGATCGCATGATCAGCTTATAAAAGGCGGCGTAAGCTGTCTG	180		
Sbjct 165	TAATACCGGATAAAGAAAGCAGATCGCATGATCAGCTTATAAAAGGCGGCGTAAGCTGTCTG	224		
Query 181	CTATGGGATGGCCCCGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAAT	240		
Sbjct 225	CTATGGGATGGCCCCGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAAT	284		
Query 241	GATGCATAGCCGAGTTGAGAGACTGAACGGCCACATTGGGACTGAGACACGGCCCAAAT	300		
Sbjct 285	GATGCATAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGCCCAAAT	344		
Query 301	CCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGC	360		
Sbjct 345	CCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGC	404		
Query 361	CGCGTGAGTGAAGAAGGTTTTTCGGATCGTAAAGCTCTGTTGTTGGTGAAGAAGGATAGAG	420		
Sbjct 405	CGCGTGAGTGAAGAAGGTTTTTCGGATCGTAAAGCTCTGTTGTTGGTGAAGAAGGATAGAG	464		
Query 421	GTAGTAAC TGCCCTTTATTTGACGGTAATCAACAGAAAGTCACGGCTAATACGTGCCA	480		
Sbjct 465	GTAGTAAC TGCCCTTTATTTGACGGTAATCAACAGAAAGTCACGGCTAATACGTGCCA	524		

Sbjct	465	GTAGTAACTGGCCTTTATTTGACGGTAATCAACCAGAAAAGTCACGGCTAACTACGTGCCA	524
Query	481	GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGC	540
Sbjct	525	GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGC	584
Query	541	GCAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATTGCATCGGA	600
Sbjct	585	GCAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATTGCATCGGA	644
Query	601	AACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACCCATGTGTAGCGGTGGAATGCG	660
Sbjct	645	AACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACCCATGTGTAGCGGTGGAATGCG	704
Query	661	TAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGACGCTGAG	720
Sbjct	705	TAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGACGCTGAG	764
Query	721	GCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGAT	780
Sbjct	765	GCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGAT	824
Query	781	GAGTGCTAAGTGTTGGGAGGTTTCCGCCTCTCAGTGCTGCAGCTAACGCATTAAGCACTC	840
Sbjct	825	GAGTGCTAAGTGTTGGGAGGTTTCCGCCTCTCAGTGCTGCAGCTAACGCATTAAGCACTC	884
Query	841	CGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAAG	900
Sbjct	885	CGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAAG	944
Query	901	CGGTGGAGCATGTGGTTTAAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCT	960
Sbjct	945	CGGTGGAGCATGTGGTTTAAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCT	1004
Query	961	AGTGCCATCCTAAGAGATTAGGAGTTCCCTTCGGGGACGCTAAGACAGGTGGTGCATGGC	1020
Sbjct	1005	AGTGCCATCCTAAGAGATTAGGAGTTCCCTTCGGGGACGCTAAGACAGGTGGTGCATGGC	1064
Query	1021	TGTCGTCAGCTCGTGTCTGTGAGATGTTGGGTAAAGTCCCGCAACGAGCGCAACCCCTTATT	1080
Sbjct	1065	TGTCGTCAGCTCGTGTCTGTGAGATGTTGGGTAAAGTCCCGCAACGAGCGCAACCCCTTATT	1124
Query	1021	TGTCGTCAGCTCGTGTCTGTGAGATGTTGGGTAAAGTCCCGCAACGAGCGCAACCCCTTATT	1080
Sbjct	1065	TGTCGTCAGCTCGTGTCTGTGAGATGTTGGGTAAAGTCCCGCAACGAGCGCAACCCCTTATT	1124
Query	1081	ATTAGTTGCCAGCATTAAGTTGGGCACTCTAATGAGACTGCCGGGGACAAACCGAGGAA	1140
Sbjct	1125	ATTAGTTGCCAGCATTAAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACCGAGGAA	1184
Query	1141	gggggggATGACGCCAAG	1158
Sbjct	1185	GGTGGGGATGACGTCAAG	1202

C.2 Blast analysis and alignment results for k2-3

☒	Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	2006	2006	99%	0.0	99.19%	NR_042111.1
☒	Lactobacillus gallinarum strain JCM 2011 16S ribosomal RNA, partial sequence	2006	2006	99%	0.0	99.19%	NR_113261.1
☒	Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	2006	2006	99%	0.0	99.19%	NR_117061.1
☒	Lactobacillus helveticus strain NBRC 15019 16S ribosomal RNA, partial sequence	1995	1995	99%	0.0	99.01%	NR_113719.1
☒	Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	1995	1995	99%	0.0	99.01%	NR_117060.1
☒	Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	1995	1995	99%	0.0	99.01%	NR_042439.1
☒	Lactobacillus crispatus strain DSM 20584 16S ribosomal RNA, partial sequence	1962	1962	99%	0.0	98.47%	NR_119274.1
☒	Lactobacillus crispatus strain DSM 20584 16S ribosomal RNA, partial sequence	1962	1962	99%	0.0	98.47%	NR_117063.1
☒	Lactobacillus crispatus strain ATCC 33820 16S ribosomal RNA, partial sequence	1962	1962	99%	0.0	98.47%	NR_041800.1
☒	Lactobacillus amylovorus DSM 20531 16S ribosomal RNA, partial sequence	1956	1956	99%	0.0	98.38%	NR_117064.1
☒	Lactobacillus amylovorus DSM 20531 16S ribosomal RNA, partial sequence	1956	1956	99%	0.0	98.38%	NR_043287.1
☒	Lactobacillus ultunensis strain CCUG 48460 16S ribosomal RNA, partial sequence	1951	1951	99%	0.0	98.29%	NR_117065.1
☒	Lactobacillus kitatonis strain JCM 1039 16S ribosomal RNA, partial sequence	1951	1951	99%	0.0	98.29%	NR_024813.1
☒	Lactobacillus ultunensis strain Kx146C1 16S ribosomal RNA, partial sequence	1945	1945	99%	0.0	98.20%	NR_042802.1
☒	Lactobacillus acidophilus strain NBRC 13951 16S ribosomal RNA, partial sequence	1940	1940	99%	0.0	98.11%	NR_113638.1
☒	Lactobacillus acidophilus strain VPI 6032 16S ribosomal RNA, partial sequence	1940	1940	99%	0.0	98.11%	NR_117062.1
☒	Lactobacillus acidophilus strain JCM 1132 16S ribosomal RNA, partial sequence	1940	1940	99%	0.0	98.11%	NR_117812.1
☒	Lactobacillus acidophilus strain BCRC10695 16S ribosomal RNA, partial sequence	1940	1940	99%	0.0	98.11%	NR_043182.1
☒	Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	1936	1936	99%	0.0	98.03%	NR_114722.1
☒	Lactobacillus intestinalis strain TH4 16S ribosomal RNA, partial sequence	1925	1925	98%	0.0	98.27%	NR_117071.1

Alignment

k2-3	GTTAGGCCACCGGCTTTGGGCAITGCAGACTTCCATGGTGTGACGGGCGGTGT-----	53
l.intest	--TAGGTGGCAAGCGTTG-----TCCGGATTATTGGGCGTAAAGCGAGCGCAGGCGGAA	53
l.gallin	--TAGGTGGCAAGCGTTG-----TCCGGATTATTGGGCGTAAAGCGAGCGCAGGCGGAA	53
helvit	--TAGGTGGCAAGCGTTG-----TCCGGATTATTGGGCGTAAAGCGAGCGCAGGCGGAA	53

k2-3	-----GTACAAGGCCCGGGAACGTATTACCGCGGCGTCTGAT--CCGCGATTACT	103
l.intest	AGATAAGTCTGATGTGAAAGCCCCCGGCTTAACCGAGGAAATGCATCGGAAACTGTGTTT	113
l.gallin	GAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAACTGCATCGGAAACTGTTTT	113
helvit	GAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAACTGCATCGGAAACTGTTTT	113
	* * * * *	
k2-3	AGCGATTCCAGCTTCGTGCAGTCGAGTTGCAGACTGCAGTCCGAACGTGAGAACAGCTTTC	163
l.intest	CTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATG	173
l.gallin	CTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATG	173
helvit	CTTGAGTGCAGAAGAGGAGAGTGGAAATCCCATGTGTAGCGGTGGAATGCGTAGATATATG	173
	* * * * *	
k2-3	AGAG-ATTGCTTGCCCTTCGCAAGGCTCGCTTCGCTGT--ACTGCC--CATTGTAGCAC	218
l.intest	GAAGAACACCAAGTGGCGAAGGCGGCTCTCTGGTCTGAACTGACGCTGAGGCTCGAAAGC	233
l.gallin	GAAGAACACCAAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGC	233
helvit	GAAGAACACCAAGTGGCGAAGGCGACTCTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGC	233
	* * * * *	

Lactobacillus intestinalis strain TH4 16S ribosomal RNA, partial sequence

Sequence ID: [NR_117071.1](#) Length: 1448 Number of Matches: 1

Range 1: 261 to 1360 [GenBank](#) [Graphics](#)

▼ [Next Match](#) ▲ [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
1925 bits(1042)	0.0	1081/1100(98%)	1/1100(0%)	Plus/Minus
Query 14	GGTTAGGCCACCGGCTTTGGGCATTGCAGACTTCCATGGTGTGACGGGCGGTGTGTACAA	73		
Sbjct 1360	GGTTAGGCCACCGGCTTCGGGCATTGCAGACTTCCATGGTGTGACGGGCGGTGTGTACAA	1301		
Query 74	GGCCCGGGAACGTATTACCGCGGCGTTCTGATCCGCGATTACTAGCGATTCCAGCTTCG	133		
Sbjct 1300	GGCCCGGGAACGTATTACCGCGGCGTCTGATCCGCGATTACTAGCGATTCCAGCTTCG	1241		
Query 134	TGCAGTCGAGTTGCAGACTGCAGTCCGAACTGAGAACAGCTTTCAGAGATTCGCTTGCCT	193		
Sbjct 1240	TGCAGTCGAGTTGCAGACTGCAGTCCGAACTGAGAACAGCTTTAAGAGATCCGCTTGCCT	1181		
Query 194	TCGCAGGCTCGCTTCTCGTTGTACTGCCATTGTAGCACGTGTGTAGCCAGGTCATAAG	253		
Sbjct 1180	TCGCAGGCTCGCTTCTCGTTGTACTGCCATTGTAGCACGTGTGTAGCCAGGTCATAAG	1121		
Query 254	GGGCATGATGACTTGACGTATCCCCACCTTCCTCCGGTTTGTACCGGCAGTCTCATTA	313		
Sbjct 1120	GGGCATGATGACTTGACGTATCCCCACCTTCCTCCGGTTTGTACCGGCAGTCTCATTA	1061		
Query 314	GAGTGCCCAACTTAATGCTGGCAACTAATAAAGGGTTGCGCTCGTTGCGGGACTTAAC	373		
Sbjct 1060	GAGTGCCCAACTTAATGCTGGCAACTAATAAAGGGTTGCGCTCGTTGCGGGACTTAAC	1001		
Query 374	CCAACATCTCACGACACGAGCTGACGACAGCCATGCACCACCTGTCTTAGCGTCCCCGAA	433		
Sbjct 1000	CCAACATCTCACGACACGAGCTGACGACAGCCATGCACCACCTGTCTTAGCGTCCCCGAA	941		
Query 434	GGGAACTCCTAATCTCTTAGGATGGCACTAGATGTCAAGACCTGGTAAGGTTCTTCGCGT	493		
Sbjct 940	GGGAACTCCTAATCTCTTAGGATGGCACTAGATGTCAAGACCTGGTAAGGTTCTTCGCGT	881		

Sbjct	940	GGGAACCTCCTAATCTCTTAGGATGGCACTAGATGTCAAGACCTGGTAAGGTTCTTCGCGT	881
Query	494	TGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCGTCAATTCCTTTGAGT	553
Sbjct	880	TGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCGTCAATTCCTTTGAGT	821
Query	554	TTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTGAG	613
Sbjct	820	TTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTGAG	761
Query	614	AGGCGGAAACCTCCCAACACTTAGCACTCATCGTTTACGGCATGGACTACCAGGGTATCT	673
Sbjct	760	AGGCGGAAACCTCCCAACACTTAGCACTCATCGTTTACGGCATGGACTACCAGGGTATCT	701
Query	674	AATCCTGTTGCTACCCATGCTTTCGAGCGCTCAGCGTCAGTTGCAGACCAGAGAGCCGCC	733
Sbjct	700	AATCCTGTTGCTACCCATGCTTTCGAGCGCTCAGCGTCAGTTGCAGACCAGAGAGCCGCC	641
Query	734	TTCGCCACTGGTGTTCTTCCATATATCTACGCATTCCACCGCTACACATGGAGTTCCTACT	793
Sbjct	640	TTCGCCACTGGTGTTCTTCCATATATCTACGCATTCCACCGCTACACATGGAGTTCCTACT	581
Query	794	CTCCTCTTCTGCACTCAAGAAAAACAGTTTCCGATGCAATTCCTCGGTTAAGCCGAGGGC	853
Sbjct	580	CTCCTCTTCTGCACTCAAGAAACACAGTTTCCGATGCAATTCCTCGGTTAAGCCGAGGGC	521
Query	854	TTTCACATCAGACTTATTCTTCCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGACAAC	913
Sbjct	520	TTTCACATCAGACTTATTCTTCCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGACAAC	461
Query	914	GCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTATTTAGCCGTGACTTTCTGGTTG	973
Sbjct	460	GCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGACTTTCTGGTTG	401
Query	974	ATTACCGTCAAATAAAGGCCAGTTACTACCTCTATCCTTCTTACCAACAACAGAGCTTT	1033
Sbjct	400	ATTACCGTCAAATAGGGGCCAGTTACTACCTCTATCCTTCTTACCAACAACAGAGCTTT	341
Query	1034	ACGATCC-AAAACCTTCTTCACTCAGGCGGCGTTGCTCCATCAAACCTTGCGTCCATTGTG	1092
Sbjct	340	ACGATCCGAAAACCTTCTTCACTCAGGCGGCGTTGCTCCATCAGGCTTTCGCCATTGTG	281
Query	1093	GAAGATTCCCTACTGCTGCC	1112
Sbjct	280	GAAGATTCCCTACTGCTGCC	261

C.3 Blast analysis and alignment results for k2-3a

Sequences producing significant alignments						Download	Manage Columns	Show	100	?
☒ select all 100 sequences selected							GenBank Graphics Distance tree of results			
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession			
☒	Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	1982	1982	100%	0.0	99.45%	NR_042111.1			
☒	Lactobacillus gallinarum strain JCM 2011 16S ribosomal RNA, partial sequence	1982	1982	100%	0.0	99.45%	NR_113261.1			
☒	Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	1982	1982	100%	0.0	99.45%	NR_117061.1			
☒	Lactobacillus helveticus strain NBRC 15019 16S ribosomal RNA, partial sequence	1977	1977	100%	0.0	99.36%	NR_113719.1			
☒	Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	1977	1977	100%	0.0	99.36%	NR_117060.1			
☒	Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	1977	1977	100%	0.0	99.36%	NR_042439.1			
☒	Lactobacillus acidophilus strain NBRC 13951 16S ribosomal RNA, partial sequence	1899	1899	100%	0.0	98.08%	NR_113638.1			
☒	Lactobacillus acidophilus strain VPI 6032 16S ribosomal RNA, partial sequence	1899	1899	100%	0.0	98.08%	NR_117062.1			



Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence

Sequence ID: [NR_042111.1](#) Length: 1546 Number of Matches: 1

Range 1: 38 to 1128 [GenBank](#) [Graphics](#)

▼ [Next Match](#) ▲ [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
1982 bits(1073)	0.0	1085/1091(99%)	0/1091(0%)	Plus/Plus
Query 1		TGCAAGTCGAGCGAGCAGAACCCAGCAGATTTACTTCGGTAATGACGCTGGGGACGCGAGC	60	
Sbjct 38		TGCAAGTCGAGCGAGCAGAACCCAGCAGATTTACTTCGGTAATGACGCTGGGGACGCGAGC	97	
Query 61		GGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCCATAGCTAGGATACCACTTGGA	120	
Sbjct 98		GGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCCATAGCTAGGATACCACTTGGA	157	
Query 121		CAGGTGCTAATACCGGATAATAAAGCAGATCGCATGATCAGCTTATAAAGGCGGCGTAA	180	
Sbjct 158		CAGGTGCTAATACCGGATAAAGAAAGCAGATCGCATGATCAGCTTATAAAGGCGGCGTAA	217	
Query 181		GCTGTCGCTATGGGATGGCCCCGCGGTGCATTAGCTAGTTGGTAAGGTAAAGGCTTACCA	240	
Sbjct 218		GCTGTCGCTATGGGATGGCCCCGCGGTGCATTAGCTAGTTGGTAAGGTAAAGGCTTACCA	277	
Query 241		AGGCAATGATGCATAGCCGAGTTGAGAGACTGAACGGCCACATTGGGACTGAGACACGGC	300	
Sbjct 278		AGGCAATGATGCATAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGC	337	
Query 301		CCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGA	360	
Sbjct 338		CCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGA	397	
Query 361		GCAACGCCGCGTGAGTGAAGAAGGTTTTTCGGATCGTAAAGCTCTGTTGTTGGTGAAGAAG	420	
Sbjct 398		GCAACGCCGCGTGAGTGAAGAAGGTTTTTCGGATCGTAAAGCTCTGTTGTTGGTGAAGAAG	457	
Query 421		GATAGAGGTAGTAAGTGGCCTTTATTTGACGGTAATCAACCAGAAAGTCACGGCTAACTA	480	
Sbjct 458		GATAGAGGTAGTAAGTGGCCTTTATTTGACGGTAATCAACCAGAAAGTCACGGCTAACTA	517	
Query 481		CGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAA	540	
Sbjct 518		CGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAA	577	

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Query 541 AGCGAGCGCAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATTG 600
Sbjct 578 AGCGAGCGCAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAC TG 637
Query 601 CATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACCTCATGTGTAGCGGTG 660
Sbjct 638 CATCGGAAACTGTTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACCTCATGTGTAGCGGTG 697
Query 661 GAATGCGTAGATATATGGAAGAACACCAAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGA 720
Sbjct 698 GAATGCGTAGATATATGGAAGAACACCAAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGA 757
Query 721 CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGT 780
Sbjct 758 CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGT 817
Query 781 AAACGATGAGTGCTAAGTGTGGGAGGTTCCGCCTCTCAGTGTGCAGCTAACGCATTA 840
Sbjct 818 AAACGATGAGTGCTAAGTGTGGGAGGTTCCGCCTCTCAGTGTGCAGCTAACGCATTA 877
Query 841 AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCC 900
Sbjct 878 AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCC 937
Query 901 GCACAAGCGGTGGAGCATGTGGTTTAATTGGAAGCAACGCGAAGAACCCTTACCAGGTCTT 960
Sbjct 938 GCACAAGCGGTGGAGCATGTGGTTTAATTGGAAGCAACGCGAAGAACCCTTACCAGGTCTT 997
Query 961 GACATCTAGTGCCATCCTAAGAGATTAGGAGTTCCTTCGGGGACGCTAAGACAGGTGGT 1020
Sbjct 998 GACATCTAGTGCCATCCTAAGAGATTAGGAGTTCCTTCGGGGACGCTAAGACAGGTGGT 1057
Query 1021 GCATGGCTGTCGTGAGCTCGTGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC 1080
Sbjct 1058 GCATGGCTGTCGTGAGCTCGTGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC 1117
Query 1081 CCTTATTATTA 1091
Sbjct 1118 CCTTGTATTA 1128

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C.4 Blast analysis and alignment results for k2-4

Sequences producing significant alignments						Download	Manage Columns	Show	100	?
☒ select all 100 sequences selected						GenBank Graphics Distance tree of results				
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession			
☒	Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial sequence	2222	2222	100%	0.0	99.67%	NR_041054.1			
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	2222	2222	100%	0.0	99.67%	NR_025880.1			
☒	Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	2218	2218	100%	0.0	99.59%	NR_113337.1			
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	2209	2209	99%	0.0	99.59%	NR_117987.1			
☒	Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	2158	2158	100%	0.0	98.69%	NR_037122.1			
☒	Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	2154	2154	100%	0.0	98.60%	NR_113333.1			
☒	Lactobacillus casei subsp. casei ATCC 393 16S ribosomal RNA, partial sequence	2152	2152	100%	0.0	98.60%	NR_041893.1			
☒	Lactobacillus casei subsp. casei ATCC 393 strain JCM 1134 16S ribosomal RNA, partial sequence	2143	2143	100%	0.0	98.44%	NR_115534.1			
☒	Lactobacillus rhamnosus strain NBRC 3425 16S ribosomal RNA, partial sequence	2128	2128	100%	0.0	98.27%	NR_113332.1			

Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial

Sequence ID: [NR_041054.1](#) Length: 1497 Number of Matches: 1

[See 1 more title\(s\)](#) ▼

Range 1: 29 to 1245 [GenBank](#) [Graphics](#)

▼ [Next Match](#) ▲ [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
2222 bits(1203)	0.0	1213/1217(99%)	3/1217(0%)	Plus/Plus
Query 1	TGC-AGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACG	59		
Sbjct 29	TGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACG	88		
Query 60	AGTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGATAACATTTGG	119		
Sbjct 89	AGTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGATAACATTTGG	148		
Query 120	AAACAGATGCTAATACCGCATAGATCCAAGAACCAGCATGGTTCTTGGCTGAAAGATGGCG	179		
Sbjct 149	AAACAGATGCTAATACCGCATAGATCCAAGAACCAGCATGGTTCTTGGCTGAAAGATGGCG	208		
Query 180	TAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA	239		
Sbjct 209	TAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA	268		
Query 240	CCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACAC	299		
Sbjct 269	CCAAGGCGATGATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACAC	328		
Query 300	GGCCCAAACCTCTACGGGAGGCGAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT	359		
Sbjct 329	GGCCCAAACCTCTACGGGAGGCGAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT	388		
Query 360	GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAACTCTGTTGTTGGAGAAG	419		
Sbjct 389	GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAACTCTGTTGTTGGAGAAG	448		
Query 420	AATGGTCGGCAGAGTAACGTGTGCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA	479		
Sbjct 449	AATGGTCGGCAGAGTAACGTGTGCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA	508		
Query 480	CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCG	539		
Sbjct 509	CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCG	568		
Query 540	TAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA	599		
Sbjct 569	TAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA	628		
Query 600	GCGCATCGGAACTGGGAACTTGAGTGCAGAAGAGGACAGTGGAACCTCATGTGTAGCG	659		
Sbjct 629	GCGCATCGGAACTGGGAACTTGAGTGCAGAAGAGGACAGTGGAACCTCATGTGTAGCG	688		
Query 660	GTGAAATGCGTAGATATATGGAAGAACACCAAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC	719		
Sbjct 689	GTGAAATGCGTAGATATATGGAAGAACACCAAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC	748		
Query 720	TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGC	779		
Sbjct 749	TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGC	808		

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Query 780 CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA 839
      |||
Sbjct 809 CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA 868

Query 840 TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG 899
      |||
Sbjct 869 TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG 928

Query 900 CCCGCACAAGCGGTGGAGCATGTGGTTTAATTGGAAGCAACGCGAAGAACCTTACCAGGT 959
      |||
Sbjct 929 CCCGCACAAGCGGTGGAGCATGTGGTTTAATTGGAAGCAACGCGAAGAACCTTACCAGGT 988

Query 960 CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCCTTCGGGGGCAAATGACAGGT 1019
      |||
Sbjct 989 CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCCTTCGGGGGCAAATGACAGGT 1048

Query 1020 GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC 1079
      |||
Sbjct 1049 GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC 1108

Query 1080 AACCCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAA 1139
      |||
Sbjct 1109 AACCCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAA 1168

Query 1140 ACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGGGCTAC-CAC 1198
      |||
Sbjct 1169 ACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGGGCTACACAC 1228

Query 1199 GTGCTACA-TGGATGGT 1214
      |||
Sbjct 1229 GTGCTACAATGGATGGT 1245

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C.5 Blast analysis and alignment results for k2-4a

Sequences producing significant alignments		Download		Manage Columns		Show	100	
☒ select all 100 sequences selected						GenBank	Graphics	Distance tree of results
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession	
☒	Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	1897	1897	100%	0.0	99.05%	NR_042111.1	
☒	Lactobacillus gallinarum strain JCM 2011 16S ribosomal RNA, partial sequence	1897	1897	100%	0.0	99.05%	NR_113261.1	
☒	Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	1897	1897	100%	0.0	99.05%	NR_117061.1	
☒	Lactobacillus helveticus strain NBRC 15019 16S ribosomal RNA, partial sequence	1886	1886	100%	0.0	98.87%	NR_113719.1	
☒	Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	1886	1886	100%	0.0	98.87%	NR_117060.1	
☒	Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	1886	1886	100%	0.0	98.87%	NR_042439.1	
☒	Lactobacillus crispatus strain DSM 20584 16S ribosomal RNA, partial sequence	1853	1853	100%	0.0	98.30%	NR_119274.1	
☒	Lactobacillus crispatus strain DSM 20584 16S ribosomal RNA, partial sequence	1853	1853	100%	0.0	98.30%	NR_117063.1	
☒	Lactobacillus crispatus strain ATCC 33820 16S ribosomal RNA, partial sequence	1853	1853	100%	0.0	98.30%	NR_041800.1	
☒	Lactobacillus amylovorus DSM 20531 16S ribosomal RNA, partial sequence	1847	1847	100%	0.0	98.20%	NR_117064.1	

Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence

Sequence ID: [NR_042111.1](#) Length: 1546 Number of Matches: 1

Range 1: 410 to 1467 [GenBank](#) [Graphics](#)

[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
1897 bits(1027)	0.0	1048/1058(99%)	1/1058(0%)	Plus/Minus
Query 1	GGCTCCTTCCCGAAGGTTAGGCCACCGGCTTTGGGCATTGCAGACTTCCATGGTGTGACG	60		
Sbjct 1467	GGCTCCTTCCCGAAGGTTAGGCCACCGGCTTTGGGCATTGCAGACTTCCATGGTGTGACG	1408		
Query 61	GGCGGTGTGTACAAGGCCCGGGAACGATTACCCGCGGCGTCTGATCCGCGATTACTAG	120		
Sbjct 1407	GGCGGTGTGTACAAGGCCCGGGAACGATTACCCGCGGCGTCTGATCCGCGATTACTAG	1348		
Query 121	CGATTCCAGCTTCGTGCAGTCGAGTTGCAGACTGCAGTCCGAAGTGAAGACAGCTTTCAG	180		
Sbjct 1347	CGATTCCAGCTTCGTGCAGTCGAGTTGCAGACTGCAGTCCGAAGTGAAGACAGCTTTCAG	1288		
Query 181	AGATTCGCTTGCCTTCGCGAGGCTCGCTTCTCGTTGTACTGCCCATTTAGTACGCTGTGTA	240		
Sbjct 1287	AGATTCGCTTGCCTTCGCGAGGCTCGCTTCTCGTTGTACTGCCCATTTAGTACGCTGTGTA	1228		
Query 241	GCCGAGGTATAGAGGGGCGATGACTTGACGTCATCCACCTTCTCCGGTTTGTGAC	300		
Sbjct 1227	GCCGAGGTATAGAGGGGCGATGACTTGACGTCATCCACCTTCTCCGGTTTGTGAC	1168		
Query 301	CGGCAGTCTCATTAGAGTGCCCAACTTAATGCTGGCAACTAATAAGGGTTGCGCTCG	360		
Sbjct 1167	CGGCAGTCTCATTAGAGTGCCCAACTTAATGCTGGCAACTAATAAGGGTTGCGCTCG	1108		
Query 361	TTGCGGGACTTAACCAACATCTCACGACACGAGCTGACGACAGCCATGCACCACTGTC	420		
Sbjct 1107	TTGCGGGACTTAACCAACATCTCACGACACGAGCTGACGACAGCCATGCACCACTGTC	1048		
Query 421	TTAGCGTCCCGAAGGGAACTCCTAATCTCTTAGGATGGCACTAGATGTCAAGACCTGGT	480		
Sbjct 1047	TTAGCGTCCCGAAGGGAACTCCTAATCTCTTAGGATGGCACTAGATGTCAAGACCTGGT	988		
Query 481	AAGGTTCTTCGCGTTGCTTCGAATTAACACACATGCTCCACCGCTTGTGCGGGCCCCGT	540		
Sbjct 987	AAGGTTCTTCGCGTTGCTTCGAATTAACACACATGCTCCACCGCTTGTGCGGGCCCCGT	928		
Query 541	CAATTCCTTTGAGTTTCAACCTTGCAGGTCGACTCCCGAGGCGGAGTGCTTAATGCGTTA	600		
Sbjct 927	CAATTCCTTTGAGTTTCAACCTTGCAGGTCGACTCCCGAGGCGGAGTGCTTAATGCGTTA	868		
Query 601	GCTGCAGCACTGAGAGGCGGAAACCTCCCAACACTTAGCACTCATCGTTACGGCATGGA	660		
Sbjct 867	GCTGCAGCACTGAGAGGCGGAAACCTCCCAACACTTAGCACTCATCGTTACGGCATGGA	808		
Query 661	CTACCAAGGATCTAATCCTGTTGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAG	720		
Sbjct 807	CTACCAAGGATCTAATCCTGTTGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAG	748		
Query 721	ACCAGAGAGCCGCTTCGCCACTGGTGTCTTCCATATATCTACGCATTCCACCGCTACA	780		
Sbjct 747	ACCAGAGAGCCGCTTCGCCACTGGTGTCTTCCATATATCTACGCATTCCACCGCTACA	688		

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Query 781 CATGGAGTTCCACTCTCTCTTCTGCACTCAAGAAAAACAGTTTCCGATGCAATTCTCTG 840
Sbjct 687 CATGGAGTTCCACTCTCTCTTCTGCACTCAAGAAAAACAGTTTCCGATGCAATTCTCTG 628

Query 841 GTTAAGCCGAGGGCTTTACATCAGACTTATTCTTCCGCCTGCGCTCGCTTTACGCCCAA 900
Sbjct 627 GTTAAGCCGAGGGCTTTACATCAGACTTATTCTTCCGCCTGCGCTCGCTTTACGCCCAA 568

Query 901 TAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTATTTAGCCG 960
Sbjct 567 TAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCG 508

Query 961 TGACTTTCTGGTTGATTACCGTCAAATAAAGGCCAGTTACTACCTCTATCCTTCTTC-CC 1019
Sbjct 507 TGACTTTCTGGTTGATTACCGTCAAATAAAGGCCAGTTACTACCTCTATCCTTCTTCACC 448

Query 1020 AACAAACAACTTTACAATCCAAAAACCTTCTTCACTC 1057
Sbjct 447 AACAAACAGAGCTTTACGATCCGAAAACCTTCTTCACTC 410

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C.6 Blast analysis and alignment results for k2-14

Sequences producing significant alignments		Download		Manage Columns		Show	100	
☒ select all 100 sequences selected		GenBank		Graphics		Distance tree of results		
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession	
☒	Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	1905	1905	100%	0.0	99.43%	NR_042111.1	
☒	Lactobacillus gallinarum strain JCM 2011 16S ribosomal RNA, partial sequence	1905	1905	100%	0.0	99.43%	NR_113261.1	
☒	Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	1905	1905	100%	0.0	99.43%	NR_117061.1	
☒	Lactobacillus helveticus strain NBRC 15019 16S ribosomal RNA, partial sequence	1893	1893	100%	0.0	99.24%	NR_113719.1	
☒	Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	1893	1893	100%	0.0	99.24%	NR_117060.1	
☒	Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	1893	1893	100%	0.0	99.24%	NR_042439.1	
☒	Lactobacillus crispatus strain DSM 20584 16S ribosomal RNA, partial sequence	1860	1860	100%	0.0	98.67%	NR_119274.1	
☒	Lactobacillus crispatus strain DSM 20584 16S ribosomal RNA, partial sequence	1860	1860	100%	0.0	98.67%	NR_117063.1	
☒	Lactobacillus crispatus strain ATCC 33820 16S ribosomal RNA, partial sequence	1860	1860	100%	0.0	98.67%	NR_041800.1	
☒	Lactobacillus amylovorus DSM 20531 16S ribosomal RNA, partial sequence	1855	1855	100%	0.0	98.57%	NR_117064.1	

Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence

Sequence ID: [NR_042111.1](#) Length: 1546 Number of Matches: 1

Range 1: 419 to 1467 [GenBank](#) [Graphics](#)

[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
1905 bits(1031)	0.0	1043/1049(99%)	0/1049(0%)	Plus/Minus
Query 1	GGCTCCTTCCCGAAGGTTAGGCCACCGGCTTTGGGCATTGCAGACTTCCATGGTGTGACG	60		
Sbjct 1467	GGCTCCTTCCCGAAGGTTAGGCCACCGGCTTTGGGCATTGCAGACTTCCATGGTGTGACG	1408		
Query 61	GGCGGTGTGTACAAGGCCCGGGAACGATTACCGCGGCGTTCTGATCCGCGATTACTAG	120		
Sbjct 1407	GGCGGTGTGTACAAGGCCCGGGAACGATTACCGCGGCGTTCTGATCCGCGATTACTAG	1348		
Query 121	CGATTCCAGCTTCGTGCAGTCGAGTTGCAGACTGCAGTCCGAAGTGAAGACAGCTTTCAG	180		
Sbjct 1347	CGATTCCAGCTTCGTGCAGTCGAGTTGCAGACTGCAGTCCGAAGTGAAGACAGCTTTCAG	1288		
Query 181	AGATTTCGCTTGCCTTCGACGGCTCGCTTCTCGTTGTACTGCCATTGTAGCACGTGTGTA	240		
Sbjct 1287	AGATTTCGCTTGCCTTCGACGGCTCGCTTCTCGTTGTACTGCCATTGTAGCACGTGTGTA	1228		
Query 241	GCCAGGTCATAAGGGGCGATGACTTGACGTCATCCACCTTCTCCGGTTTGTGAC	300		
Sbjct 1227	GCCAGGTCATAAGGGGCGATGACTTGACGTCATCCACCTTCTCCGGTTTGTGAC	1168		
Query 301	CGGCAGTCTCATTAGAGTGCCCAACTTAATGCTGGCAACTAATAAGGGTTGCGCTCG	360		
Sbjct 1167	CGGCAGTCTCATTAGAGTGCCCAACTTAATGCTGGCAACTAATAAGGGTTGCGCTCG	1108		
Query 361	TTGCGGGACTTAACCAACATCTCACGACACGAGCTGACGACAGCCATGCACCACTGTC	420		
Sbjct 1107	TTGCGGGACTTAACCAACATCTCACGACACGAGCTGACGACAGCCATGCACCACTGTC	1048		
Query 421	TTAGCGTCCCGAAGGGAACCTCTAATCTCTTAGGATGGCACTAGATGTCAAGACCTGGT	480		
Sbjct 1047	TTAGCGTCCCGAAGGGAACCTCTAATCTCTTAGGATGGCACTAGATGTCAAGACCTGGT	988		
Query 481	AAGGTTCTTCGCGTTGCTTCGAATTAACCAACATGCTCCACCGCTTGTGCGGGCCCCGT	540		
Sbjct 987	AAGGTTCTTCGCGTTGCTTCGAATTAACCAACATGCTCCACCGCTTGTGCGGGCCCCGT	928		
Query 541	CAATTCCTTTGAGTTTCAACCTTGCAGTCTACTCCCGAGGCGGAGTGCTTAATGCGTTA	600		
Sbjct 927	CAATTCCTTTGAGTTTCAACCTTGCAGTCTACTCCCGAGGCGGAGTGCTTAATGCGTTA	868		
Query 601	GCTGCAGCACTGAGAGGCGGAAACCTCCCAACACTTAGCACTCATCGTTTACGGCATGGA	660		
Sbjct 867	GCTGCAGCACTGAGAGGCGGAAACCTCCCAACACTTAGCACTCATCGTTTACGGCATGGA	808		
Query 661	CTACCAGGGTATCTAATCTGTTGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAG	720		
Sbjct 807	CTACCAGGGTATCTAATCTGTTGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAG	748		
Query 721	ACCAGAGAGCCGCTTCGCCACTGGTGTCTTCCATATATCTACGCATTCCACCGCTACA	780		
Sbjct 747	ACCAGAGAGCCGCTTCGCCACTGGTGTCTTCCATATATCTACGCATTCCACCGCTACA	688		

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Query 781 CATGGAGTTCCACTCTCCTCTTCTGCACTCAAGAAAAACAGTTTCCGATGCAATTCTCTG 840
Sbjct 687 CATGGAGTTCCACTCTCCTCTTCTGCACTCAAGAAAAACAGTTTCCGATGCAATTCTCTG 628

Query 841 GTTAAGCCGAGGGCTTTACATCAGACTTATTCTTCCGCCTGCGCTCGCTTTACGCCCAA 900
Sbjct 627 GTTAAGCCGAGGGCTTTACATCAGACTTATTCTTCCGCCTGCGCTCGCTTTACGCCCAA 568

Query 901 TAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTATTTAGCCG 960
Sbjct 567 TAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCG 508

Query 961 TGACTTTCTGGTTGATTACCGTCAAATAAAGGCCAGTTACTACCTCTATCCTTCTTCACC 1020
Sbjct 507 TGACTTTCTGGTTGATTACCGTCAAATAAAGGCCAGTTACTACCTCTATCCTTCTTCACC 448

Query 1021 AACACAAAGCTTTACGATCCGAAAACCT 1049
Sbjct 447 AACACAGAGCTTTACGATCCGAAAACCT 419

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C.7 Blast analysis and alignment results for k3-2a

Sequences producing significant alignments

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	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☒	Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial sequence	2021	2021	100%	0.0	99.73%	NR_041054.1
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	2021	2021	100%	0.0	99.73%	NR_025880.1
☒	Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	2017	2017	100%	0.0	99.64%	NR_113337.1
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	2015	2015	100%	0.0	99.64%	NR_117987.1
☒	Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	1986	1986	100%	0.0	98.64%	NR_037122.1
☒	Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	1963	1963	100%	0.0	98.55%	NR_113333.1
☒	Lactobacillus casei subsp. casei ATCC 393 16S ribosomal RNA, partial sequence	1949	1949	100%	0.0	98.55%	NR_041893.1
☒	Lactobacillus casei subsp. casei ATCC 393 strain JCM 1134 16S ribosomal RNA, partial sequence	1941	1941	100%	0.0	98.37%	NR_115534.1
☒	Lactobacillus rhamnosus strain NBRC 3425 16S ribosomal RNA, partial sequence	1927	1927	100%	0.0	98.19%	NR_113332.1

Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial sequen

Sequence ID: [NR_041054.1](#) Length: 1497 Number of Matches: 1

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Range 1: 39 to 1141 [GenBank](#) [Graphics](#)

▼ [Next Match](#) ▲ [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
2021 bits(1094)	0.0	1100/1103(99%)	0/1103(0%)	Plus/Plus
Query 1	ACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACGAGTGGCGGAC	60		
Sbjct 39	ACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACGAGTGGCGGAC	98		
Query 61	GGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGATAACATTGGAAACAGATGC	120		
Sbjct 99	GGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGATAACATTGGAAACAGATGC	158		
Query 121	TAATACCGCATAGATCCAAGAACCAGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCG	180		
Sbjct 159	TAATACCGCATAGATCCAAGAACCAGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCG	218		
Query 181	CTTTTGGATGGACCCCGCGGTATTAGCTAGTTGGTGAGGTAAATGGCTACCAAGGCGAT	240		
Sbjct 219	CTTTTGGATGGACCCCGCGGTATTAGCTAGTTGGTGAGGTAAATGGCTACCAAGGCGAT	278		
Query 241	GATACGTAGCCGAACAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAAT	300		
Sbjct 279	GATACGTAGCCGAACAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAAT	338		
Query 301	CCTACGGGAGGCGAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGC	360		
Sbjct 339	CCTACGGGAGGCGAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGC	398		
Query 361	CGCGTGAGTGAAGAAGGCTTTCCGGTCGTAAGCTCTGTTGTTGGAGAAGAATGGTCGGC	420		
Sbjct 399	CGCGTGAGTGAAGAAGGCTTTCCGGTCGTAAGCTCTGTTGTTGGAGAAGAATGGTCGGC	458		
Query 421	AGAGTAACTGTTGCCGCGTGACGGTATCCAACAGAAAGCCACGGCTAACTACGTGCCA	480		
Sbjct 459	AGAGTAACTGTTGCCGCGTGACGGTATCCAACAGAAAGCCACGGCTAACTACGTGCCA	518		
Query 481	GCAGCCGCGTAATACGTAGGTGGCAAGCGTTATCCGGATTATTGGGCGTAAAGCGAGC	540		
Sbjct 519	GCAGCCGCGTAATACGTAGGTGGCAAGCGTTATCCGGATTATTGGGCGTAAAGCGAGC	578		
Query 541	GCAGGCGGTTTTTAAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGA	600		
Sbjct 579	GCAGGCGGTTTTTAAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGA	638		
Query 601	AACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCG	660		
Sbjct 639	AACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCG	698		
Query 661	TAGATATATGGAAGAACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAGTACGCTGAG	720		
Sbjct 699	TAGATATATGGAAGAACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAGTACGCTGAG	758		

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Query 721 GCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAACGAT 780
Sbjct 759 GCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAACGAT 818

Query 781 GAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGAGCTAACGCATTAAGCATTTC 840
Sbjct 819 GAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGAGCTAACGCATTAAGCATTTC 878

Query 841 CGCCTGGGGAGTACGACCGCAAGGTTGAACTCAAAGGAATTGACGGGGGCCCGACAAG 900
Sbjct 879 CGCCTGGGGAGTACGACCGCAAGGTTGAACTCAAAGGAATTGACGGGGGCCCGACAAG 938

Query 901 CGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCT 960
Sbjct 939 CGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCT 998

Query 961 TTTGATCACCTGAGAGATCAGGTTTCCCTTCGGGGGCAAAATGACAGGTGGTGCATGGT 1020
Sbjct 999 TTTGATCACCTGAGAGATCAGGTTTCCCTTCGGGGGCAAAATGACAGGTGGTGCATGGT 1058

Query 1021 TGTCTGTCAGCTCGTGTCTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCTTATG 1080
Sbjct 1059 TGTCTGTCAGCTCGTGTCTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCTTATG 1118

Query 1081 ACTAGTTGCCAGCATTTAGTTGG 1103
Sbjct 1119 ACTAGTTGCCAGCATTTAGTTGG 1141

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C.8 Blast analysis and alignment results for k3-3a

Sequences producing significant alignments

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	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☒	Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	2008	2008	99%	0.0	99.19%	NR_042111.1
☒	Lactobacillus gallinarum strain JCM 2011 16S ribosomal RNA, partial sequence	2008	2008	99%	0.0	99.19%	NR_113261.1
☒	Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	2008	2008	99%	0.0	99.19%	NR_117081.1
☒	Lactobacillus helveticus strain NBRC 15019 16S ribosomal RNA, partial sequence	1997	1997	99%	0.0	99.01%	NR_113719.1
☒	Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	1997	1997	99%	0.0	99.01%	NR_117080.1
☒	Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	1997	1997	99%	0.0	99.01%	NR_042439.1
☒	Lactobacillus acidophilus strain NBRC 13951 16S ribosomal RNA, partial sequence	1945	1945	99%	0.0	98.20%	NR_113838.1
☒	Lactobacillus acidophilus strain VPI 6032 16S ribosomal RNA, partial sequence	1945	1945	99%	0.0	98.20%	NR_117082.1
☒	Lactobacillus acidophilus strain JCM 1132 16S ribosomal RNA, partial sequence	1945	1945	99%	0.0	98.20%	NR_117812.1

Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence

Sequence ID: [NR_042111.1](#) Length: 1546 Number of Matches: 1

Range 1: 77 to 1191 [GenBank](#) [Graphics](#)

[Next Match](#) [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
2008 bits(1087)	0.0	1106/1115(99%)	1/1115(0%)	Plus/Plus
Query 4	AATGACGCTGGGGACGCGAGCGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCCATA	63		
Sbjct 77	AATGACGCTGGGGACGCGAGCGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCCATA	136		
Query 64	GTCTAGGATACCACTTGGAAACAGGTGCTAATACCGGATAATAAGCAGATCGCATGATC	123		
Sbjct 137	GTCTGGGATACCACTTGGAAACAGGTGCTAATACCGGATAATAAGCAGATCGCATGATC	196		
Query 124	AGCTTATAAAAGGCGGCGTAAGCTGTCGCTATGGGATGGCCCCGCGTGCTAGCTAGT	183		
Sbjct 197	AGCTTATAAAAGGCGGCGTAAGCTGTCGCTATGGGATGGCCCCGCGTGCTAGCTAGT	256		
Query 184	TGGTAAGGTAAACGGCTTACCAAGGCAATGATGCATAGCCGAGTTGAGAGACTGAACGGCC	243		
Sbjct 257	TGGTAAGGTAAACGGCTTACCAAGGCAATGATGCATAGCCGAGTTGAGAGACTGATCGGCC	316		
Query 244	ACATTGGGACTGAGACACGGCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTCCAC	303		
Sbjct 317	ACATTGGGACTGAGACACGGCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTCCAC	376		
Query 304	AATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAAGTGAAGAGGTTTCGGATCGTAAA	363		
Sbjct 377	AATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAAGTGAAGAGGTTTCGGATCGTAAA	436		
Query 364	GCTCTGTTGTTGGTGAAGAAGGATAGAGGTAGTAACCTGGCCTTTATTTGACGGTAATCAA	423		
Sbjct 437	GCTCTGTTGTTGGTGAAGAAGGATAGAGGTAGTAACCTGGCCTTTATTTGACGGTAATCAA	496		
Query 424	CCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTT	483		
Sbjct 497	CCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTT	556		
Query 484	GTCCGGATTTATTGGGCGTAAAGCAGCGCAGGCGGAAGAATAAGTCTGATGTGAAAGCC	543		
Sbjct 557	GTCCGGATTTATTGGGCGTAAAGCAGCGCAGGCGGAAGAATAAGTCTGATGTGAAAGCC	616		
Query 544	CTCGGCTTAACCGAGGAATTGCATCGGAACTGTTTTCTTGAGTGCAGAAGAGGAGAGT	603		
Sbjct 617	CTCGGCTTAACCGAGGAATTGCATCGGAACTGTTTTCTTGAGTGCAGAAGAGGAGAGT	676		
Query 604	GGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAACACCACTGGCGAAGGC	663		
Sbjct 677	GGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAACACCACTGGCGAAGGC	736		
Query 664	GGCTCTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGA	723		
Sbjct 737	GGCTCTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGA	796		
Query 724	TACCCTGGTAGTCCATGCCGTAACGATGAGTGCTAAGTGTGGGAGGTTTCCGCCTCTC	783		
Sbjct 797	TACCCTGGTAGTCCATGCCGTAACGATGAGTGCTAAGTGTGGGAGGTTTCCGCCTCTC	856		

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Query 784 AGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACT 843
Sbjct 857 AGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACT 916

Query 844 CAAAGGAATTGACGGGGGCCCCGACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACG 903
Sbjct 917 CAAAGGAATTGACGGGGGCCCCGACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACG 976

Query 904 CGAAGAACCCTTACCAGGCTTGACATCTAGTGCCATCCTAAGAGATTAGGAGTTCTCTTC 963
Sbjct 977 CGAAGAACCCTTACCAGGCTTGACATCTAGTGCCATCCTAAGAGATTAGGAGTTCCCTTC 1036

Query 964 GGGGACGCTAAGACAGGTGGTGATGGCTGTCGTGAGCTCGTGTGAGATGTTGGGTT 1023
Sbjct 1037 GGGGACGCTAAGACAGGTGGTGATGGCTGTCGTGAGCTCGTGTGAGATGTTGGGTT 1096

Query 1024 AAGTCCCGCAACGAGCGCAACCCTTATTATTAGTTGCCAGCATTAAAGTTGGGCACTCTAA 1083
Sbjct 1097 AAGTCCCGCAACGAGCGCAACCCTTATTATTAGTTGCCAGCATTAAAGTTGGGCACTCTAA 1156

Query 1084 TGAGACTGCCGGTGACAAACCGGAGGAAAG-GGGG 1117
Sbjct 1157 TGAGACTGCCGGTGACAAACCGGAGGAAAGGTTGGGG 1191

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C.9 Blast analysis and alignment results for k3-8b2

Sequences producing significant alignments		Download		Manage Columns		Show	100	
☒ select all 100 sequences selected		GenBank		Graphics		Distance tree of results		
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession	
☒	Lactobacillus paracasei subsp. tolerans strain NBRC 15808 16S ribosomal RNA, partial sequence	2209	2209	100%	0.0	99.51%	NR_041054.1	
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	2209	2209	100%	0.0	99.51%	NR_026880.1	
☒	Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	2206	2206	100%	0.0	99.42%	NR_113337.1	
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	2198	2198	99%	0.0	99.42%	NR_117887.1	
☒	Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	2145	2145	100%	0.0	98.52%	NR_037122.1	
☒	Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	2141	2141	100%	0.0	98.44%	NR_113333.1	
☒	Lactobacillus casei subsp. casei ATCC 393 16S ribosomal RNA, partial sequence	2139	2139	100%	0.0	98.44%	NR_041893.1	
☒	Lactobacillus casei subsp. casei ATCC 393 strain JCM 1134 16S ribosomal RNA, partial sequence	2130	2130	100%	0.0	98.28%	NR_115534.1	
☒	Lactobacillus rhamnosus strain NBRC 3425 16S ribosomal RNA, partial sequence	2115	2115	100%	0.0	98.11%	NR_113332.1	
☒	Lactobacillus casei strain ATCC 393 16S ribosomal RNA, partial sequence	2108	2108	100%	0.0	97.95%	NR_115533.1	

Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial sequence

Sequence ID: [NR_041054.1](#) Length: 1497 Number of Matches: 1

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Range 1: 29 to 1245 [GenBank](#) [Graphics](#)

[▾ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
2209 bits(1196)	0.0	1211/1217(99%)	5/1217(0%)	Plus/Plus
Query 1	TGC-AGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGACCCGAGATTCAACATGGAACG	59		
Sbjct 29	TGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGACCCGAGATTCAACATGGAACG	88		
Query 60	AGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTGG	119		
Sbjct 89	AGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTGG	148		
Query 120	AAACAGATGCTAATACCGCATAGATCCAAGAACCAGCATGGTTCTTGGCTGAAAGATGGCG	179		
Sbjct 149	AAACAGATGCTAATACCGCATAGATCCAAGAACCAGCATGGTTCTTGGCTGAAAGATGGCG	208		
Query 180	TAAGCTATCGCTTTTGGATGGACCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA	239		
Sbjct 209	TAAGCTATCGCTTTTGGATGGACCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA	268		
Query 240	CCAAGGCGATGATACGTAGCCGAAGTGAAGGTTGATCGGCCACATTGGGACTGAGACAC	299		
Sbjct 269	CCAAGGCGATGATACGTAGCCGAAGTGAAGGTTGATCGGCCACATTGGGACTGAGACAC	328		
Query 300	GGCCCAAACCTCTACGGGAGGCGAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT	359		
Sbjct 329	GGCCCAAACCTCTACGGGAGGCGAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT	388		
Query 360	GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTGCGGTCGTAAACTCTGTTGTTGGAGAAG	419		
Sbjct 389	GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTGCGGTCGTAAACTCTGTTGTTGGAGAAG	448		
Query 420	AATGGTCGGCAGAGTAACCTGTTGCCGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA	479		
Sbjct 449	AATGGTCGGCAGAGTAACCTGTTGCCGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA	508		
Query 480	CTACGTGCCAGCAGCCGCGTAATACGTAGGTGGCAAGCGTTATCCGGATTATTGGGCG	539		
Sbjct 509	CTACGTGCCAGCAGCCGCGTAATACGTAGGTGGCAAGCGTTATCCGGATTATTGGGCG	568		
Query 540	TAAAGCGAGCGCAGGCGGTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA	599		
Sbjct 569	TAAAGCGAGCGCAGGCGGTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA	628		
Query 600	GCGCATCGGAAACTGGGAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG	659		
Sbjct 629	GCGCATCGGAAACTGGGAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG	688		
Query 660	GTGAAATGCGTAGATATATGGAAGAACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC	719		
Sbjct 689	GTGAAATGCGTAGATATATGGAAGAACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC	748		

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Query 720 TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGC 779
Sbjct 749 TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGC 808

Query 780 CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGACGCTAACGCA 839
Sbjct 809 CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGACGCTAACGCA 868

Query 840 TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG 899
Sbjct 869 TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG 928

Query 900 CCCGCACAAGCGGTGGAGCATGTGGTTTAAATTCGAAGCAACGCGAAGAACCTTACCAGGT 959
Sbjct 929 CCCGCACAAGCGGTGGAGCATGTGGTTTAAATTCGAAGCAACGCGAAGAACCTTACCAGGT 988

Query 960 CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGT 1019
Sbjct 989 CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCCTTCGGGGGCAAAATGACAGGT 1048

Query 1020 GGTGCATGGTTGTCGTGAGCTCGTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC 1079
Sbjct 1049 GGTGCATGGTTGTCGTGAGCTCGTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC 1108

Query 1080 AACCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGAC-A 1138
Sbjct 1109 AACCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAA 1168

Query 1139 ACCGGAGGAAGGTGGGGATGACGTC-AATCATCATGCCCTTATGACCTGGGCTAC-CAC 1196
Sbjct 1169 ACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGGGCTACACAC 1228

Query 1197 GTGCTACA-TGGATGGT 1212
Sbjct 1229 GTGCTACAATGGATGGT 1245

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C.10 Blast analysis and alignment results for k3-13b

Sequences producing significant alignments

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	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☒	Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial sequence	2091	2091	100%	0.0	99.23%	NR_041054.1
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	2091	2091	100%	0.0	99.23%	NR_026880.1
☒	Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	2087	2087	100%	0.0	99.14%	NR_113337.1
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	2085	2085	100%	0.0	99.14%	NR_117987.1
☒	Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	2028	2028	99%	0.0	98.44%	NR_037122.1
☒	Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	2025	2025	99%	0.0	98.35%	NR_113333.1
☒	Lactobacillus casei subsp. casei ATCC 393 16S ribosomal RNA, partial sequence	2023	2023	99%	0.0	98.38%	NR_041893.1
☒	Lactobacillus casei subsp. casei ATCC 393 strain JCM 1134 16S ribosomal RNA, partial sequence	2013	2013	99%	0.0	98.18%	NR_115534.1
☒	Lactobacillus rhamnosus strain NBRC 3425 16S ribosomal RNA, partial sequence	2002	2002	98%	0.0	98.34%	NR_113332.1

Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial sequence

Sequence ID: [NR_041054.1](#) Length: 1497 Number of Matches: 1

[See 1 more title\(s\) ▾](#)

Range 1: 42 to 1196 [GenBank](#) [Graphics](#)

[▽ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
2091 bits(1132)	0.0	1154/1163(99%)	8/1163(0%)	Plus/Plus
Query 1	AGTGTCTCGTTGATGATCGGTGCTTGACCGAGATTCAACATGGAACGAGTGGCGGACGG	60		
Sbjct 42	AGT-TCTCGTTGATGATCGGTGCTTGACCGAGATTCAACATGGAACGAGTGGCGGACGG	100		
Query 61	GTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATGCTA	120		
Sbjct 101	GTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATGCTA	160		
Query 121	ATACCGCATAGATCCAAGAACCAGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCT	180		
Sbjct 161	ATACCGCATAGATCCAAGAACCAGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCT	220		
Query 181	TTTGGATGGACCGCGCGCTATTAGCTAGTTGGTGAGGTAAATGGCTACCAAGGCGATGA	240		
Sbjct 221	TTTGGATGGACCGCGCGCTATTAGCTAGTTGGTGAGGTAAATGGCTACCAAGGCGATGA	280		
Query 241	TACGTAGCCGAACAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCAAACTCC	300		
Sbjct 281	TACGTAGCCGAACAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCAAACTCC	340		
Query 301	TACGGGAGGCGAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCG	360		
Sbjct 341	TACGGGAGGCGAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCG	400		
Query 361	CGTGAGTGAAGAAGGCTTTCGGGTCGTAAACTCTGTTGTTGGAGAAGAAATGGTCGGCAG	420		
Sbjct 401	CGTGAGTGAAGAAGGCTTTCGGGTCGTAAACTCTGTTGTTGGAGAAGAAATGGTCGGCAG	460		
Query 421	AGTAACTGTTGCCGCGGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGC	480		
Sbjct 461	AGTAACTGTTGCCGCGGTGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGC	520		
Query 481	AGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGC	540		
Sbjct 521	AGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGC	580		
Query 541	AGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAA	600		
Sbjct 581	AGGCGGTTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAA	640		
Query 601	CTGGGAAACTTGAGTGCAGAAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTA	660		
Sbjct 641	CTGGGAAACTTGAGTGCAGAAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTA	700		
Query 661	GATATATGGAAGAACACAGTGGCGAAGGCGGCTGTCTGGTCTGTAACGACGCTGAGGC	720		
Sbjct 701	GATATATGGAAGAACACAGTGGCGAAGGCGGCTGTCTGGTCTGTAACGACGCTGAGGC	760		

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Query 721 TCGAAAGCATGGGTAGCGAACAGGATTAGATACCTGGTAGTCCATGCCGTAACGATGA 780
      |||
Sbjct 761 TCGAAAGCATGGGTAGCGAACAGGATTAGATACCTGGTAGTCCATGCCGTAACGATGA 820

Query 781 ATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCACTAACGCATTAAGCATTCCG 840
      |||
Sbjct 821 ATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCACTAACGCATTAAGCATTCCG 880

Query 841 CCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAAGCG 900
      |||
Sbjct 881 CCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAAGCG 940

Query 901 GTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCCTTACCAGGTCTTGACATCTTT 960
      |||
Sbjct 941 GTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCCTTACCAGGTCTTGACATCTTT 1000

Query 961 TGATCACCTGAGAGATCAGGTTTCCCTTCGGGGGCAAAATGACAGGTGGTGATGGTTG 1020
      |||
Sbjct 1001 TGATCACCTGAGAGATCAGGTTTCCCTTCGGGGGCAAAATGACAGGTGGTGATGGTTG 1060

Query 1021 TCGTCAGCTCGTGTCTGAGATGTTGGGTTAAGTCCGCAACGAGCGCAACCCTTATGAC 1080
      |||
Sbjct 1061 TCGTCAGCTCGTGTCTGAGATGTTGGGTTAAGTCCGCAACGAGCGCAACCCTTATGAC 1120

Query 1081 TAGTTGCCAGCATTTAGTTGGGGCACTCTAGTAAGAACTGCCGGGTGACAAACCGGGAAG 1140
      |||
Sbjct 1121 TAGTTGCCAGCATTTAGTTGGGGCACTCTAGTAAGAACTGCCGGGTGACAAACCGGGAAG 1175

Query 1141 GAAGGTGGGGGATGACGTCCAAA 1163
      |||
Sbjct 1176 GAAGGTGGGG-ATGACGTC-AAA 1196

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C.11 Blast analysis and alignment results for k3-20

Sequences producing significant alignments

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Graphics

Distance tree of results

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☒	Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	1704	1794	100%	0.0	99.80%	NR_113337.1
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	1704	1794	100%	0.0	99.80%	NR_117987.1
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	1794	1794	100%	0.0	99.80%	NR_025880.1
☒	Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	1790	1790	100%	0.0	99.69%	NR_037122.1
☒	Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	1788	1788	100%	0.0	99.69%	NR_113333.1
☒	Lactobacillus rhamnosus strain NBRC 3425 16S ribosomal RNA, partial sequence	1788	1788	100%	0.0	99.69%	NR_113332.1
☒	Lactobacillus casei subsp. casei ATCC 393 16S ribosomal RNA, partial sequence	1788	1788	100%	0.0	99.69%	NR_041893.1
☒	Lactobacillus casei subsp. casei ATCC 393 strain JCM 1134 16S ribosomal RNA, partial sequence	1788	1788	100%	0.0	99.69%	NR_115534.1
☒	Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial sequence	1784	1784	100%	0.0	99.59%	NR_041054.1
☒	Lactobacillus rhamnosus strain JCM 1136 16S ribosomal RNA, partial sequence	1784	1784	100%	0.0	99.59%	NR_043408.1

Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequenceSequence ID: [NR_113337.1](#) Length: 1495 Number of Matches: 1Range 1: 487 to 1463 [GenBank](#) [Graphics](#)[▼ Next Match](#) [▲ Prev](#)

Score	Expect	Identities	Gaps	Strand
1794 bits(971)	0.0	975/977(99%)	0/977(0%)	Plus/Minus
Query 1	GCTCGCTCCCTAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCATGGTGTGA	60		
Sbjct 1463	GCTCGCTCCCTAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCATGGTGTGA	1404		
Query 61	CGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTGTGATCCGCGATTACT	120		
Sbjct 1403	CGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTGTGATCCGCGATTACT	1344		
Query 121	AGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACTGAGAATGGCTTTA	180		
Sbjct 1343	AGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACTGAGAATGGCTTTA	1284		
Query 181	AGAGATTAGCTTGACCTCGCGGTCGCGAACTCGTTGTACCATCCATTGTAGCACGTGTG	240		
Sbjct 1283	AGAGATTAGCTTGACCTCGCGGTCGCGAACTCGTTGTACCATCCATTGTAGCACGTGTG	1224		
Query 241	TAGCCCAAGGTATGAAGGGGATGATGATTTGACGTATCCCCACCTTCCTCCGGTTTGTC	300		
Sbjct 1223	TAGCCCAAGGTATGAAGGGGATGATGATTTGACGTATCCCCACCTTCCTCCGGTTTGTC	1164		
Query 301	ACCGGCAGTCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGCT	360		
Sbjct 1163	ACCGGCAGTCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGCT	1104		
Query 361	CGTTGCGGGACTTAACCCAACATCTCAGGACACGAGCTGACGACAACCATGCACCACCTG	420		
Sbjct 1103	CGTTGCGGGACTTAACCCAACATCTCAGGACACGAGCTGACGACAACCATGCACCACCTG	1044		
Query 421	TCATTTTGCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCTG	480		
Sbjct 1043	TCATTTTGCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCTG	984		
Query 481	GTAAGGTTCTTCGCGTTGCTTCGAATTAACACATGCTCCACCGCTTGTGCGGGCCCCC	540		
Sbjct 983	GTAAGGTTCTTCGCGTTGCTTCGAATTAACACATGCTCCACCGCTTGTGCGGGCCCCC	924		
Query 541	GTCAATTCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAATGCTTAATGCGT	600		
Sbjct 923	GTCAATTCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAATGCTTAATGCGT	864		
Query 601	TAGCTGCGGCACTGAAGGGCGGAAACCTTCAACACCTAGCATTATCGTTTACGGCATG	660		
Sbjct 863	TAGCTGCGGCACTGAAGGGCGGAAACCTTCAACACCTAGCATTATCGTTTACGGCATG	804		
Query 661	GACTACCAGGGTATCTAATCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTAC	720		
Sbjct 803	GACTACCAGGGTATCTAATCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTAC	744		
Query 721	AGACCAGACAGCCGCTTCGCCACTGGTGTCTTCCATATATCTACGCATTTACCGCTA	780		
Sbjct 743	AGACCAGACAGCCGCTTCGCCACTGGTGTCTTCCATATATCTACGCATTTACCGCTA	684		

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Query 781  CACATGGAGTTCCTACTGTCCTCTTCTGCACTCAAGTTTCCAGTTTCCGATGCGCTTCCT 840
           |||
Sbjct 683  CACATGGAGTTCCTACTGTCCTCTTCTGCACTCAAGTTTCCAGTTTCCGATGCGCTTCCT 624

Query 841  CGGTTAAGCCGAGGGCTTTACATCAGACTTAAAAAACCGCTGCGCTCGCTTTACGCCC 900
           |||
Sbjct 623  CGGTTAAGCCGAGGGCTTTACATCAGACTTAAAAAACCGCTGCGCTCGCTTTACGCCC 564

Query 901  AATAAATCCGGATAACGCTTGCCACCTACATATTACCGCGGCTGCTGGCACGTAATTAGC 960
           |||
Sbjct 563  AATAAATCCGGATAACGCTTGCCACCTACGTAATTACCGCGGCTGCTGGCACGTAATTAGC 504

Query 961  CGTGGCTTTCTGGTTGG 977
           |||
Sbjct 503  CGTGGCTTTCTGGTTGG 487

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C.12 Blast analysis and alignment results for k3-20 a

Sequences producing significant alignments

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[Graphics](#)

[Distance tree of results](#)

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☒	Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial sequence	1450	1450	100%	0.0	99.75%	NR_041054.1
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	1450	1450	100%	0.0	99.75%	NR_025880.1
☒	Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	1447	1447	100%	0.0	99.62%	NR_113337.1
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	1445	1445	100%	0.0	99.62%	NR_117987.1
☒	Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	1411	1411	100%	0.0	98.88%	NR_037122.1
☒	Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	1410	1410	100%	0.0	98.74%	NR_113333.1
☒	Lactobacillus casei subsp. casei ATCC 393 16S ribosomal RNA, partial sequence	1408	1408	100%	0.0	98.74%	NR_041893.1
☒	Lactobacillus casei subsp. casei ATCC 393 strain JCM 1134 16S ribosomal RNA, partial sequence	1399	1399	100%	0.0	98.49%	NR_115534.1
☒	Lactobacillus rhamnosus strain NBRC 3425 16S ribosomal RNA, partial sequence	1389	1389	100%	0.0	98.36%	NR_113332.1

Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial sequence

Sequence ID: [NR_041054.1](#) Length: 1497 Number of Matches: 1

[See 1 more title\(s\) ▼](#)

Range 1: 60 to 850 [GenBank](#) [Graphics](#)

[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
1450 bits(785)	0.0	790/792(99%)	1/792(0%)	Plus/Plus
Query 1	GTGCTTGCACCGAGATTCAACATGGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTA	60		
Sbjct 60	GTGCTTGCACCGAGATTCAACAT-GGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTA	118		
Query 61	ACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAG	120		
Sbjct 119	ACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAG	178		
Query 121	AACCGCATGGTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCGCGGC	180		
Sbjct 179	AACCGCATGGTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCGCGGC	238		
Query 181	GTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACGTAGCCGAAGTGA	240		
Sbjct 239	GTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGATGATACGTAGCCGAAGTGA	298		
Query 241	GGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACCTCTACGGGAGGCAGCAGTAG	300		
Sbjct 299	GGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACCTCTACGGGAGGCAGCAGTAG	358		
Query 301	GGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTT	360		
Sbjct 359	GGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTT	418		
Query 361	TCGGGTCGTAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACGTGTCGGCGT	420		
Sbjct 419	TCGGGTCGTAAACTCTGTTGTTGGAGAAGAATGGTCGGCAGAGTAACGTGTCGGCGT	478		
Query 421	GACGGTATCCAACAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG	480		
Sbjct 479	GACGGTATCCAACAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG	538		
Query 481	GTGGCAAGCGTTATCCGGATTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTAAAGTCT	540		
Sbjct 539	GTGGCAAGCGTTATCCGGATTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTAAAGTCT	598		
Query 541	GATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAACTTGAGTGCA	600		
Sbjct 599	GATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAACTTGAGTGCA	658		
Query 601	GAAGAGGACAGTGGAAGTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACC	660		
Sbjct 659	GAAGAGGACAGTGGAAGTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACC	718		
Query 661	AGTGGCGAAGGCGGCTGTCTGGTCTGTAAGTACGCTGAGGCTCGAAAGCATGGGTAGCG	720		
Sbjct 719	AGTGGCGAAGGCGGCTGTCTGGTCTGTAAGTACGCTGAGGCTCGAAAGCATGGGTAGCG	778		
Query 721	AACAGGATTAGATACCTGGTAGTCCATGCCGTAACGATGAATGCTAGGTGTTGGAGGG	780		
Sbjct 779	AACAGGATTAGATACCTGGTAGTCCATGCCGTAACGATGAATGCTAGGTGTTGGAGGG	838		
Query 781	TTTCCGCCCTTC	792		
Sbjct 839	TTTCCGCCCTTC	850		

C.13 Blast analysis and alignment results for k3-28

Sequences producing significant alignments							Download	Manage Columns	Show	100	?
☒ select all 100 sequences selected											
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession	GenBank	Graphics	Distance tree of results	
☒	Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial sequence	2191	2191	100%	0.0	99.92%	NR_041054.1				
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	2191	2191	100%	0.0	99.92%	NR_025880.1				
☒	Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	2187	2187	100%	0.0	99.83%	NR_113337.1				
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	2178	2178	100%	0.0	99.75%	NR_117987.1				
☒	Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	2126	2126	100%	0.0	98.91%	NR_037122.1				
☒	Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	2122	2122	100%	0.0	98.82%	NR_113333.1				
☒	Lactobacillus casei subsp. casei ATCC 393 16S ribosomal RNA, partial sequence	2119	2119	100%	0.0	98.82%	NR_041893.1				
☒	Lactobacillus casei subsp. casei ATCC 393 strain JCM 1134 16S ribosomal RNA, partial sequence	2111	2111	100%	0.0	98.66%	NR_115534.1				
☒	Lactobacillus rhamnosus strain NBRC 3426 16S ribosomal RNA, partial sequence	2097	2097	100%	0.0	98.49%	NR_113332.1				
☒	Lactobacillus rhamnosus strain NBRC 3426 16S ribosomal RNA, partial sequence	2097	2097	100%	0.0	98.49%	NR_113332.1				

Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial sequence

Sequence ID: [NR_041054.1](#) Length: 1497 Number of Matches: 1

[See 1 more title\(s\)](#) ▼

Range 1: 35 to 1223 [GenBank](#) [Graphics](#)

▼ [Next Match](#) ▲ [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
2191 bits(1186)	0.0	1188/1189(99%)	0/1189(0%)	Plus/Plus
Query 1	TCGAACGAGTTCCTCGTTGATGATCGGTGCTTGACCGAGATTCAACATGGAACGAGTGGC	60		
Sbjct 35	TCGAACGAGTTCCTCGTTGATGATCGGTGCTTGACCGAGATTCAACATGGAACGAGTGGC	94		
Query 61	GGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAG	120		
Sbjct 95	GGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGGAAACAG	154		
Query 121	ATGCTAATACCGCATAGATCCAAGAACCAGCATGGTTCTTGGCTGAAAGATGGCGTAAGCT	180		
Sbjct 155	ATGCTAATACCGCATAGATCCAAGAACCAGCATGGTTCTTGGCTGAAAGATGGCGTAAGCT	214		
Query 181	ATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAGG	240		
Sbjct 215	ATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAGG	274		
Query 241	CGATGATACGTAGCCGAAGTGAAGAGTTGATCGGCCACATTGGGACTGAGACACGGCCCA	300		
Sbjct 275	CGATGATACGTAGCCGAAGTGAAGAGTTGATCGGCCACATTGGGACTGAGACACGGCCCA	334		
Query 301	AACTCCTACGGGAGGAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCA	360		
Sbjct 335	AACTCCTACGGGAGGAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCA	394		
Query 361	ACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAACTCTGTTGTTGGAGAAGATGGT	420		
Sbjct 395	ACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAACTCTGTTGTTGGAGAAGATGGT	454		
Query 421	CGGCAGAGTAACGTGTTGCCGGCGTGACGGTATCCAACAGAAAGCCACGGCTAATACGT	480		
Sbjct 455	CGGCAGAGTAACGTGTTGCCGGCGTGACGGTATCCAACAGAAAGCCACGGCTAATACGT	514		
Query 481	GCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTATTGGGCGTAAAGC	540		
Sbjct 515	GCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTATTGGGCGTAAAGC	574		
Query 541	GAGCGCAGGCGGTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCAT	600		
Sbjct 575	GAGCGCAGGCGGTTTTTAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCAT	634		
Query 601	CGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAA	660		
Sbjct 635	CGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAA	694		
Query 661	TGCGTAGATATATGGAAGAACACCAAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGC	720		
Sbjct 695	TGCGTAGATATATGGAAGAACACCAAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGC	754		

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Query 721   TGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAA   780
Sbjct 755   TGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAA   814

Query 781   CGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAAGC   840
Sbjct 815   CGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAAGC   874

Query 841   ATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCA   900
Sbjct 875   ATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCA   934

Query 901   CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGAC   960
Sbjct 935   CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGAC   994

Query 961   ATCTTTTGATCACCTGAGAGATCAGGTTTCCCTTCGGGGGCAAAATGACAGGTGGTGCA   1020
Sbjct 995   ATCTTTTGATCACCTGAGAGATCAGGTTTCCCTTCGGGGGCAAAATGACAGGTGGTGCA   1054

Query 1021  TGGTTGTCGTCAGCTCGTGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCT   1080
Sbjct 1055  TGGTTGTCGTCAGCTCGTGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCT   1114

Query 1081  TATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAAACCGGA   1140
Sbjct 1115  TATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAAACCGGA   1174

Query 1141  GGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGGGCTA   1189
Sbjct 1175  GGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGGGCTA   1223

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C.14 Blast analysis and alignment results for k4-6a

Sequences producing significant alignments						Download ▾	Manage Columns ▾	Show 100 ▾	?
☒ select all 100 sequences selected							GenBank Graphics Distance tree of results		
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession		
☒	Lactobacillus helveticus strain NBRC 15019 16S ribosomal RNA, partial sequence	2137	2137	100%	0.0	99.86%	NR_113719.1		
☒	Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	2137	2137	100%	0.0	99.86%	NR_042111.1		
☒	Lactobacillus gallinarum strain JCM 2011 16S ribosomal RNA, partial sequence	2137	2137	100%	0.0	99.86%	NR_113281.1		
☒	Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	2137	2137	100%	0.0	99.86%	NR_117081.1		
☒	Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	2137	2137	100%	0.0	99.86%	NR_117080.1		
☒	Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	2137	2137	100%	0.0	99.86%	NR_042439.1		
☒	Lactobacillus acidophilus strain NBRC 13951 16S ribosomal RNA, partial sequence	2054	2054	100%	0.0	98.37%	NR_113838.1		

k4-6a helveticus l.gallinarium	CATCGGAAACTGTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTG CATCGGAAACTGTTTTCTTGAGTGCAGAAGAGGAGAGTGGAAATCCATGTGTAGCGGTG CATCGGAAACTGTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTG *****
k4-6a helveticus l.gallinarium	GAATGCGTAGATATATGGAAGAACACCACTGGCGAAGGCGACTCTCTGGTCTGCAACTGA GAATGCGTAGATATATGGAAGAACACCACTGGCGAAGGCGACTCTCTGGTCTGCAACTGA GAATGCGTAGATATATGGAAGAACACCACTGGCGAAGGCGGCTCTCTGGTCTGCAACTGA *****
k4-6a helveticus l.gallinarium	CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGT CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGT CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGT *****
k4-6a helveticus l.gallinarium	AAACGATGAGTGCTAAGTGTGGGAGGTTTCCGCTCTCAGTGCTGCAGCTAACGCATTA AAACGATGAGTGCTAAGTGTGGGAGGTTTCCGCTCTCAGTGCTGCAGCTAACGCATTA AAACGATGAGTGCTAAGTGTGGGAGGTTTCCGCTCTCAGTGCTGCAGCTAACGCATTA *****



Lactobacillus helveticus strain NBRC 15019 16S ribosomal RNA, partial sequence

Sequence ID: [NR_113719.1](#) Length: 1487 Number of Matches: 1

Range 1: 28 to 1196 [GenBank](#) [Graphics](#)

[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
2137 bits(1157)	0.0	1165/1169(99%)	0/1169(0%)	Plus/Plus
Query 1	TGCAAGTCGAGCGAGCAGAACAGCAGATTACTTCGGTAATGACGCTGGGGACGCGAGC	60		
Sbjct 28	TGCAAGTCGAGCGAGCAGAACAGCAGATTACTTCGGTAATGACGCTGGGGACGCGAGC	87		
Query 61	GGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCCATAGTCTGGGATACCACTTGGAAA	120		
Sbjct 88	GGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCCATAGTCTGGGATACCACTTGGAAA	147		
Query 121	CAGGTGCTAATACCGGATAAGAAAGCAGATCGCATGATCAGCTTATAAAAGGCGGCTAA	180		
Sbjct 148	CAGGTGCTAATACCGGATAAGAAAGCAGATCGCATGATCAGCTTATAAAAGGCGGCTAA	207		
Query 181	GCTGTCGCTATGGGATGGCCCCGCGGTGATTAGCTAGTTGGTAAGGTAACGGCTTACCA	240		
Sbjct 208	GCTGTCGCTATGGGATGGCCCCGCGGTGATTAGCTAGTTGGTAAGGTAACGGCTTACCA	267		
Query 241	AGGCAATGATGATAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGC	300		
Sbjct 268	AGGCAATGATGATAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGC	327		
Query 301	CCAAACTCCTACGGGAGGCGAGTAGGGAATCTTCCCAATGGACGCAAGTCTGATGGA	360		
Sbjct 328	CCAAACTCCTACGGGAGGCGAGTAGGGAATCTTCCCAATGGACGCAAGTCTGATGGA	387		
Query 361	GCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTGGTGAAGAAG	420		
Sbjct 388	GCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTGGTGAAGAAG	447		
Query 421	GATAGAGGCGAGTAAGTGGCCTTTATTTGACGGTAATCAACCAGAAAGTCACGGCTAATA	480		
Sbjct 448	GATAGAGGCGAGTAAGTGGCCTTTATTTGACGGTAATCAACCAGAAAGTCACGGCTAATA	507		
Query 481	CGTGCCAGCAGCGCGGTAATACGTAGGTGGCAAGCGTTGTCGGGATTTATTGGGCGTAA	540		
Sbjct 508	CGTGCCAGCAGCGCGGTAATACGTAGGTGGCAAGCGTTGTCGGGATTTATTGGGCGTAA	567		
Query 541	AGCGAGCGCAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATG	600		
Sbjct 568	AGCGAGCGCAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATG	627		
Query 601	CATCGGAAACTGTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTG	660		
Sbjct 628	CATCGGAAACTGTTTTCTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTG	687		
Query 661	GAATGCGTAGATATATGGAAGAACCAGTGGCGAAGGCGACTCTCTGGTCTGCAACTGA	720		
Sbjct 688	GAATGCGTAGATATATGGAAGAACCAGTGGCGAAGGCGACTCTCTGGTCTGCAACTGA	747		
Query 721	CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCTTGGTAGTCCATGCCGT	780		
Sbjct 748	CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCTTGGTAGTCCATGCCGT	807		

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Query 781 AAACGATGAGTGCTAAGTGTGGGAGGTTCCGCCTCTCAGTGCTGCAGCTAACGCATTA 840
Sbjct 808 AAACGATGAGTGCTAAGTGTGGGAGGTTCCGCCTCTCAGTGCTGCAGCTAACGCATTA 867

Query 841 AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCC 900
Sbjct 868 AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCC 927

Query 901 GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTT 960
Sbjct 928 GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTT 987

Query 961 GACATCTAGTGCCATCCTAAGAGATTAGGAGTTCCTTCGGGGACGCTAAGACAGGTGGT 1020
Sbjct 988 GACATCTAGTGCCATCCTAAGAGATTAGGAGTTCCTTCGGGGACGCTAAGACAGGTGGT 1047

Query 1021 GCATGGCTGTGTCAGCTCGTGTCTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC 1080
Sbjct 1048 GCATGGCTGTGTCAGCTCGTGTCTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC 1107

Query 1081 CCTTGTTATTAGTTGCCAGCATTAAAGTTGGGCACTCTAATGAAACTGCCGGTGACAAACC 1140
Sbjct 1108 CCTTGTTATTAGTTGCCAGCATTAAAGTTGGGCACTCTAATGAGACTGCCGGTGATAAAACC 1167

Query 1141 GGAGGAAGGTGGGGATGACGTCAAGTCAT 1169
Sbjct 1168 GGAGGAAGGTGGGGATGACGTCAAGTCAT 1196

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C.15 Blast analysis and alignment results for k4-16

Sequences producing significant alignments							Download	Manage Columns	Show	100	
☒ select all 100 sequences selected											
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession				
☒	Lactobacillus paracasei subsp. tolerans strain NBRC 15908 16S ribosomal RNA, partial sequence	1447	1447	100%	0.0	99.87%	NR_041064.1				
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	1447	1447	100%	0.0	99.87%	NR_025880.1				
☒	Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	1443	1443	100%	0.0	99.75%	NR_113337.1				
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	1441	1441	100%	0.0	99.75%	NR_117987.1				
☒	Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	1408	1408	100%	0.0	98.98%	NR_037122.1				
☒	Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	1406	1406	100%	0.0	98.85%	NR_113333.1				

Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial seque

Sequence ID: [NR_041054.1](#) Length: 1497 Number of Matches: 1

[See 1 more title\(s\) ▼](#)

Range 1: 62 to 847 [GenBank](#) [Graphics](#)

[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
1447 bits(783)	0.0	785/786(99%)	0/786(0%)	Plus/Plus
Query 1	GCTTGCACCGAGATTCAACATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACC	60		
Sbjct 62	GCTTGCACCGAGATTCAACATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACC	121		
Query 61	TGCCCTTAAGTGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAAC	120		
Sbjct 122	TGCCCTTAAGTGGGGGATAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAAC	181		
Query 121	CGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGCGGCGTA	180		
Sbjct 182	CGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGCGGCGTA	241		
Query 181	TTAGCTAGTTGGTGAAGTAATGGCTCACCAAGGCGATGATACGTAGCCGAACAGAGGT	240		
Sbjct 242	TTAGCTAGTTGGTGAAGTAATGGCTCACCAAGGCGATGATACGTAGCCGAACAGAGGT	301		
Query 241	TGATCGGCCACATTGGGACTGAGACACGGCCCAAACCTCTACGGGAGGCAGCAGTAGGGA	300		
Sbjct 302	TGATCGGCCACATTGGGACTGAGACACGGCCCAAACCTCTACGGGAGGCAGCAGTAGGGA	361		
Query 301	ATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCG	360		
Sbjct 362	ATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCG	421		
Query 361	GGTCGTAAAACCTCTGTTGTTGGAGAAGAAATGGTCGGCAGAGTAACGTGTCGGCGTGAC	420		
Sbjct 422	GGTCGTAAAACCTCTGTTGTTGGAGAAGAAATGGTCGGCAGAGTAACGTGTCGGCGTGAC	481		
Query 421	GGTATCCAACAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAAACGTAGGTG	480		
Sbjct 482	GGTATCCAACAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAAACGTAGGTG	541		
Query 481	GCAAGCGTTATCCGGATTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTAAGTCTGAT	540		
Sbjct 542	GCAAGCGTTATCCGGATTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTAAGTCTGAT	601		
Query 541	GTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAA	600		
Sbjct 602	GTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAAACTTGAGTGCAGAA	661		
Query 601	GAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACCAGT	660		
Sbjct 662	GAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACCAGT	721		
Query 661	GGCGAAGGCGGCTGTCTGGTCTGTAACGTGACGCTGAGGCTCGAAAGCATGGGTAGCGAAC	720		
Sbjct 722	GGCGAAGGCGGCTGTCTGGTCTGTAACGTGACGCTGAGGCTCGAAAGCATGGGTAGCGAAC	781		
Query 721	AGGATTAGATACCTGGTAGTCCATGCCGTAACGATGAATGCTAGGTGTTGGAGGGTTT	780		
Sbjct 782	AGGATTAGATACCTGGTAGTCCATGCCGTAACGATGAATGCTAGGTGTTGGAGGGTTT	841		
Query 781	CCGCCC 786			
Sbjct 842	CCGCCC 847			

C.16 Blast analysis and alignment results for k4-17

Sequences producing significant alignments							Download	Manage Columns	Show	100	
☒ select all 100 sequences selected											
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession				
☒	Lactobacillus paracasei strain NBRC 15839 16S ribosomal RNA, partial sequence	1936	1936	100%	0.0	99.81%	NR_113337.1				
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	1936	1936	100%	0.0	99.81%	NR_117987.1				
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	1936	1936	100%	0.0	99.81%	NR_025880.1				
☒	Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	1932	1932	100%	0.0	99.72%	NR_037122.1				
☒	Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	1930	1930	100%	0.0	99.72%	NR_113333.1				
☒	Lactobacillus rhamnosus strain NBRC 3425 16S ribosomal RNA, partial sequence	1930	1930	100%	0.0	99.72%	NR_113332.1				
☒	Lactobacillus casei subsp. casei ATCC 393 16S ribosomal RNA, partial sequence	1930	1930	100%	0.0	99.72%	NR_041893.1				



Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence

Sequence ID: [NR_113337.1](#) Length: 1495 Number of Matches: 1

Range 1: 409 to 1463 [GenBank](#) [Graphics](#)

[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
1936 bits(1048)	0.0	1053/1055(99%)	1/1055(0%)	Plus/Minus
Query 1	GCTCGCTCCCT-AAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCATGGTGTGA	59		
Sbjct 1463	GCTCGCTCCCTAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCATGGTGTGA	1404		
Query 60	CGGGCGGTGTGTACAAGGCCCGGGAACGTATTACCGCGGCGTGTGATCCGCGATTACT	119		
Sbjct 1403	CGGGCGGTGTGTACAAGGCCCGGGAACGTATTACCGCGGCGTGTGATCCGCGATTACT	1344		
Query 120	AGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACTGAGAATGGCTTTA	179		
Sbjct 1343	AGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACTGAGAATGGCTTTA	1284		
Query 180	AGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGTG	239		
Sbjct 1283	AGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGTG	1224		
Query 240	TAGCCAGGTCTAAGGGGATGATGATTTGACGTATCCCACTTCTCCGGTTTGTG	299		
Sbjct 1223	TAGCCAGGTCTAAGGGGATGATGATTTGACGTATCCCACTTCTCCGGTTTGTG	1164		
Query 300	ACCGGCGAGTCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGCT	359		
Sbjct 1163	ACCGGCGAGTCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGCT	1104		
Query 360	CGTTGCGGGACTTAACCAACATCTCAGGACGAGCTGACGACAACCATGCACACCTG	419		
Sbjct 1103	CGTTGCGGGACTTAACCAACATCTCAGGACGAGCTGACGACAACCATGCACACCTG	1044		
Query 420	TCATTTTGGCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCTG	479		
Sbjct 1043	TCATTTTGGCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCTG	984		
Query 480	GTAAGGTTCTTCGCGTTGCTTCGAATTAACACATGCTCCACCGCTTGTGCGGGCCCCC	539		
Sbjct 983	GTAAGGTTCTTCGCGTTGCTTCGAATTAACACATGCTCCACCGCTTGTGCGGGCCCCC	924		
Query 540	GTCAATTCTTTGAGTTTCAACCTTGCAGTCTGCTACCTCCAGGCGGAATGCTTAATGCGT	599		
Sbjct 923	GTCAATTCTTTGAGTTTCAACCTTGCAGTCTGCTACCTCCAGGCGGAATGCTTAATGCGT	864		
Query 600	TAGCTGCGGCATGAAGGGCGGAAACCTCCAACACCTAGCATTTCATCGTTTACGGCATG	659		
Sbjct 863	TAGCTGCGGCATGAAGGGCGGAAACCTCCAACACCTAGCATTTCATCGTTTACGGCATG	804		
Query 660	GACTACAGGGTATCTAATCCTGTTTCGCTACCCATGCTTTGAGCCTCAGCGTCAGTTAC	719		
Sbjct 803	GACTACAGGGTATCTAATCCTGTTTCGCTACCCATGCTTTGAGCCTCAGCGTCAGTTAC	744		
Query 720	AGACCAGACAGCCGCTTCGCCACTGGTGTCTTCCATATATCTACGCATTTACCGCTA	779		
Sbjct 743	AGACCAGACAGCCGCTTCGCCACTGGTGTCTTCCATATATCTACGCATTTACCGCTA	684		

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Query 780  CACATGGAGTTCCTACTGTCCTCTTCTGCACTCAAGTTTCCAGTTTCCGATGCGCTTCCT 839
          |||
Sbjct 683  CACATGGAGTTCCTACTGTCCTCTTCTGCACTCAAGTTTCCAGTTTCCGATGCGCTTCCT 624

Query 840  CGGTTAAGCCGAGGGCTTTACATCAGACTTAAAAACCGCTGCGCTCGCTTTACGCCC 899
          |||
Sbjct 623  CGGTTAAGCCGAGGGCTTTACATCAGACTTAAAAACCGCTGCGCTCGCTTTACGCCC 564

Query 900  AATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGC 959
          |||
Sbjct 563  AATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGC 504

Query 960  CGTGGCTTTCTGGTTGGATACCGTCACGCCGGCAACAGTTACTCTGCCGACCATTCTTCT 1019
          |||
Sbjct 503  CGTGGCTTTCTGGTTGGATACCGTCACGCCGGCAACAGTTACTCTGCCGACCATTCTTCT 444

Query 1020 CCAACAACAGAGTTTTACGACCCGAAAGCCTTCTT 1054
          |||
Sbjct 443  CCAACAACAGAGTTTTACGACCCGAAAGCCTTCTT 409

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C.17 Blast analysis and alignment results for k4-28

Sequences producing significant alignments

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100

☒ select all 100 sequences selected

[GenBank](#)

[Graphics](#)

[Distance tree of results](#)

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☒	Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial sequence	2172	2172	100%	0.0	99.50%	NR_041054.1
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	2172	2172	100%	0.0	99.50%	NR_025880.1
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	2170	2170	99%	0.0	99.58%	NR_117987.1
☒	Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	2169	2169	100%	0.0	99.41%	NR_113337.1
☒	Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	2108	2108	100%	0.0	98.49%	NR_037122.1
☒	Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	2104	2104	100%	0.0	98.41%	NR_113333.1

Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial sequence

Sequence ID: [NR_041054.1](#) Length: 1497 Number of Matches: 1

[See 1 more title\(s\)](#) ▼

Range 1: 29 to 1223 [GenBank](#) [Graphics](#)

▼ [Next Match](#) ▲ [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
2172 bits(1176)	0.0	1189/1195(99%)	2/1195(0%)	Plus/Plus
Query 1	TGC-AGTCG-ACGAGTTCTCGTTGATGATCGGTGCTTGACCCGAGATTCAACATGGAACG	58		
Sbjct 29	TGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGACCCGAGATTCAACATGGAACG	88		
Query 59	AGTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGATAACATTTGG	118		
Sbjct 89	AGTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGATAACATTTGG	148		
Query 119	AAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCG	178		
Sbjct 149	AAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCG	208		
Query 179	TAAGCTATCGCTTTTGGATGGACCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA	238		
Sbjct 209	TAAGCTATCGCTTTTGGATGGACCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA	268		
Query 239	CCAAGGCGATGATACGTAGCCGAACGAGAGGTTGATCGGCCACATTGGGACTGAGACAC	298		
Sbjct 269	CCAAGGCGATGATACGTAGCCGAACGAGAGGTTGATCGGCCACATTGGGACTGAGACAC	328		
Query 299	GGCCCAAACCTCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT	358		
Sbjct 329	GGCCCAAACCTCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT	388		
Query 359	GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAACTCTGTTGTTGGAGAAG	418		
Sbjct 389	GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAACTCTGTTGTTGGAGAAG	448		
Query 419	AATGGTCGCGCAGAGTAAGTGTGCGGCGTGACGGTATCCAACAGAAAGCCACGGCTAA	478		
Sbjct 449	AATGGTCGCGCAGAGTAAGTGTGCGGCGTGACGGTATCCAACAGAAAGCCACGGCTAA	508		
Query 479	CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTATTGGGCG	538		
Sbjct 509	CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTATTGGGCG	568		
Query 539	TAAAGCGAGCGCAGGCGGTTTTTAAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA	598		
Sbjct 569	TAAAGCGAGCGCAGGCGGTTTTTAAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA	628		
Query 599	GCGCATCGGAAACTGGGAACTTGAGTGCAGAAAGAGGACAGTGGAACTCCATGTGTAGCG	658		
Sbjct 629	GCGCATCGGAAACTGGGAACTTGAGTGCAGAAAGAGGACAGTGGAACTCCATGTGTAGCG	688		
Query 659	GTGAAATGCGTAGATATATGGAAGAACACAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC	718		
Sbjct 689	GTGAAATGCGTAGATATATGGAAGAACACAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC	748		

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Query 719  TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCTGGTAGTCCATGC 778
Sbjct 749  TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCTGGTAGTCCATGC 808

Query 779  CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA 838
Sbjct 809  CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA 868

Query 839  TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG 898
Sbjct 869  TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG 928

Query 899  CCCGCACAAGCGGTGGAGCATGTGGTTTAATTGGAAGCAACGCGAAGAACCCTTACCAGGT 958
Sbjct 929  CCCGCACAAGCGGTGGAGCATGTGGTTTAATTGGAAGCAACGCGAAGAACCCTTACCAGGT 988

Query 959  CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCTTCGGGGGCAAAATGACAGGT 1018
Sbjct 989  CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCTTCGGGGGCAAAATGACAGGT 1048

Query 1019  GGTGCATGGTTGTCGTCAGCTCGTGTCTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC 1078
Sbjct 1049  GGTGCATGGTTGTCGTCAGCTCGTGTCTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC 1108

Query 1079  AACCTTATGACTAGTTGCCAGCATTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAA 1138
Sbjct 1109  AACCTTATGACTAGTTGCCAGCATTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAA 1168

Query 1139  ACCGGAGGAAggggggATGACGTCAAATCATCATGCCCTTATGACTGGGGCTA 1193
Sbjct 1169  ACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGACTGGGGCTA 1223

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C.18 Blast analysis and alignment results for k5-3

Sequences producing significant alignments		Download	Manage Columns	Show	100	
☒ select all	100 sequences selected					
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident
☒	Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial sequence	2224	2224	100%	0.0	99.43%
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	2224	2224	100%	0.0	99.43%
☒	Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	2220	2220	100%	0.0	99.35%
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	2211	2211	99%	0.0	99.35%
☒	Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	2150	2150	100%	0.0	98.45%
☒	Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	2150	2150	100%	0.0	98.37%
☒	Lactobacillus casei subsp. casei ATCC 393 16S ribosomal RNA, partial sequence	2152	2152	100%	0.0	98.37%
☒	Lactobacillus casei subsp. casei ATCC 393 strain JCM 1134 16S ribosomal RNA, partial sequence	2145	2145	100%	0.0	98.21%
☒	Lactobacillus rhamnosus strain NBRC 3425 16S ribosomal RNA, partial sequence	2130	2130	100%	0.0	98.05%

Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial seq

Sequence ID: [NR_041054.1](#) Length: 1497 Number of Matches: 1

[See 1 more title\(s\)](#) ▼

Range 1: 29 to 1256 [GenBank](#) [Graphics](#)

▼ [Next Match](#) ▲ [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
2224 bits(1204)	0.0	1221/1228(99%)	6/1228(0%)	Plus/Plus
Query 1	TGC-AGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACG	59		
Sbjct 29	TGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACG	88		
Query 60	AGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGG	119		
Sbjct 89	AGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAACATTTGG	148		
Query 120	AAACAGATGCTAATACCGCATAGATCCAAGAACCAGCATGGTCTTGGCTGAAAGATGGCG	179		
Sbjct 149	AAACAGATGCTAATACCGCATAGATCCAAGAACCAGCATGGTCTTGGCTGAAAGATGGCG	208		
Query 180	TAAGCTATCGCTTTTGGATGGACCGCGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA	239		
Sbjct 209	TAAGCTATCGCTTTTGGATGGACCGCGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA	268		
Query 240	CCAAGGCGATGATACGTAGCCGAACAGAGAGTTGATCGGCCACATTGGGACTGAGACAC	299		
Sbjct 269	CCAAGGCGATGATACGTAGCCGAACAGAGAGTTGATCGGCCACATTGGGACTGAGACAC	328		
Query 300	GGCCCAAACCTCTACGGGAGGCGAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT	359		
Sbjct 329	GGCCCAAACCTCTACGGGAGGCGAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT	388		
Query 360	GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACCTCTGTTGTTGGAGAAG	419		
Sbjct 389	GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACCTCTGTTGTTGGAGAAG	448		
Query 420	AATGGTCGGCAGAGTAACGTGTTGCCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA	479		
Sbjct 449	AATGGTCGGCAGAGTAACGTGTTGCCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA	508		
Query 480	CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTATTGGGCG	539		
Sbjct 509	CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTATTGGGCG	568		
Query 540	TAAAGCGAGCGCAGGCGGTTTTTAAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA	599		
Sbjct 569	TAAAGCGAGCGCAGGCGGTTTTTAAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA	628		
Query 600	GCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG	659		
Sbjct 629	GCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG	688		
Query 660	GTGAAATGCGTAGATATATGGAAGAACCACAGTGGCGAAGGCGGCTGTCTGGTCTGTAAAC	719		
Sbjct 689	GTGAAATGCGTAGATATATGGAAGAACCACAGTGGCGAAGGCGGCTGTCTGGTCTGTAAAC	748		

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Query 720 TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCTGGTAGTCCATGC 779
Sbjct 749 TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCTGGTAGTCCATGC 808

Query 780 CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA 839
Sbjct 809 CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA 868

Query 840 TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG 899
Sbjct 869 TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG 928

Query 900 CCCGCACAAGCGGTGGAGCATGTGGTTAATTGGAAGCAACGCGAAGAACCTTACCAGGT 959
Sbjct 929 CCCGCACAAGCGGTGGAGCATGTGGTTAATTGGAAGCAACGCGAAGAACCTTACCAGGT 988

Query 960 CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCTTCGGGGGCAAAATGACAGGT 1019
Sbjct 989 CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCTTCGGGGGCAAAATGACAGGT 1048

Query 1020 GGTGCATGGTTGTCGTGAGCTCGTGTCTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC 1079
Sbjct 1049 GGTGCATGGTTGTCGTGAGCTCGTGTCTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC 1108

Query 1080 AACCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAA 1139
Sbjct 1109 AACCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAA 1168

Query 1140 ACCGGAGGAAGGTGGG-ATGACGTC-AATCATCATGCCCCCTTATGACCTGGGCTAC-CAC 1196
Sbjct 1169 ACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCCTTATGACCTGGGCTACACAC 1228

Query 1197 GTGCTACA-TGGATGGTACA-CGAGTTG 1222
Sbjct 1229 GTGCTACAATGGATGGTACAACGAGTTG 1256

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C.19 Blast analysis and alignment results for k5-3 a

Sequences producing significant alignments

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	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☒	Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	2206	2206	100%	0.0	99.75%	NR_113337.1
☒	Lactobacillus paracasei subsp. tolerans strain NBRC 15909 16S ribosomal RNA, partial sequence	2204	2204	100%	0.0	99.75%	NR_041054.1
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	2204	2204	100%	0.0	99.75%	NR_026880.1
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	2191	2191	99%	0.0	99.87%	NR_117987.1
☒	Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	2156	2156	100%	0.0	99.00%	NR_037122.1
☒	Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	2152	2152	100%	0.0	98.92%	NR_113333.1
☒	Lactobacillus casei subsp. casei ATCC 393 16S ribosomal RNA, partial sequence	2150	2150	100%	0.0	98.92%	NR_041893.1
☒	Lactobacillus casei subsp. casei ATCC 393 strain JCM 1134 16S ribosomal RNA, partial sequence	2141	2141	100%	0.0	98.75%	NR_115534.1
☒	Lactobacillus rhamnosus strain NBRC 3425 16S ribosomal RNA, partial sequence	2126	2126	100%	0.0	98.59%	NR_113332.1

Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence

Sequence ID: [NR_113337.1](#) Length: 1495 Number of Matches: 1

Range 1: 19 to 1221 [GenBank](#) [Graphics](#)

[Next Match](#) [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
2206 bits(1194)	0.0	1200/1203(99%)	1/1203(0%)	Plus/Plus
Query 1		GCCTAATACATGCAAGTCGAACGAGTTCTTGTGATGATCGGTGCTTGCACCGAGATTCA		60
Sbjct 19		GCCTAATACATGCAAGTCGAACGAGTTCTTGTGATGATCGGTGCTTGCACCGAGATTCA		78
Query 61		ACATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGA		120
Sbjct 79		ACATGGAACGAGTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGA		138
Query 121		TAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAACCAGATGGTTCTTGGCTG		180
Sbjct 139		TAACATTTGGAAACAGATGCTAATACCGCATAGATCCAAGAACCAGATGGTTCTTGGCTG		198
Query 181		AAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGCGCGTATTAGCTAGTTGGTGAGG		240
Sbjct 199		AAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGCGCGTATTAGCTAGTTGGTGAGG		258
Query 241		TAACGGCTCACCAAGGCGATGATACGTAGCCGAACAGAGGTTGATCGGCCACATTGGG		300
Sbjct 259		TAACGGCTCACCAAGGCGATGATACGTAGCCGAACAGAGGTTGATCGGCCACATTGGG		318
Query 301		ACTGAGACACGGCCAACTCCTACGGGAGGAGCAGTAGGGAATCTTCCACAATGGACG		360
Sbjct 319		ACTGAGACACGGCCAACTCCTACGGGAGGAGCAGTAGGGAATCTTCCACAATGGACG		378
Query 361		CAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAACTCTGTT		420
Sbjct 379		CAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAACTCTGTT		438
Query 421		GTTGGAGAAGAAATGGTCGGCAGAGTAAGTGTGTCGGCGTGACGGTATCCAACAGAAAG		480
Sbjct 439		GTTGGAGAAGAAATGGTCGGCAGAGTAAGTGTGTCGGCGTGACGGTATCCAACAGAAAG		498
Query 481		CCACGGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGTGGCAAGCGTTATCCGGAT		540
Sbjct 499		CCACGGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGTGGCAAGCGTTATCCGGAT		558
Query 541		TTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTAAGTCTGATGTGAAAGCCCTCGGCTT		600
Sbjct 559		TTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTAAGTCTGATGTGAAAGCCCTCGGCTT		618
Query 601		AACCGAGGAAGCGCATCGGAACTGGGAACTTGAGTGCAGAAGAGGACAGTGGAACTCC		660
Sbjct 619		AACCGAGGAAGCGCATCGGAACTGGGAACTTGAGTGCAGAAGAGGACAGTGGAACTCC		678
Query 661		ATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACCAGTGGCGAAGGCGGCTGTCT		720
Sbjct 679		ATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACCAGTGGCGAAGGCGGCTGTCT		738
Query 721		GGTCTGTAAGTACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCTGG		780
Sbjct 739		GGTCTGTAAGTACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCTGG		798

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Query 781 TAGTCCATGCCGTAACGATGAATGCTAGGTGTTGGAGGGTTCCGCCCTTCAGTGCCGC 840
      |||
Sbjct 799 TAGTCCATGCCGTAACGATGAATGCTAGGTGTTGGAGGGTTCCGCCCTTCAGTGCCGC 858

Query 841 AGCTAACGCATTAAAGCATTCCGCCCTGGGGAGTACGACCACAAGGTTGAAACTCAAAGGAA 900
      |||
Sbjct 859 AGCTAACGCATTAAAGCATTCCGCCCTGGGGAGTACGACCACAAGGTTGAAACTCAAAGGAA 918

Query 901 TTGACGGGGGCCCGCACAAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAAC 960
      |||
Sbjct 919 TTGACGGGGGCCCGCACAAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAAC 978

Query 961 CTTACCAGGTCTTGACATCTTTGATCACCTGAGAGATCAGGTTTCCCTTCGGGGGCAA 1020
      |||
Sbjct 979 CTTACCAGGTCTTGACATCTTTGATCACCTGAGAGATCAGGTTTCCCTTCGGGGGCAA 1038

Query 1021 AATGACAGGTGGTGCATGGTTGTCGTGAGCTCGTGTGAGATGTTGGGTTAAGTCCCG 1080
      |||
Sbjct 1039 AATGACAGGTGGTGCATGGTTGTCGTGAGCTCGTGTGAGATGTTGGGTTAAGTCCCG 1098

Query 1081 CAACGAGCGCAACCCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACCTAGTAAGACTG 1140
      |||
Sbjct 1099 CAACGAGCGCAACCCCTTATGACTAGTTGCCAGCATTTAGTTGGGCACCTAGTAAGACTG 1158

Query 1141 CCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAAT-ATCATGCCCTTATGACCTG 1199
      |||
Sbjct 1159 CCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTG 1218

Query 1200 GGC 1202
      |||
Sbjct 1219 GGC 1221

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C.20 Blast analysis and alignment results for k5-5 a

Sequences producing significant alignments

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	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☒	Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	2025	2025	100%	0.0	99.91%	NR_113337.1
☒	Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial sequence	2023	2023	100%	0.0	99.91%	NR_041054.1
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	2023	2023	100%	0.0	99.91%	NR_025880.1
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	2017	2017	100%	0.0	99.82%	NR_117987.1
☒	Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	1973	1973	100%	0.0	99.09%	NR_037122.1
☒	Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	1971	1971	100%	0.0	99.00%	NR_113333.1
☒	Lactobacillus casei subsp. casei ATCC 393 16S ribosomal RNA, partial sequence	1967	1967	100%	0.0	99.00%	NR_041893.1
☒	Lactobacillus casei subsp. casei ATCC 393 strain JCM 1134 16S ribosomal RNA, partial sequence	1960	1960	100%	0.0	98.82%	NR_115534.1
☒	Lactobacillus rhamnosus strain NBRC 3425 16S ribosomal RNA, partial sequence	1940	1940	100%	0.0	98.54%	NR_113332.1

Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence

Sequence ID: [NR_113337.1](#) Length: 1495 Number of Matches: 1

Range 1: 29 to 1126 [GenBank](#) [Graphics](#)

[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
2025 bits(1096)	0.0	1097/1098(99%)	0/1098(0%)	Plus/Plus
Query 1		TGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGACCCGAGATTCAACATGGAACG	60	
Sbjct 29		TGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGACCCGAGATTCAACATGGAACG	88	
Query 61		AGTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGATAACATTTGG	120	
Sbjct 89		AGTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGATAACATTTGG	148	
Query 121		AAACAGATGCTAATACCGCATAGATCCAAGAACCAGCATGGTTCTTGGCTGAAAGATGGCG	180	
Sbjct 149		AAACAGATGCTAATACCGCATAGATCCAAGAACCAGCATGGTTCTTGGCTGAAAGATGGCG	208	
Query 181		TAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAACGGCTCA	240	
Sbjct 209		TAAGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCTAGTTGGTGAGGTAANGGCTCA	268	
Query 241		CCAAGGCGATGATACGTAGCCGAAGTGAAGGTTGATCGGCCACATTGGGACTGAGACAC	300	
Sbjct 269		CCAAGGCGATGATACGTAGCCGAAGTGAAGGTTGATCGGCCACATTGGGACTGAGACAC	328	
Query 301		GGCCCAAACCTCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT	360	
Sbjct 329		GGCCCAAACCTCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGAT	388	
Query 361		GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTTCGGGTCGTAAACTCTGTTGTTGGAGAAG	420	
Sbjct 389		GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTTCGGGTCGTAAACTCTGTTGTTGGAGAAG	448	
Query 421		AATGGTCGGCAGAGTAAGTGTGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA	480	
Sbjct 449		AATGGTCGGCAGAGTAAGTGTGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA	508	
Query 481		CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCG	540	
Sbjct 509		CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCG	568	
Query 541		TAAAGCGAGCGCAGGCGGTTTTTAAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA	600	
Sbjct 569		TAAAGCGAGCGCAGGCGGTTTTTAAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA	628	
Query 601		GCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG	660	
Sbjct 629		GCGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG	688	
Query 661		GTGAAATGCGTAGATATATGGAAGAACACAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC	720	
Sbjct 689		GTGAAATGCGTAGATATATGGAAGAACACAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC	748	
Query 721		TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCTTGGTAGTCCATGC	780	
Sbjct 749		TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCTTGGTAGTCCATGC	808	

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Query 781  CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA 840
Sbjct 809  CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCA 868

Query 841  TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG 900
Sbjct 869  TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG 928

Query 901  CCCGCACAAGCGGTGGAGCATGTGGTTAATTCGAAGCAACGCGAAGAACCTTACCAGGT 960
Sbjct 929  CCCGCACAAGCGGTGGAGCATGTGGTTAATTCGAAGCAACGCGAAGAACCTTACCAGGT 988

Query 961  CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCTTCGGGGGCAAAATGACAGGT 1020
Sbjct 989  CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCTTCGGGGGCAAAATGACAGGT 1048

Query 1021  GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCACAACGAGCGC 1080
Sbjct 1049  GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCACAACGAGCGC 1108

Query 1081  AACCCCTTATGACTAGTTG 1098
Sbjct 1109  AACCCCTTATGACTAGTTG 1126

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C.21 Blast analysis and alignment results for k5-11

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	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☒	Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	2071	2071	100%	0.0	99.56%	NR_113337.1
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	2071	2071	100%	0.0	99.56%	NR_025880.1
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	2069	2069	99%	0.0	99.56%	NR_117897.1
☒	Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	2067	2067	100%	0.0	99.47%	NR_037122.1
☒	Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	2065	2065	100%	0.0	99.47%	NR_113333.1
☒	Lactobacillus rhamnosus strain NBRC 3425 16S ribosomal RNA, partial sequence	2065	2065	100%	0.0	99.47%	NR_113332.1
☒	Lactobacillus casei subsp. casei ATCC 393 16S ribosomal RNA, partial sequence	2065	2065	100%	0.0	99.47%	NR_041893.1
☒	Lactobacillus paracasei subsp. tolerans strain NBRC 15908 16S ribosomal RNA, partial sequence	2061	2061	100%	0.0	99.39%	NR_041054.1
☒	Lactobacillus casei subsp. casei ATCC 393 strain JCM 1134 16S ribosomal RNA, partial sequence	2060	2060	100%	0.0	99.38%	NR_115534.1
☒	Lactobacillus rhamnosus strain JCM 1136 16S ribosomal RNA, partial sequence	2052	2052	100%	0.0	99.21%	NR_043408.1

Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence

Sequence ID: [NR_113337.1](#) Length: 1495 Number of Matches: 1

Range 1: 328 to 1464 [GenBank](#) [Graphics](#)

[Next Match](#) [Prev](#)

Score	Expect	Identities	Gaps	Strand
2071 bits(1121)	0.0	1132/1137(99%)	1/1137(0%)	Plus/Minus
Query 1	GGCTCGCTCCCTAAA-GGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCATGGTGTG	59		
Sbjct 1464	GGCTCGCTCCCTAAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCATGGTGTG	1405		
Query 60	ACGGGCGGTGTGTACAAGGCCGGGAACGTATTACCGCGGCGTGTGATCCGCGATTAC	119		
Sbjct 1404	ACGGGCGGTGTGTACAAGGCCGGGAACGTATTACCGCGGCGTGTGATCCGCGATTAC	1345		
Query 120	TAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACGAGAATGGCTTT	179		
Sbjct 1344	TAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACGAGAATGGCTTT	1285		
Query 180	AAGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGT	239		
Sbjct 1284	AAGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGT	1225		
Query 240	GTAGCCAGGTGATGAAGGGGATGATGATTTGACGTCATCCCACTTCCTCCGGTTTGT	299		
Sbjct 1224	GTAGCCAGGTGATGAAGGGGATGATGATTTGACGTCATCCCACTTCCTCCGGTTTGT	1165		
Query 300	CACCGGCGAGTCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGC	359		
Sbjct 1164	CACCGGCGAGTCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGC	1105		
Query 360	TCGTTGCGGGACTTAACCAACATCTCACGACACGAGCTGACGACAACCATGCACACCT	419		
Sbjct 1104	TCGTTGCGGGACTTAACCAACATCTCACGACACGAGCTGACGACAACCATGCACACCT	1045		
Query 420	GTCATTTTGCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCT	479		
Sbjct 1044	GTCATTTTGCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCT	985		
Query 480	GGTAAGGTTCTTCGCGTTGCTTCGAATTAACACATGCTCCACCGCTTGTGCGGGCCCC	539		
Sbjct 984	GGTAAGGTTCTTCGCGTTGCTTCGAATTAACACATGCTCCACCGCTTGTGCGGGCCCC	925		
Query 540	CGTCAATTCCTTTGAGTTTCAACCTTGCGGTCTGACTCCCAAGGCGGAATGCTTAATGCG	599		
Sbjct 924	CGTCAATTCCTTTGAGTTTCAACCTTGCGGTCTGACTCCCAAGGCGGAATGCTTAATGCG	865		
Query 600	TTAGTGCGGCACTGAAGGGCGGAACCTCCAACACCTAGCATTATCGTTTACGGCAT	659		
Sbjct 864	TTAGTGCGGCACTGAAGGGCGGAACCTCCAACACCTAGCATTATCGTTTACGGCAT	805		
Query 660	GGACTACAGGGTATCTAATCCTGTTGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTA	719		
Sbjct 804	GGACTACAGGGTATCTAATCCTGTTGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTA	745		
Query 720	CAGACCAGACAGCCGCTTCGCCACTGGTGTCTTCCATATATCTACGCATTTACCGCT	779		
Sbjct 744	CAGACCAGACAGCCGCTTCGCCACTGGTGTCTTCCATATATCTACGCATTTACCGCT	685		

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Query 780 ACACATGGAGTTCCACTGTCTCTTCTGCACTCAAGTTTCCAGTTTCCGATGCGCTTCC 839
      |||
Sbjct 684 ACACATGGAGTTCCACTGTCTCTTCTGCACTCAAGTTTCCAGTTTCCGATGCGCTTCC 625

Query 840 TCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAAACCGCTGCGCTCGCTTTACGCC 899
      |||
Sbjct 624 TCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAAACCGCTGCGCTCGCTTTACGCC 565

Query 900 CAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAG 959
      |||
Sbjct 564 CAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAG 505

Query 960 CCGTGGCTTTCTGGTTGGATACCGTCACGCCGGCAACAGTTACTTGCCGACCATTCTTC 1019
      |||
Sbjct 504 CCGTGGCTTTCTGGTTGGATACCGTCACGCCGGCAACAGTTACTTGCCGACCATTCTTC 445

Query 1020 TCCAACAACAGAGTTTACGACCCGAAAGCCTTCTTCACTACGGCGGCTTGCTCCATCA 1079
      |||
Sbjct 444 TCCAACAACAGAGTTTACGACCCGAAAGCCTTCTTCACTACGGCGGCTTGCTCCATCA 385

Query 1080 AACTTGCCTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAATTTGGGCCG 1136
      |||
Sbjct 384 GACTTGCCTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAATTTGGGCCG 328

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C.22 Blast analysis and alignment results for k5-14

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	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☒	Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	2050	2050	100%	0.0	99.30%	NR_113337.1
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	2050	2050	100%	0.0	99.30%	NR_025880.1
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	2049	2049	99%	0.0	99.29%	NR_117987.1
☒	Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	2047	2047	100%	0.0	99.21%	NR_037122.1
☒	Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	2045	2045	100%	0.0	99.21%	NR_113333.1
☒	Lactobacillus rhamnosus strain NBRC 3425 16S ribosomal RNA, partial sequence	2045	2045	100%	0.0	99.21%	NR_113332.1

```

1.zeae      GCGGCGTGCCTAATACATGCAAGTCGAACGAGTTTGGTTCGATGAACGGTGCCTGCATCG
1.paracasei GCGGCGTGCCTAATACATGCAAGTCGAACGAGTTCGTTGATGATCGGTGCCTGCACCG
k5-14      --GGCTCGCTCCCTAAAGGGTTACGCCACCGGCTTCGGTG--TTACAAACTCTCATGGTG
          ***  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

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Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence

Sequence ID: [NR_037122.1](#) Length: 1522 Number of Matches: 1

Range 1: 330 to 1464 [GenBank](#) [Graphics](#)

[▼ Next Match](#) [▲ Previous](#)

Score	Expect	Identities	Gaps	Strand
2047 bits(1108)	0.0	1126/1135(99%)	2/1135(0%)	Plus/Minus
Query 1	GGCTCGCTCCCTAAA-GGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCATGGTGTG	59		
Sbjct 1464	GGCTCGCTCCCTAAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCATGGTGTG	1405		
Query 60	ACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTGCTGATCCGCGATTAC	119		
Sbjct 1404	ACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCGTGCTGATCCGCGATTAC	1345		
Query 120	TAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAAGTGAAGTGGCTTT	179		
Sbjct 1344	TAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAAGTGAAGTGGCTTT	1285		
Query 180	AAGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGT	239		
Sbjct 1284	AAGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGT	1225		
Query 240	GTAGCCAGGTCTAAGGGGCGATGATGATTGACGTCATCCCCACCTTCTCCGGTTTGT	299		
Sbjct 1224	GTAGCCAGGTCTAAGGGGCGATGATGATTGACGTCATCCCCACCTTCTCCGGTTTGT	1165		
Query 300	CACCGGCGAGTCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGC	359		
Sbjct 1164	CACCGGCGAGTCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGC	1105		
Query 360	TCGTTGCGGGACTTAACCAACATCTCAGACACGAGCTGACGACAACCATGCACACCT	419		
Sbjct 1104	TCGTTGCGGGACTTAACCAACATCTCAGACACGAGCTGACGACAACCATGCACACCT	1045		
Query 420	GTCATTTTCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCT	479		
Sbjct 1044	GTCATTTTCCCCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCT	985		
Query 480	GGTAAGGTTCTTCGCGTTGCTTCGAATTAACACATGCTCCACCGCTTGTGCGGGCCCC	539		
Sbjct 984	GGTAAGGTTCTTCGCGTTGCTTCGAATTAACACATGCTCCACCGCTTGTGCGGGCCCC	925		
Query 540	CGTCAATTCTTTGAGTTTCAACCTTTCGGTCTACTCCCCAGGCGGAATGCTTAATGCG	599		
Sbjct 924	CGTCAATTCTTTGAGTTTCAACCTTTCGGTCTACTCCCCAGGCGGAATGCTTAATGCG	865		
Query 600	TTAGCTGCGGCACTGAAGGGCGGAAACCTCCAACACCTAGCATTTCATCGTTTACGGCAT	659		
Sbjct 864	TTAGCTGCGGCACTGAAGGGCGGAAACCTCCAACACCTAGCATTTCATCGTTTACGGCAT	805		
Query 660	GGACTACAGGGTATCTAATCCTGTTTCGCTACCATGCTTTGAGCCTCAGCGTCAGTTA	719		
Sbjct 804	GGACTACAGGGTATCTAATCCTGTTTCGCTACCATGCTTTGAGCCTCAGCGTCAGTTA	745		
Query 720	CAGACCAGACAGCCGCTTCGCCACTGGTGTCTTCCATATATCTACGCATTTACCGCT	779		
Sbjct 744	CAGACCAGACAGCCGCTTCGCCACTGGTGTCTTCCATATATCTACGCATTTACCGCT	685		

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Query 780 ACACATGGAGTTCACCTGTCTCTCTGCACTCAAGTTTCCAGTTTCCGATGCGCTTCC 839
      |||
Sbjct 684 ACACATGGAGTTCACCTGTCTCTCTGCACTCAAGTTTCCAGTTTCCGATGCGCTTCC 625

Query 840 TCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAACCGCTGCGCTCGCTTACGCC 899
      |||
Sbjct 624 TCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAACCGCTGCGCTCGCTTACGCC 565

Query 900 CAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAG 959
      |||
Sbjct 564 CAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAG 505

Query 960 CCGTGGCTTTCTGGTTGGATACCGTCACGCCGGCAACAGTTACTCTGCCGACCATTCCTC 1019
      |||
Sbjct 504 CCGTGGCTTTCTGGTTGGATACCGTCACGCCGGCAACAGTTACTCTGCCGACCATTCCTC 445

Query 1020 TCCAACAACAGAGTTTTACAACCCAAAGCCTTCTTCACTCAGCGGCGTTGCTCCATCA 1079
      |||
Sbjct 444 TCCAACAACAGAGTTTTACGACCCGAAAGCCTTCTTCACTCAGCGGCGTTGCTCCATCA 385

Query 1080 AACTTGCGTCCATTGTGGAAGATCCCTACTGCTGCCTCCCGTAGGA-TTTGGGC 1133
      |||
Sbjct 384 GACTTGCGTCCATTGTGGAAGATCCCTACTGCTGCCTCCCGTAGGAGTTTGGGC 330

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C.23 Blast analysis and alignment results for k5-15

Sequences producing significant alignments

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[Graphics](#)

[Distance tree of results](#)

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☒	Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	2202	2202	100%	0.0	98.79%	NR_113337.1
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	2200	2200	100%	0.0	98.79%	NR_025880.1
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	2198	2198	99%	0.0	98.79%	NR_117987.1
☒	Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	2196	2196	100%	0.0	98.71%	NR_037122.1
☒	Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	2194	2194	100%	0.0	98.71%	NR_113333.1
☒	Lactobacillus casei subsp. casei ATCC 393 16S ribosomal RNA, partial sequence	2194	2194	100%	0.0	98.71%	NR_041893.1
☒	Lactobacillus casei subsp. casei ATCC 393 strain JCM 1134 16S ribosomal RNA, partial sequence	2193	2193	100%	0.0	98.71%	NR_115534.1
☒	Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial sequence	2191	2191	100%	0.0	98.63%	NR_041054.1
☒	Lactobacillus rhamnosus strain NBRC 3425 16S ribosomal RNA, partial sequence	2189	2189	100%	0.0	98.63%	NR_113332.1

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k5-15      TTACGCC-ACCGGCTGCGGT-GTTACAACTCTCA-TGG--TGGACGGGCGGTGT-TACAAGGCCCGGGAACGTATT
l.zeae     GCAAGTCGAACGAGTTTTGTCG--ATGACGGTGCTTGCAATCGA--TTCAACTTAAACGAGTGGCGGACGGGTGAG
l.paracasei GCAAGTCGAACGAGTTCTCGTTG--ATGATCGGTGCTTGCAACGAGA--TTCAACATGAACGAGTGGCGGACGGGTGAG
          * * * * *

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Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence

Sequence ID: [NR_037122.1](#) Length: 1522 Number of Matches: 1

Range 1: 228 to 1464 [GenBank](#) [Graphics](#)

[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
2196 bits(1189)	0.0	1221/1237(99%)	2/1237(0%)	Plus/Minus
Query 1	GGCTCGCTCCCTAAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCATGGTGTG	60		
Sbjct 1464	GGCTCGCTCCCTAAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCATGGTGTG	1405		
Query 61	ACGGGCGGTGTGTACAAGGCCGGGAACGTATTCACCGCGCGTGTGATCCGCGATTAC	120		
Sbjct 1404	ACGGGCGGTGTGTACAAGGCCGGGAACGTATTCACCGCGCGTGTGATCCGCGATTAC	1345		
Query 121	TAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACGAGAATGGCTTT	180		
Sbjct 1344	TAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACGAGAATGGCTTT	1285		
Query 181	AAGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGT	240		
Sbjct 1284	AAGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGT	1225		
Query 241	GTAGCCAGGTGATGAAGGGGATGATGATTTGACGTATCCACCTTCCTCCGGTTTGT	300		
Sbjct 1224	GTAGCCAGGTGATGAAGGGGATGATGATTTGACGTATCCACCTTCCTCCGGTTTGT	1165		
Query 301	CACCGGCGAGTCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGC	360		
Sbjct 1164	CACCGGCGAGTCTTACTAGAGTGCCCAACTRAATGCTGGCAACTAGTCATAAGGGTTGCGC	1105		
Query 361	TCGTTGCGGGACTTAACCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCT	420		
Sbjct 1104	TCGTTGCGGGACTTAACCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCT	1045		
Query 421	GTCATTTTGCCCCGAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCT	480		
Sbjct 1044	GTCATTTTGCCCCGAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCT	985		
Query 481	GGTAAGGTTCTTCGCGTTGCTTCGAATTAACCACATGCTCCACCGCTTGTGCGGGCCCC	540		
Sbjct 984	GGTAAGGTTCTTCGCGTTGCTTCGAATTAACCACATGCTCCACCGCTTGTGCGGGCCCC	925		
Query 541	CGTCAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCGAGGCGAATGCTTAATGCG	600		
Sbjct 924	CGTCAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCGAGGCGAATGCTTAATGCG	865		
Query 601	TTAGCTGCGGCACTGAAGGGCGGAAACCTCCAACACCTAGCATTATCGTTTACGGCAT	660		
Sbjct 864	TTAGCTGCGGCACTGAAGGGCGGAAACCTCCAACACCTAGCATTATCGTTTACGGCAT	805		
Query 661	GGACTACGAGGTATCTAATCCTGTTGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTA	720		
Sbjct 804	GGACTACGAGGTATCTAATCCTGTTGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTA	745		
Query 721	CAGACCAGACAGCCGCTTCGCCACTGGTGTCTTCCATATATCTACGCATTTACCGCT	780		
Sbjct 744	CAGACCAGACAGCCGCTTCGCCACTGGTGTCTTCCATATATCTACGCATTTACCGCT	685		

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Query 781  ACACATGGAGTTTCACTGTCTCTTCTGCACTCAAGTTTCCAGTTTCCGATGCGCTTCC 840
Sbjct 684  ACACATGGAGTTTCACTGTCTCTTCTGCACTCAAGTTTCCAGTTTCCGATGCGCTTCC 625

Query 841  TCGGTTAAGCCGAGGGCTTTACATCAGACTTAAAAAACCGCTGCGCTCGCTTTACGCC 900
Sbjct 624  TCGGTTAAGCCGAGGGCTTTACATCAGACTTAAAAAACCGCTGCGCTCGCTTTACGCC 565

Query 901  CAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAG 960
Sbjct 564  CAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAG 505

Query 961  CCGTGGCTTTCTGGTTGGATACCGTCACGCCGACAACAGTTACTCTGCCGACCATTCCTC 1020
Sbjct 504  CCGTGGCTTTCTGGTTGGATACCGTCACGCCGACAACAGTTACTCTGCCGACCATTCCTC 445

Query 1021  TCCAACAACAGAGTTTTACGACCCGAAAGCCTTCTTCACTCAGGCGGCGTTGCTCCATCA 1080
Sbjct 444  TCCAACAACAGAGTTTTACGACCCGAAAGCCTTCTTCACTCAGGCGGCGTTGCTCCATCA 385

Query 1081  GACTTGCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCTAAGAATTTGGGCCGTGT 1140
Sbjct 384  GACTTGCGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCTAGGAGTTTGGGCCGTGT 325

Query 1141  CTCAGTCCCAATGTGGCCGATCAACCTCTCAATTCGGCTACATATCATCCCTTGGTGAA 1200
Sbjct 324  CTCAGTCCCAATGTGGCCGATCAACCTCTCAGTTCGGCTACGTATCATCGCCTTGGTGAG 265

Query 1201  CCCTTACCTCCCCA-CTAGCTTAAACCCC-CGGGTTC 1235
Sbjct 264  CCGTTACCTCACCAACTAGCTAATACGCCGCGGGTTC 228

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C.24 Blast analysis and alignment results for k6-1

Sequences producing significant alignments						Download	Manage Columns	Show	100	
☒ select all 100 sequences selected										
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession	GenBank Graphics Distance tree of results		
☒	Lactobacillus paracasei subsp. tolerans strain NBRC 15908 16S ribosomal RNA, partial sequence	1832	1832	99%	0.0	97.06%	NR_041054.1			
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA, partial sequence	1832	1832	99%	0.0	97.06%	NR_025880.1			
☒	Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	1829	1829	99%	0.0	96.97%	NR_113337.1			
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	1816	1816	99%	0.0	96.79%	NR_117987.1			
☒	Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	1772	1772	99%	0.0	96.06%	NR_037122.1			
☒	Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	1770	1770	99%	0.0	95.98%	NR_113333.1			
☒	Lactobacillus casei subsp. casei ATCC 393 16S ribosomal RNA, partial sequence	1766	1766	99%	0.0	95.97%	NR_041893.1			
☒	Lactobacillus casei subsp. casei ATCC 393 strain JCM 1134 16S ribosomal RNA, partial sequence	1759	1759	99%	0.0	95.78%	NR_115534.1			
☒	Lactobacillus rhamnosus strain NBRC 3425 16S ribosomal RNA, partial sequence	1738	1738	99%	0.0	95.50%	NR_113332.1			

Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial s

Sequence ID: [NR_041054.1](#) Length: 1497 Number of Matches: 1

[See 1 more title\(s\) ▼](#)

Range 1: 27 to 1115 [GenBank](#) [Graphics](#)

[▼ Next Match](#) [▲ Previous](#)

Score	Expect	Identities	Gaps	Strand
1832 bits(992)	0.0	1058/1090(97%)	4/1090(0%)	Plus/Plus
Query 1	CATGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAA	60		
Sbjct 27	CATGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAA	86		
Query 61	CGAGTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGATAACATTT	120		
Sbjct 87	CGAGTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGATAACATTT	146		
Query 121	GGAAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGG	180		
Sbjct 147	GGAAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGG	206		
Query 181	CGTAAGCTATCGCTTTTGGATGGACCCGCGCGTATTAGCTAGTTGGTGAGGTAATGGCT	240		
Sbjct 207	CGTAAGCTATCGCTTTTGGATGGACCCGCGCGTATTAGCTAGTTGGTGAGGTAATGGCT	266		
Query 241	CACCAAGGCGATGATACGTAGCCGAACCTGAGAGGTTGATCGGCCACATTGGGACTGAGAC	300		
Sbjct 267	CACCAAGGCGATGATACGTAGCCGAACCTGAGAGGTTGATCGGCCACATTGGGACTGAGAC	326		
Query 301	ACGGCCCAAACCTCTACGGGAGGAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTG	360		
Sbjct 327	ACGGCCCAAACCTCTACGGGAGGAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTG	386		
Query 361	ATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTGCGGTCGTAAACTCTGTTGTTGGAGA	420		
Sbjct 387	ATGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTGCGGTCGTAAACTCTGTTGTTGGAGA	446		
Query 421	AGAATGGTCGGCAGAGTAAGTGTGCGGCGTGACGGTATCCAACAGAAAGCCACGGCT	480		
Sbjct 447	AGAATGGTCGGCAGAGTAAGTGTGCGGCGTGACGGTATCCAACAGAAAGCCACGGCT	506		
Query 481	AACTACGTGCCAGCAGCCGCGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGG	540		
Sbjct 507	AACTACGTGCCAGCAGCCGCGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGG	566		
Query 541	CGTAAAGCGAGCGCAGGCGGTTTTTAAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGG	600		
Sbjct 567	CGTAAAGCGAGCGCAGGCGGTTTTTAAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGG	626		
Query 601	AAGCGCATCGGAAACTGGGAACTTGAGTGCAGAAGAAGACAGTGGAACTCCATGTGTAG	660		
Sbjct 627	AAGCGCATCGGAAACTGGGAACTTGAGTGCAGAAGAAGACAGTGGAACTCCATGTGTAG	686		
Query 661	CGGTGAAATGCGTAGATATATGGAAGAACCAGTGGCGAAAGCGGCTGTCTGGTCTGTA	720		
Sbjct 687	CGGTGAAATGCGTAGATATATGGAAGAACCAGTGGCGAAAGCGGCTGTCTGGTCTGTA	746		

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Query 721 ACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCAT 780
      |||
Sbjct 747 ACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCAT 806

Query 781 GCCGTAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAATGCCGCAGCTAACG 840
      |||
Sbjct 807 GCCGTAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAATGCCGCAGCTAACG 866

Query 841 CATTAAAGCATTCCGCCTGGGAGTACGACCGCCAGGTTGAAACTCAAAGGAATTGACGGG 900
      |||
Sbjct 867 CATTAAAGCATTCCGCCTGGGAGTACGACCGCCAGGTTGAAACTCAAAGGAATTGACGGG 926

Query 901 GGCCCCACAACCGGTGGAGCATGTGGTTTAATTC-AAGCAACCCGAGAAACCTTACCAG 959
      |||
Sbjct 927 GGCCCCACAACCGGTGGAGCATGTGGTTTAATTCGAAGCAACCGAGAAACCTTACCAG 986

Query 968 GGCTTGACTTCTTTTATCACCCTGAAAAATCAGGTTTCCCTTCGGGGGCAAAATGACAG 1019
      |||
Sbjct 987 GTCTTGACATCTTTTATCACCCTGAGAGATCAGGTTTCCCTTCGGGGGCAAAATGACAG 1046

Query 1020 ggggggCATGGTTGCTCCACCCCGTCCGGGAAAT-TTGGGTTAATTCCTCCACCAAG 1078
      |||
Sbjct 1047 GTGGTGATGGTTGTCGTGAGCTCGTGTC-GTGAGATGTTGGGTTAAGTCCCGCAACGAG 1105

Query 1079 -GCAACCCCTT 1087
      |||
Sbjct 1106 CGCAACCCCTT 1115

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C.25 Blast analysis and alignment results for k6-3a

Sequences producing significant alignments

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[GenBank](#)[Graphics](#)[Distance tree of results](#)

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☒	Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	1786	1786	100%	0.0	99.49%	NR_042111.1
☒	Lactobacillus gallinarum strain JCM 2011 16S ribosomal RNA, partial sequence	1786	1786	100%	0.0	99.49%	NR_113281.1
☒	Lactobacillus gallinarum strain ATCC 33199 16S ribosomal RNA, partial sequence	1786	1786	100%	0.0	99.49%	NR_117081.1
☒	Lactobacillus helveticus strain NBRC 15019 16S ribosomal RNA, partial sequence	1780	1780	100%	0.0	99.38%	NR_113719.1
☒	Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	1780	1780	100%	0.0	99.38%	NR_117080.1
☒	Lactobacillus helveticus DSM 20075 = CGMCC 1.1877 16S ribosomal RNA, partial sequence	1780	1780	100%	0.0	99.38%	NR_042439.1
☒	Lactobacillus acidophilus strain NBRC 13951 16S ribosomal RNA, partial sequence	1716	1716	100%	0.0	98.56%	NR_113838.1
☒	Lactobacillus acidophilus strain VPI 6032 16S ribosomal RNA, partial sequence	1716	1716	100%	0.0	98.56%	NR_117082.1

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1.helvat      CATCGGAAACTGTTTTCTTGAGTGCAGAAGAGGAGAGTGGAAATCCATGTGTAGCGGTG
k6-3a         CATCGGAAACTGTTTTCTTGAGTGCAGAAGAGGAGAGTGGAAATCCATGTGTAGCGGTG
1.gallinarium CATCGGAAACTGTTTTCTTGAGTGCAGAAGAGGAGAGTGGAAATCCATGTGTAGCGGTG
*****

1.hevat      GAATGCGTAGATATATGGAAGAACACCAAGTGGCGAAGGCGACTCTCTGGTCTGCAACTGA
k6-3a         GAATGCGTAGATATATGGAAGAACACCAAGTGGCGAAGGCGACTCTCTGGTCTGCAACTGA
1.gallinarium GAATGCGTAGATATATGGAAGAACACCAAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGA
*****

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Lactobacillus helveticus strain NBRC 15019 16S ribosomal RNA, partial sequence

Sequence ID: [NR_113719.1](#) Length: 1487 Number of Matches: 1

Range 1: 28 to 1181 [GenBank](#) [Graphics](#)

[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
2080 bits(1126)	0.0	1145/1154(99%)	1/1154(0%)	Plus/Plus
Query 1	TGCAAGTCGAGCGAGCAGAACAGCAGATTACTTCGGTAATGACGCTGGGGACGCGAGC	60		
Sbjct 28	TGCAAGTCGAGCGAGCAGAACAGCAGATTACTTCGGTAATGACGCTGGGGACGCGAGC	87		
Query 61	GGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCATAGCTAGGATACCACTTGGAAA	120		
Sbjct 88	GGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCATAGCTAGGATACCACTTGGAAA	147		
Query 121	CAGGTGCTAATACCGGATAATAAGCAGATCGCATGATCAGCTTATAAAGGCGGCGTAA	180		
Sbjct 148	CAGGTGCTAATACCGGATAAGAAAGCAGATCGCATGATCAGCTTATAAAGGCGGCGTAA	207		
Query 181	GCTGTCGCTATGGGATGGCCCCGCGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCA	240		
Sbjct 208	GCTGTCGCTATGGGATGGCCCCGCGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCA	267		
Query 241	AGGCAATGATGCATAGCCGAGTTGAGAGACTGAACGGCCACATTGGGACTGAGACACGGC	300		
Sbjct 268	AGGCAATGATGCATAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGC	327		
Query 301	CCAAACTCCTACGGGAGGAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGA	360		
Sbjct 328	CCAAACTCCTACGGGAGGAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGA	387		
Query 361	GCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTGGTGAAGAAG	420		
Sbjct 388	GCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTGGTGAAGAAG	447		
Query 421	GATAGAGGTAGTAAGTGGCTTTTATTTGACGGTAATCAACAGAAAGTACCGGCTAACTA	480		
Sbjct 448	GATAGAGGTAGTAAGTGGCTTTTATTTGACGGTAATCAACAGAAAGTACCGGCTAACTA	507		
Query 481	CGTGCCAGCAGCCGCGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAA	540		
Sbjct 508	CGTGCCAGCAGCCGCGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAA	567		
Query 541	AGCGAGCGCAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATTG	600		
Sbjct 568	AGCGAGCGCAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATTG	627		
Query 601	CATCGGAAACTGTTTTCTTGAGTGCAGAAAGAGGAGAGTGGAACTCCATGTGTAGCGGTG	660		
Sbjct 628	CATCGGAAACTGTTTTCTTGAGTGCAGAAAGAGGAGAGTGGAACTCCATGTGTAGCGGTG	687		
Query 661	GAATGCGTAGATATATGGAAGAACACAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGA	720		
Sbjct 688	GAATGCGTAGATATATGGAAGAACACAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGA	747		
Query 721	CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCTGGTAGTCCATGCCGT	780		
Sbjct 748	CGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCTGGTAGTCCATGCCGT	807		

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Query 781 AAACGATGAGTGCTAAGTGTGGGAGGTTTCCGCCCTCAGTGCTGCAGCTAACGCATTA 840
Sbjct 808 AAACGATGAGTGCTAAGTGTGGGAGGTTTCCGCCCTCAGTGCTGCAGCTAACGCATTA 867

Query 841 AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCC 900
Sbjct 868 AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCC 927

Query 901 GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCCTTACCAGGTCTT 960
Sbjct 928 GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCCTTACCAGGTCTT 987

Query 961 GACATCTAGTGCCATCCTAAGAGATTAGGAGTTCCCTTCGGGGACGCTAAGACAGGTGGT 1020
Sbjct 988 GACATCTAGTGCCATCCTAAGAGATTAGGAGTTCCCTTCGGGGACGCTAAGACAGGTGGT 1047

Query 1021 GCATGGCTGTCGTGAGCTCGTGTGTCGTGAGATGTTGGGTTAAGTCCCACAACGAGCGCAAC 1080
Sbjct 1048 GCATGGCTGTCGTGAGCTCGTGTGTCGTGAGATGTTGGGTTAAGTCCCACAACGAGCGCAAC 1107

Query 1081 CCTTATTATTAGTTGCCAGCATTAAAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACC 1140
Sbjct 1108 CCTTGTATTAGTTGCCAGCATTAAAGTTGGGCACTCTAATGAGACTGCCGGTGATAAACC 1167

Query 1141 GGAGGAAGG-GGGG 1153
Sbjct 1168 GGAGGAAGGTGGG 1181

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Alignment

C.26 Blast analysis and alignment results for k6-6a

Sequences producing significant alignments

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	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☒	Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial sequence	1881	1881	99%	0.0	99.52%	NR_041054.1
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	1881	1881	99%	0.0	99.52%	NR_025880.1
☒	Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	1877	1877	99%	0.0	99.42%	NR_113337.1
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	1869	1869	99%	0.0	99.32%	NR_117987.1
☒	Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	1820	1820	99%	0.0	98.45%	NR_037122.1
☒	Lactobacillus casei strain NBRC 16883 16S ribosomal RNA, partial sequence	1818	1818	99%	0.0	98.36%	NR_113333.1
☒	Lactobacillus casei subsp. casei ATCC 393 16S ribosomal RNA, partial sequence	1816	1816	99%	0.0	98.36%	NR_041893.1
☒	Lactobacillus casei subsp. casei ATCC 393 strain JCM 1134 16S ribosomal RNA, partial sequence	1807	1807	99%	0.0	98.16%	NR_115534.1
☒	Lactobacillus rhamnosus strain NBRC 3426 16S ribosomal RNA, partial sequence	1786	1786	99%	0.0	97.87%	NR_113332.1

Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial sequence

Sequence ID: [NR_041054.1](#) Length: 1497 Number of Matches: 1

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Range 1: 35 to 1068 [GenBank](#) [Graphics](#)

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Score	Expect	Identities	Gaps	Strand
1881 bits(1018)	0.0	1029/1034(99%)	1/1034(0%)	Plus/Plus
Query 1	TCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACGAGTGGC	60		
Sbjct 35	TCGAACGAGTTCTCGTTGATGATCGGTGCTTGCACCGAGATTCAACATGGAACGAGTGGC	94		
Query 61	GGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGATAACATTTGGAAACAG	120		
Sbjct 95	GGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGATAACATTTGGAAACAG	154		
Query 121	ATGCTAATACCGCATAGATCCAAGAACCAGCATGGTTCTTGGCTGAAAGATGGCGTAAGCT	180		
Sbjct 155	ATGCTAATACCGCATAGATCCAAGAACCAGCATGGTTCTTGGCTGAAAGATGGCGTAAGCT	214		
Query 181	ATCGCTTTTGGATGGACCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGG	240		
Sbjct 215	ATCGCTTTTGGATGGACCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGG	274		
Query 241	CGATGATACGTAGCCGAAGTGAAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCA	300		
Sbjct 275	CGATGATACGTAGCCGAAGTGAAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCA	334		
Query 301	AACTCCTACGGGAGGCGAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCA	360		
Sbjct 335	AACTCCTACGGGAGGCGAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCA	394		
Query 361	ACGCCGCGTGAGTGAAGAAGGCTTTCGGGTGTAAGAACTCTGTTGTTGGAGAAGAATGGT	420		
Sbjct 395	ACGCCGCGTGAGTGAAGAAGGCTTTCGGGTGTAAGAACTCTGTTGTTGGAGAAGAATGGT	454		
Query 421	CGGCAGAGTAACGTGTCGGCGTGACGGTATCCAACAGAAAGCCACGGCTAACTACGT	480		
Sbjct 455	CGGCAGAGTAACGTGTCGGCGTGACGGTATCCAACAGAAAGCCACGGCTAACTACGT	514		
Query 481	GCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTATTGGGCGTAAAGC	540		
Sbjct 515	GCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTATTGGGCGTAAAGC	574		
Query 541	GAGCGCAGGCGGTTTTTAAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCAT	600		
Sbjct 575	GAGCGCAGGCGGTTTTTAAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCAT	634		
Query 601	CGGAAACTGGGAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAA	660		
Sbjct 635	CGGAAACTGGGAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAA	694		
Query 661	TGCGTAGATATATGGAAGAACACAGTGGCGAAGGCGGCTGTCTGGTCTGTAAGTACGAC	720		
Sbjct 695	TGCGTAGATATATGGAAGAACACAGTGGCGAAGGCGGCTGTCTGGTCTGTAAGTACGAC	754		

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Query 721  TGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCTGGTAGTCCATGCCGTAAA 780
          |||
Sbjct 755  TGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCTGGTAGTCCATGCCGTAAA 814

Query 781  CGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAAGC 840
          |||
Sbjct 815  CGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAAGC 874

Query 841  ATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCA 900
          |||
Sbjct 875  ATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCA 934

Query 901  CAAGCGGTGGAGCATGTGGTTTAAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGAC 960
          |||
Sbjct 935  CAAGCGGTGGAGCATGTGGTTTAAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGAC 994

Query 961  ATCTTTTGATCACCTGAAAGATCAGGTTTCCCTTCGGGGGC-AAATGACAGGGGGTGCA 1019
          |||
Sbjct 995  ATCTTTTGATCACCTGAGAGATCAGGTTTCCCTTCGGGGGCAAAATGACAGGTGGTGCA 1054

Query 1020  TGGTTGCCGTCAGC 1033
          |||
Sbjct 1055  TGGTTGTCGTCAGC 1068

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C. 27 Blast analysis and alignment results for k6-7

Sequences producing significant alignments

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	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☒	Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial sequence	2204	2204	100%	0.0	99.42%	NR_041054.1
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	2204	2204	100%	0.0	99.42%	NR_025880.1
☒	Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	2200	2200	100%	0.0	99.34%	NR_113337.1
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	2191	2191	99%	0.0	99.34%	NR_117987.1
☒	Lactobacillus zeae strain RIA 492 16S ribosomal RNA, partial sequence	2139	2139	100%	0.0	98.44%	NR_037122.1
☒	Lactobacillus casei strain NBRC 16883 16S ribosomal RNA, partial sequence	2135	2135	100%	0.0	98.36%	NR_113333.1
☒	Lactobacillus casei subsp. casei ATCC 393 16S ribosomal RNA, partial sequence	2132	2132	100%	0.0	98.36%	NR_041893.1
☒	Lactobacillus casei subsp. casei ATCC 393 strain JCM 1134 16S ribosomal RNA, partial sequence	2124	2124	100%	0.0	98.19%	NR_115534.1
☒	Lactobacillus rhamnosus strain NBRC 3425 16S ribosomal RNA, partial sequence	2109	2109	100%	0.0	98.03%	NR_113332.1

Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial seq

Sequence ID: [NR_041054.1](#) Length: 1497 Number of Matches: 1

[See 1 more title\(s\) ▼](#)

Range 1: 29 to 1245 [GenBank](#) [Graphics](#)

[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
2204 bits(1193)	0.0	1210/1217(99%)	6/1217(0%)	Plus/Plus
Query 1		TGC-AGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGACCCGAGATTCAACATGGAACG		59
Sbjct 29		TGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGACCCGAGATTCAACATGGAACG		88
Query 60		AGTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGATAACATTTGG		119
Sbjct 89		AGTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGATAACATTTGG		148
Query 120		AAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCG		179
Sbjct 149		AAACAGATGCTAATACCGCATAGATCCAAGAACCGCATGGTTCTTGGCTGAAAGATGGCG		208
Query 180		TAAGCTATCGCTTTTGGATGGACCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA		239
Sbjct 209		TAAGCTATCGCTTTTGGATGGACCGCGGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA		268
Query 240		CCAAGGCGATGATACGTAGCCGAAGTGAAGGTTGATCGGCCACATTGGGACTGAGACAC		299
Sbjct 269		CCAAGGCGATGATACGTAGCCGAAGTGAAGGTTGATCGGCCACATTGGGACTGAGACAC		328
Query 300		GGCCCAAACCTCTACGGGAGGCGAGCAGTAGGGAACTCTCCACAATGGACGCAAGTCTGAT		359
Sbjct 329		GGCCCAAACCTCTACGGGAGGCGAGCAGTAGGGAACTCTCCACAATGGACGCAAGTCTGAT		388
Query 360		GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAACTCTGTTGTTGGAGAAG		419
Sbjct 389		GGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAACTCTGTTGTTGGAGAAG		448
Query 420		AATGGTCGGCAGAGTAAGTGTTCGGGCGTGACGGTATCAACCAGAAAGCCACGGCTAA		479
Sbjct 449		AATGGTCGGCAGAGTAAGTGTTCGGGCGTGACGGTATCAACCAGAAAGCCACGGCTAA		508
Query 480		CTACGTGCCAGCAGCCGCGTAATACGTAGGTGGCAAGCGTTATCCGGATTATTGGGCG		539
Sbjct 509		CTACGTGCCAGCAGCCGCGTAATACGTAGGTGGCAAGCGTTATCCGGATTATTGGGCG		568
Query 540		TAAAGCGAGCGCAGGCGGTTTTTAAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA		599
Sbjct 569		TAAAGCGAGCGCAGGCGGTTTTTAAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA		628
Query 600		GCGCATCGGAAACTGGGAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG		659
Sbjct 629		GCGCATCGGAAACTGGGAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG		688
Query 660		GTGAAATGCGTAGATATATGGAAGAACACCAAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC		719
Sbjct 689		GTGAAATGCGTAGATATATGGAAGAACACCAAGTGGCGAAGGCGGCTGTCTGGTCTGTAAC		748

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Query 728 TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCTGGTAGTCCATGC 779
      |||
Sbjct 749 TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCTGGTAGTCCATGC 808

Query 788 CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCGAGCTAACGCA 839
      |||
Sbjct 809 CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCGAGCTAACGCA 868

Query 848 TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG 899
      |||
Sbjct 869 TTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG 928

Query 908 CCCGCACAAGCGGTGGAGCATGTGGTTAATTGGAAGCAACGCGAAGAACCTTACCAGGT 959
      |||
Sbjct 929 CCCGCACAAGCGGTGGAGCATGTGGTTAATTGGAAGCAACGCGAAGAACCTTACCAGGT 988

Query 968 CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCTTCGGGGGCAAAATGACAGGT 1019
      |||
Sbjct 989 CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCTTCGGGGGCAAAATGACAGGT 1048

Query 1028 GGTGCATGGTTGTCGTGAGCTCGTGTGAGATGTTGGGTTAAGTCCGCAACGAGCGC 1079
      |||
Sbjct 1049 GGTGCATGGTTGTCGTGAGCTCGTGTGAGATGTTGGGTTAAGTCCGCAACGAGCGC 1108

Query 1088 AACCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGAC-A 1138
      |||
Sbjct 1109 AACCTTATGACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAA 1168

Query 1139 ACCGGAGGAAGGTGGGGATGACGTC-AATCATCATGCCCTTATGACC-GGGCTAC-CAC 1195
      |||
Sbjct 1169 ACCGGAGGAAGGTGGGGATGACGTC-AATCATCATGCCCTTATGACC-GGGCTACACAC 1228

Query 1196 GTGCTACA-TGGATGGT 1211
      |||
Sbjct 1229 GTGCTACAATGGATGGT 1245

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C.28 Blast analysis and alignment results for k6-9a

Sequences producing significant alignments

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	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☒	Lactobacillus paracasei subsp. tolerans strain NBRC 15808 16S ribosomal RNA, partial sequence	2274	2274	100%	0.0	98.98%	NR_041054.1
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	2274	2274	100%	0.0	98.98%	NR_025880.1
☒	Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	2270	2270	100%	0.0	98.90%	NR_113337.1
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	2257	2257	100%	0.0	98.75%	NR_117987.1
☒	Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	2209	2209	100%	0.0	98.04%	NR_037122.1
☒	Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	2206	2206	100%	0.0	97.98%	NR_113333.1
☒	Lactobacillus casei subsp. casei ATCC 393 16S ribosomal RNA, partial sequence	2204	2204	100%	0.0	97.98%	NR_041893.1
☒	Lactobacillus casei subsp. casei ATCC 393 strain JCM 1134 16S ribosomal RNA, partial sequence	2194	2194	100%	0.0	97.80%	NR_115534.1
☒	Lactobacillus rhamnosus strain NBRC 3426 16S ribosomal RNA, partial sequence	2180	2180	100%	0.0	97.65%	NR_113332.1

Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial se

Sequence ID: [NR_041054.1](#) Length: 1497 Number of Matches: 1

[See 1 more title\(s\) ▼](#)

Range 1: 29 to 1301 [GenBank](#) [Graphics](#)

[▼ Next Match](#) [▲ Previous](#)

Score	Expect	Identities	Gaps	Strand
2274 bits(1231)	0.0	1261/1274(99%)	7/1274(0%)	Plus/Plus
Query 1		TGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGACCCGAGATTCAACATGGAACG		60
Sbjct 29		TGCAAGTCGAACGAGTTCTCGTTGATGATCGGTGCTTGACCCGAGATTCAACATGGAACG		88
Query 61		AGTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGATAACATTTGG		120
Sbjct 89		AGTGGCGGACGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGATAACATTTGG		148
Query 121		AAACAGATGCTAATACCGCATAGATCCAAGAACCAGCATGGTTCTTGGCTGAAAGATGGCG		180
Sbjct 149		AAACAGATGCTAATACCGCATAGATCCAAGAACCAGCATGGTTCTTGGCTGAAAGATGGCG		208
Query 181		TAAGCTATCGCTTTTGGATGGACCGCGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA		240
Sbjct 209		TAAGCTATCGCTTTTGGATGGACCGCGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA		268
Query 241		CCAAGGCGATGATACGTAGCCGAACGAGAGGTTGATCGGCCACATTGGGACTGAGACAC		300
Sbjct 269		CCAAGGCGATGATACGTAGCCGAACGAGAGGTTGATCGGCCACATTGGGACTGAGACAC		328
Query 301		GGCCCAAACCTCTACGGGAGGAGCAGTAGGGAACTTCCACAATGGACGCAAGTCTGAT		360
Sbjct 329		GGCCCAAACCTCTACGGGAGGAGCAGTAGGGAACTTCCACAATGGACGCAAGTCTGAT		388
Query 361		GGAGCAACGCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAACTCTGTTGTTGGAGAAG		420
Sbjct 389		GGAGCAACGCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAACTCTGTTGTTGGAGAAG		448
Query 421		AATGGTCGGCAGAGTAACGTGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA		480
Sbjct 449		AATGGTCGGCAGAGTAACGTGTCGGCGTGACGGTATCCAACCAGAAAGCCACGGCTAA		508
Query 481		CTACGTGCCAGCAGCCGCGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCG		540
Sbjct 509		CTACGTGCCAGCAGCCGCGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCG		568
Query 541		TAAAGCGAGCGCAGGCGGTTTTTAAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA		600
Sbjct 569		TAAAGCGAGCGCAGGCGGTTTTTAAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAA		628
Query 601		GCGCATCGGAAACTGGGAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG		660
Sbjct 629		GCGCATCGGAAACTGGGAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCG		688
Query 661		GTGAAATGCGTAGATATATGGAAGAACACCAAGTGGCGAAGGCGGCTGTCTGGTCTGTAAAC		720
Sbjct 689		GTGAAATGCGTAGATATATGGAAGAACACCAAGTGGCGAAGGCGGCTGTCTGGTCTGTAAAC		748

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Query 721   TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGC 780
Sbjct 749   TGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGC 808

Query 781   CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGACGCTAACGCA 840
Sbjct 809   CGTAAACGATGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGACGCTAACGCA 868

Query 841   TTAAGCATTCCGCCTGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG 900
Sbjct 869   TTAAGCATTCCGCCTGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGG 928

Query 901   CCCGCACAAGCGGTGGAGCATGTGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGT 960
Sbjct 929   CCCGCACAAGCGGTGGAGCATGTGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGT 988

Query 961   CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCTTCGGGGGCAAAATGACAGGT 1020
Sbjct 989   CTTGACATCTTTTGATCACCTGAGAGATCAGGTTTCCCTTCGGGGGCAAAATGACAGGT 1048

Query 1021  GGTGCATGGTTGTCGTGAGCTCGTGTCTGAGAGATGTTGGGTTAAGTCCCGCAACGAGCGC 1080
Sbjct 1049  GGTGCATGGTTGTCGTGAGCTCGTGTCTGAGAGATGTTGGGTTAAGTCCCGCAACGAGCGC 1108

Query 1081  AACCCTTATGACTAGTTGCCAGCATTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAA 1140
Sbjct 1109  AACCCTTATGACTAGTTGCCAGCATTAGTTGGGCACTCTAGTAAGACTGCCGGTGACAA 1168

Query 1141  -CCGGAAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGGGCTAC-CA 1198
Sbjct 1169  ACCGG-AGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGGGCTACACA 1227

Query 1199  CGTGCTAC-ATGGATGGTACACCAA-TTGCAAAACCGCGAGGTC-AGCTAATCTCTTAAA 1255
Sbjct 1228  CGTGCTACAAATGGATGGTACAAACGAGTTCGAGACCGCGAGGTCAGCTAATCTCTTAAA 1287

Query 1256  GCCTTTC-CAGTTC 1268
Sbjct 1288  GCCATTCTCAGTTC 1301

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C.29 Blast analysis and alignment results for k6-12

Sequences producing significant alignments

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	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	2145	2145	100%	0.0	99.25%	NR_117987.1
☒	Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	2141	2141	100%	0.0	99.16%	NR_113337.1
☒	Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial sequence	2139	2139	100%	0.0	99.16%	NR_041054.1
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	2139	2139	100%	0.0	99.16%	NR_025880.1
☒	Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	2091	2091	100%	0.0	98.41%	NR_037122.1
☒	Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	2087	2087	100%	0.0	98.32%	NR_113333.1
☒	Lactobacillus casei subsp. casei ATCC 393 16S ribosomal RNA, partial sequence	2085	2085	100%	0.0	98.32%	NR_041893.1
☒	Lactobacillus casei subsp. casei ATCC 393 strain JCM 1134 16S ribosomal RNA, partial sequence	2076	2076	100%	0.0	98.16%	NR_115534.1
☒	Lactobacillus rhamnosus strain NBRC 3425 16S ribosomal RNA, partial sequence	2080	2080	98%	0.0	98.47%	NR_113332.1

Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence

Sequence ID: [NR_117987.1](#) Length: 1441 Number of Matches: 1

Range 1: 18 to 1201 [GenBank](#) [Graphics](#)

[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
2145 bits(1161)	0.0	1184/1193(99%)	9/1193(0%)	Plus/Plus
Query 1	CGAGTTTCTCGTTGATGATCGGTGCTTGACCGAGATTCAACATGGGAACGAGTGGCGGA	60		
Sbjct 18	CGAG-TTCTCGTTGATGATCGGTGCTTGACCGAGATTCAACAT-GGAACGAGTGGCGGA	75		
Query 61	CGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATG	120		
Sbjct 76	CGGGTGAGTAACACGTGGGTAACTGCCCTTAAGTGGGGGATAACATTTGGAAACAGATG	135		
Query 121	CTAATACCGCATAGATCCAAGAACCAGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATC	180		
Sbjct 136	CTAATACCGCATAGATCCAAGAACCAGCATGGTTCTTGGCTGAAAGATGGCGTAAGCTATC	195		
Query 181	GCTTTTGGATGGACCCGCGCGTATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGA	240		
Sbjct 196	GCTTTTGGATGGACCCGCGCGTATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGA	255		
Query 241	TGATACGTAGCCGAACAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCAAAC	300		
Sbjct 256	TGATACGTAGCCGAACAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCAAAC	315		
Query 301	TCCTACGGGAGGCGAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACG	360		
Sbjct 316	TCCTACGGGAGGCGAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACG	375		
Query 361	CCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAGCTCTGTTGTTGGAGAAGAATGGTCGG	420		
Sbjct 376	CCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAGCTCTGTTGTTGGAGAAGAATGGTCGG	435		
Query 421	CAGAGTAACGTGTTGTCGGCGTGACGGTATCCAACAGAAAGCCACGGCTAACTACGTGCC	480		
Sbjct 436	CAGAGTAACGTGTTGTCGGCGTGACGGTATCCAACAGAAAGCCACGGCTAACTACGTGCC	495		
Query 481	AGCAGCCGCGGTAAACGTAGGTGGCAAGCGTTATCCGGATTATTGGGCGTAAAGCGAG	540		
Sbjct 496	AGCAGCCGCGGTAAACGTAGGTGGCAAGCGTTATCCGGATTATTGGGCGTAAAGCGAG	555		
Query 541	CGCAGGCGGTTTTTAAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGG	600		
Sbjct 556	CGCAGGCGGTTTTTAAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAAGCGCATCGG	615		
Query 601	AAACTGGGAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGC	660		
Sbjct 616	AAACTGGGAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGC	675		
Query 661	GATAGATATGGAAGAACACCAAGTGGCGAAGGCGGCTGTCTGGTCTGTAAGTACGCTGA	720		
Sbjct 676	GATAGATATGGAAGAACACCAAGTGGCGAAGGCGGCTGTCTGGTCTGTAAGTACGCTGA	735		
Query 721	GGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGA	780		
Sbjct 736	GGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGA	795		

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Query 781 TGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGCATT 840
Sbjct 796 TGAATGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGCATT 855

Query 841 CCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACA 900
Sbjct 856 CCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACA 915

Query 901 GCGGTGGAGCATGTGGTTTAAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATC 960
Sbjct 916 GCGGTGGAGCATGTGGTTTAAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATC 975

Query 961 TTTTGATCACCTGAGAGATCAGGTTTCCCTTCGGGGGCAAAATGACAGGTGGTGATGG 1020
Sbjct 976 TTTTGATCACCTGAGAGATCAGGTTTCCCTTCGGGGGCAAAATGACAGGTGGTGATGG 1035

Query 1021 TTGTCGTAGCTCGTGTCTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTTAT 1080
Sbjct 1036 TTGTCGTAGCTCGTGTCTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTTAT 1095

Query 1081 GACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCCCGGTGACAAACCCGGAG 1140
Sbjct 1096 GACTAGTTGCCAGCATTTAGTTGGGCACTCTAGTAAGACTGCC-GGTGACAAA-CCGGAG 1153

Query 1141 GAAGGTGGGGGATGAACGTCAAATCATCATGCCCTTATGAACCTGGGGCTA 1193
Sbjct 1154 GAAGGTGGGG-ATG-ACGTCAAATCATCATGCCCTTATGA-CTTGGG-CTA 1201

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C.30 Blast analysis and alignment results for k6-14

Sequences producing significant alignments

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[Distance tree of results](#)

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☒	Lactobacillus paracasei strain NBRC 15889 16S ribosomal RNA, partial sequence	1993	1993	100%	0.0	99.54%	NR_113337.1
☒	Lactobacillus paracasei strain R094 16S ribosomal RNA gene, partial sequence	1993	1993	100%	0.0	99.54%	NR_025880.1
☒	Lactobacillus paracasei strain ATCC 25302 16S ribosomal RNA, partial sequence	1991	1991	99%	0.0	99.54%	NR_117987.1
☒	Lactobacillus zeae strain RIA 482 16S ribosomal RNA, partial sequence	1989	1989	100%	0.0	99.45%	NR_037122.1
☒	Lactobacillus casei strain NBRC 15883 16S ribosomal RNA, partial sequence	1988	1988	100%	0.0	99.45%	NR_113333.1
☒	Lactobacillus rhamnosus strain NBRC 3426 16S ribosomal RNA, partial sequence	1988	1988	100%	0.0	99.45%	NR_113332.1
☒	Lactobacillus casei subsp. casei ATCC 393 16S ribosomal RNA, partial sequence	1988	1988	100%	0.0	99.45%	NR_041893.1
☒	Lactobacillus casei subsp. casei ATCC 393 strain JCM 1134 16S ribosomal RNA, partial sequence	1988	1988	100%	0.0	99.45%	NR_115534.1
☒	Lactobacillus paracasei subsp. tolerans strain NBRC 15906 16S ribosomal RNA, partial sequence	1984	1984	100%	0.0	99.38%	NR_041054.1
☒	Lactobacillus rhamnosus strain JCM 1136 16S ribosomal RNA, partial sequence	1980	1980	100%	0.0	99.27%	NR_043408.1

```

L.paracasei      CGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAACTTGAGTGCAGAAGAGGACAGTGG
L.zeae          CGGCTTAACCGAGGAAGCGCATCGGAAACTGGGAACTTGAGTGCAGAAGAGGACAGTGG
L.rhamnosus     CGGCTTAACCGAGGAAGTGCATCGGAAACTGGGAACTTGAGTGCAGAAGAGGACAGTGG
k6-14           TGGTGTGAC--GGGCGGTGTGTACAAGGCCCGGAACGTATTACCGCGGCGTGC--TGA
                ** * * * * * * * * * * * * * * * * * * * * * *

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Lactobacillus rhamnosus strain NBRC 3425 16S ribosomal RNA, partial sequence

Sequence ID: [NR_113332.1](#) Length: 1495 Number of Matches: 1

Range 1: 370 to 1464 [GenBank](#) [Graphics](#)

[▼ Next Match](#) [▲ Previous](#)

Score	Expect	Identities	Gaps	Strand
1988 bits(1076)	0.0	1089/1095(99%)	2/1095(0%)	Plus/Minus
Query 1		GGCTCGCTCCCTAAA-GGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCATGGTGTG		59
Sbjct 1464		GGCTCGCTCCCTAAAAGGGTTACGCCACCGGCTTCGGGTGTTACAAACTCTCATGGTGTG		1405
Query 60		ACGGGCGGTGTGTACAAGGCCCGGGAACGTATTACCGCGCGTGTGATCCGCGATTAC		119
Sbjct 1404		ACGGGCGGTGTGTACAAGGCCCGGGAACGTATTACCGCGCGTGTGATCCGCGATTAC		1345
Query 120		TAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACGTAGAATGGCTTT		179
Sbjct 1344		TAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAGTCCGAACGTAGAATGGCTTT		1285
Query 180		AAGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGT		239
Sbjct 1284		AAGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTTGTACCATCCATTGTAGCACGTGT		1225
Query 240		GTAGCCAGGTCTAAGGGGCGATGATGATTGACGTATCCCCACCTTCCTCCGGTTTGT		299
Sbjct 1224		GTAGCCAGGTCTAAGGGGCGATGATGATTGACGTATCCCCACCTTCCTCCGGTTTGT		1165
Query 300		CACCGGCGAGTCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGC		359
Sbjct 1164		CACCGGCGAGTCTTACTAGAGTGCCCAACTAAATGCTGGCAACTAGTCATAAGGGTTGCGC		1105
Query 360		TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGTGACGACAAACATGCACACCT		419
Sbjct 1104		TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGTGACGACAAACATGCACACCT		1045
Query 420		GTCATTTTGCCTCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCT		479
Sbjct 1044		GTCATTTTGCCTCCGAAGGGGAAACCTGATCTCTCAGGTGATCAAAAGATGTCAAGACCT		985
Query 480		GGTAAGGTTCTTCGCGTTGCTTCGAATTAACACCATGCTCCACCGCTTGTGCGGGCCCC		539
Sbjct 984		GGTAAGGTTCTTCGCGTTGCTTCGAATTAACACCATGCTCCACCGCTTGTGCGGGCCCC		925
Query 540		CGTCAATTCTTTGAGTTTCAACCTTGCGGTGCTACTCCCCAGGCGGAATGCTTAATGCG		599
Sbjct 924		CGTCAATTCTTTGAGTTTCAACCTTGCGGTGCTACTCCCCAGGCGGAATGCTTAATGCG		865
Query 600		TTAGTGCAGGCACTGAAGGGCGGAAACCTCCAACACCTAGCATTATCGTTTACGGCAT		659
Sbjct 864		TTAGTGCAGGCACTGAAGGGCGGAAACCTCCAACACCTAGCATTATCGTTTACGGCAT		805
Query 660		GGACTACGAGGATCTAATCCTGTTGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTA		719
Sbjct 804		GGACTACGAGGATCTAATCCTGTTGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTA		745
Query 720		CAGACCAGACAGCCGCTTCGCCACTGGTGTCTTCCATATATCTACGCATTTACCGCT		779
Sbjct 744		CAGACCAGACAGCCGCTTCGCCACTGGTGTCTTCCATATATCTACGCATTTACCGCT		685

Query	600	TTAGCTGCGGCACTGAAGGGCGGAAACCTCCAACACCTAGCATTATCGTTTACGGCAT	659
Sbjct	864	TTAGCTGCGGCACTGAAGGGCGGAAACCTCCAACACCTAGCATTATCGTTTACGGCAT	805
Query	660	GGACTACCAGGGTATCTAATCCTGTTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTA	719
Sbjct	804	GGACTACCAGGGTATCTAATCCTGTTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTA	745
Query	720	CAGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTACCGCT	779
Sbjct	744	CAGACCAGACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTACCGCT	685
Query	780	ACACATGGAGTTCACCTGTCTCTTCTGCACTCAAGTTTCCAGTTTCCGATGCGCTTCC	839
Sbjct	684	ACACATGGAGTTCACCTGTCTCTTCTGCACTCAAGTTTCCAGTTTCCGATGCGCTTCC	625
Query	840	TCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAAACCGCCTGCGCTCGCTTTACGCC	899
Sbjct	624	TCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAAACCGCCTGCGCTCGCTTTACGCC	565
Query	900	CAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAG	959
Sbjct	564	CAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAG	505
Query	960	CCGTGGCTTTCTGGTTGGATACCGTCACGCCGGCAACAGTTACTCTGCCGACCATTTCTC	1019
Sbjct	504	CCGTGGCTTTCTGGTTGGATACCGTCACGCCGGCAACAGTTACTCTGCCGACCATTTCTC	445
Query	1020	TCCA-CAACAAAATTTTACGACCCGAAAGCCTTCTTCACTCACGCGGCGTTGCTCCATCA	1078
Sbjct	444	TCCAACAAACAGAGTTTACGACCCGAAAGCCTTCTTCACTCACGCGGCGTTGCTCCATCA	385
Query	1079	GACTTGCGTCCATTG	1093
Sbjct	384	GACTTGCGTCCATTG	370



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EDUCATION

Degree	Institution	Year of Graduation
MS	Egypt, Zagazig University, Faculty of Science Department of Food Microbiology.	2008
BS	Egypt, Zagazig University, Faculty of Science, Department of Food Microbiology.	2003
High School	Zagazig secondary High School ,Egypt.	1999

WORK EXPERIENCE

Year	Place	Enrollment
2003-2008	Egypt, Zagazig University	Research Assistant

FOREIGN LANGUAGES

Advanced English

PUBLICATIONS

-Eman Y. Tohamy and Manal S. El said. 2007. Detoxification of aflatoxin B1 by certain microorganisms. Egyptian journal of biotechnology.

HOBBIES

Computer technologies, Tennis, Travel, Reading.